Journal of Trace Elements and Minerals 2 (2022) 100015

Contents lists available at ScienceDirect

Journal of Trace Elements and Minerals

journal homepage: www.elsevier.com/locate/jtemin

Potential adverse effects of heavy metals on clinical health parameters of *Caretta caretta* from a nesting area affected by mining tailings in Brazil

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A R T I C L E I N F O

Keywords:
Biochemical parameters
Doce River
Hematology
Loggerhead sea turtle
Scutes
trace elements

A B S T R A C T

Background: In 2015, Brazil had its most notorious ecological disaster due to an iron mine dam collapse. It was considered the world's largest environmental disaster associated with mining industry. The tailings composed mostly of iron ore and silica, but with high levels of heavy metals reached the Atlantic Ocean, through the Doce River, sixteen days after the rupture. The region where the mud ran into the ocean is an important nesting area for loggerhead turtles (*Caretta caretta*) in Brazil (Povoação, Espírito Santo state). The aim of this study is to evaluate the heavy metal effects on the health parameters of loggerhead turtles that nest in the exposed area (Povoação, Doce river estuary) and compare them with turtles from another coastal area that was not affected by the tailings (Praia do Forte, Bahia state).
Methods: Animals' health status was determined by a physical examination during which blood samples used to perform biochemical and hematological analysis were also obtained. Heavy metal concentrations were evaluated in the blood and carapace scutes.
Results: Sea turtles from Povoação had a worse body condition with higher ectoparasitic and epibiont loads compared to Praia do Forte animals. They also showed eye lesions and more frequent fibropapillomatosis suggestive tumors. Several correlations between the contaminants and hematological and plasma biochemical analytes were found in loggerhead sea turtles from Povoação. Blood metal concentrations were distinct between nesting areas, with higher As, Cd, Cr, Fe, Pb, and Zn levels in animals from the affected area. In the carapace scute, the heavy metal levels were similar between sites.
Conclusion: The occurrence of eye lesions, suggestive fibropapillomatosis tumors, worse body condition, higher ectoparasite count, greater epibiont loads, large number of correlations between health parameters and heavy metal levels, and higher levels of some metals in the blood of PV turtles indicates that the tailings could have a negative impact on the health and reproduction of these turtles. The long-term monitoring of the exposed area is important to quantify the direct and indirect impacts of the tailings on the health of these animals. Additionally, the data reported here are important for temporal and spatial comparative studies in the future.

1. Introduction

On November 5th, 2015, Brazil suffered its worst ecological disaster when an iron mine dam (Fundão dam) collapsed in the municipality of Mariana, State of Minas Gerais (20°12′38.56"S, 43°27′37.10"W), releasing metal-rich tailings waste. Approximately 43 million cubic meters of tailings were unleashed, producing mud waves 10 m high, and causing irreversible environmental damage to hundreds of watercourses in the Doce River basin and its associated ecosystems [1]. The released tailings volume is the largest ever registered, followed by previous occurrences registered in the Philippines (32.2 million m^3) and Canada (23.6 million m^3) [2]. Within two days, the mine slurry caused instant sediment accumulation, burying, and killing benthic organisms and increasing by several orders of magnitude heavy metal concentrations in Doce River [3]. Twenty-one fish species were dead in large numbers. The death of invertebrates, reptiles, birds, and large mammals was also reported [4].

The mud traveled about 668 km along the Doce river basin, causing massive mortality of its biota, and reaching the Atlantic Ocean on November 22nd, 2015. It currently represents the longest traveled distance by tailings in a dam event (the previous record was reported in

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https://doi.org/10.1016/j.jtemin.2022.100015
Received 30 May 2022; Received in revised form 2 August 2022; Accepted 4 August 2022
Available online 5 August 2022

Mexico, 420 km) [4]. The catastrophe exhibited distinct characteristics that make it the largest environmental disaster in the world mining industry, both in terms of the volume of tailings released and the geographic extent of socioeconomic and environmental damage [1,2].

