



Metal and Metalloid Contamination in Green Sea Turtles (*Chelonia mydas*) Found Stranded in Southeastern Brazil

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Sea turtles tend to accumulate high metal levels in their tissues and are considered excellent pollution bioindicators. Studies concerning metal contamination in hatchlings, however, are non-existent for one of the most abundant species in Brazil, green sea turtles, while several other metal assessments in juvenile muscles are still scarce. In this context, this study aimed to analyze the concentrations of 12 elements in kidney and muscle samples from green sea turtles (*Chelonia mydas*; $n = 24$) found stranded in Rio de Janeiro, southeastern Brazil. Elemental concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS). The presence of fibropapillomatosis, an increasingly common disease in sea turtles which has been associated to metal contamination, was also evaluated. Most elements (Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, and Pb) were significantly higher in liver, while Al and As were significantly higher in muscle tissue, indicating bioaccumulation of the latter two elements. No differences between juvenile male and female green turtles were observed for either liver or kidney concerning the investigated elements. Literature comparisons of studies conducted in other areas throughout the Brazilian coast indicate higher concentrations of Cd, Mn, As, Hg, and Zn in kidneys, of probable anthropogenic origin. Several statistically significant inter-elemental correlations were observed between toxic elements, indicating similar sources for these environmental contaminants. Significant correlations between Hg in muscle and kidney and As in the same organs suggest bioaccumulation of both elements in muscle. Three individuals

assessed herein exhibited fibropapillomatosis, and further assessments in this regard and potential correlations to the detected metal concentrations are currently being carried out. In addition, evaluations concerning other toxic compounds, as well as deleterious cellular effects, are also underway, since total metal concentrations do not reflect total elemental bioavailability.

Keywords: metal contamination, *Chelonia mydas*, fibropapillomatosis, environmental health, bioindicator

INTRODUCTION

Among environmental pollutants generated by anthropogenic activities, metals are of significant concern due to their environmental persistence and potential toxicity (Marins et al., 1998; Marcovecchio, 2000; Baird, 2002; Miller et al., 2002; Melo, 2003).

Long lived animals, such as sea turtles, tend to accumulate higher levels of metals in their tissues compared to the water column (De la Lanza-Espino et al., 2000; Aguilar et al., 2002), and are, thus, considered competent chemical contamination indicators (Caurant et al., 1999). Exposure to metals, in general, has been associated with turtle enzyme inactivation and protein denaturing, causing deleterious effects such as developmental disorders, neurological damage, carcinogenic effects, and death (Fossi and Marsili, 2003; Decataldo et al., 2004; De Jesus and de Carvalho, 2008; Marijić et al., 2016). In addition to deleterious biochemical effects, sea turtles have also been reported to develop fibropapillomatosis due to contaminant exposure, including metals (Keller et al., 2014; Da Silva et al., 2016), and higher frequencies of fibropapillomatosis have been reported in contaminated environments (Formia et al., 2007; Guimarães et al., 2013), indicating a potential link between chemical environmental contamination, oxidative stress, dysregulation of metabolic functions and the consequent development of this disease. This condition is caused by a herpesvirus and, in turtles, is characterized by the appearance of tumor masses on different parts of the body, such as the oral cavity, eyes, skin, carapace and, in about 25–30% of affected individuals, internal organs (Herbst, 1994; Aguirre et al., 2002; Aguirre and Lutz, 2004).

Of the seven species of sea turtle found worldwide, five are found throughout the Brazilian coast, constituting two families, Cheloniidae, comprising *Chelonia mydas* (green turtles), *Eretmochelys imbricata* (hawksbill turtles), *Caretta caretta* (loggerhead turtles), and *Lepidochelys olivacea* (olive ridley turtles), and Dermochelyidae, comprising *Dermochelys coriacea* (leatherback turtles) (Marcovaldi and Dei Marcovaldi, 1999). All are classified as vulnerable, threatened or critically endangered, according to the International Union for Conservation of Nature (IUCN, 2020). The most frequent and abundant species in Brazil are green sea turtles (Bezerra et al., 2012). Green sea turtle hatchlings exhibit a pelagic phase lasting for about 5–10 years, during which they remain associated with pelagic currents (Musick and Limpus, 1997), while juveniles generally recruit to developmental habitats prior to moving to adult feeding grounds (Arthur et al., 2008). Unfortunately, many sea turtle nesting and feeding areas in Southeastern Brazil are located in highly urbanized areas (De Macêdo et al., 2015), exposing these animals

to contaminants originated from anthropogenic activities during all development stages. Studies concerning metal contamination during different turtle development stages, however, are scarce in general. Furthermore, assessments concerning metal levels in kidney and muscle samples from green sea turtle hatchlings are extremely infrequent in Brazil, while a scarcity of metal assessments for juvenile muscle tissue in general is noted. In this context, this study aimed to analyze the concentrations of 12 metals and metalloids in kidney and muscle samples of male and female hatchling and juvenile green sea turtle individuals found stranded in the state of Rio de Janeiro, southeastern Brazil and perform preliminary associations between the presence of these contaminants and the development of fibropapillomatosis.

METHODOLOGY

Study Area

The study area is located in the state of Rio de Janeiro, 200 southeastern Brazil, in the Região dos Lagos region, which 201 comprises the municipalities of Maricá, Saquarema, Araruama, 202 São Pedro da Aldeia, Cabo Frio, Arraial do Cabo, Iguaba Grande, 203 Armação dos Búzios, Casimiro de Abreu, and Rio das Ostras (**Figure 1**). This region suffers the influence of ports and oil platforms in adjacent areas, as well as increasing urbanization in recent years. In addition, during certain periods of the year, the Região dos Lagos receives a high number of tourists, significantly impacting the marine environment, as high volumes sewage, solid waste and contaminants are directly discharged into local water bodies (Da Silva et al., 2018).

