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Daphne de Albuquerque Bruno

Contaminação de metais e metalóides em tartarugas-verdes (*Chelonia mydas*) encontradas encalhadas no sudeste do Brasil

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Orientador: Prof. Dr. Fábio Veríssimo Correia.

Coorientador: Prof.ª Drª. Rachel Ann Hauser-Davis e Prof. Dr. Enrico Mendes Saggioro.

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"A compaixão para comos animais éa mais nobre virtude da natureza humana."

DARWIN

RESUMO

As tartarugas-marinhas tendem a acumular altos níveis de metais em seustecidos e são consideradas excelentes bioindicadores de poluição. Estudos sobre contaminação por metais em filhotes, no entanto, são inexistentes para uma das espéciesmais abundantes no Brasil, as tartarugas verdes, enquanto avaliações de metais em músculos juvenis ainda são escassas. Nesse contexto, o presenteestudo teve como objetivo analisar as concentrações de 12 elementos em amostras renais emusculares detartarugas-verdes (*Chelonia mydas*; n = 24) encontradas encalhadas no Rio de Janeiro, sudeste do Brasil. As concentrações de metais totais foram determinadas por espectrometriade massa com plasma indutivamente acoplado (ICP-MS). Também foi avaliada a presença de fibropapilomatose, doença cada vez mais comum em tartarugasmarinhas e associada à contaminação por metais. A maioria dos elementos (Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg e Pb) foram significativamente maiores no fígado, enquanto Ale As foram significativamente maiores no tecidomuscular, indicando bioacumulação dos dois últimos elementos. Não foram observadas diferenças entre os juvenis de tartarugas verdes machos e fêmeas para o fígado ou rim em relação aos elementos investigados. Comparações na literatura de estudos realizados em outras áreas da costabrasileira indicam maiores concentrações de Cd, Mn, As, Hg e Zn nos rins, de provável origem antropogênica. Várias correlações inter-elementares estatisticamente significativas foram observadas entre os elementos tóxicos, indicando fontes semelhantes para esses contaminantes ambientais. Correlaçõessignificativas entre Hg no músculo e rim e As nos mesmos órgãos sugerem bioacumulação de ambos os elementos no músculo. Três indivíduos avaliados neste estudo apresentavam fibropapilomatose,e avaliações adicionais a este respeito e potenciais correlações com as concentrações de metal detectadas estão sendo realizadas atualmente. Além disso, avaliações sobre outros compostos tóxicos, bem como efeitos celulares deletérios, também estão em andamento, uma vez que as concentrações totais de metais não refletem a biodisponibilidade total do elemento.

Palavras-chave:contaminação, *Chelonia mydas*, fibropapilomatose, saúde ambiental, bioindicador.

ABSTRACT

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 inkidney 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 presenceof 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 observedfor either liver or kidney concerning the investigated elements. Literature comparisonsof 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 cellulareffects, are also underway, since total metal concentrations do not reflect total elemental bioavailability.

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

SUMÁRIO

1. INTRODUÇÃO

As atividades humanas têm causado cada vez mais impactos significativos sobre o ambiente aquático, provocando diversas alterações em seus processos ecológicos, sendo a relação entre estas atividades, a saúde destes ambientes e a saúde pública um consenso (Fleming;Laws, 2006). Dentre os diversos aspectosimportantes para a saúde humana, estes ambientes representam fontes significativas de água, produção debiomassa e oxigênio e diversidade biológica. Além disso, a qualidade destes ecossistemas é indispensávelpara a manutenção do planeta e, consequentemente, da saúde pública (Moura et al., 2011; NRC, 1999; Sandifer et al., 2004).

Diversos impactos negativos têm sido observados nestes ecossistemas comoa destruição do habitat e a consequente extinção de diversas espécies, levando a perda da biodiversidade e mobilização de diversos poluentes (Andersen, 1997), doenças na populaçãohumana devido à poluição (Bassil et al., 2007; Dhillon et al.,2008) e agentes infecciosos, incluindo agentes bacterianos, virais e protozoários (Santos et al., 2017),com reflexos em atividades socioeconômicas e culturais (PNUMA, 2004).