Among the organisms exposed to the tailings are the sea turtles that use the beaches located on the right and left bank at the Doce River mouth to nest. At Linhares municipality in Espírito Santo state are the Regência and Povoação beaches (south and north of Doce River mouth, respectively), that together sum up 80 km of coastline that support the second largest aggregation of nesting loggerhead sea turtle (*Caretta caretta*) in the southeast Atlantic. This area is also the only region where leatherback sea turtle (*Dermochelys coriacea*) regularly nests in Brazil. In the same nesting season, a single turtle can lay its eggs on both sides of the Doce river mouth [5].

The tailings were composed mostly of iron ore and silica, but also presented high levels of arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), mercury (Hg), manganese (Mn), nickel (Ni), and selenium (Se) [6,7]. Biologically essential elements such as Al, As, Co, Cr, Cu, Se, and Zn, are generally maintained within relatively narrow optimal circulating ranges that are regulated by physiological processes. These elements are therefore expected to show little variation within individuals of the same species unless poor health or elevated environmental exposure affects the body's ability to maintain homeostasis [8]. In contrast, non-essential elements (e.g. As, Cd, Hg, Pb) are not under homeostatic control and their clearance can be slower than their intake rates, resulting in bioaccumulation in sea turtles.

Contaminants' distribution and concentration in the body can be significantly affected by age since poorly metabolized metals increase their levels over time. Some metals may accumulate to concentrations that could cause harm, even at low exposure regimens [9]. The trophic level also influences the accumulation of pollutants once some compounds are biomagnified through the food chain. As *C. caretta* grow older, they progressively change their diet to higher trophic levels [10], which makes them especially vulnerable to ingestion of pollutants. Migrations, fasting, breeding, and nesting represent a high energy demand on the body. During these periods, lipid stores are mobilized along with associated chemicals and may influence liver and kidney metabolic capacity, thus affecting the toxicokinetic of the pollutants [11].

Blood sampling in sea turtles during nesting is an accessible strategy to evaluate circulating heavy metals and other hematological and biochemical parameters. With elimination rates of many trace elements generally on the order of weeks to months (depending on the element and its speciation), blood is considered a suitable matrix for evaluating relatively recent exposure [12,13]. Considering this, abrupt changes in exposure are rapidly reflected in blood and can be observed depending on the time of sampling relative to the time of exposure [14,15]. Loggerhead sea turtles can lay one to six egg clutches per season, with an internesting period of 12-16 days [16]. Consequently, they can stay in the nesting area for 12 days to three months, being exposed to the environmental contaminants of that area through food, sediment, and water. Nesting sea turtles have rarely fed, however, to reduce their body temperature and ensure egg production (albumen is mainly composed of water), they ingest a significant water volume [17,18]. This could contribute to their contamination during this period.

As already mentioned, blood concentrations can be a proxy for recent exposure, while keratinous scutes reflect a longer-term signature. Thereby, each carapace keratinous layer is composed of contaminant exposures over the past 1.4-2.8 years [19] and it has been shown that toxic elements in keratin correlate with various health indicators, which enhances the value of this tissue analysis [20].

Short and long-term exposure of sea turtles to pollutants can increase the incidence of diseases and cause adverse effects like compromised physiology, chronic stress, and impaired immune function [21]. Understanding the prevalence of diseases in sea turtle populations provides a critical link between sea turtle health and ecosystem health. It is therefore important to investigate relationships between contaminant exposure and declines in sea turtle health. Currently, the impact of the tailings on nesting sea turtles is unknown. The only article that studied the effects of mining tailings on sea turtles' health is Miguel et al. [22]. They evaluated juvenile green sea turtles (*Chelonia mydas*) from a foraging area and noticed that the animals from the affected area had a notably worse nutritional conditions, as well as a higher incidence of anemia, immunosuppression, fibropapillomatosis tumors, and ectoparasite load, in addition to possible hepato-renal pathologies. In this context, the present study aims to analyze heavy metals levels in adult female loggerhead turtles from blood and carapace scute and their prospective effects on biochemical, hematological, and health parameters. In addition, the area contaminated by the mining tailings (Povoação) will be compared with an area that did not receive the mud (Praia do Forte, further up north) to address differences that could explain environmental aspects and other factors that could influence metal profiles.

