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Trace metals concentration in *Fregata magnificens*: A case study of Coroa Grande Mangrove in Sepetiba Bay, Rio de Janeiro, Brazil

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Concentrations of trace metals, cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb), chromium (Cr), and nickel (Ni) were determined in Magnificent Frigatebird (*Fregata magnificens*) harvested in Sepetiba Bay, Rio de Janeiro, Brazil. Mean concentration levels in liver and kidney ($\mu g.g^{-1}$ dry weight) were 8.04 and 5.57 (Cd); 66.44 and 54.64 (Zn); 45.29 and 46.89 (Cu); 44.67 and 37.35 (Pb); 2.94 and 5.02 (Cr); and 8.12 and 5.98 (Ni), respectively. Nowadays, there is growing conviction that seabird surveys provide important geographical and temporal evidence patterns of aquatic ecosystems conditions, and for this reason the present study provides new insights to use seabirds as bio-sentinel for aquatic pollutants. The results reflect higher concentrations of metals in *F. magnificens*. These findings are highly the importance of heavy metals accumulation in the aquatic system and portend a serious health risk to human consumption and along marine trophic chains.

Key words: Trace metals, concentrations, Sepetiba Bay, *Fregata magnificens*.

INTRODUCTION

Seabirds gather expressive levels of trace elements due to their position in marine food webs and their long life duration (Seebaugh et al., 2005). Heavy metals enter the aquatic environment through the earth crust which has led to steady state background level in aquatic environment. In addition to the geological weathering, human activities such as Municipal, industrial wastes, beside the atmospheric route have also introduced large quantities of heavy metals to localized area (Amado et al., 1999; Santos et al., 2008).

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. Seabirds are good sentinel species because they are observable,

sensitive to toxicants, and live in different trophic positions (Ferreira and Horta, 2010). Consequently, studies assessing avian population status, reproductive success, and toxicological importance of metal exposures can be extrapolated to other wildlife and probably humans.

Heavy metals are naturally present in various natural segments. However, human activity exerted for stimulus provided by industrial development, has changed the biogeochemical cycles influencing the transfer of these elements (Pereira and Kuch, 2005). A number of metal ions are essential for biological systems as Na, K, Ca, Mn, Fe, Co, Cu, Zn, and Mo. Small quantities of B, Si, V, As, Se, Ni, Nb, Rb, Sr, and Ti were found essential for living organism. Some other metals are non essential such as Cd, Pb, Hg (Johansen et al., 2006). Quantification of trace element levels in different tissues of the organism is an indicator of the bioavailable fraction of the element in the environment (Spalding et al., 1997;

Esteban and Castaño, 2009).

Pollution in the marine environment has become an issue of great concern, especially to coastal states (Pereira and Ebecken, 2009). The oceans cannot provide an infinite sink for anthropogenic wastes but insufficient attention has been given to evaluating the limits of capacity of coastal areas for waste assimilation (Juresa and Blanusa, 2003). Consequently, instances of fisheries shortage, spoiled beaches, destroyed coral reefs and wildlife habitat, toxic blooms and lost coastal ecological communities are widespread, with a corresponding deter-mination of cost benefit (Costanza and Farley, 2007). Recent concerns about connectivity of ocean health issues and the relationship to human disease highlight an important area for study (Lacerda and Molisani, 2006).

Knowledge of the ocean and the impact of human activities on it can reveal the complexity and interdepen-dence of all aspects of the system (Costanza and Farley, 2007). Improved acquaintance and predictive capabilities are required for more effective and sustained develop-ment of the marine environment to obtain associated economic benefits and to preserve marine resources.

Nowadays, the increasing use of the waste chemical and agricultural drainage systems represents the most dangerous chemical pollution (Karez et al., 1994; Lacerda and Molisani, 2006). The concentrations of cer-tain metals in marine waters have reached levels which cause damage to wildlife populations and created serious human health problems (Ferreira, 2009). 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).