Um dos principais efeitos danosos nos ambientes aquáticos, devido a atividades antrópicas é a contaminação por poluentes químicos (Cohen,1995), que, além de contribuir para a degradação de habitatsde diversas espécies, pode também levar ao consumode espécies com altos níveis de contaminantes químicos (CAEM, 2003). Assim, é de extremaimportância monitorar condições ambientais dos ecossistemas aquáticos, tanto para realizar ações de preservação da biodiversidade, quanto ações relacionadas com a saúde pública. Istopode ser feito através de espécies sentinela (Espino, 2000).

Dentre os diversos organismos utilizados para este fim estão as tartarugas marinhas, consideradas excelentes indicadores biológicos de poluição química no ambiente marinho (Caurant, 1999). Com o biomonitoramento e controle de qualidade ambiental é possível, a partir da perspectiva de One Health, prevenir e mitigar danos causadas na biota, saúde ambiental, animal e humana. Neste contexto, este estudo teve como objetivo analisar as concentrações de metais e metalóides em amostras renais e musculares de tartarugas- verdes (*Chelonia mydas*) encontradas encalhadas no Rio de Janeiro, sudeste do Brasil, e também avaliar a presença de fibropapilomatose, doença cada vez mais comum em tartarugasmarinhas e associada à contaminação por metais, em um contexto de One Health.

De acordo com tal conceito, há apenas um tipo de saúde existente, composta por três linhas: saúde ambiental, saúde animal e saúde humana. Neste sentido, não é possívelque haja excelência em apenas um dos pilares, visto que, no caso de um desequilibrio, as trêsserão prejudicadas. Desta forma, para se entender, prevenir e recuperar um dos pilares, é necessário que haja um estudo profundo e interdisciplinar, visando o todo (Pérez; Wise Sr, 2018).

2. REFERENCIAL TEÓRICO

2.1. POLUIÇÃO MARINHA

Como consequência do crescimento populacional descontrolado, há falta de planejamento urbano eficaz, nos quais, muitas vezes, não estão contidos planos de instalação de redes de esgoto e de estações de tratamento de efluentes e, devido à disposição inadequada de resíduos, muitos contaminantes entram continuamente no ambiente aquático (Derraik, 2002).

Dentre os diversos contaminantes ambientais presentes nestes ecossistemas, os de maior preocupação são os que apresentam persistência ambiental, biodisponibilidade, tendência de bioacumulação na cadeia trófica e efeitos tóxicos, como os plásticos, os poluentes orgânicos persistentes (POPs) (Jones; Voogt, 1999) e os metais (Singh et al. 2011).

Com isso, a matéria orgânica e inorgânica continua disponível, sendo depositada nos sedimentos demares ou ficando em suspensão na coluna d'água, levando a consequências à fauna e flora do locale arredores, além da morte de animais e impactodireto na produtividade pesqueira (Silva et al., 2018). Dessa forma, os emissários submarinos são opções viáveis com relação à diminuição do despejo de efluente em regiões próximas à costa, as quais são mais utilizadas para fins de recreação(Heller, 1998). Entretanto, são passíveis de grandes impactos causados ao meio ambiente, comoa intoxicação de animais e seres humanos por substâncias tóxicas, como desreguladoresendócrinos, contaminantes emergentes e metais (Pérez; Wise Sr, 2018).

2.2. METAIS

Os metais são usualmente encontrados nos sedimentos de ambientes marinhos, contudo, podem também ser encontrados solúveis na coluna d'água. A sua toxicidade pode ser considerada relativa, pois além de sua assimilação na cadeiaalimentar depender da forma química na qual se encontra noambiente aquático, os seus efeitos tóxicos dependem da dose e do tempo de exposição dosorganismos aos metais (Melo, 2003; Baird, 2002).

Alguns metais são essenciais para o funcionamento dos organismos, incluindo os seres humanos, entretanto, em excesso, se tornam tóxicos, podendo levar a distúrbios metabólicos (Tavares; Carvalho, 1992). Ademais, devido à grande capacidade destes contaminantes de ocuparem diversoscompartimentos de um mesmo ambiente, sua biodisponibilidade é aumentada, tornando- os mais acessíveis e disponíveis à biota, causando diversos efeitos ao longo de toda a cadeia trófica (Costa et al., 2008). Nesse caso, a intoxicação por metais pode ser capaz de inativar enzimas edesnaturar proteínas (Decataldo et al., 2004; Jesus; Carvalho, 2008; Marijić et al., 2016), além de causar neoplasias, lesões em diversos tecidos, problemas reprodutivos, danos neurológicose morte (Fossi;Marsili, 2003).

