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Concentrations of metals in liver of Guiana dolphins (*Sotalia guianensis*) from an estuary in Southeast of Brazil

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Abstract

Concentrations of Fe, Zn, Cu, Mn, Pb, Cr, Co and Ni were measured in the liver of 21 Guiana dolphins from the Estuarine-Lagoon Complex of Iguape-Cananéia, in order to indicate the impact of their habitat and to verify if these concentrations differ between the sexes and age classes. The concentrations followed the order: Fe>Zn>Cu>Mn>Pb>Cr>Co>Ni, and with the exception of lead, all concentrations were in the same order of magnitude observed for the species. The accumulations between the sexes were similar, while copper were higher in infants, suggesting that the organ can act as a reservoir for Co in a period of rapidly postnatal growth. Lead mean concentration (3.17 $\mu g\ g^{-1}$) were the highest described for the species, which from a toxicological point of view may be a matter of concern. This accumulation suggests a trophic transfer, indicating the contamination of the food chain, which possibly reflects the local environmental contamination caused by ancient lead mining.

Key words: Bioaccumulation. Cetaceans. Pb. Sentinel. *Sotalia guianensis.* Trace elements.

INTRODUCTION

Metals have great ecological and biological interest, as they are able to pass through bioaccumulation and biomagnification processes. Many of them are essential for the metabolism and life, as others, such as Hg and Pb, have unknown biological function (Das *et al*., 2000). In both cases, at high concentrations they become toxic and thus harmful to living beings (O´shea, 1999). They occur naturally in the environment, however, human activities have also released them into the ecosystems, changing their distribution patterns in the globe, which has eventually contaminated several aquatic environments, both continentals and coastal (Yi *et al*., 2011).

Chemical contamination is considered a major threat to aquatic mammals, especially cetaceans, because of their biological and ecological characteristics, which offer great potential for the accumulation of high levels of contaminants in their tissues (Siciliano *et al*., 2005; Seixas *et al*., 2009). The balance between exposure, absorption and elimination of metals by aquatic organisms heads its input and bioaccumulation into the food chain, and the biomagnification to the top predators' species (Kunito *et al*., 2004; Moura *et al*., 2011). These accumulations occur in all species of cetaceans and have different degrees depending on the biological and ecological factors of the species (Lemos *et al*., 2013; Moura *et al*., 2014).

The effects of the metals on the body may be lethal or sub-lethal to all aquatic organisms, which may exhibit responses ranging from subtle adaptations to drastic effects (Reijnders *et al.,* 1999; Das *et al.,* 2000; Yi *et al*., 2011). However, the potential toxic effects of metals in marine mammals remain unclear since these animals have been exposed to these natural elements for a very long time in evolutionary terms, and because they present mechanisms to control and/or mitigate their toxic effects (Law *et al*., 1991; O'Shea, 1999; Das *et al*., 2000; Caurant *et al.,* 2006). These mechanisms include the presence of metallothioneins,

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cytosolic metal-binding proteins, that in mammal's species are primally synthesized in the liver, kidney and intestinal mucosa, whose biological function are related with the regulation of essential metals, such Zn and Cu, and with the detoxification of toxic metal such Cd and Hg (Mason *et al.,* 1980).

The liver has greater cumulative potential for metals due to its function in the metabolism of toxicants (Law *et al*., 1991; Das *et al*., 2000). Factors such as geographic location, exposure pathways, nutritional status, eating habits, age and sex, visceral distribution, the metabolic rate and the rate of excretion, influence the accumulation of metals in the liver of cetaceans (Das *et al*., 2000; Moura *et al*., 2014). It is expected that the concentrations of the essential metals in the liver, such as Fe, Zn and Mn, are regulated and present low variability among the animals of the same species (Law *et al*., 1991; 1992). However, the concentrations of the non-essential metals in the liver of cetaceans are usually scarce, with the exception of cases of exposure to environmental contamination (Law *et al*., 1991; 1992; Moura *et al*., 2014).

The Guiana dolphin (*Sotalia guianensis* - Van Bénéden, 1864) is a small odontocete that can reach up to 2.10 m, with estimated longevity about 30-35 years (Rosas *et al*., 2003). The diet of the species is based on teleost fish, crustaceans and cephalopods (Lopes *et al*., 2012; Cremer *et al*., 2012), which in many cases are also intended for human consumption. The distribution of the Guiana dolphin extends from Honduras, in Central America, to Santa Catarina State in southern Brazil (Flores & Da Silva, 2009). The species displays a coastal distribution with preference for estuarine environments (Flores & Da Silva, 2009), where they end up being more susceptible to the consequences of the anthropic activities as their habitat exposes these small dolphins to chemicals and pathogens, which can affect their health and therefore their survival (Moura *et al*., 2014).

Residence patterns are observed in the region of Iguape-Cananéia (Santos *et al*., 2001; Oshima *et al.,* 2010), where the animals are seen throughout the year with an estimated population of about 200 animals (Havukainen *et al*., 2011). Features such as a relatively long lifetime, diet and residential patters make the species a suitable model to monitor the environmental contamination by metals and also the concentrations to which man may be exposed on the consumption of seafood, since, like humans, they can be characterized as final victims of the chemical accumulation process (Moura *et al*., 2014).

The Estuarine-Lagoon Complex of Iguape-Cananéia is one of the most important estuarine ecosystems of the Brazilian coast, recognized by UNESCO as part of the Biosphere Reserve of the Atlantic Rainforest. However, this system passed through dramatic changes in the last centuries that affected the quality of this environment. The opening of an artificial channel (Valo Grande Channel), in Iguape in the 19th century, diverted about 70% of the waters of the Ribeira de Iguape River to inside the estuarine system (Mahiques *et al*., 2013). This river housed several mines of Pb, Zn, Au and Ag, that operated dumping tailing and metallurgical slags of blast furnace directly into the river until 1995, contaminating the water and the sediments along its course and part of the estuary, especially with Pb (Guimarães & Sígolo, 2008; Mahiques *et al*., 2013). After the closure of the mines the residues were deposited on the riverbanks, exposed to the weathering and consequently to lixiviation (Guimarães & Sígolo, 2008).

