

Assessment of heavy metals in *Egretta thula*. Case study: Coroa Grande mangrove, Sepetiba Bay, Rio de Janeiro, Brazil

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(With 2 figures)

Abstract

This study focuses on metals analysis in kidney and liver tissues of *Egretta thula* which were collected prostrate or newly dead in Coroa Grande mangrove, Sepetiba Bay, Rio de Janeiro, Brazil, between March 2005 and October 2008. Kidney and liver were collected and analysed to evaluate heavy metal pollution. High values and widest range were detected for all metals in liver and kidney tissues. Geometric mean differences from metals concentrations for Zn, Cd, Ni, Pb, Cu, and Cr, respectively, were found in both organs. Results from linear regression analysis were non-significant in kidney ($r = -0.79975$, $P = 0.10428$), and in liver ($r = -0.53193$, $P = 0.35618$). With ANOVA analysis for metal accumulation differences (kidney*liver), at the 0.05 level, the results were significantly different ($F = 33.17676$, $P = 0.00000$; $F = 12.47880$, $P = 0.00000$). These results indicate that Sepetiba Bay shows worrying levels of metals in this study with *E. thula*, showing potential power of widespread biological and mutagenic adverse effects in trophic levels, and therefore, signalling risk to human health.

Keywords: heavy metal, contamination, Sepetiba Bay, *Egretta thula*.

Avaliação de metais pesados em *Egretta thula*. Estudo de caso: Manguezal de Coroa Grande, Baía de Sepetiba, Rio de Janeiro, Brasil

Resumo

Este estudo centra-se na análise de metais nos rins e fígado de tecidos de *Egretta thula* que foram recolhidas prostradas ou recém mortas no manguezal de Coroa Grande, Baía de Sepetiba, Rio de Janeiro, Brasil, entre março de 2005 e outubro de 2008. Valores elevados e ampla faixa foram detectados para todos os metais tanto no fígado quanto no rim. Foram encontradas diferenças nas concentrações médias geométricas de Zn, Cd, Ni, Pb, Cu e Cr, respectivamente, em ambos os órgãos. Resultados de análise de regressão linear foram não significativos no rim ($r = -0,79975$, $P = 0,10428$), e no fígado ($r = -0,53193$, $P = 0,35618$). Com a análise ANOVA para avaliar diferenças de acumulação de metais (rim*fígado), no nível 0,05, os resultados foram significativamente diferentes ($F = 33,17676$, $P = 0,00000$; $F = 12,47880$, $P = 0,00000$). Estes resultados indicam que a Baía de Sepetiba apresenta níveis preocupantes de metais, baseando-se neste estudo com *E. thula* como indicador da avifauna local, evidenciando potencial poder de generalização adversa de efeitos biológicos e mutagênicos em níveis tróficos, e por conseguinte, sinalizando risco para a saúde humana.

Palavras-chave: metais pesados, contaminação, Baía de Sepetiba, *Egretta thula*.

1. Introduction

The pollution of aquatic systems is due not only to natural causes but above all to anthropogenic activity such as discharges of domestic or industrial effluents, leaching and runoff of pesticides in agricultural lands, among others (Amado Filho et al., 1999). The nature of metals from both natural and anthropogenic sources combined with their necessity in biological processes produces a multifaceted system for assessment. Metal distributions in abiotic and

biotic systems should be examined to precisely evaluate impact on ecosystems. Wildlife studies of exposure and effect can be challenging, but the results are more complete than evaluation of only metal concentrations.

Birds are good sentinel species because they are observable, sensitive to toxicants, and live in different trophic positions (Custer and Osborn, 1977; Walsh, 1990; Prichard et al., 1997; Spalding et al., 1997). Consequently,

studies assessing avian population status, reproductive success, and toxicological importance of metal exposures can be extrapolated to other wildlife and probably humans.

Egretta thula (Molina, 1782) is a heron species that occurs in temperate and tropical America with a wide distribution in Brazil, mainly at the study site, and due to present characteristics, it is favourable as an important indicator. The data found is expressive and could help in future decisions that can interfere and help in environment control. Moreover this research corroborates with other studies in the same domain (White and Cromartie, 1985; Honda et al., 1986; Shaw-Allen et al., 2005).