Sea Turtle Sampling and Processing

Green sea turtles were found stranded during regular beach monitoring, carried out twice a day from January to December 2019 at Região dos Lagos. Geographic coordinates, time, and carcass conditions were recorded. Carcass weight, curved carapace length (CCL), and curved carapace width (CCW) were determined (Frazer and Ehrhart, 1985). Juveniles were categorized as those displaying over 20 cm CCL and less than 90–100 cm, when sexual maturity is reached (Heppell et al., 2003; Reich et al., 2007). Fibropapillomatosis was detected through visual inspection of external masses by experienced veterinarians (presence or absence of external tumor masses). Sex was determined through visual macroinspection of the gonads during necropsies, according to Wyneken (2001). After the biometric analyses, the animals were dissected through standardized procedures (Wyneken, 2001), and muscle and kidney samples were obtained. The tissue samples were stored

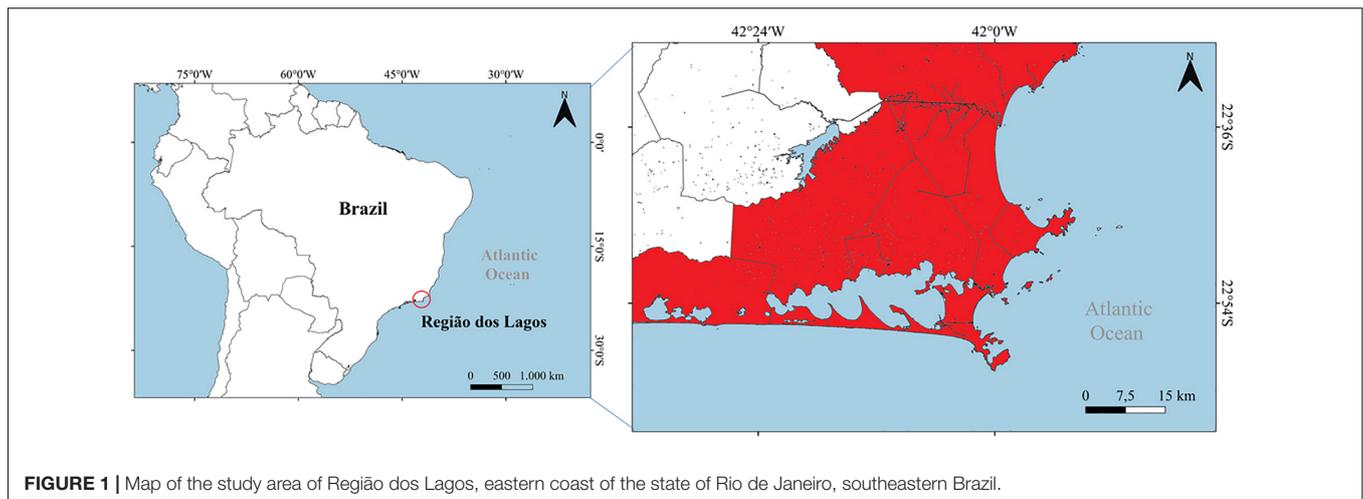


FIGURE 1 | Map of the study area of Região dos Lagos, eastern coast of the state of Rio de Janeiro, southeastern Brazil.

TABLE 1 | ICP-MS instrumental operating conditions applied in elemental determinations in green turtle (*Chelonia mydas*) muscle and kidney samples.

ICP-MS parameter	Condition
RF power	1100 W
Plasma gas flow rate	17.0 L min ⁻¹
Auxiliary gas flow rate	1.2 L min ⁻¹
Carrier gas flow rate	0.98 L min ⁻¹
Sampling and skimmer cones	Pt
Dwell time	50 ms per isotope
Number of readings	5

separately in sterile polypropylene tubes and frozen at -80°C until further processing. Authorization for sampling and analyses of the stranded sea turtles was given by the Brazilian Ministry of Environment (ABIO license no. 861/2017). No ethics committee authorization in Brazil is required for the analyses of animals found dead.

Total Elemental Determinations

Samples were thawed and approximately 150 mg of each were weighed, transferred to new sterile 15 mL polypropylene tubes and mixed with 1.5 mL of bidistilled nitric acid (HNO_3 , 67% v/v). The samples were then heated to 100°C in the capped (closed) polypropylene tubes for approximately 4 h, avoiding loss of volatile elements, such as As and Hg (USP, 2013). After cooling, samples were adequately diluted with Milli-Q water (resistivity $>18.0\ \text{M}\Omega\ \text{cm}$) obtained from a Merck Millipore water purifying system (Darmstadt, Germany), for subsequent analysis by inductively coupled plasma mass spectrometry (ICP-MS), employing a NexIon 300X spectrometer (PerkinElmer, United States). Multielemental external calibration was applied by appropriate dilutions of a mixed standard solution (Merck IV) and ^{103}Rh was used as the internal standard from a $20\ \text{mg}\ \text{L}^{-1}$ solution introduced online. All determinations were performed in triplicate. Analytical curve correlation coefficients were always above 0.995. The instrumental ICP-MS conditions are displayed in **Table 1**.