Fregata magnificens is distributed widely in Brazil colonies found in Fernando de Noronha, Bahia, Rio de Janeiro, São Paulo, Parana and Santa Catarina (Coelho et al., 1991; Alves and Vecchi, 2009). This area has been selected because a large number of the birds harbour this place. The purpose of this study was to evaluate some metal concentrations in different tissues of *F. magnificens* collected from Sepetiba Bay, which is situated in the southern Atlantic Coast of Rio de Janeiro State, Brazil (Figure 1). This area constitutes an important natural breeding place for molluscs, crustaceans, fish, and at Coroa Grande mangrove, many species of seabirds, being *F. magnificens* presenting a great number (Amado Filho et al., 1999; Dittmar et al., 2006). However, several environmental problems has been brought to the Bay, due to poor sanitation con-ditions, including domestic and industrial sewage effluent, potentiating injuries mainly that related to heavy metals bioaccumulation in trophic level (Copeland et al., 2003; Lacerda and Molisani, 2006). The purpose goal of this study was to evaluate selected metal concentrations in different tissues of *F. magnificens* collected from Sepetiba Bay.

MATERIALS AND METHODS

The 43 analysed specimens (adults) of *F. magnificens* found stranded or dead were recorded in Coroa Grande mangrove, Sepetiba Bay, Rio de Janeiro State, between April 2007 and October 2010. After collection the birds were brought up to the laboratory, and immediately the carcasses were subjected to necropsy according to Jauniaux et al. (1998), while putrefactive specimens were discarded. Organs were collected (liver and kidney), weighed, and kept frozen (at -20°C) prior analysis.

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

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% HNO_3 and 0.3 ml of 70% $HClO_4$ at 80°C for 24 h (Bustamante et al., 2003).

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 $\mu g.g^{-1}$ wet weight (w.wt.).

Statistical analysis was performed using Origin 7.5 software package (OriginLab Corporation). The average distribution of heavy metal by the *F. magnificens* was assessed using analysis of variance (ANOVA), testing inter and intragroup mean differences between organs. In order to determine which organ was significantly different from each other, a post-hoc comparison with the Turkey's multiple comparison tests was carried out. For all tests, p-values of < 0.05 were used to determine significant differences.

RESULTS

Concentrations of the elements in liver and kidney of *F. magnificens* are presented in Figure 2, Tables 1 and 2. Mean concentration levels and range in liver was 5.52 (1.34 to 8.43)(Cd); 43.78 (19.63 to 62.65)(Zn); 38.94 (13.49 to 51.43)(Cu); 39.95 (20.82 to 59.13)(Pb); 3,99 (1.12 to 7.64)(Cr); and 5.05 (2.44 to 8.34)(Ni). Mean concentration levels and range in kidney were 5.57 (1.22 to 9.46)(Cd); 54.64 (27.72 to 77.15)(Zn); 46.89 (21.69 to 67.43)(Cu); 37.35 (15.24 to 55.67)(Pb); 5.02 (1.36 to 7.55) (Cr); and 5.98 (2.15 to 9.636) (Ni).

According to the ANOVA test, significant differences (P=0.05) were found for all metals in liver (F = 441.557; P= 0.00000), and in kidney (F= 545.69223; P= 0.00000). In individual analysis for each metal in liver and kidney, Zn (F= 25.35756; P= 0.00000), Cu (F= 21.35442; P= 0.00001), Cr (F= 9.94342; P= 0.00224), and Ni (F=9.09480; P= 0.00339), exerted significant differences; Cd
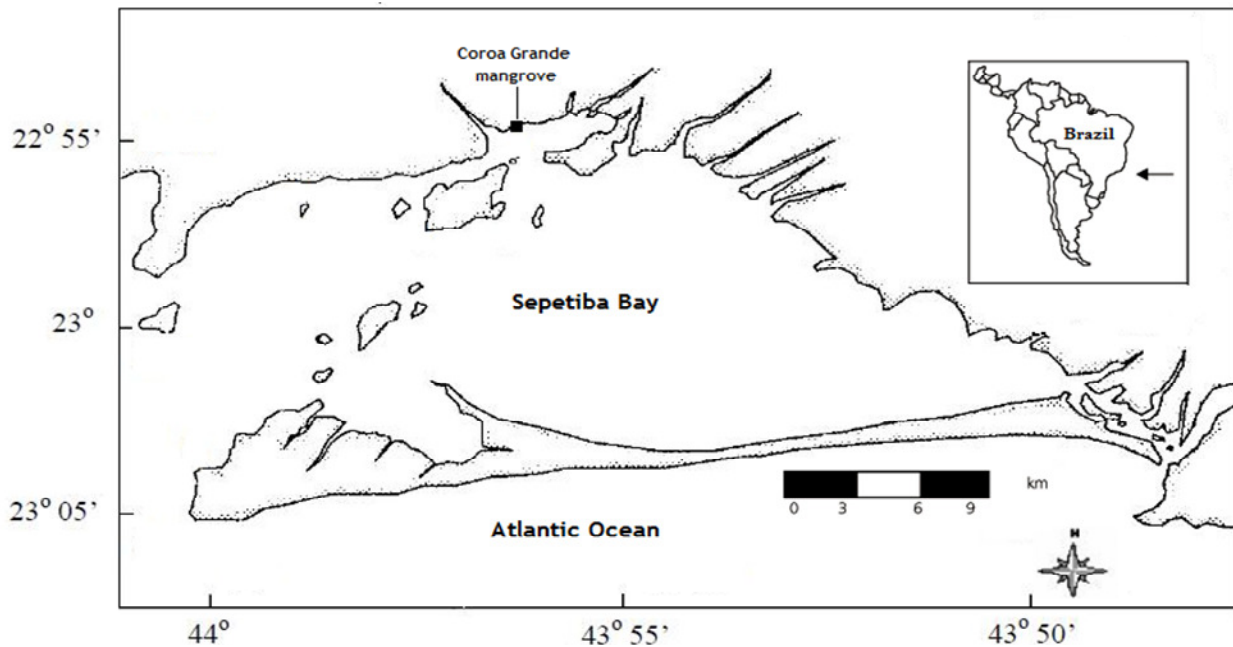
Figure 1. Study area- Coroa Grande Mangrove, Sepetiba Bay, Rio de Janeiro, Brazil.