Over the years the concentrations of Pb in the sediments and in the water have decreased considerably (Mahiques *et al*., 2013; Abessa *et al*., 2014), however the system remains weakened by the load of mining residues received. The metals suffer mobility along the drainage course of the river and in the estuarine system through suspended solids, being bioavailable and assimilated by the local biota (Guimarães & Sígolo, 2008; Rodrigues *et al*., 2012; Choueri, 2015). This fact allows the accumulation of metals in high concentrations in animals of higher levels of the food chain, as marine mammals.

Despite of the growing number of studies with contaminants in marine mammals in Brazil, little is known about the health of these animals and how much chemical contamination is affecting the many populations of cetaceans that occur in these waters (Siciliano *et al*., 2005; Lemos *et al*., 2013; Moura *et al*., 2014). The *Sotalia guianensis* conservation status is defined as "data deficient" in both the National Action Plan for the Conservation of Aquatic Mammals of ICMBio (2011) and the IUCN (2018), therefore, more research is necessary. Quantifying the levels of metals in the species can help to diagnose the impact of their habitat over different populations and to maintain assistance in developing strategies for the conservation of these dolphins on the Brazilian coast (Lemos *et al*., 2013).

This study aimed to determine the concentrations of Fe, Zn, Mn, Cr, Cu, Co, Pb and Ni in liver samples of Guiana dolphins of the Estuarine-Lagoon Complex of Iguape-Cananéia and verify if these concentrations differ between the sexes and age classes. These results were compared to the literature of the same species from other geographical areas to evaluate the environmental quality of their habitat and increase the knowledge about the Guiana dolphin on Brazilian waters.

MATERIALS AND METHODS

Study area

The Estuarine-Lagoon Complex of Iguape-Cananéia (Figure 1) is located in the south of the São Paulo State, southeastern Brazil. It corresponds to a system formed by four large islands (Cardoso, Cananéia, Comprida and Iguape), located between latitudes of 24º 50` to 25º 10`S and longitudes of 47º 25` to 48º 00`W (Tessler & Souza, 1998).

Sampling

Samples were obtained from stranded dead Guiana dolphins on the beaches of that region between 2009 and 2012, through the beaches monitoring program of the Instituto de Pesquisas Cananéia - IPeC (SISBio License No. 22840-

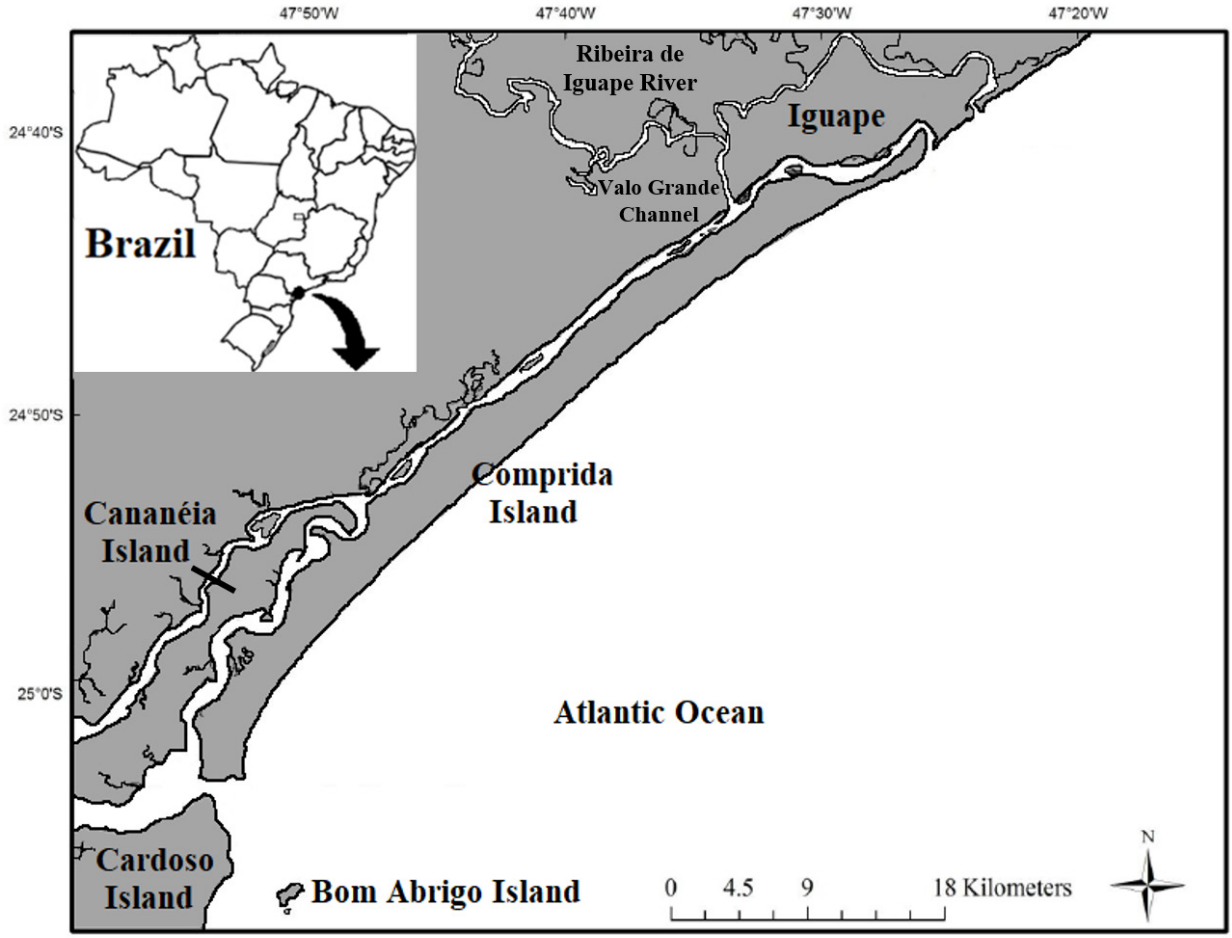

Figure 1. Map of the Estuarine-Lagoon Complex of Iguape-Cananéia and its location in Brazil.