A maioria dos estudos relativos a metais avaliam concentrações totais demetais específicos, não levando em conta a investigação da biodisponibilidade intracelular desses contaminantes. Como metais são capazes de atravessar membranas celulares e sofrer compartimentalização no interior dascélulas, há aumento da sua biodisponibilidade e o seu potencial causador de efeitos deletérios (Ferreira; Matsubara,

1997; Jesus; Carvalho, 2008). Com isso, os resultados de metais totais não refletem, em muitas ocasiões a concentração e biodisponibilidade de metais em um organismo (Decataldo et al., 2004; Marijić et al., 2016). Assim, também é de extrema importânciaavaliar as concentrações intracelulares de metais, e não apenas os totais.

2.3. FIBROPAPILOMATOSE

O aparecimento e o crescimento de massas tumorais de variados tamanhos no corpo de tartarugas marinhas, como na região ocular, cavidade bucal, pele,carapaça e, em casos mais graves, em órgãosinternos (Herbst, 1994; Aguirre et al., 2002) caracterizam a doença infecciosa denominada fibropapilomatose. Neste contexto, concentrações de HPAs e metais em tecidos de tartarugas marinhas, associados à presença de herpesvírus, já foram postulados como sendo a etiologia do desenvolvimento destas massas tumorais (Da Silva et al., 2016; Keller et al., 2014). O crescimento de tumores parece ser mais frequente em ambientes com condições estressantes, em regiões com baixa qualidade de água, como por exemplo regiões compresença de indústrias e de contaminantes(Formia et al., 2007; Guimarães et al., 2013).Indo mais além da tentativa de avaliar a causa desta condição em tartarugas marinhas, estudos preliminares com objetivos de relatar a presença de plásticos e fibropapilomasem tartarugas-marinhas, foram realizados na costa do estado do Rio de Janeiro (Awabdiet al., 2013; McKinlay, 2018; Reis et al., 2009; Reis et al., 2010; Tagliolatto et al.,2016), os quais mostraram a presença de diversas composições e tamanhos de materialplásticos, além de morte de indivíduos por obstrução intestinal e/ou rompimento do trato gastrointestinal. Entretanto, a questão ainda carece de informações em função dosefeitos de longo prazo da exposição a tais contaminantes.

A fibropapilomatose compartilha diversas vulnerabilidades genéticas com alguns tipos de câncer humano, como o pancreático e neuronais, como meduloblastoma e neuroblastoma (Duffyet al., 2018), revelando-se passível de tratamento com terapias antineoplásicas humanas. Assim, demonstra-se também a importância de compreender os mecanismos causativos desta doença em tartarugas marinhas, com relação direta com a saúde pública, já que alguns tipos de câncer estão entre as dez primeiras causas de morte em todo o mundo (OPAS, 2020).

2.4 TARTARUGAS MARINHAS COMO ESPÉCIES-SENTINELA

Das sete espécies de tartarugas marinhas de ocorrência mundial, cinco podem ser encontradas no Atlântico Sul e no Brasil, constituindo duas famílias:Cheloniidae, à qual pertencem as espécies *Chelonia mydas* (tartaruga-verde), *Eretmochelys imbricata* (tartaruga-de-pente), *Caretta caretta* (tartaruga cabeçuda) e *Lepidochelys olivacea* (tartaruga-oliva), e família Dermochelyidae, à qual pertence a espécie *Dermochelys coriacea* (tartaruga-de-couro) (Marcovaldi; Marcovaldi, 1999).

As regiões estuarinas, costeiras e oceânicas do litoral brasileiro são utilizadas por estas espécies para fins reprodutivos, alimentares e de desenvolvimento, apresentando locais específicos para cada fim (Marcovaldi; Marcovaldi, 1999). Todas as espécies que ocorrem no Brasil estão incluídas na Lista Nacional das Espécies da Fauna Brasileira Ameaçadas de Extinção e são classificadas segundo a IUCN como vulneráveis, ameaçadas ou criticamente ameaçadas de extinção (IUCN, 2020), constando também nos Apêndices I e II da Convenção sobre o Comércio Internacionalde Espéciesde Fauna e Flora Selvagens em Perigo de Extinção (CITES).