(F= 0.01723; P= 0.89589) and Pb showed no significant differences (F= 2.12510; P= 0.14863).

Means comparison using Turkey test indicated that in liver (P=0.05), Cd concentrations was changed significantly for Ni and Cr, and for Zn, Cu and Pb were statistically significant; Zn concentration was not altered significantly for Pb, and differed significantly for Cu, Cr, and Ni; Cu concentration did not show up any significance for Cr, and differed significantly for Ni; Pb concentration differed significantly for Cr and Ni; Cr concentrations did not differed significantly for Ni. Comparison of means using Turkey test indicated that in kidney (P=0.05), Cd concentration did not show any significance for Cr and Ni, but was significant for Zn, and Pb; Zn concentration showed marked difference for Cu, Pb, Cr, and Ni; Cu concentration showed difference for Pb, Cr, and Ni; Pb concentration statistically differ for Cr and Ni; Cr concentration did not differed significantly for Ni.

DISCUSSION

Despite the problems associated with using seabirds as indicators and interpreting data on abundance and extent for the potential risk to public health, there are still reasons for incorporating them in an assessment of environmental conditions. First, they satisfy the criterion of general importance and public reliability, as well as any indicator. Moreover, growth and reproduction of higher organisms is a clear indication of a functioning food web

and operating environment that meets the minimal habitat requirements, providing relevant data for assessments (Birungi et al., 2007). Examination of metal uptake and accumulation in *F. magnificens* 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 (Kalay et al., 1999; Birungi et al., 2007), a fact that initially guided the selection of metals to be included in this study. Data show warning levels of some metals in some fish species and it was possible to expect the same levels in the analysed samples. The notable increase of Cr, Cd, Zn, Pb, Cu, and Ni found in seabirds occur as a result of two main processes: bioaccumulation through food and bio-degradation of environmental processes (Walsh, 1990; Johansen et al., 2006). According to Schmitt-Jansen et al. (2008) the sub-lethal effects on birds include growth retardation, suppression of egg production, compression of egg shell and changes in behaviour.