Heavy metals are naturally present in various natural segments. However, human activity related to industrial development has changed the biogeochemical cycles influencing the transfer of these elements (Savinov et al., 2003; Pereira and Kuch, 2005). Many of them are able to form complexes with organic substances achieving concentrations up to 100 times higher than their assimilation and fixation in tissues, becoming toxic to organisms. The effects of these metals can be bioaccumulative, sublethal or lethal to all components of the biota, such as phytoplankton, zooplankton, benthos, fish, birds, and finally humans. These effects can be carcinogenic, teratogenic and mutagenic (Esteban and Castaño, 2009).

Knowledge of the ocean and the impact of human activities on it can reveal the complexity and interdependence of all aspects of the system (Costanza and Farley, 2007). Improved acquaintance and predictive capabilities are required for more effective and sustained development in marine environment to obtain associated economic benefits and to preserve marine resources. Recent concerns about

connectivity of ocean health issues and the relationship to human disease highlight an important area for study (Juresa and Blanusa, 2003; Ferreira, 2009; Pereira and Ebecken, 2009).

The aquatic environment with its water quality is considered the main factor controlling the state of health and disease in both man and animal (Carvalho et al., 2000). Nowadays, the increasing use of the waste chemical and agricultural drainage systems represents the most dangerous chemical pollution. The most important heavy metals from the point of view of water pollution are Zn, Cu, Pb, Cd, Ni and Cr. Some of these metals (e.g. Cu, Ni, Cr and Zn) are essential trace metals for living organisms, but become toxic at higher concentrations. Others, such as Pb and Cd, have no known biological function but are toxic elements (Andreani et al., 2008).

The purpose of this work was to evaluate selected metal concentrations in different tissues of *E. thula* collected from Sepetiba Bay, which is situated in the southern Atlantic Coast of Rio de Janeiro state, Brazil (Figure 1), and to establish comparisons with maximum permissible concentration standards. This area constitutes an important natural breeding place for molluscs, crustaceans, fish, and at Coroa Grande mangrove, many species of seabirds, with *E. thula* presenting a great number (Amado Filho et al., 1999; Dittmar et al., 2006). However, several environmental problems have been brought to the Bay, due to poor sanitation conditions, including domestic and industrial sewage effluent, bringing about injuries mainly related to heavy metals bioaccumulation in trophic levels (Copeland et al., 2003; Lacerda and Molisani, 2006), motivating this study.

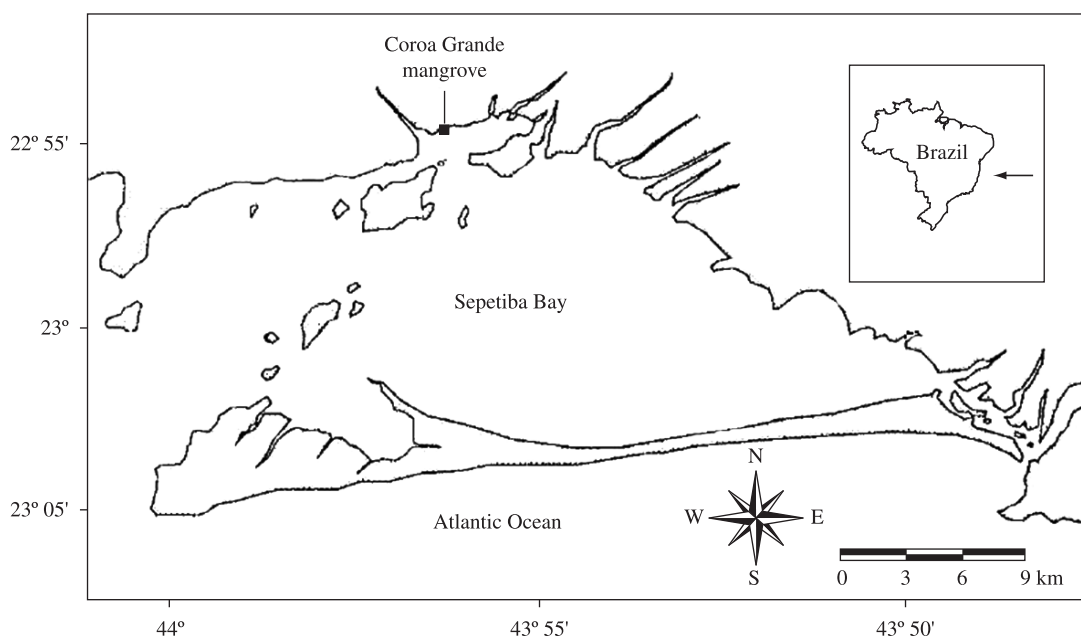


Figure 1. Study area: Coroa Grande mangrove, Sepetiba Bay, Rio de Janeiro (■).