Assim como as aves e mamíferos marinhos, as tartarugas marinhas têm uma longa expectativa de vida e podem atingir diversos níveis tróficos na cadeia alimentar (Aguilar; Borrell, 1997). Além disso, apresentam papel ecológico extremamente importante na teia alimentar aquática, por ocuparem diversos níveis tróficos e por serem animais topos de cadeia, possuindo importante função para o ciclode energia e nutrientes nos diferentes ambientes e no controle da população de espéciesdas quais se alimenta, sendo importantes elos entre ambiente, contaminantes e populações humanas (Espino, 2000). Dessa forma, devido ao logo tempo de vida, ocupação de diferentes níveis na cadeiae por sua alta locomoção, estes animais são considerados excelentes indicadores biológicos de poluição química no ambiente marinho (Caurant et al., 1999), espécies-sentinela ou bioindicadores, retratando a situação ambiental nos níveis de contaminantes corporais. Porém, é importante ressaltarque a utilização de tartarugas-marinhas como espécies-sentinela no país ainda é escassa e, mesmo nestes casos, a maioria dos estudos realizados contempla poucas espécies, geralmente a tartaruga- cabeçuda (*Caretta caretta*) e a tartaruga verde (*Chelonia mydas*) (Ross et al., 2017; Reis et al., 2010).

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3 ARTIGO

Metal and Metalloid Contaminationin 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 lattertwo 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 reportedin contaminated environments (Formia et al., 2007; Guimarãeset 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 nestingand 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 levelsin 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

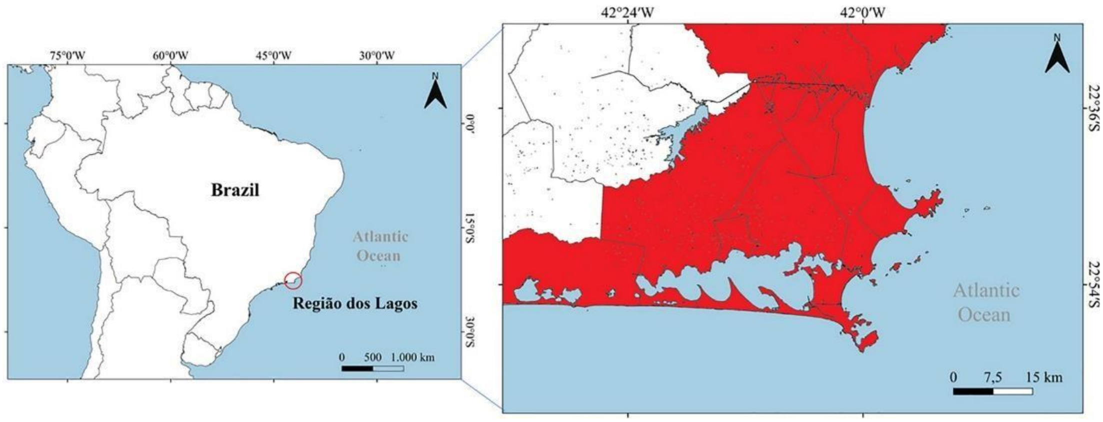

FIGURE 1 | Map of the study area of Região dos Lagos, eastern coast of the state of Rio de Janeiro, southeastern Brazil.

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 samp 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 M▲ 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 mg 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 (mg kg^{-1})	LOQ (mg 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 (mg 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: LOQ = (3 × DSDP × df)/slope of the line

LOQ = (10 × SD × df)/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 analysisof 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 consideredadequate 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 normalitytest. 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 exhibitedin **Table 4**. Concerning sex, 75% of the individuals were female and 25% males. Regarding maturity, most (87.5%) were juvenilesand 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