2. Material and methods

2.1. Study sites

Povoação (PV) is located on the left bank at the mouth of the Doce River, in Espírito Santo state (19°34'43.05" S, 39°46'49.85" W) (Fig. 1). It is a little village with approximately 3200 inhabitants that used to rely on fishing as their main economic activity, before the disaster. Povoação beach is 39 km long and it is patrolled every night in the nesting season by Projeto TAMAR members. Povoação was within the direct impact area of the tailings in 2015 and periodically affected by the resuspension of sediments caused by changes in weather over the Doce river basin and the adjacent coastal areas [23,24].

Praia do Forte (PF) is a touristic village inhabited by traditional communities on the north coast of Bahia state (12°33'30.54" S, 37°59'28.05" W), with 1800 habitants and it is also the main nesting area of loggerhead sea turtles in Brazil. It was chosen as a reference due to its proximity to Povoação (795 km straight distant from each other) whilst not affected by the tailings [24].

2.2. Turtle sampling

The beaches of Povoação and Praia do Forte were patrolled from 8 pm to 3 am for three and one month, respectively, in the 2018/2019 nesting season. Once a turtle was encountered, the team remained in silent, with the head lamp off and far enough to not disturb it, allowing it to begin the nesting process. The researchers only approached when the animal laid at least 50 eggs. If the turtle was found when finishing nesting, it was restrained manually, without sedation. Blood samples were collected with an 18G needle and a 10 mL- syringe from the cervical venous sinus, only after the female had laid a minimum of 50 eggs. The blood was transferred to a heparinized tube (Vacutainer©, Greiner Bio-one, Brazil), kept in a cooler at 4-8°C without direct contact with the ice to prevent hemolysis, until arrive at the laboratory in the field station. For hematology, whole blood smears were air-dried at the time of sampling. Heparinized blood was centrifuged (5000 rpm for 5 min, Centribio® TDL-80-2B) to separate plasma then, frozen in liquid nitrogen until analysis. For heavy metal quantification, blood samples were placed into Vacuette® Trace Elements Sodium Heparin tube (Greiner Bio-one, Brazil), frozen by immersion in liquid nitrogen, and transported to the laboratory where they were stored at −80°C. Superficial scraping of the carapace scutes was collected and stored in plastic Zip-lock® bags at −80°C until analyses were conducted. The study was performed under the licenses SISBIO 61063 and 60430 of the Chico Mendes Biodiversity Institute (ICMBIO).

2.3. Morphometric and health assessment

Morphometrics and health assessment were performed as detailed in Miguel et al. [22]. Briefly, each sea turtle was measured with a flex-