3). Twenty-one animals, being six males, thirteen females and two unidentified, were analyzed. Biometrics and macroscopic evaluation was held to assess the conditions of the carcasses and to identify their sex and the age class, when possible. Information about the age of the animals were not available so the age classes were arbitrarily estimated according to the length of the animals. Animals of a length varying between 90 and 100cm were classified as infants; animals between 111 and 165cm as juveniles; and animals longer than 165cm as adults, adapting to the species' propositions made by Rosas & Monteiro-Filho (2002). Samples of the liver were collected from fresh carcasses (code 2) or when in early decomposition phase (code 3), according to the classification recommended by Geraci & Lounsbury (1993). The necropsy of the animals was held following the conduct protocol of the Rede de Encalhe de Mamíferos Aquáticos do Nordeste - REMANE (IBAMA, 2005). Approximately 20 grams of the hepatic tissue was removed using a stainless-steel scalpel, stored in plastic containers and frozen at -20°C until analysis.

Analytical procedures

Analytical procedures were held in the Laboratório de Estudos Avançados em Química Ambiental - LEAQUA in the Universidade Tecnológica Federal do Paraná - UTFPR. Analytical procedures has followed one of the methods presented by Más-Rosa (2009), with some adaptations, in which the samples were homogenized by maceration and divided into triplicates of approximately 5g (wet weight). Acid digestion was performed with 30ml of aqueous solution of HNO_3 1:1 under reflux on a heating plate at 95°C for 12 hours until complete oxidation, evidenced by limpid extract. Next, the samples were reduced to an equivalent volume of 5mL on the heating plate. After cooling, the samples were filtered and had their volume gauged to 50mL with deionized water. All the reagents used were of an analytic degree and the materials used during the procedures were previously decontaminated by washing with neutral detergent and by bathing in a solution of nitric acid (10%) for 24h.

The quantification of Fe, Zn, Mg, Cr, Cu, Co, Pb and Ni was done by Flame Atomic Absorption Spectrometry (FAAS) model GBC, Avanta, respecting the prepositions of the method to the different metals (Table 1). Standard curves were prepared with successive dilutions using a stock solution of each metal of analytical grade (1.000 mg L^{-1}) Merck. The quality of the results was accompanied with the use of an analytical blank and the determination performed in triplicates. Due to the lack of certified reference material (CRM) of aquatic mammal

Table 1. Linear correlation coefficient (r^2), limits of detection (LOD) and limits of quantification (LOQ) for the studied metals.

Metals	r^2	LOD (µg L^{-1})	LOQ (µg L^{-1})
Fe	0.9997	5	15
Zn	0.9995	3	10
Mn	0.9954	6	18
Co	0.9985	4	15
Cu	0.9963	4	13
Cr	0.9896	5	16
Pb	0.9954	5	16
Ni	0.9979	2	6

tissues, the exactitude of the method was verified by addition and recovery tests performed in fish tissues, used to guarantee the internal standards in the laboratory. Recovery rates ranged from 82% to 108% in all investigations. Metal concentrations were expressed in µg g^{-1} wet weight.

Statistical analysis

Statistical analyses were conducted to verify if the mean concentrations of the metals in liver differ between the sexes and the age classes. The normality test Shapiro Wilk was applied, followed by the homogeneity test of Levene. For data with normal distribution ANOVA test was used. For data with no normal distribution the non-parametric test Kruskal-Wallis was applied. The level of statistical significance was defined at $p < 0.05$.

RESULTS

Biological data of sampled animals are shown in Table 2. The concentrations of the metals in the liver of each analyzed animal (in µg g^{-1} wet weight) are shown in Table 3. Mean concentrations of the metals in liver (in µg g^{-1} wet weight) were obtained in the following order of magnitude: Fe>Zn>Cu>Mn>Pb>Cr>Co>Ni (Figure 2).

The iron and zinc had the highest mean concentrations in livers, with values of 124.82 and 41.52 µg g^{-1}, respectively. The variations in livers (minimum and maximum values among the animals) ranged from 55.28 to 233.51 µg g^{-1} for iron and from 25.76 µg g^{-1} to 100.75 µg g^{-1} for zinc. There was no influence of the gender (Fe: $r^2 = 0.1782$; $F_{1:17} = 3.03$; $p = 0.0998$; Zn: $\chi^2 = 0.0692$; df = 1; $p = 0.7925$), nor the age classes (Fe: $r^2 = 0.5269$; $F_{2:18} = 0.50$; $p = 0.6144$; Zn: $\chi^2 = 4.530$; df = 2; $p > 0.0987$) in the livers concentrations for both metals.