TABLE 2 | Limits of detection (LOD) and limits of quantification (LOQ) for each element investigated herein in green turtle (*Chelonia mydas*) muscle and kidney samples.

Element	LOD ($\text{mg}\ \text{kg}^{-1}$)	LOQ ($\text{mg}\ \text{kg}^{-1}$)
Al	0.29	0.99
As	0.002	0.008
Ag	0.0005	0.0017
Cd	0.0001	0.0002
Co	0.0002	0.0006
Cu	0.017	0.055
Fe	0.50	1.65
Hg	0.009	0.028
Mg	0.29	0.99
Mn	0.002	0.006
Na	0.050	0.168
Ni	0.0015	0.0050
Pb	0.010	0.035
Zn	0.013	0.042

TABLE 3 | Observed and certified concentrations ($\text{mg}\ \text{kg}^{-1}$) for the ERMBB422 certified reference material and elemental recoveries (%).

Element	Certified value	Observed value	% Recovery
As	12.7 ± 0.7	15.7 ± 0.7	124
Cd	0.0075 ± 0.0018	0.0049 ± 0.0024	66
Cu	1.67 ± 0.16	1.62 ± 0.09	97
Fe	9.4 ± 1.4	9.8 ± 2.8	104
Hg	0.601 ± 0.030	0.701 ± 0.049	116
Mg	1370	1330 ± 38.74	97
Na	2800	2665 ± 114	95
Zn	16 ± 1.1	16 ± 0.7	99

The limits of detection (LOD) and limits of quantification (LOQ) for each investigated element were calculated according to the Brazilian National Institute of Metrology, Quality and Technology (Inmetro, 2016) using the following equations: $\text{LOQ} = (3 \times \text{DSDP} \times \text{df})/\text{slope of the line}$ and

$LOQ = (10 \times SD \times df)/\text{slope}$ of the line, where SD is the standard deviation of the ratio of the analytical signal to the internal standard signal of 10 blanks and df is the applied sample dilution factor. The determined LOD and LOQ for each investigated element in the present study are displayed in **Table 2**.

Method accuracy was verified by the parallel analysis of procedural blanks and of a certified reference material (ERMBB422 – fish tissue, European Commission), in triplicate. **Table 3** displays the observed and certified values for the ERMBB422 certified reference material and elemental recovery percentages. The volatile elements determined herein (As and Hg) presented slightly higher concentrations than the certified values, demonstrating that the sample preparation procedure is efficient and not prone to losses. In addition, the concentrations are also higher than the limits of quantification of the technique obtained through direct introduction of the sample solution. Therefore, it is not necessary to employ the vapor generation technique, which is more time- and reagent-consuming. Certified reference material recovery values were considered adequate for this type of study, as per Eurachem standards (Eurachem, 1998; Ishak et al., 2015).

Statistical Analyses

Data normality was assessed by the Shapiro–Wilk normality test. As data displayed a non-gaussian distribution, Spearman's correlation test was used to evaluate the degree of associations between the determined elements and CCL, and between

element pairs. Only strong and very strong correlations were evaluated, according to Bryman and Cramer (2011). The Mann–Whitney test was used to assess differences between elemental concentrations among organs and between males and females. Potential differences regarding metal concentrations between the three juvenile individuals presenting fibropapillomatosis and the other juveniles were evaluated by the Kruskal–Wallis test. The significance level for all statistical tests was set at $p < 0.05$. The statistical analyses were performed using the R software (version 3.5.0) R Core Team (2019).

RESULTS

Sea Turtle Stranding Locations

A total of 24 stranded green sea turtles were obtained. The sea turtle stranding locations within the oil fields and exploration blocks located at the Campos Basin are exhibited in **Figure 2**.

Morphobiometric Results

The morphobiometric data and the presence or absence of fibropapillomatosis of the assessed green turtles are exhibited in **Table 4**. Concerning sex, 75% of the individuals were female and 25% males. Regarding maturity, most (87.5%) were juveniles and 12.5% were hatchlings. Of the 24 individuals, only three (12.5%) presented fibropapillomatosis. CCL and CCW means for hatchlings were of 26.87 ± 0.47 cm and