2. Material and Methods

A total of forty-two adult *E. thula*, stranded and dead, were collected in Coroa Grande mangrove, Sepetiba Bay, Rio de Janeiro state, between March 2006 and October 2008. The carcasses were necropsied according to Jauniaux et al. (1998), while putrefactive specimens were discarded. Organs were collected (liver, kidney), weighed, and kept frozen (-20 °C) prior to analysis.

In the laboratory, two identical samples were prepared from the liver and kidney, weighing approximately 100 mg each. One sample from each tissue was kept for the toxicological analysis and the other was taken to dry in an oven at 60 °C until a constant weight to allow turning the concentrations of various elements, obtained primarily in terms of wet weight of sample concentrations on the dry weight of the sample analysed.

Separated tissue samples were dried to a constant weight for several days at 60 °C and then homogenised. Whenever possible, two aliquots of approximately 300 mg of each homogenised dry sample were digested with 5 ml of 65% HNO₃ and 0.3 ml of 70% HClO₄ at 80 °C for 24 hours.

Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used for the determination of chromium, copper, nickel, lead, zinc, and cadmium; with the advantage of determining multi-elements in larger amounts, minors, and traces without changes in experimental parameters. The following absorption lines were used: Cr 267.716 nm, Cu 324.754 nm, Ni 231.604 nm, Pb 220.353 nm, Zn 213.856 nm, and Cd 226.502 nm. Accuracy and reproducibility of the methods were tested using muscle (Dorm-2, National Research Council, Canada) certified material. Standard and blanks were analysed along with each set of samples. Concentrations are expressed as µg.g⁻¹ wet weight (w.wt.).

Statistical analysis was performed using Origin 7.5 software package (Origin Lab Corporation). Linear regression was used to check for significant relationships between metal concentrations (Zn, Pb, Cd, Ni, Cr, and Cu), liver and kidney. In all cases, the level of significance was set at Po0:05. One-way variance analysis (Anova) was utilised in order to verify a possible difference among the metal and organs.

3. Results and Discussion

The geometric mean concentrations of heavy metals for each organ are summarised in Table 1. The highest heavy metal values in kidney were recorded for chromium. Chromium also had the widest range of concentrations in liver; likewise for all other metals in the organs studied, the levels found also had high values. Geometric mean concentrations varied from 1.5 to 132.1 in the kidney and from 1.4 to 112.7 in liver. The maximum permissible concentrations (MPC) were those established by the Brazilian Ministry of Health (Decree Law N° 55871/65, Decree Law N° 685/98) (Brasil, 1977, 1988). The limits established for the metals Cd, Pb, Zn, Cr, Cu, and Ni, are respectively, 1.0, 8.0, 50.0, 0.10, 30.0, 5.0 µg.g⁻¹.

Figure 2 shows the results from linear regression analysis denoting high negative correlation in kidney ($r = -0.79975$, $P = 0.10428$), and median correlation ($r = -0.53193$, $P = 0.35618$) in liver.

Table 2 shows the metal accumulation differences (kidney*liver) of *E. thula*, from the Anova analysis. In relation to analysis in kidney and metals, at the 0.05 level, the metals presence is significantly different ($F = 33.17676$, $P = 0.00000$). Also this fact occurs in analysis done in liver and metals, where at the 0.05 level, the metals presence is significantly different ($F = 12.47880$, $P = 0.00000$). When the analysis was done with each metal tested in kidney and liver, all the results demonstrated that, at the 0.05 level, they were not significantly different ($F = 3.58167$, $P = 0.09506$; $F = 0.82051$, $P = 0.39149$; $F = 0.04889$, $P = 0.83054$; $F = 0.67931$, $P = 0.43371$; $F = 4.89743$, $P = 0.05781$), for Zn, Cd, Ni, Pb, Cu, and Cr, respectively.

Examination of metal uptake and accumulation in *E. thula* inhabiting the site provided useful information about metal availability, uptake, and distribution that can be used for health effects assessments to determine risk and effects from such exposures. There are scarce available data on levels of metals in birds in Sepetiba Bay. Most of the research focuses on fish, a fact that initially guided the selection of metals to be included in this study. Data show worrying levels of some metals in some fish species and similar levels were expected for the analysed samples. However, all searched metals showed higher concentrations.