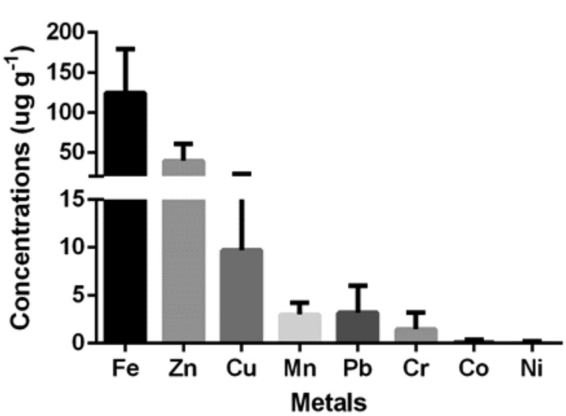

Figure 2. Mean concentrations and standard deviation of the metals Fe, Zn, Cu, Mn, Pb, Cr, Co and Ni in liver of *Sotalia guianensis* from the Estuarine-Lagoon Complex of Iguape-Cananéia (in mg g^{-1} wet weight).

The concentration of copper varied from 1.28 to 52.08 µg g^{-1}, with a mean value of 9.71 µg g^{-1} among the animals. The accumulations between the sexes were also similar ($\chi^2 = 1.6187$; df = 1; $p = 0.2033$), while infants showed higher concentrations of the metal in the liver ($\chi^2 = 5.499$; df = 2; $p = 0.0533$).

For manganese, the mean concentration obtained was of 3.00 µg g^{-1}, varying from 0.73 to 5.55 µg g^{-1}. For this metal, no differences in the accumulation between the sexes ($r^2 = 0.1205$; $F_{1:17} = 2.04$; $p = 0.17$) and the age classes ($r^2 = 0.2858$; $F_{2:18} = 3.60$; $p = 0.0483$) were observed.

For lead, a mean concentration of 3.17 µg g^{-1}, varying from lower than the limit of quantification (<LOQ) to 9.62 µg g^{-1} was found. This is the highest mean concentration and variation observed for the species in the Brazilian coast compared to the previous studies (Table 3), showing some concern from the toxicology point of view. There was no influence of the

Table 2. Data about *Sotalia guianensis* sampled from August 2009 to July 2012 in the Estuarine-Lagoon Complex of Iguape-Cananéia.

Sample ID	Date	Sex	Body length (cm)	Age Class	Conditional phase of the carcasses
Sg01	2009/08/30	n.a.	96	Infant	3
Sg02	2009/09/10	M	208	Adult	3
Sg03	2010/01/09	F	169	Adult	3
Sg04	2010/02/19	F	149	Juvenile	3
Sg05	2010/03/29	F	198	Adult	3
Sg06	2010/05/23	F	204	Adult	3
Sg07	2011/01/09	M	155	Juvenile	2
Sg08	2011/03/20	F	198	Adult	2
Sg09	2011/03/30	M	164	Juvenile	3
Sg10	2011/04/15	F	195	Adult	2
Sg11	2011/05/11	F	208	Adult	2
Sg12	2011/08/08	F	177	Adult	3
Sg13	2011/08/26	F	201	Adult	3
Sg14	2011/09/02	F	98	Infant	3
Sg15	2011/09/13	n.a.	99	Infant	3
Sg16	2011/09/22	F	208	Adult	2
Sg17	2011/10/07	F	185	Adult	2
Sg18	2011/10/27	F	198	Adult	2
Sg19	2012/05/11	M	205	Adult	2
Sg20	2012/06/14	M	189	Adult	2
Sg21	2012/07/07	M	195	Adult	3

n.a., Data not available.

* Conditional phase of the carcasses: Code 1 - Live stranded animals. Code 2 - Fresh carcass.

Code 3 - Carcasses in early phases of decomposition. Code 4 - Carcasses at advanced phases of decomposition. Code 5 - Mummified carcasses or skeletal remains (Geraci & Lounsbury, 1993).

Table 3. Concentrations of the studied metals (in µg g^{-1}) in *Sotalia guianensis* sampled from August 2009 to July 2012 in the Estuarine-Lagoon Complex of Iguape-Cananéia.

Sample ID	Fe	Zn	Cu	Mn	Pb	Cr	Co	Ni
Sg01	105.10	78.03	6.12	5.55	6.03	<LOQ	0.17	0.26
Sg02	61.76	29.54	3.83	2.81	<LOQ	<LOQ	<LOQ	<LOQ
Sg03	55.28	26.79	5.87	2.54	5.42	<LOQ	<LOQ	<LOQ
Sg04	162.59	100.75	15.46	4.16	3.82	1.88	0.93	<LOQ
Sg05	203.87	35.81	6.72	2.60	1.22	0.37	<LOQ	0.43
Sg06	175.20	28.10	6.28	2.11	8.93	0.71	0.88	<LOQ
Sg07	75.55	63.61	6.76	4.03	7.15	<LOQ	<LOQ	<LOQ
Sg08	233.51	33.48	9.69	2.91	3.91	<LOQ	<LOQ	<LOQ
Sg09	101.65	32.91	4.20	2.98	9.62	<LOQ	<LOQ	<LOQ
Sg10	85.83	33.90	4.08	1.59	1.15	3.09	<LOQ	<LOQ
Sg11	184.09	29.11	6.31	1.70	1.46	3.08	<LOQ	<LOQ
Sg12	183.86	56.81	4.37	3.95	<LOQ	4.55	<LOQ	<LOQ
Sg13	75.00	25.76	3.62	3.32	0.88	4.77	<LOQ	<LOQ
Sg14	98.55	48.08	52.08	3.53	0.80	2.23	<LOQ	<LOQ
Sg15	92.30	44.55	43.59	3.64	1.09	2.33	0.27	<LOQ
Sg16	201.05	26.70	7.08	2.02	0.99	4.92	0.08	<LOQ
Sg17	72.62	25.64	1.28	0.73	2.93	<LOQ	<LOQ	<LOQ
Sg18	120.59	30.51	4.20	2.52	4.70	2.15	0.07	<LOQ
Sg19	72.16	50.05	4.55	5.54	2.76	0.12	0.21	<LOQ
Sg20	139.26	42.10	3.53	2.97	1.64	<LOQ	<LOQ	<LOQ
Sg21	121.38	29.72	4.36	1.89	2.01	0.49	0.14	0.48

<LOQ – Lower than the limit of quantification.

gender in the concentrations found in the livers ($\chi^2 = 0.4330$; df = 1; p = 0.5105), nor the age classes ($\chi^2 = 4.522$; df = 2; p = 0.1015).