The most common health problem is due to Cr which involved the respiratory tract. These health effects include irritation of the lining of the nose, runny nose, and breathing problems (asthma, cough, shortness of breath, wheezing) (Savinov et al., 2003). Cd is known for its long half-life in biological systems (decades in humans and years in birds), and 0.1 to 1.0% of ingested Cd is absorb through the avian gastrointestinal tract to be distributed to the kidney and liver. Also, Cd can connect the liver to
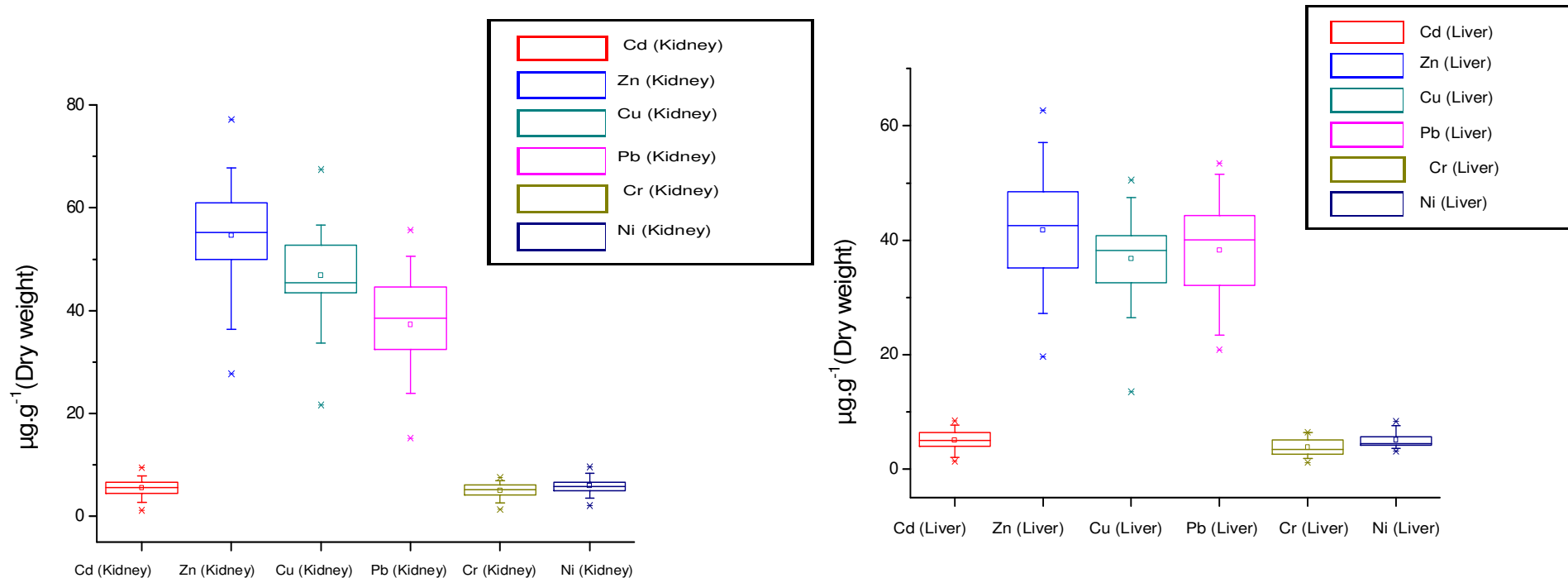
Figure 2. Cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb), chromium (Cr) and nickel (Ni) concentrations (in µg.g^{-1} dry weight) in liver and kidney of *F. magnificens*. Box-plots illustrate the 10, 25, 50 (median), 75 and 90% percentiles and the outliers (*).

metallothionein (Seebaugh et al., 2005), which is responsible for a liaison in alleviating the potential toxic effects. The high levels of Cd detected in the kidneys are higher than expected, and when compared to the liver, may indicate high exposure (Thompson et al., 2007). Metallothionein provide a protection for the effects of certain toxic metals, sequestering them and decreasing the amount of free metal ion (Bustamante et al., 2008). Zn is metabolically regulated in seabirds' tissues (White and Cromartie, 1985). And 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. Pb absorption from the gastrointestinal tract ranges from 40 to 70% depending on the form of Pb ingested and the age of the exposed individual. A large amount of Pb is deposited into bone, which acts as a depot that provides a reliable indication of long-term exposure. Cu is essential for good health. However, exposure to higher doses can be harmful. Long-term exposure can irritate nose, mouth, and eyes, and cause headaches, dizziness, nausea, and diarrhea (Thompson et al.,

2007). The harmful health effects from exposure to Ni, such as chronic bronchitis, reduced lung function, and cancer of the lung and nasal sinus. Exposure to high levels of nickel compounds that dissolve easily in water (soluble) may also result in cancer when nickel compounds that are hard to dissolve (less soluble) are present, or when other chemicals that can produce cancer are present (Karez et al., 1994).

International legislation is yet to have threshold parameters for the presence of heavy metals in seabirds, and for that reason, researchers have used national standards that can be applied to

Table 1. Concentrations of elements analyzed (µg.g⁻¹ metal, wet weight) in liver of *F. magnificens* (n = 43).