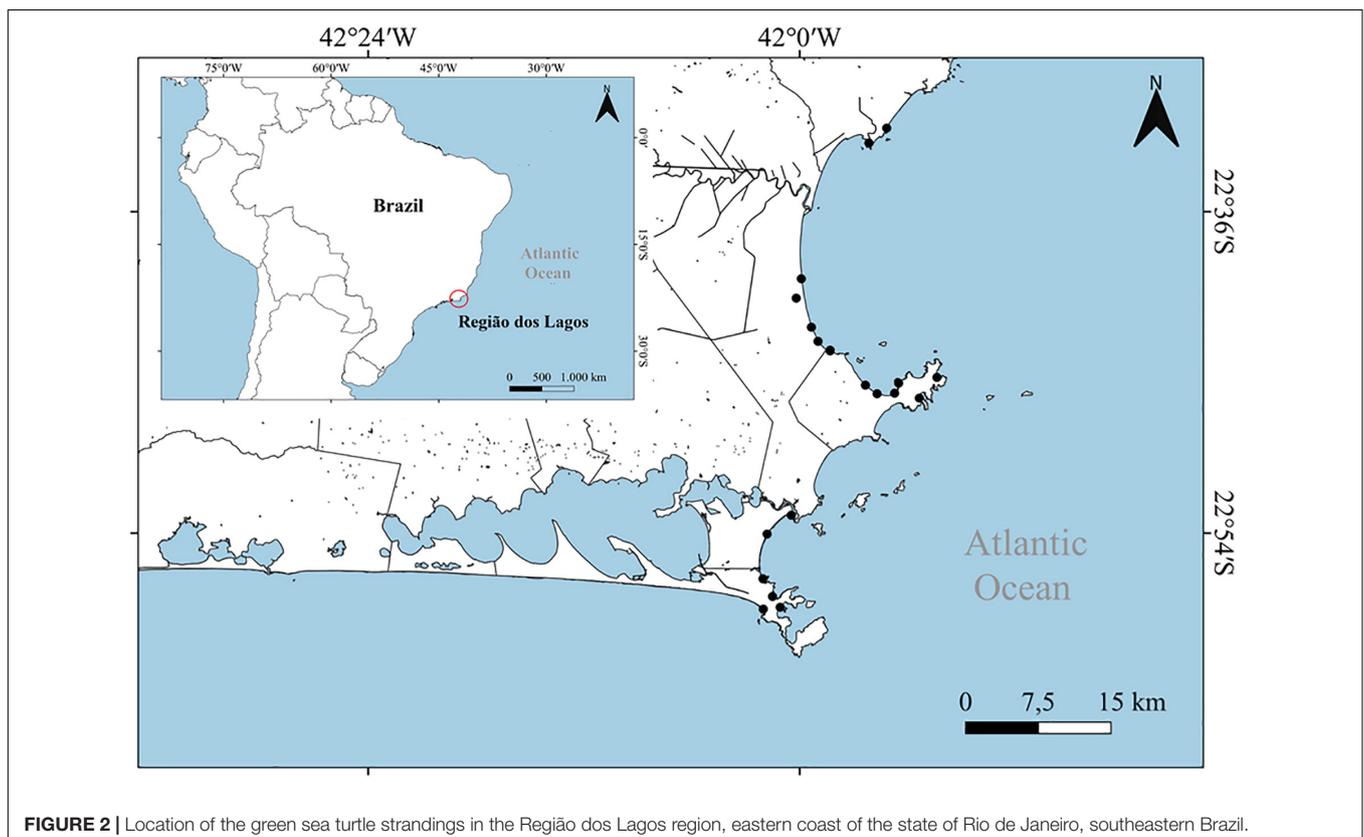


FIGURE 2 | Location of the green sea turtle strandings in the Região dos Lagos region, eastern coast of the state of Rio de Janeiro, southeastern Brazil.

TABLE 4 | Morphobiometric results of the evaluated sea turtles, indicating, sex, maturation (according to CCL), curved carapace length (CCL, in cm), curved carapace width (CCW, in cm), and presence or absence of fibropapillomatosis.

Code	Sex	Maturation stage	CCL (cm)	CCW (cm)	Fibropapillomatosis	Decomposition scale
T1	Female	Juvenile	32	27.8	Absent	2
T2	Female	Juvenile	29.8	28.3	Absent	2
T3	Female	Juvenile	35.9	35.5	Absent	2
T4	Female	Juvenile	35.2	31.2	Absent	2
T5	Female	Juvenile	38.9	34.6	Absent	2
T6	Female	Hatchling	26.5	25.2	Absent	2
T7	Female	Juvenile	57.4	50.7	Present	3
T8	Female	Juvenile	35.5	32.5	Absent	2
T9	Female	Juvenile	34.5	30.4	Absent	3
T10	Female	Juvenile	32.5	31.5	Absent	4
T11	Female	Juvenile	39.9	35.7	Absent	3
T12	Female	Juvenile	29.8	28.4	Absent	2
T13	Male	Hatchling	26.7	24.9	Absent	3
T14	Male	Juvenile	33.8	30.5	Absent	2
T15	Female	Juvenile	39.9	36.1	Absent	3
T16	Female	Juvenile	34.7	31	Absent	2
T17	Male	Juvenile	40.6	36.3	Absent	2
T18	Female	Hatchling	27.4	25.2	Absent	2
T19	Male	Juvenile	32.2	29.3	Absent	2
T20	Male	Juvenile	52.9	45.8	Present	2
T21	Female	Juvenile	38.9	36.4	Absent	2
T22	Female	Juvenile	33.7	29.8	Absent	2
T23	Female	Juvenile	34	30.3	Present	3
T24	Male	Juvenile	37.2	34.2	Absent	2

25.10 ± 0.17 cm, while juveniles measured 37.11 ± 6.8 cm and 33.63 ± 5.67 cm, respectively.