Chromium concentrations varied from <LOQ to 4.77 µg g^{-1}, with a mean value of 1.46 µg g^{-1}. The accumulations between the sexes ($\chi^2 = 5.4686$; df = 1; p = 0.0193) and age classes ($\chi^2 = 1.298$; df = 2; p = 0.5513) were similar for this metal.

For cobalt and nickel the results varied from <LOQ to 0.93 µg g^{-1} and <LOQ to 0.48 µg g^{-1}, with a mean concentration of 0.13 µg g^{-1} and 0.06 µg g^{-1}, respectively. The concentrations of these metals in the liver were similar between the sexes (Co: $\chi^2 = 0.0113$; df = 1; p = 0.9153; Ni: $\chi^2 = 0.4340$; df = 1; p = 0.5105) and the age classes (Co: $\chi^2 = 1.506$; df = 2; p = 0.5961; Ni: $\chi^2 = 1.058$; df = 2; p = 0.9999).

DISCUSSION

Table 4 shows a comparison among the mean values ± standard deviation (SD) of the concentrations for the studied metals in livers and the variation of the means (minimum and maximum values) found in this and in other studies with the species on the Brazilian coast.

The concentrations obtained for Fe, Zn, Cu, Mn, Cr, Co and Ni, compared with the literature, were in the same order of variation described before for the species (Table 3). In general, the concentrations of these essential metals are expected to be regulated (Law *et al.,* 1991).

In marine species, higher concentrations of Fe and Zn in the liver, compared with other metals, occur due to its abundance in the environment and to the fact that these metals actively participate in the metabolism (O'Shea, 1999; Kunito *et al.,* 2004). However, environmental contamination may increase the bioavailability, and consequently the absorption and the accumulation of these elements. This situation was observed in the north coast of Rio de Janeiro State by Lemos *et al.* (2013), where high Zn levels in the livers of the animals were related to local contamination. Although the studied area was affected by ancient mining activities, that also enriched the estuary with Zn (Guimarães & Sígolo, 2008; Mahiques *et al.,* 2013), the concentrations found by this research were lower than the ones described by Lemos *et al.* (2013), suggesting that it is not a concern for the species in the area.

The higher Cu concentrations observed in the liver of infant animals corroborate with the study made by Más-Rosa (2009) in the coast of Bahia State, which also found higher concentrations of this metal in infants. Kunito *et al.* (2004) also found high concentrations of Cu in the liver of animals of this same species under the age of one year. This may be for the fact that newborns have a specific requirement for the metal, and present lower excretion rate (Caurant *et al.,* 1994). Moreover, the liver can act as a storage for this element in a period of rapidly postnatal growth (Mason *et al.,* 1980), and elevated concentrations of Cu in neonates and infants of marine mammals have been extensively observed (Caurant *et al.,* 1994; Law *et al.,* 1991; Kunito *et al.,* 2004; Bilandžić *et al.,* 2016).

The concentrations of Mn resembled the one found by Lemos *et al.* (2013), also in wet weight samples. Nevertheless, the Mn was the element detected in higher concentrations in the liver when analyzed by dry weight in the same study (Lemos *et al.,* 2013), being similar to the concentrations found by Kunito *et al.* (2004) and Más-Rosa (2009).

The evaluation of Cr, Co and Ni in cetacean tissues are rather scarce and there are few data in the literature for the Guiana dolphin. Generally, the concentrations of these metals in the liver of dolphins are low, and in some cases, it does not overcome the limits of quantification of the method (Das *et al.,* 2000; Kunito *et al.,* 2004), as observed in this study. The variation of the Cr concentrations was similar to the variation presented by Kunito *et al.* (2004) in animals of the coast of Paraná and São Paulo states. For Ni, the values found were similar to those presented by Más-Rosa (2009) in the study on the coast of Bahia State. For Co, the concentrations were similar to both studies (Kunito *et al.,* 2004; Más-Rosa, 2009).

In general, variations of concentrations among the studies are common and expected as a result of the various factors that affect the accumulation of metals in marine mammals and the techniques applied to the studies. However, the variations presented in this study, combined with the other values reported in the literature, can represent general guides for the accumulation of these metals for the species in the Brazilian coast, reflecting its metabolic needs and exposure.

Nonetheless, two important points must be considered. The presented data came from stranded animals, therefore, not necessarily representatives of healthy conditions. In addition,

Table 4. Comparison of the concentrations of metals (mean ± SD in µg g-1) in livers of *Sotalia guianensis* obtained in this study with the Brazilian literature for the species.