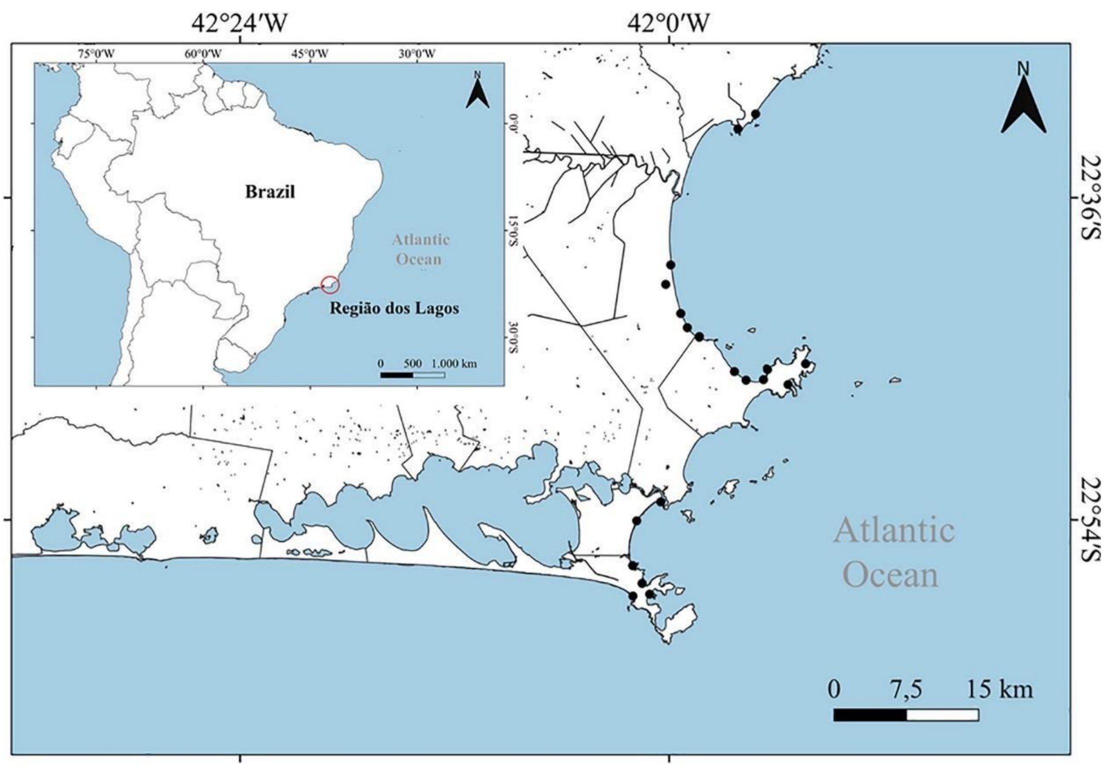

FIGURE 2 | Location of the green sea turtle strandings in the Região dos Lagos region, eastern coast of the state of Rio de Janeiro, southeastern Brazil.

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 onlythen, 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