Elemental Determinations

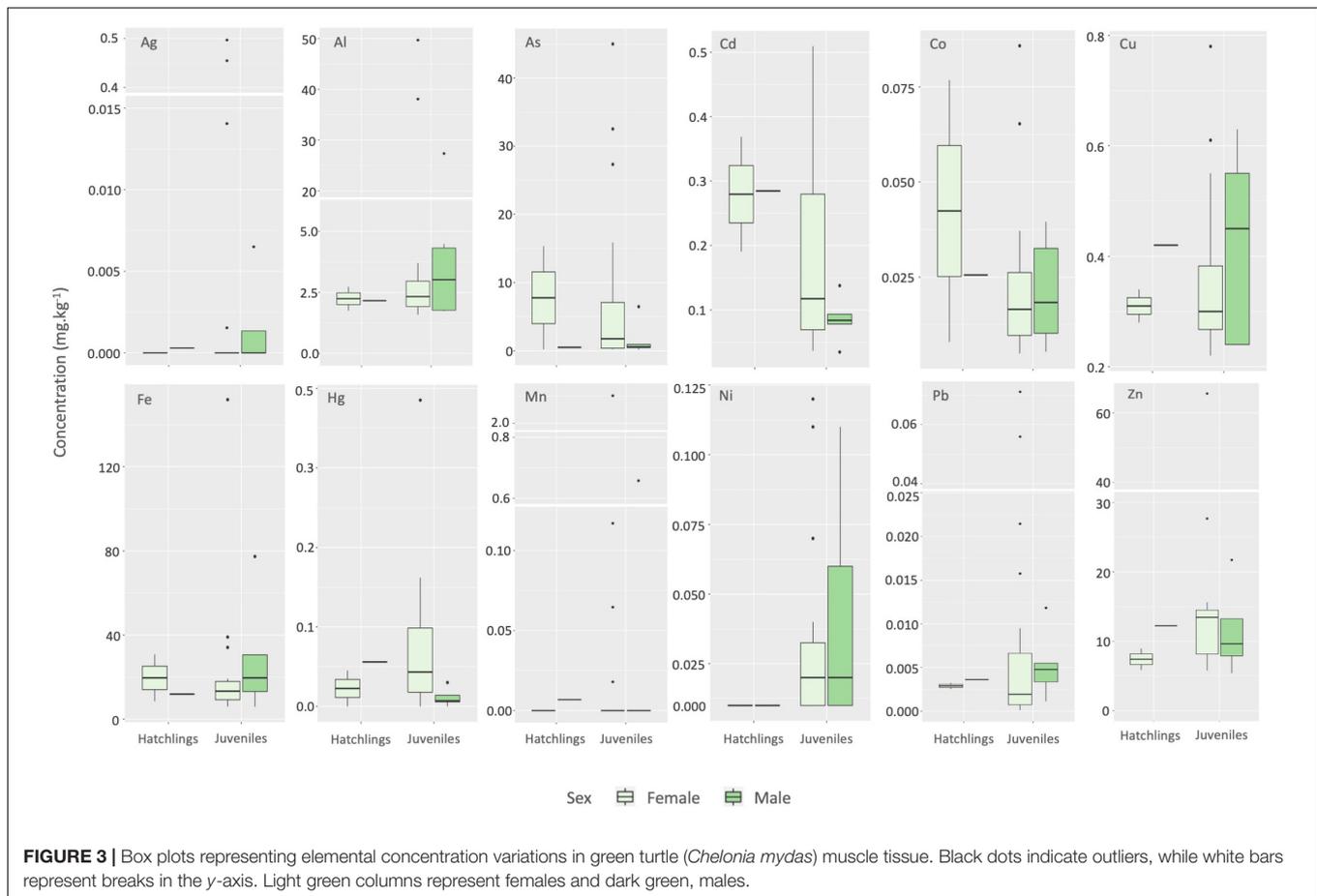
Elemental concentrations in green turtle muscle tissue and kidneys are exhibited in **Figures 3, 4**. The concentrations of most elements (Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, and Pb) were significantly ($p < 0.05$) higher in kidney when compared to muscle samples. In contrast, Al and As were significantly ($p < 0.05$) higher in muscle tissue. This seems to indicate inefficient detoxification of these elements by the liver and kidney routes, thus resulting in muscle bioaccumulation. This is due to the fact that these contaminants are generally lipophilic and usually detoxified first by the liver, followed by kidneys and only then, if present in high concentrations above organic-specific detoxification thresholds, do they accumulate in muscle (Hall et al., 1989; Becker and Bigham, 1995; Taylor et al., 2017).

No significant differences in concentrations were observed between male and female green turtle juveniles in liver or kidney for all investigated elements in the present study. Most studies report no differences in feeding strategies between adult males and females (Barbieri, 2009; Prior et al., 2015; Stokes et al., 2019). One of these studies compared the diets of males, gravid females and non-gravid females from the Western Indian Ocean (Republic of Seychelles), and reported only differences between the gravid and non-gravid females, where seagrass accounted

for 95% of gut content biomass for both males and non-breeding females, but only 58% for gravid females, alongside substrate (14%), and macroalgae (13%) contents (Stokes et al., 2019). Juvenile assessments, however, are rarer. One assessment indicated no difference between green turtle juvenile and adult feeding habits in seagrass habitats by applying last-bite diet and stable isotope analyses within Port Curtis, QLD, Australia (Prior et al., 2015), although the authors did not assess differences between male and female juveniles.

Generally higher concentrations in juveniles compared to hatchlings were observed for Ag, Al, Cu, Fe, Hg, Ni, Pb, and Zn. This is expected, as green turtle hatchlings, which remain associated with pelagic currents for a number of years (Musick and Limpus, 1997), shift their dietary niche from omnivorous, feeding on neustonic material, to herbivorous in their juvenile and adult stages, when they feed on macroalgae, sea grass and/or mangrove material (Bjorndal, 1997; Cardona et al., 2010), favoring environmental contaminant biomagnification. In addition, juveniles inhabit a slightly differential niche than adult individuals, of shallower habitats and amongst mangroves, with higher abundance of macroalgae, while adults inhabit deeper channels, feeding on deeper seagrass beds (Limpus et al., 2005).