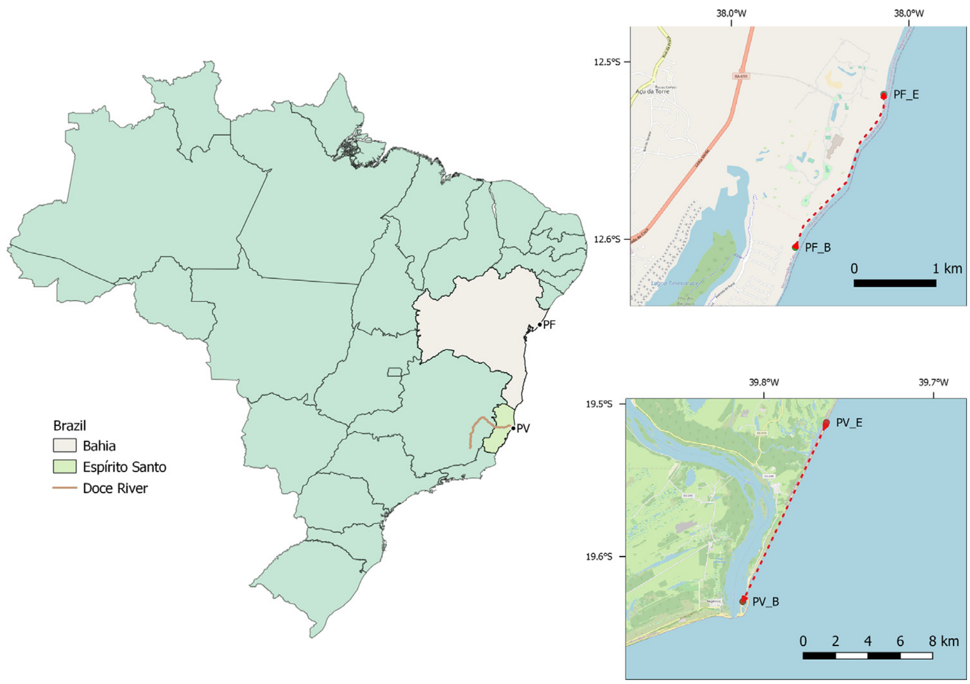

Fig. 1. Map of study areas in Brazil. Povoação (PV) north of Doce River mouth, Espírito Santo state, affected by the mining tailings, and Praia do Forte (PF), Bahia state, not affected by the tailings. Dashed red line denotes the monitoring area.
PF_B: beginning point of the monitoring in Praia do Forte; PF_E: ending point of the monitoring in Praia do Forte; PV_B: beginning point of the monitoring in Povoação; PV_E: ending point of the monitoring in Povoação.

ible plastic tape over the curved carapace length (CCL) and the curved carapace width (CCW). For sea turtles' identification, Inconel tags were applied with pliers to each of the anterior flippers, between the first and second scales. A physical examination was performed to establish the body condition (BC), scored as poor (1), average (2), or good (3); and to identify other external health indicators (injuries and obvious signs of illness). Epibiont percentual cover on the carapace and ectoparasites were categorized using an ordinal scale of 1 (mild), 2 (moderate), and 3 (heavy). Eye swabs were taken from sea turtles with eye lesions and sent to the microbiology laboratory, University of Vila Velha, Brazil, for bacterial and fungi culture and identification.

2.4. Hematology

Neubauer counting chamber (Neubauer chamber, New Optics, São Paulo, Brazil) and Natt and Herrick solution were used to determining red blood cell (RBC) count and total white blood cell (WBC) count [25]. At a magnification of 40x, all four large corner squares of the chamber were counted for WBCs and inside the central large square, 5 small squares (four corner squares and the center square) were counted for the RBCs. Complete blood count was performed within 6 hours of collection. Blood smears made with blood without anticoagulant, during the blood sample collection, were used to estimate the differential white cell count. The smears were stained with a Romanovsky like kit (Instant-Prov Kit®, Newprov, Brazil). A hundred cells were counted using a binocular light microscope and a 100x oil immersion lens. Cells were classified as heterophils, lymphocytes, monocytes, eosinophils, and basophils [26,27]. Hemoparasites were also screened in the blood smears.

Hematocrit (Hct) was calculated by the volume percentage of red blood cells in the blood, using a StatSpin® Microhematocrit Tube (IRIS USA, Inc.) Hemoglobin (Hb) was determined by the cianometahemoglobin method, using a commercial Labtest® Kit in a spectrophotometer (Quimis Q898DPT, Brasil). The erythrocytic index was calculated according to Campbell [25].

2.5. Plasma biochemistry

Plasma biochemical analytes albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), calcium, creatine phosphokinase (CPK), glucose, globulin, triglycerides, total cholesterol, total protein, iron, phosphorus, magnesium, potassium, sodium, urea, uric acid were measured using an automated biochemical analyzer (Humastar 200, In Vitro Diagnóstica, Brasil) with specific reagents according to manufacturer instructions.

2.5. Heavy metal analysis

For heavy metals quantification, 1mL of plasma and 1g of the superficial scraping of the carapace scutes were microwave-digested in 6 ml of nitric acid using a Multiwave 3000 (Anton Paar®) system, according to Miguel et al. [22]. High-Resolution Continuum Source Graphite Furnace Atomic Absorption Spectrometer (HR-CS GF AAS, Analytik Jena, Jena, Germany) was used to quantify arsenic, cadmium, copper, chromium, iron, manganese, lead and zinc and an atomic fluorescence spectrometer Mercur Duo Plus (Analytik Jena, Jena, Germany) was used in mercury quantification. The analytical quantification was determined from the calibration curve using standard solutions (Multi-Element Standards Certipur®, Merck, Darmstadt, Germany). National Research Council Canada (NCR - CNRC) certificated reference materials (TORT-3, DOLT-5, DORM-4) were analyzed to check the accuracy of the method. All the values of the reference materials were within certified limits. Recovery values were: As 94%; Cd 92%; Cr 89%; Cu 90%; Fe 88%; Hg 97%; Mn 88%; Pb 89%; and Zn 85%. Metal levels are expressed as μg/g of w.w.