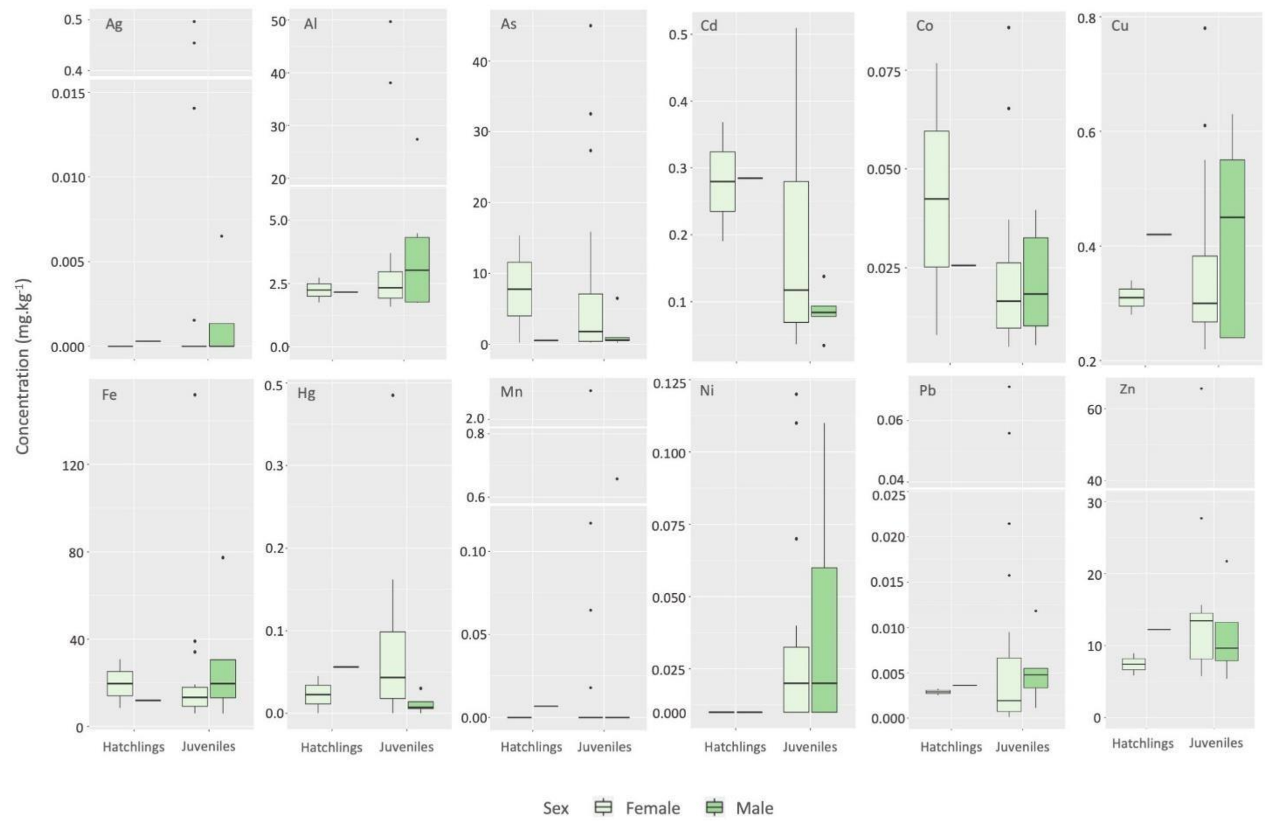

FIGURE 3 | Box plots representing elemental concentration variations in green turtle (*Chelonia mydas*) muscle tissue. Black dots indicate outliers, while white bars represent breaks in the *y*-axis. Light green columns represent females and dark green, males.

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 mg kg^{-1}, significantly lower than the mean for healthy individuals, of 1.82 $\pm$0.20 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 $\mu g\ g^{-1}$ and 1.44 $\mu g\ g^{-1}$, respectively) in the blood of sea turtle individuals positively correlated to the inhibitionof 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 belownormal 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 standto reason that the decreased Cu concentrations observed in the three individuals presenting fibropapillomatosis are related