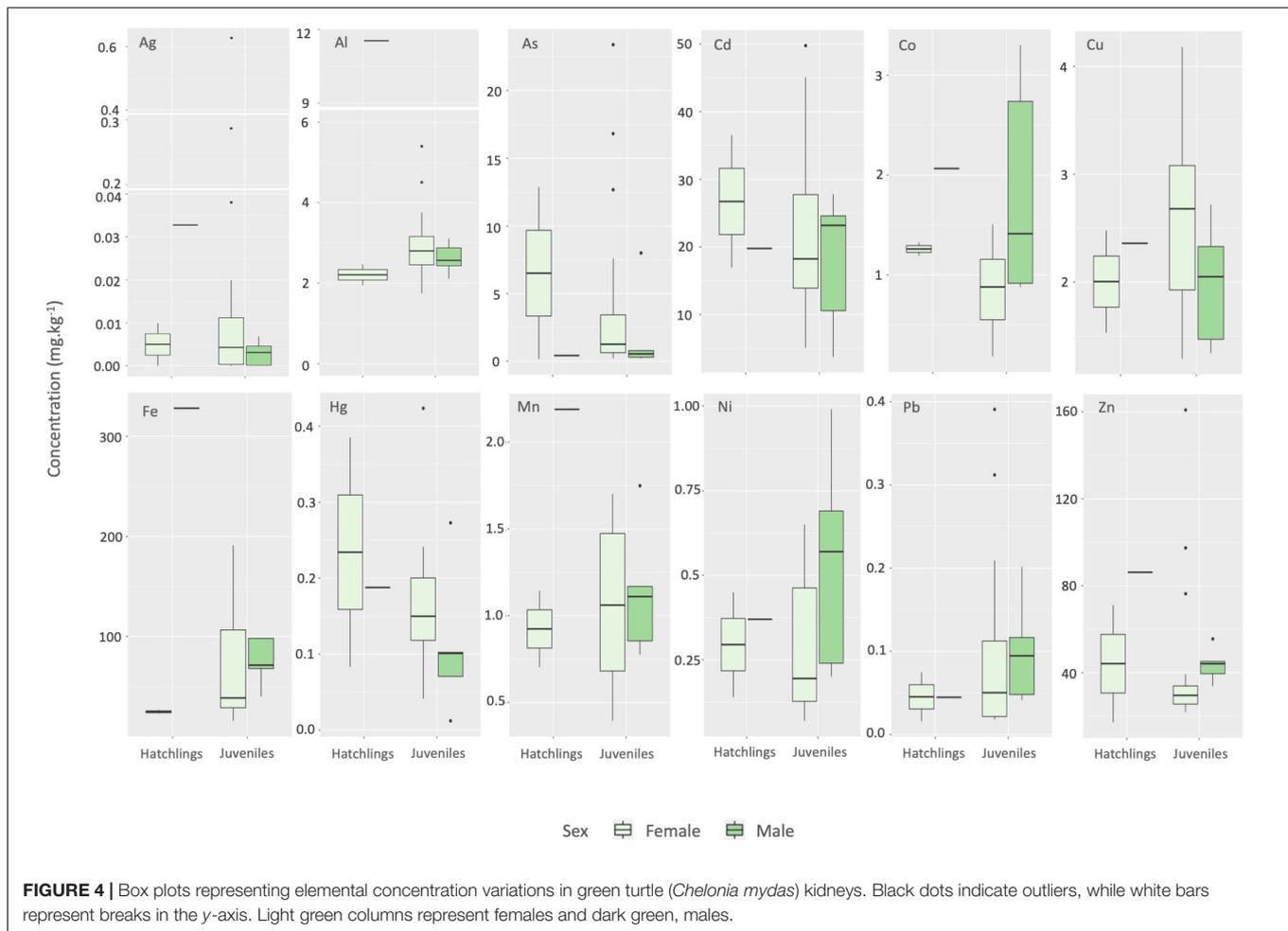
No statistically significant correlations were observed between the concentrations of the determined elements in both muscle and kidney and turtle CCL. This is in agreement with previous assessments (Gardner et al., 2006), but in contrast with other reports indicating negative correlations between CCL and Cd



and Cu concentrations in muscle (Da Silva et al., 2014), CCL and Cd and Zn concentrations in kidney (Gordon et al., 1998), CCL and As in muscle and kidney (Saeki et al., 2000), as well as negative correlations between CCL and Zn, Cu and Cd in kidney, positive correlation between CCL and Mn concentration in the same tissue, negative correlations between CCL and Mn and Hg concentration in muscle and positive correlation between CCL and Cu concentrations (Sakai et al., 2000) in green turtles. A trend toward negative correlations between elemental concentrations in kidney and muscle and body size in green turtles has been attributed to the previously mentioned ontogenic shift in diet observed in this species McKenzie et al. (1999). Thus, exposure to metals and a bioaccumulation trend may occur only in the initial phase of this species life, and subsequent growth and development would decrease contaminant loads, due to the “dilution effect” (Kamunde and Wood, 2003).