Table 1. Ranges of element concentrations (µg.g⁻¹ metal, w.wt.) in the organs (liver and kidney) of *E. thula*.

Element	MPC (µg.g ⁻¹)	Organ			
		Kidney		Liver	
		Geometric mean (µg.g ⁻¹)	Range (µg.g ⁻¹)	Geometric mean (µg.g ⁻¹)	Range (µg.g ⁻¹)
Cd	1.0	4.83	2.15-11.09	7.03	3.04-10.87
Zn	50.0	101.45	77.7-132.1	57.26	28.3-112.7
Cu	30.0	72.40	43.11-115.7	43.91	27.1-60.17
Pb	8.0	41.45	24.1-67.15	51.24	39.2-68.33
Cr	0.10	3.45	1.5-9.44	2.58	1.4-4.21
Ni	5.0	9.86	7.23-14.15	8.76	4.43-16.7

MPC = Maximum Permitted Concentrations.

Table 2. Metal accumulation differences (kidney*livrer) of *E. thula*, from the ANOVA analysis.

Kidney				Liver			
Dataset	Mean	SD	SE	Dataset	Mean	SD	SE
Data1_CdK	5,732	3,67246	1,64237	Data1_CdL	7,648	2,98052	1,33293
Data1_CrK	4,428	3,37445	1,5091	Data1_CrL	2,756	1,03433	0,46256
Data1_CuK	76,742	28,68972	12,83043	Data1_CuL	45,572	12,99295	5,81062
Data1_NiK	10,238	3,09586	1,38451	Data1_NiL	9,68	4,71783	2,10988
Data1_PbK	44,378	17,96886	8,03592	Data1_PbL	52,156	11,06378	4,94787
Data1_znk	102,93	19,6004	8,76557	Data1_ZnL	66,534	38,27608	17,11759
ANOVA							
Source	DoF	Sum of Squares	Mean Square	F Value	P Value		
Model	20	43257,3427	8651,46855	33,17676	0,00000		
Error	24	6258,45420	260,768925				
At the 0,05 level, the concentrations in kidney are significantly different.							
Zn kidney*livrer							
Source	DoF	Sum of Squares	Mean Square	F Value	P Value		
Model	1	3311,67204	3311,67204	3,58167	0,09506		
Error	8	7396,93752	924,617190				
At the 0,05 level, Zn are not significantly different.							
Ni kidney*livrer							
Source	DoF	Sum of Squares	Mean Square	F Value	P Value		
Model	1	0,778410000	0,778410000	0,04889	0,83054		
Error	8	127,369280	15,9211600				
At the 0,05 level, Ni are not significantly different.							
Cu kidney*livrer							
Source	DoF	Sum of Squares	Mean Square	F Value	P Value		
Model	1	2428,92225	2428,92225	4,89743	0,05781		
Error	8	3967,66576	495,958220				
At the 0,05 level, Cu are not significantly different.							
Pb kidney*livrer							
Source	DoF	Sum of Squares	Mean Square	F Value	P Value		
Model	1	151,243210	151,243210	0,67931	0,43371		
Error	8	1781,14760	222,643450				
At the 0,05 level, Pb are not significantly different.							
Cr kidney*livrer							
Source	DoF	Sum of Squares	Mean Square	F Value	P Value		
Model	1	6,98896000	6,98896000	1,12212	0,32040		
Error	8	49,8270000	6,22837500				
At the 0,05 level, Cr not significantly different.							