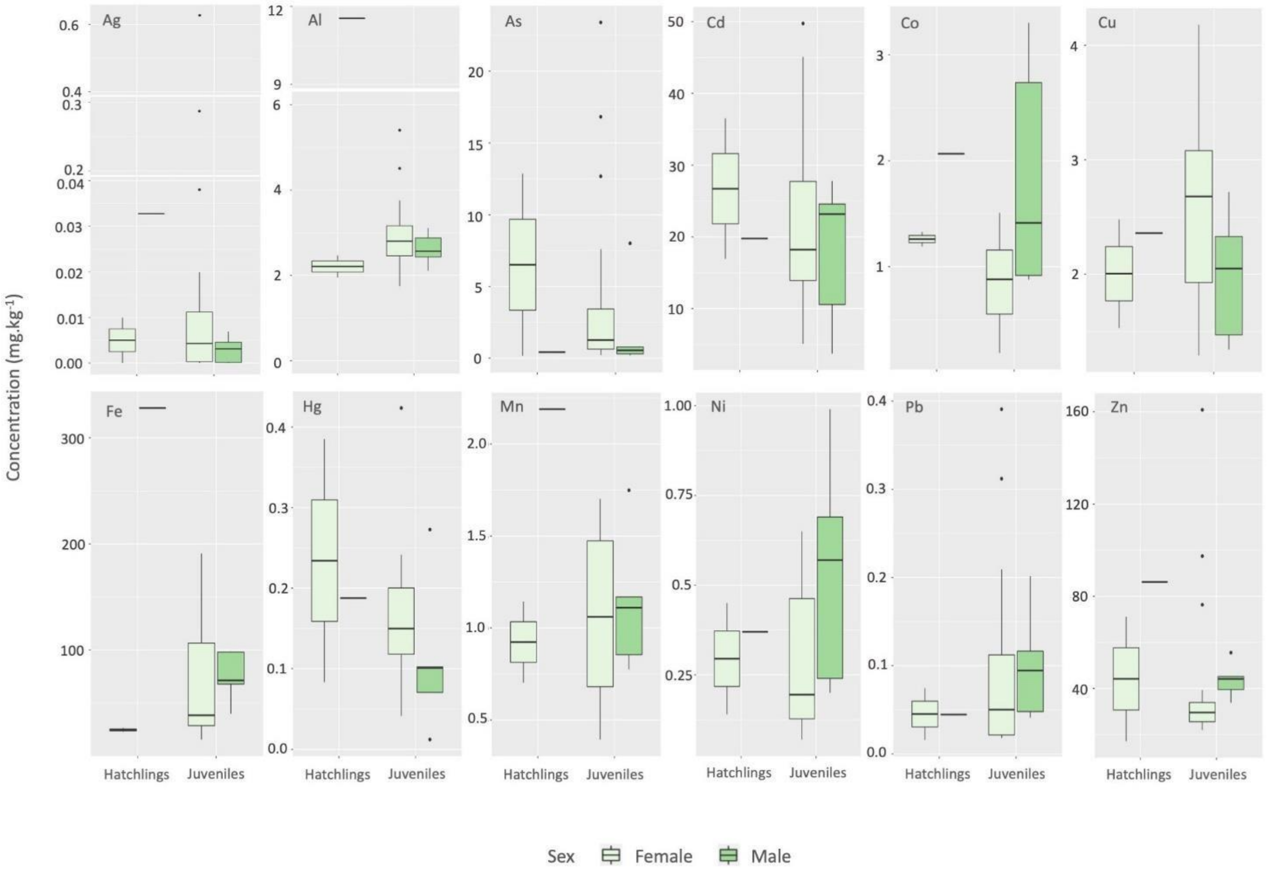

FIGURE 4 | Box plots representing elemental concentration variations in green turtle (*Chelonia mydas*) kidneys. Black dots indicate outliers, while white bars represent breaks in the *y*-axis. Light green columns represent females and dark green, males.

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 Cananeia 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 physic-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,	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
Brazil		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,	Kidney	Hatchlings (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Barbieri, 2009
Brazil		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,	Kidney	Hatchlings (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	De Macêdo et al., 2015
Brazil		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,	Kidney	Hatchlings (males and females)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Da Silva et al., 2014
Brazil		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, althoughonly 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 an 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 agein greenturtle 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 thethree juvenile individuals presenting fibropapillomatosis wereobserved compared to healthy juveniles, which may indicate decreased immune functions and adequate herpesvirus activationin 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 approvedthe submitted version.

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Sea turtles were sampled through the beach monitoring project (PMP-BC/ES) between the states of Rio de Janeiro and Espírito Santo, Brazil. This project is part of the requirements established by the federal environmental licensing process of the Brazilian Environmental Agency (IBAMA), for the exploration of oil and gas by PETROBRAS at the Campos Basin Province. The metal and metalloid results were obtained directly in research using different laboratory and techniques from those adoptedby PMP-BC/ES. TS acknowledges CNPq and FAPERJ for the granted scholarships.

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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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4 CONCLUSÃO

Neste contexto, o estudo em questão reforça a importância de estudos ecotoxicológicos queenglobem o ambiente como um todo, considerando todos os níveis tróficos. Este estudo foi também capaz de ceder importantes informações de base e inéditas para o desenvolvimento de mais pesquisas e de programas de monitoramento e preservação ambiental. Além disso, puderam ser discutidas informações de extrema importância para o desenvolvimento de análises de riscos à saúde humana.

Este estudo serve como base pra futuras pesquisas e corrobora outros estudos sobre as influências da contaminação ambiental em organimos marinhos. Assim, torna-se extremamente necessárioo desenvolvimento de mais estudos acerca do tema e a criação de medidas mitigatórias, visando diminuir os impactos sobre os diversos ambientes e organismos. Tornam-se necessários da mesma forma, estudos que realizem o aprofundamento dasa consequencias físicas, mentais e materiais, causados pela poluição ambiental, destruição de hábitats e perda de biodiversidade na população humana, reforçando os conceitos de One Health. Tal conceito defende a existência de uma saúde única, onde não é possível separar a qualidade do ambiental, da saúde animal, da saúde humana, estando os três níveis interligados e conectados(Zinsstag, J., et al, 2011; Gibbs, E.P., 2014).

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