2.6. Statistical analysis

All statistical analyses were carried out in Statistical Package for Social Science (SPSS) 20.0. To evaluate differences between areas (Povoação and Praia do Forte) and between samples (carapace and blood) parametric (Student *t*-test) or nonparametric (Mann-Whitney *U*-test) analyses were performed, as appropriate.

The relationship between each blood parameter and curved carapace length (as an indicator of body size) and heavy metals were evaluated using Pearson's or Spearman's correlation test, depending on whether both parameters exhibited a normal distribution. Principal component analysis (PCA) was used to assess the overall trends of contaminant loadings and their relationships with health markers (package FactoMineR in R software).

Table 1
Visual health assessment of loggerhead sea turtles from Povoação ($n = 66$) and Praia do Forte ($n = 37$), Brazil.

Parameter	Povoação		Praia do Forte	
	n	%	n	%
Body condition				
Good	34	51.5	30	81.1
Average	31	46.9	7	18.9
Poor	1	1.6	0	0
Epibiont load				
None	7	10.6	7	18.9
Mild	12	18.2	20	54.1
Moderate	22	33.3	8	21.6
Heavy	25	37.9	2	5.4
Ectoparasites				
None	46	69.7	28	75.7
Mild	14	21.2	9	24.3
Moderate	4	6.1	0	0
Heavy	2	3.0	0	0
Fibropapillomas				
None	63	95.4	37	100
Mild	3	4.6	0	0
Eye lesion				
None	60	90.9	37	100
Present	6	9.1	0	0

3. Results

A total of 66 loggerhead sea turtles were sampled in Povoação (PV), with three recaptures. At Praia do Forte (PF) 37 animals were sampled and none was recaptured. The Brazilian Sea Turtle Conservation Program (Projeto TAMAR) has a detailed inventory of female turtles by tagging all nesting individuals, which enables the identification of first-time nesters or remigrants. Within the sea turtles sampled in PV, thirty-one (49.2%) females were identified as remigrants while thirty-two (50.8%) may be nesting for the first time. At PF, seventeen (45.9%) females were remigrants, and twenty (54.1%) were tagged for the first time.

3.1. Morphometric and health assessment

The mean (± SD) CCL was 99 ± 5.0 cm (interval: 89-114 cm) and 98 ± 5 cm (interval: 82-108 cm) in females from PV and PF, respectively, and mean (± SD) CCW was 90 ± 4.0 cm (interval: 82-104 cm) in PV and 89 ± 3.0 cm (interval: 79-99 cm) in PF. Visual health assessments of the nesting loggerhead females in each area are shown in Table 1. A significant difference in body condition was found between locations ($U = 856.5$, $p < 0.0041$). Turtles from PV were mostly in average (46.9%), or good conditions (51.5%) and only one animal showed poor condition. In PF good and average conditions accounted for 81.1% and 18.9%, respectively. Loggerhead sea turtles from PF were not identified with fibropapillomatosis tumors or eye lesions, while at PV three (4.6%) animals had suggestive fibropapillomatosis tumors (Fig. S1) and six (9.1%) showed eye lesions (Figs. S2 and S3).

As only one turtle was in poor body condition, differences in epibiont loads and ectoparasite counts among BC were calculated only for average and good condition. Yet, this turtle (poor BC) had heavy epibiont loads and heavy ectoparasite counts. Epibiont loads varied significantly among turtle body conditions ($U = 951$, $p = 0.028$). All loggerhead sea turtles in average condition had epibionts and showed a greater epibiont load than turtles with good BC. A significant difference in epibiont load was also found between locations ($U = 631.5$, $p < 0.0001$), at PV 89.4% of the turtles had epibionts compared to 81.1% in PF. Ectoparasite counts did not vary among turtle BC ($U = 1168$, $p = 0352$) nor study areas ($U = 1157$, $p = 0.293$). However, twenty (30.3%) loggerhead sea turtles from PV had ectoparasites compared to nine (24.3%) from PF. Six turtles from PV had their carapace covered by mud.