Element	Liver ($\mu g \cdot g^{-1}$)											
	Mean	Sd (yEr±)	Se (yEr±)	P25	P75	P95	Min	Max	Range	Median	Var	Coef Var
Cd	5.52	1.71667	0.26179	4.5	7.04	7.67	1.34	8.43	7.09	5.62	2.94696	0.31097
Zn	43.78	9.80954	1.49594	35.49	50.29	57.39	19.63	62.65	43.02	45.27	96.22706	0.22404
Cu	38.94	7.83488	1.19481	34.37	44.67	49.32	13.49	51.43	37.94	39.61	61.38527	0.20122
Pb	39.95	8.02909	1.22443	35.45	45.56	51.5	20.82	59.13	38.31	40.09	64.46633	0.20096
Cr	3.99	1.6118	0.2458	2.66	5.17	6.53	1.12	7.64	6.52	3.94	2.5979	0.40349
Ni	5.05	1.30678	0.19928	4.16	5.67	7.53	2.44	8.34	5.9	4.77	1.70768	0.25897

Sd = Standard deviation of data. Se = Standard error of the mean. P25 = 25 percentile. P75 = 75 percentile. P95 = 95 percentile. Var = Variance. Coef Var = Coefficient of variation.

Table 2. Concentrations of elements analyzed (µg.g⁻¹ metal, wet weight) in kidney of *F. magnificens* (n = 43).

Element	Kidney ($\mu g \cdot g^{-1}$)											
	Mean	Sd (yEr±)	Se (yEr±)	P25	P75	P95	Min	Max	Range	Median	Var	Coef Var
Cd	5.57	1.61755	0.24667	4.46	6.64	7.83	1.22	9.46	8.24	5.62	2.61647	0.29053
Zn	54.64	10.18417	1.55307	49.93	60.93	67.78	27.72	77.15	49.43	55.25	103.71722	0.18637
Cu	46.89	8.13233	1.24017	43.41	52.71	56.66	21.69	67.43	45.74	45.45	66.13476	0.17341
Pb	37.35	8.55426	1.30451	32.45	44.57	50.61	15.24	55.67	40.43	38.56	73.17541	0.22905
Cr	5.02	1.39592	0.21288	4.15	6.17	6.93	1.36	7.55	6.19	5.22	1.94859	0.27807
Ni	5.98	1.55975	0.23786	4.97	6.65	8.45	2.15	9.63	7.48	5.77	2.43283	0.26075

Sd = Standard deviation of data. Se = Standard error of the mean. P25 = 25 percentile. P75 = 75 percentile. P95 = 95 percentile. Var = Variance. Coef Var = Coefficient of variation.

these biological indicators (Ferreira, 2009; Ferreira and Horta, 2010).

Average concentrations of metals found in birds ranged from 1.09 to 39.9 times above the limit in the liver and 1.2 to 50.1 times above the limit in the kidney (Tables 1 and 2), based on the maximum tolerance limits for inorganic contaminants in fish and fishery products established by Brazilian legislation - National Health Surveillance Agency (ANVISA). The limits 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 (Brazil Ordinance, 685/98).

Seabirds are top consumers in marine foodchains which offer opportunities to detect and assess the toxicological effects of different inorganic elements on the marine ecosystem. This study presents a scientific approach for assessing the ecological condition of the Sepetiba Bay and the impacts caused by heavy metals in a particular species of bird used as indicator, which has a big value in Latin America. The key assumptions underlying the approach are:

(1) The importance of putting analysis on ecosystems attributes of public importance.
(2) The consistent with scientific understanding of what is important to sustain ecosystems structure and function.
(3) Measurement of environmental indicators must be scientifically defensible.
(4) Are there any implications on health risk on human and along marine trophic chains public health?

The assessment of environmental variables and biological effects in seabirds will provide critical insights into the level and extent in human 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 to marine ecosystems. Human health impacts of water contact are among the most well understood pollution problems by the public given the severe impact of waterborne diseases throughout human history. There are established state and federal standards for assessing water quality in relation to identify potential health hazards. The existence of standard methods makes sampling straightforward, and standards have been used to generate an assessment of condition of marine environment (Ferreira and Horta, 2010).

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 depend are critically influenced by the ocean (Pereira and Ebecken, 2009).

Heavy metals comprise a significant part of pollutants in bay waters. 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 (Birungi et al., 2007) , providing risks to human health posed by metals in seafood. The basis of toxicity for some metals support and anticipate problems due to its speciation and more effort should be focused on researches in the future. The focus of research in the assessment of heavy metals in birds as a representative of the top of trophic chains shows that despite being sedentary species, the degree of bioaccumulation of metals studied may be reflected also in the migratory species due to exposure. The metal contamination in one area can have harmful effects on an entire region, particularly in areas of active feeding or reproduction, or migration routes and breeding sites.

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