Metal	Measurement weight	Mean concentration + SD (mg g-1)	Variation Min-Max (mg g-1)	Site	Reference
Fe	Wet weight	**124.82 ± 54.07**	**55.28 – 233.51**	**SP – Brazil**	**This study**
	Dry weight	-	567 – 879	BA – Brazil	Más-Rosa, 2009
	Dry weight	794 ± 370	330 – 1470	SP/PR – Brazil	Kunito et al., 2004
Zn	Wet weight	**41.52 ± 19.48**	**25.76 – 100.75**	**SP – Brazil**	**This study**
	Wet weight	65.85 ± 51.11	26.20 – 209.3	RJ – Brazil	Lemos et al., 2013
	Dry weight	192.6 ± 124.1	96.74 – 530.8	RJ – Brazil	Lemos et al., 2013
	Dry weight	-	161 – 255	BA – Brazil	Más-Rosa, 2009
	Wet weight	85.40	65.90 – 107	RJ – Brazil	Carvalho et al., 2008
	Dry weight	192 ± 76	117 – 348	SP/PR – Brazil	Kunito et al., 2004
Cu	Wet weight	**9.71 ± 13.05**	**1.28 – 52.08**	**SP – Brazil**	**This study**
	Wet weight	8.120 ± 4.6	1.180 – 18.03	RJ - Brazil	Lemos et al., 2013
	Dry weight	23.66 ± 11.13	4.880 – 46.05	RJ - Brazil	Lemos et al., 2013
	Dry weight	-	18.2 – 50.1	BA - Brazil	Más-Rosa, 2009
	Dry weight	26.48 ± 19.89	13.44 – 83.77	RJ - Brazil	Seixas et al., 2009
	Dry weight	157 ± 436	14.5 – 1970	SP/PR - Brazil	Kunito et al., 2004
Mn	Wet weight	**3.00 ± 1.21**	**0.73 – 5.55**	**SP – Brazil**	**This study**
	Wet weight	2.51 ± 1.13	0.66 – 5.11	RJ – Brazil	Lemos et al., 2013
	Dry weight	7.34 ± 2.53	2.73 – 13.05	RJ – Brazil	Lemos et al., 2013
	Dry weight	**-**	8.64 – 13.7	BA – Brazil	Más-Rosa, 2009
	Dry weight	9.84 ± 2.75	5.91 – 15.1	SP/PR – Brazil	Kunito et al., 2004
Pb	Wet weight	**3.17 ± 2.84**	**<LOQ – 9.62**	**SP – Brazil**	**This study**
	Dry weight	-	0.040 – 0.3	BA – Brazil	Korn et al., 2010
	Dry weight	<LOQ	<LOQ	BA – Brazil	Más-Rosa, 2009
	Dry weight	1.55 ± 0.75	0.74 – 2.73	RJ – Brazil	Seixas et al., 2009
	Dry weight	0.063 ± 0.06	0.028 – 0.197	SP/PR – Brazil	Kunito et al., 2004
	Dry weight	0.11 ± 0.02	0.10 – 0.12	CE – Brazil	Monteiro-Neto et al., 2003
Cr	Wet weight	**1.46 ± 1.75**	**<LOQ – 4.77**	**SP - Brazil**	**This study**
	Dry weight	-	0.49 – 0.68	BA - Brazil	Más-Rosa, 2009
	Dry weight	0.93 ± 1.29	0.26 – 5.1	SP/PR - Brazil	Kunito et al., 2004
Co	Wet weight	**0.13 ± 0.26**	**<LOQ – 0.93**	SP - Brazil	This study
	Dry weight	<LOQ	<LOQ	BA - Brazil	Más-Rosa, 2009
	Dry weight	0.027 ± 0.012	0,016 – 0.051	SP/PR - Brazil	Kunito et al., 2004
Ni	Wet weight	**0.06 ± 0.14**	**<LOQ – 0.48**	**SP – Brazil**	**This study**
	Dry weight	-	<LOQ – 0.30	BA – Brazil	Más-Rosa, 2009

<LOQ – Lower than the limit of quantification.

the sampling is conducted in unknown postmortem time and some metal loss may occur. Moreover, this species is highly captured by fisheries in the Cananéia region (Zappes *et al.,* 2009), and some of the individuals analyzed in this study may be from incidental capture, there so being healthy animals.

Differently from the other studied metals, Pb has a high toxic potential. This metal is not distributed homogeneously throughout the body. As Pb is qualitatively a biological analogue of calcium (Moreira & Moreira, 2004), the highest accumulation of Pb in aquatic mammals occurs in hard tissues, as bones and teeth (O'Shea, 1999; O'Hara & O'Shea, 2001; Das *et al.,* 2000, Caurant *et al.,* 2006). In general, the Pb concentrations found in soft tissues are often lower than 1 μg g^{-1} (O'Hara & O'Shea, 2001; Caurant *et al.,* 2006). In this research, the highest mean concentration of Pb in the liver for the species was described (3.17 μg g^{-1}) arousing some concern and indicating that Pb concentrations in bones or teeth of the analyzed individuals could have been even higher.

Among the animals, six specimens (Sg01, Sg03, Sg06, Sg07, Sg09, and Sg18) had elevated mean concentrations exceeding 4 μg g^{-1} of Pb in the liver (Table 4). These animals form a heterogenic group that contemplates both sex and different age classes. The specimen Sg01 was an infant that had a 6.03 μg g^{-1} Pb mean concentration in the liver. The specimens Sg07 and Sg09 were juvenile males, which had mean concentrations of 7.15 μg g^{-1} and 9.62 μg g^{-1} of Pb in the liver, respectively. This last one being the most elevated mean concentration of the metal among the studied animals. The specimens Sg03, Sg06 and Sg18 were adult females that had mean concentrations of 5.42 μg g^{-1}, 8.93 μg g^{-1} and 4.70 μg g^{-1} of Pb in the liver, respectively. The female Sg18 had been producing milk, which indicates that it was probably nursing an infant from a recent pregnancy. Due to the limitations imposed by the lack of available information, a profound analysis could not be held in these animals.

In order to understand these elevated accumulations, the bioavailability of the element in the environment must be observed, as the concentrations of non-essential metals are expected to be high only in cases of exposure to environmental contamination. In these cases, the eating habits of the species and the complexity of the food chain are also important, once the primary route of exposure to metals is through the feeding (Law *et al.,* 1991; Das *et al.,* 2000).