Statistically significant elemental concentration differences between the three juvenile individuals presenting fibropapillomatosis and healthy juveniles were observed only for Cu, an essential element, in muscle. In this case, animals presenting the condition exhibited a means of $0.26 \pm 0.02 \text{ mg kg}^{-1}$, significantly lower than the mean for healthy individuals, of $1.82 \pm 0.20 \text{ mg kg}^{-1}$. Although the exact etiology of fibropapillomatosis is not yet fully known, its multicausal

nature is a consensus, and many studies suggest genetic predispositions, contamination by environmental contaminants, mainly polycyclic aromatic hydrocarbons and metals, alterations in chemical water parameters and the presence of the herpesvirus in the environment (Aguirre and Lutz, 2004; Da Silva et al., 2016). For example, reported high Cu and Pb concentrations (means of $1.32 \mu\text{g g}^{-1}$ and $1.44 \mu\text{g g}^{-1}$, respectively) in the blood of sea turtle individuals positively correlated to the inhibition of the activity of 3-hydroxy-3-methylglutaryl-CoA reductase, an enzyme that participates in the biosynthesis process of sterols, including cholesterol, which was detected at levels below normal in turtles affected by fibropapillomatosis. In addition, the authors suggest that oxidative stress caused by high Fe and Pb concentrations may be directly related to the cause and development of the disease. It is known that Cu is essential for optimal innate immune function, and that its deficiency results in decreased immune responses in several groups, such as mollusks, birds, and mammals (Blanco et al., 2004; Wang et al., 2009; Djoko et al., 2015), and that the reactivation of herpesvirus is closely correlated with the immune system, as the disease mainly occurs in immunocompromised individuals in several organisms (Schmader and Dworkin, 2008). Thus, it may stand to reason that the decreased Cu concentrations observed in the three individuals presenting fibropapillomatosis are related



to decreased immune responses, activating this herpesvirus in green turtles, although further evaluations are required to verify this hypothesis.

Literature comparisons of studies conducted throughout the Brazilian coast are presented in **Table 5**. The kidney samples analyzed herein exhibited higher Cd, Mn, and Ni concentrations, with a notable difference related to Cd concentrations, and lower Cu concentrations when compared to Barbieri (2009), who evaluated samples obtained from the Cananea estuary, a reportedly pristine area, with little or no metal pollution, which is probably the cause of the low metal concentrations detected in most of the assessed samples. Cd, Hg, Pb, and Zn, on the other hand, were considerably higher than reported by De Macêdo et al. (2015), which is interesting as those authors assessed a highly industrialized region on the northern coast of Bahia and expected to present high metal contamination levels. Cd and Zn levels were also considerably higher when compared to the kidney samples analyzed. Muscle samples analyzed herein contained lower Ag and Pb concentrations compared to values reported by Da Silva et al. (2014), and higher Cd, Cu, and Zn. All studies grouped both males and females, due to the aforementioned lack of statistical differences between feeding strategies. The study area evaluated by Da Silva et al. (2014) has seen a significant

increase in urban, industrial and port activities in recent years, which may have contributed to increased metal concentration in the surrounding marine environment and, consequently, in sea turtles. In addition, the region suffers from the continuous and growing input of agricultural waste from the adjacent plantation area.

Statistical Correlations Between Variables

The strong and very strong significant ($p < 0.05$) inter-elemental Spearman correlations observed among sea turtle organs are exhibited in **Table 6**.

Metal and metalloid interactions within organisms are related to several physico-chemical and biotic aspects, such as chemical speciation in the environment, which may lead to preferential competitive environmental uptake and differential tissue affinity, as well as different metal detoxification routes, resulting in differential distribution patterns in animal tissues, which indicate that inter-elemental interactive effects should be considered in environmental monitoring and risk assessment evaluations (Norwood et al., 2003; Peterson et al., 2009; Yang et al., 2010). Therefore, investigations of statistically significant inter-

TABLE 5 | Literature comparisons of metal concentrations (mg kg⁻¹) in green turtle (*Chelonia mydas*) tissues conducted in other areas throughout the Brazilian coast.

Sampling site	Tissue	Age	Ag	Al	As	Cd	Co	Cu	Fe	Hg	Mn	Ni	Pb	Zn	References
Região dos Lagos, Rio de Janeiro, Brazil	Kidney	Hatchlings (males and females)	0.014 ± 0.017	5.321 ± 5.394	4.482 ± 7.259	24.418 ± 10.573	1.530 ± 0.470	2.122 ± 0.516	125.849 ± 175.052	0.219 ± 0.153	1.345 ± 0.763	0.321 ± 0.165	0.045 ± 0.029	58.204 ± 36.257	Present Study
		Juveniles (males and females)	0.049 ± 0.146	2.893 ± 0.848	3.850 ± 6.387	20.676 ± 12.109	1.096 ± 0.742	2.492 ± 0.826	73.153 ± 55.937	0.153 ± 0.093	1.071 ± 0.402	0.349 ± 0.254	0.100 ± 0.101	43.824 ± 32.622	
	Muscle	Hatchlings (males and females)	0.000 ± 0.000	2.217 ± 0.492	5.359 ± 8.659	0.281 ± 0.089	0.037 ± 0.036	0.345 ± 0.072	17.029 ± 12.018	0.034 ± 0.030	0.002 ± 0.004	0.011 ± 0.005	0.003 ± 0.001	9.024 ± 3.190	
		Juveniles (males and females s)	0.046 ± 0.143	7.745 ± 13.321	6.981 ± 12.546	0.166 ± 0.152	0.023 ± 0.205	0.377 ± 0.160	25.112 ± 36.266	0.060 ± 0.089	0.148 ± 0.503	0.036 ± 0.037	0.011 ± 0.019	14.773 ± 12.810	
Cananeia Estuary, São Paulo, Brazil	Kidney	Hatchlings (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Barbieri, 2009
		Juveniles (males and females)	ND	ND	ND	0.280 ± 0.090	ND	3.514 ± 0.291	ND	ND	1.070 ± 0.204	0.025 ± 0.003	ND	ND	
	Muscle	Hatchlings (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
		Juveniles (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Arembepe Beach, Bahia, Brazil	Kidney	Hatchlings (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	De Macêdo et al., 2015
		Juveniles (males and females)	ND	19.488 ± 16.156	337.400 ± 295.120	15.260 ± 5.936	1.243 ± 0.655	3.808 ± 1.820	121.800 ± 64.960	0.101 ± 0.039	1.694 ± 0.787	0.538 ± 0.395	0.042 ± 0.039	42.280 ± 5.880	
	Muscle	Hatchlings (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
		Juveniles (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Rio Grande do Sul, Brazil	Kidney	Hatchlings (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Da Silva et al., 2014
		Juveniles (males and females)	0.115 ± 0.014	ND	ND	7.924 ± 0.644	ND	3.416 ± 0.308	ND	ND	ND	ND	1.512 ± 0.112	15.204 ± 1.148	
	Muscle	Hatchlings (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
		Juveniles (males and females)	0.084 ± 0.021	ND	ND	0.084 ± 0.021	ND	0.252 ± 0.042	ND	ND	ND	ND	0.882 ± 0.063	3.486 ± 0.273	