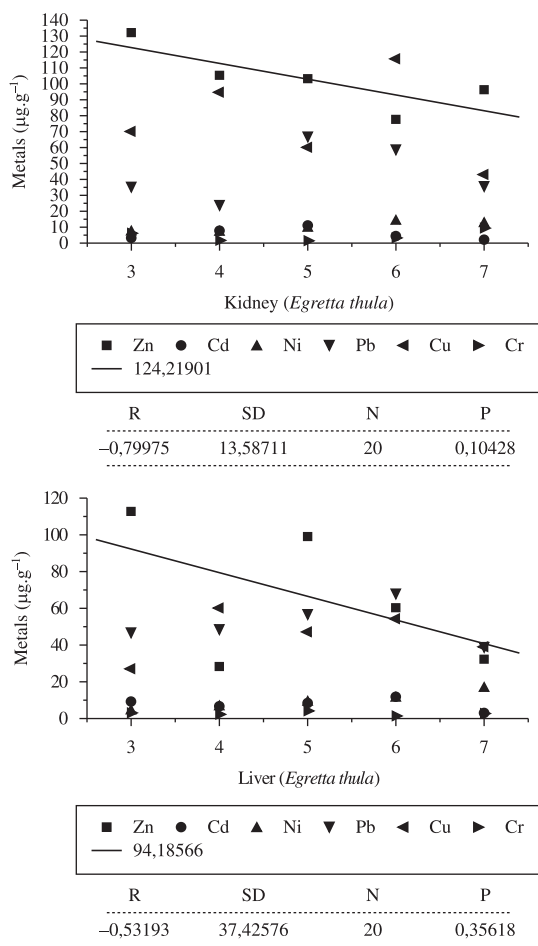


Figure 2. Linear regression analysis between metal concentrations (Zn, Pb, Cd, Ni, Cr, Cu), liver and kidney of *E. thula*.

The levels of Cd, Zn, Pb, and Cu found in seabirds are the result of two main processes: bioaccumulation through food and biodegradation of environmental processes (Johansen et al., 2006). According to Schmitt-Jansen et al. (2008) the sub-lethal effects on birds include growth retardation, suppression of egg production, thinner egg shell and changes in behaviour. Cadmium is known for its long half-life in biological systems (decades in humans and years in birds), and 0.1-1.0% of ingested Cd is absorbed through the avian gastrointestinal tract to be distributed to the kidney and liver. Also, the *metallothionein* binds the Cd to the liver (Seebaugh et al., 2005), a bond which enables the reduction of potential toxic effects.

The high levels of Cd detected in the kidneys are greater than expected, and on the other hand, when compared to the liver, may indicate high exposure (Thompson et al., 2007). The *metallothionein* is a group of soluble molecules of low molecular weight, characterised by its stability at high temperatures, high content in cysteine and the absence of flavoured amino acids. These proteins act in the transport and storage of elements. They also provide protection for the effects of certain toxic metals, sequestering them and

decreasing the amount of free metal ion (Bustamante et al., 2008).

The levels of essential metals such as Zn are metabolically regulated in seabird tissues. Zn has an important role in many metabolic processes, especially in the activation of enzymes and the regulation of gene expression, and therefore its higher concentration (Savinov et al., 2003). In fact, levels of Zn were higher in both the kidney and in liver.

Lead absorption from the gastrointestinal tract ranges from 4-70% depending on the form of Pb ingested and the age of the exposed individual. A large amount of lead is deposited into bone, which acts as a depot that provides a reliable indication of long-term exposure.

Heavy metals comprise a significant part of pollutants in the marine environment. It is important to distinguish between the introduction of these metals from anthropogenic activities and those from natural weathering processes. Although sources of heavy metals in the marine environment are relatively diverse, in Sepetiba Bay there is great evidence of widespread adverse biological effects in fish, providing risks to human health posed by metals in seafood (Karez et al., 1994). The basis of toxicity for some metals support and anticipate problems due to its speciation and more effort should be focused on research in the future.

At the present time there are unprecedented pressures on natural resources (Lauwerys and Hoet, 1993). Sustainable development of these resources is hindered by an inability to detect emerging environmental problems at an early stage when remedial measures can still be effective. Nowhere is this inadequacy so pronounced as in the marine environment. Global energy cycles and the biological processes upon which all life depends are critically influenced by the ocean (Pain et al., 1998; Pereira and Ebecken, 2009).

The aquatic environment with its water quality is considered the main factor controlling the state of health and disease in both man and animal. Nowadays, the increasing use of waste chemicals and agricultural drainage systems represents the most dangerous chemical pollution (Karez et al., 1994; Lacerda and Molisani, 2006). Heavy metals are natural components of marine ecosystems, but their levels may be elevated as a result of increased input into the oceans resulting from industrial activities. In some cases the concentrations of certain metals in marine waters have reached levels which cause damage to wildlife populations and created serious human health problems. Identifying levels in wildlife which are elevated as a result of pollution is difficult, since very few data have been reported concerning the natural levels of metals in any species of marine vertebrates (Storelli et al., 2007).

The assessment of environmental variables and biological effects in seabirds will provide critical insights into the level and extent in public health effects associated with marine areas and resources. Also, the direct contaminant loads and exposure will assist regional, and consequently, national decision makers in efforts to ensure the sustained protection of marine ecosystems.

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