Regarding theses aspects, the local history and previous studies elucidate the bioavailability of the element in the area. The contamination of the sediments of the Ribeira de Iguape River and of the northern and central part of the Estuarine-Lagoon Complex of Iguape-Cananéia by lead caused by ancient mining activities may have decreased over the years (Guimarães & Sígolo, 2008; Mahiques *et al.,* 2013; Abessa *et al.,* 2014). However, the metal is still bioavailable through the suspended solids being assimilated by the local biota, with the elevated accumulation of the metal reported in the tissues of different species of mollusks of the Ribeira de Iguape River (Guimarães & Sígolo, 2008; Rodrigues *et al.,* 2012) and in fishes from the estuarine region (Choueri, 2015).

This direct measurement of the bioavailability of Pb in the liver of the dolphins suggests the trophic transfer of the metal. Their exposition through the animals of lower trophic levels, as mollusks and fishes, would allow the biomagnification of lead in higher trophic levels and may be reflected in the concentrations found in the liver of the Guiana dolphins. As so, indicating that the contamination of the aquatic food chain in the studied area, may be of concern for the health of this small dolphin population and for the local human population on the consumption of aquatic organisms.

Previous investigations demonstrate the influence of the environmental contamination on the exposition of dolphins to Pb, and the importance to investigate their habitat conditions. Law *et al.* (1991; 1992) found high concentrations of this metal, up to 7 μg $g^{-1 \text{ in the liver}}$ of dolphins, and the author evidenced that these higher concentrations of Pb were observed in the animals that live close to anthropogenic sources. Kemper *et al.* (1994) reported the highest concentration of Pb, in the amount of 62 μg g^{-1}, in bones of an infant bottlenose dolphin (*Tursiops truncatus*) stranded dead near a lead smelter in the Australian coast.

However, it is still necessary to evaluate how physiological parameters and the environmental conditions interfere in the process of bioaccumulation, since the total amount of these elements in the liver does not provide enough information about the origin of the contamination and the toxicity of the analyzed samples.

The very wide variation of Pb concentrations in the analyzed animals (<LOQ – 9.62 μg g^{-1}), suggests that the eating habits of the species also may affect the intake of the metal. The Guiana dolphin has most of its diet based on teleost fish, displaying different proportions of items and different prey items according to the location and the availability of food (Cremer *et al.,* 2012). This may affect the exposure of Pb of each animal individually, similarly to the Cd, were the eating habits of each sampled individual may influence the intake and, consequently, the accumulation of the metal (Salgado *et al.,* 2015).

Thus, the intake of different food items may explain the variations in the concentrations of the metal among the animals from the same location, since it varies with the age and the sex of the individuals and the occupancy of different areas between the specimens, allowing the accumulation of the metal in different levels. Additionally, factors such as the effect of individual metabolism would influence Pb concentrations in dolphins, this being probably the most difficult factor to understand when working with wildlife fauna such as marine mammals.

In infants (as observed in the specimen Sg01), high concentrations of Pb can be explained by the mother to the infant transfers, which can occur by the capacity of the metal to penetrate the placenta and during the lactation. This transfer possible reflects the concentration of maternal skeleton and in humans this transfer may determine more than 50% of Pb level transferred to the fetus, causing the fetal blood to contain almost the same metal levels that the mother´s blood (Chen *et al.,* 2014). This deposit also contributes significantly as a source of lead to the fetus, in fetal skeleton training period (Chen *et al.,* 2014).

Moreover, there is the possibility that these animals could be natural from others areas and that these concentrations may reflect the bioavailability of nearby areas. The studied region is located between two nominated impacted regions, the Paranaguá Estuary in the south and the Santos Estuary in the north, which houses two of the most important harbors of the Brazilian coast. However, there is no information about the concentrations of metals in Guianas dolphins from these areas.

Regarding the Pb toxicity and it possible adverse effects, in general, clinical signs of poisoning by Pb vary according to the degree and the duration of the exposure (Moreira & Moreira, 2004). The case described by Shlosberg *et al.* (1997) shows the direct effects of lead poisoning resulting in death within a short period. The authors analyzed a bottlenose dolphin that died in an aquarium in Israel, few weeks after ingesting inadvertently 55 lead bullets (25g) and verified a concentration of 3.6 µg g^{-1} in the liver. According to the authors, this concentration has been responsible for several dysfunctions in the animal's body with the observation of damages in the liver, kidneys, brain and the optic nerve.

However, chronic intoxication, which occurs through continuous ingestion of low concentrations as seen in wild animals, leads it to more subtle effects evident only on continuous observation, which may over time, induce sub-lethal effects and exceptionally lead it to death (Reijnders *et al.,* 1999). In addition, the Pb stored in the skeleton can be remobilized into the circulation in situations that promote bone resorption as in physiological conditions (rapid growth of infants, pregnancy, lactation) and pathological conditions (infections, osteoporosis), thus providing a mechanism for delayed toxicity (Smith *et al.,* 1996).

Metals can cause immunosuppression, secondary diseases and tumors (Reijnders *et al.,* 1999; Das *et al.,* 2000). They can influence the function of immunocompetent cells resulting in the humoral immunity suppression, as well as acute or chronic inflammatory processes leading to hypersensitivity and autoimmune diseases (Reijnders *et al.,* 1999; Kakuschke & Prange, 2007). Pellisso *et al.* (2008) demonstrated the adverse effects of Pb in the immune functions. The authors observed the suppression of lymphocyte proliferation *in vitro* in blood cells of captive dolphins (*Tursiops truncatus*) exposed to concentrations lower than 10 mg L^{-1}, which may result in reduced resistance to infectious diseases (Pellisso *et al.*, 2008).