ND, Non-determined. Results reported as dry weight were transformed to wet weight considering 72% and 79% water content for kidney and muscle water content, respectively (Godley et al., 1999).

TABLE 6 | Significant ($p < 0.05$) inter-elemental Spearman correlations observed between sea turtle organs.

Association	Correlation coefficient	Strength
As K × As M	0.924	Very strong
Hg K × Hg M	0.833	Strong
Ni M × Pb M	0.808	Strong
As K × Hg K	0.733	Strong

K, kidney; M, muscle.

and intra-organ correlations are paramount in performing biological inferences. These associations have been previously suggested as indicative of common exposure sources, storage pathways and/or detoxification routes (Ribeiro et al., 2009; Jerez et al., 2013).

In the present study, all statistically significant associations were positive and either strong or very strong, although only between toxic elements (As, Ni, Hg, and Pb). Elemental associations between different organs indicate transport between tissues (Erasmus, 2004; Hauser-Davis et al., 2020) and are important to assess potentially deleterious effects and bioaccumulation or excretion processes. As all associations detected herein were positive, probable synergistic effects and similar sources for different elemental pairs in the same organ are postulated (e.g., Ni and Pb in muscle, and As and Hg in kidney). On the other hand, As and Hg present in both, kidney and muscle, seem to indicate inter-tissue transport and muscle bioaccumulation, as described previously.

CONCLUSION

The data reported herein includes baseline levels for both green turtle hatchlings and for Al, As, Co, Fe, Hg, Mn, and Ni in muscle tissue for juvenile and hatchling green sea turtles, as no studies concerning metal levels in hatchling kidney and muscle samples are available for Brazil and Al, As, Co, Fe, Hg, Mn, and Ni have not yet been determined in muscle for any age in green turtle specimens in Brazil. The establishment of these baseline elemental data is extremely valuable and paramount for biomonitoring efforts and, consequently, conservation measures for this threatened species, furthering knowledge on environmental elemental contamination in this species geographic distribution range.

No differences were observed between male and female element loads in kidney or liver, corroborating other literature reports, and generally higher metal concentrations in juveniles were observed compared to hatchlings for Ag, Al, Cu, Fe, Hg, Ni, Pb, and Zn, probably due to the ontogenic dietary shifts that occur in this species.

Statistically significant lower Cu concentrations in the three juvenile individuals presenting fibropapillomatosis were observed compared to healthy juveniles, which may indicate decreased immune functions and adequate herpesvirus activation in green turtles. Further assessments concerning other contaminants, such as polycyclic aromatic hydrocarbons, also

implicated in the etiology of this disease are, thus, required to further evaluate potentially associated factors to this condition.

In addition, probable synergistic effects and similar sources for the Ni-Pb and As-Hg toxic element pairs in green turtle muscle and kidney, respectively, are postulated, as well as inter-tissue transport between kidney and muscle and muscle bioaccumulation of As and Hg, which may be the result of a highly contaminated environment in the Campos Basin.

In sum, constant green sea turtles monitoring in this region is required, in order to aid in the conservation this species and maintain the ecological balance of marine environments. Furthermore, contaminant monitoring is also necessary aiming at both environmental and human health risk assessments and decision-making.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because no ethics committee authorization in Brazil is required for the analyses of animals found dead. Authorization for sampling and analyses of the stranded sea turtles was given by the Brazilian Ministry of Environment (ABIO license no. 861/2017).

AUTHOR CONTRIBUTIONS

DB: conceptualization, resources, data curation, visualization, investigation, formal analysis, and writing – original draft. IW and LP: data curation, visualization, and formal analysis. RR: validation, data curation, and formal analysis. TS: validation, resources, funding acquisition, supervision, and writing – draft reviewing. PB: data curation, resources, funding acquisition, and writing – draft reviewing. AS and AT: data curation, resources, and validation. FC and ES: conceptualization, resources, project administration, supervision, and writing – draft reviewing. LL: formal analysis, conceptualization, supervision, writing – original draft, and writing – draft reviewing. SS: conceptualization, resources, funding acquisition, project administration, supervision, and writing – draft reviewing. RH-D: conceptualization, resources, investigation, validation, data curation, formal analysis, project administration, supervision, writing – original draft, and writing – draft reviewing. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: PB was employed by BW Consultoria Veterinária. AS and AT were employed by CTA Serviços em Meio Ambiente.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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