Three species of bacteria were identified in the eye lesions *Myroides odonatum, Staphylococcus aureus, Bacillus subtilis* and two species of Fungi *Aspergillus terreus, Cryptococcus albidus.*

3.2. Hematology

Hematological and biochemical parameters from animals from both areas are showed in Table 2. No hemoparasites were found in blood smear examinations. Sea turtles from PV had higher counts of RBC ($U = 948.5$, $p = 0.038$), Hb ($U = 840$, $p = 0.0112$), Hct ($t = 3.383$, df=100, $p = 0.0010$), leukocytes ($U = 701$, $p = 0.0004$), and lymphocytes ($U = 891.5$, $p = 0.019$), and significantly lower counts of eosinophils ($U = 910.5$, $p = 0.026$) than animals from PF.

3.3. Plasma Biochemistry

Considering biochemical analytes, PV turtles had significantly higher levels of calcium ($t = 3.924$, df= 99, $p = 0.0002$), sodium ($t = 2.38$, df= 99, $p = 0.019$), potassium ($t = 2.97$, df= 99, $p = 0.0037$), total protein ($U = 623.5$, $p < 0.0001$), globulin ($t = 4.799$, df= 99, $p < 0.0001$), albumin ($t = 1.805$, df= 99, $p = 0.037$), uric acid (U=758, $p = 0.003$), and alkaline phosphatase ($t = 3.15$, df= 99, $p = 0.002$), and significantly lower ALT ($U = 505.5$, $p < 0.0001$) and AST ($U = 692$, $p = 0.0006$) activity, compared to PF turtles.

3.4. Heavy metal analysis

The results of elements determination in the blood and carapace of loggerhead sea turtles from both regions are summarized in Table 3. Trace element distribution in blood of PV and PF turtles was Zn > Fe > Cu > As > Mn > Cd > Cr > Pb > Hg and Zn > Fe > Cu> Mn > As > Cr > Cd > Pb > Hg, respectively. Higher levels of As ($U = 652$, $p = 0.0001$), Cd ($U = 536.5$, $p < 0.0001$), Cr ($U = 585.5$, $p = 0.0027$), Fe ($U = 788.5$, $p = 0.0027$), Pb ($U = 847$, $p = 0.029$), and Zn ($U = 952$, $p = 0.03$) were found in the blood of PV turtles, while PF turtles had higher levels of Cu ($U = 711$, $p = 0.0004$) and Mn ($U = 529.5$, $p < 0.0001$). In the scute samples, heavy metals levels were Fe> Zn> Mn> As> Cu> Cr> Pb> Hg> Cd in PV turtles and Fe> Zn> Mn> As> Cu> Cr> Pb> Cd> Hg in PF turtles. The only significant difference in carapace levels between areas was for Hg ($U = 391.5$, $p = 0.022$), its concentration was higher in PV turtles.

Comparing heavy metal levels in blood and scutes samples of animals from the same area, it is possible to note that PV turtles had significant higher levels of As ($U = 1245$, $p = 0.015$), Cd ($U = 535$, $p < 0.0001$), and Cu ($U = 180$, $p < 0.0001$) in the blood than in the carapace. On the other hand, levels of Cr ($U = 399$, $p < 0.0001$), Fe ($U = 13$, $p < 0.0001$), Hg ($U = 900$, $p = 0.0004$), Mn ($U = 0$, $p < 0.0001$), Pb ($U = 731$, $p < 0.0001$), and Zn ($U = 191$, $p < 0.0001$) were significant higher in the carapace. PF turtles had significantly higher levels of Cd ($U = 259$, $p = 0.010$) and Cu ($U = 47$, $p < 0.0001$) in the blood, and Cr ($U = 47$, $p < 0.0001$), Fe ($U = 0$, $p < 0.0001$), Mn ($U = 0$, $p < 0.0001$), and Zn ($U = 133$, $p < 0.0001$) in the carapace compared to the blood.