Many researches have highlighted the possibility of a single kind of contaminant or the synergistic role of different substances in the increase of the susceptibility of affected animals to diseases (Das *et al.,* 2000; Van Bressem *et al.,* 2009a; Moura *et al.,* 2011; Moura *et al.,* 2014). Immunosuppressants contaminants seem to assist in the emergence of skins diseases (Van Bressem *et al.,* 2009 a; Van Bressem *et al.,* 2009b) and aggravate parasite infections in dolphins (Measures, 2001).

There are no studies regarding the incidence of skin diseases in the Guiana dolphins' population from the studied area. However, some cutaneous lesions observed in the animals by the Instituto de Pesquisas Cananéia (IPeC), including specimens used in this study (Figure 3 A), suggests that these pathological conditions are already present among these dolphins. Others skin diseases, as lobomycosis-like disease and nodular skin disease were observed in Guiana dolphins from a very near coastal area, as a possible indicator of a compromised marine environment (Van Bressem *et al.,* 2009 b).

The presence of the parasites, *Braunina cordiformis* in the stomach and *Halocercus brasiliensis* in the lungs, have been frequently observed in *Sotalia guianensis* (Marigo *et al.,* 2010), and also seen in the population of the estuary (Figure 3 B and C). When in large numbers, they can cause significant lesions that may affect the health and, subsequently, the animal's survival. Marigo *et al.* (2010), found the same two species of parasites in dolphins of the Cananéia region and in the coast of Paraná State and concluded that the presence of small dots of gastric lesions caused by *B. cordiformis*, it probably would not affect the health of the animals. However, the high amount of *H. brasiliensis* found in the lung and the evidence of parasitic pneumonias through histopathological analyzes in some animals indicated that this could be an important debilitating condition for the species.

It is still unclear how the level of these Pb concentrations may be affecting the health of this Guiana dolphin population. The effects of the chronic exposure are difficult to observe and may go unnoticed for years in natural populations and so far, cause-effects relations have not been established.

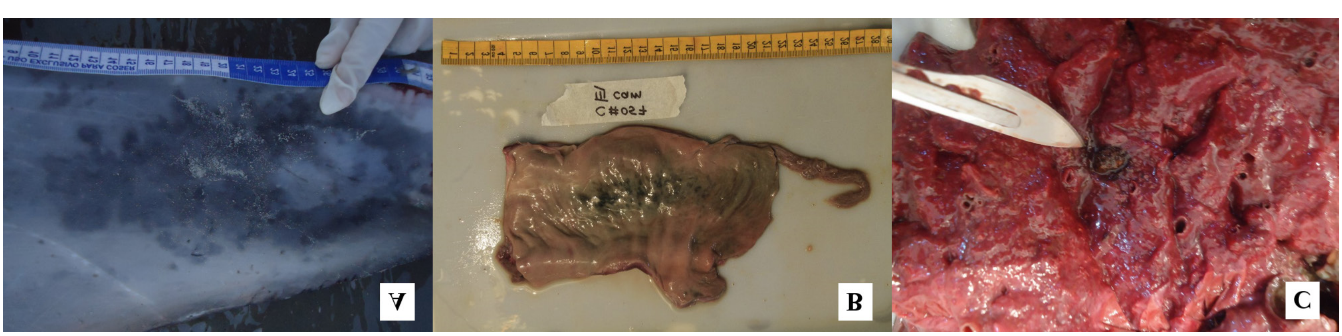

Figure 3. Pathological conditions observed in *S. guianensis* of the Estuarine-Lagoon Complex of Iguape-Cananéia. Images corresponds to Sg18 specimen. A) Dermal lesions on the caudal peduncle, indicating a cutaneous disease of unknown etiology. B) Necrosis in the third chamber of the stomach that may have been caused by the presence of a large number of *B. cordiformis*. C) *H. brasiliensis* cysts in lung bronchi. Source: IPeC File.

In relation to the accumulation patters observed for the studies metals, marine mammals generally do not present significant differences between the sexes (O´shea, 1999; Caurant *et al.,* 2006). This similarity in the accumulations of metals in liver between the genders was also observed for *S. guianensis* by Monteiro-Neto *et al.* (2003), Kunito *et al.* (2004) and Seixas *et al.* (2009). Moreover, the studies held by Carvalho *et al.* (2008), Seixas *et al.* (2009) and Lemos *et al.* (2013) found no correlation between the accumulation of the studied metals in the liver of the species and the body length, which is directly related to the age class (Rosas & Monteiro-Filho, 2002). However, high copper concentrations have been extensively observed in very young animals in marine mammals (Caurant *et al.,* 1994; Kunito *et al.,* 2004; Más-Rosa, 2009; Bilandžić *et al.,* 2016).

FINAL CONSIDERATIONS

This study provides new data of concentrations of metals in liver of Guiana Dolphin, contributing to a better understanding of the accumulations in the specie and the impact of their habitat in the Brazilian coast.

We described the highest mean concentration of lead for the species, revealing the existence of an extensive environmental contamination by this element in the studied area. This direct measurement of the bioavailability of the metal in the dolphins may reflect their exposition through the lower trophic levels animals, highlighting the contamination of the food chain, which may be a matter of concern for the health of this small dolphin population. However, it is still necessary to evaluate how physiological parameters and the environmental conditions interfere in the process of bioaccumulation, since the total amount of these elements in the liver does not provide enough information about the origin of the contamination and the toxicity of the analyzed samples.

Thus, the author's incentives news research on the way to analyze the potential link between the level of these concentrations and depletory effects in these mammals health. For that, a long-term monitoring program is required, using detailed pathological studies combined with the consideration of the burden of chemical contaminants, in order to elucidate the forms of this metal in the environment, involving the evaluation of the food chain and establishing relationships between the availability and its toxicity. Moreover, we recommend the use of the species as a sentinel of environmental quality of their habitat due its biological and ecological peculiarities on the way to contribute to the conservation status of these animals in Brazil

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