All significant positive and negative correlations between heavy metals and health parameters are summarized in Table 4, and between metals in the blood and scutes in Table 5. The principal component analysis of health parameters and heavy metal levels showed two principal components that explained 28.1% of the variations in the data set. The PC1 explains 16.5% of the variability and the PC2 explains 11.6% (Fig. 2). A separation of the samples can be observed in PCA, being a group formed by PV individuals and hematological parameters (RBC, Hct, Hb, leukocytes thrombocytes, and lymphocytes) and diseases (fibropapillomatosis and eye lesion); and another group formed by PF individuals and body condition. Considering heavy metals, two groups are also formed, one by As, Cr, Cu, Zn and Fe in Povoação; and another with Cd and Mn levels in Praia do Forte.

Table 2
Morphometric, hematological, and plasma biochemical parameters for loggerhead sea turtles from Povoação and Praia do Forte, Brazil.

Parameter	Povoação (n = 66)				Praia do Forte (n = 37)			
	mean	SD	Min	Max	mean	SD	Min	Max
Morphometry								
CCL (m)	0.99	0.05	0.89	1.14	0.98	0.05	0.82	1.08
CCW (m)*	0.90	0.04	0.82	1.04	0.89	0.03	0.79	0.99
Hematology								
Red blood cell (x10^6/µL)**	0.39	0.23	0.16	1.19	0.29	0.05	0.17	0.40
Hemoglobin (g/dL)**	8.76	1.55	5.30	14.8	7.78	2.15	3.90	12.0
Hematocrit (%)*	33.5	5.01	19.0	44.0	29.2	7.77	10.0	47.0
MCV (fl)	1040	416	258	1899	1015	333	337	1880
MCH (pg)	276	122	68.2	689	266	78.9	98.1	520
MCHC (g/dL)	26.3	4.54	15.3	41.1	27.8	10.0	11.4	77.6
Leukocytes /µL**	5954	2828	1125	14750	4385	1892	2000	9375
Thrombocytes /µL	5584	3263	82	14750	4801	1552	1750	8625
Heterophils (%)	58.5	14.6	6	85	61.3	8.73	47	83
Heterophils /µL	3572	2240	264	10768	3206	1046	1120	5261
Lymphocytes (%)**	22.8	12.8	3	60	18.1	5.65	7	34
Lymphocytes /µL**	1284	927	82.5	4744	1001	527	140	2253
Monocytes (%)	5.12	3.23	0	17	4.86	3.20	0	15
Monocytes /µL	280	180	0	786	265	216	0	1050
Eosinophils (%)**	12.1	6.37	0	31	15.7	5.54	6	26
Eosinophils /µL**	722	547	0	2803	839	435	228	2094
Basophils (%)	0	0	0	0	0	0	0	0
Basophils /µL	0	0	0	0	0	0	0	0
Biochemistry								
Uric Acid (mg/dL)**	0.56	0.23	0.15	1.37	0.43	0.14	0.19	0.79
Urea (mg/dL)	23.3	8.96	2.00	55.0	22.5	11.9	3.00	69.0
Calcium (mg/dL)*	8.96	2.27	1.41	13.7	6.82	3.18	1.14	12.1
Phosphorus (mg/dL)	7.28	1.67	3.90	11.4	7.75	1.89	3.50	11.1
Sodium (mEq/L)*	142	4.85	133	152	139	7.45	123	150
Potassium (mEq/L)*	4.14	0.47	3.15	5.30	3.86	0.41	2.85	4.66
Glucose (mg/dL)	98.7	16.1	59.0	141	91.8	17.0	47.0	118
Total Cholesterol (mg/dL)	258	64.9	124	446	226	73.6	104	374
Triglycerides (mg/dL)	653	349	57.0	1429	641	395	100	1510
Total Protein (g/dL)**	3.75	0.65	1.90	5.10	3.09	0.69	1.40	4.10
Albumin (g/dL)*	1.35	0.27	0.70	2.00	1.25	0.25	0.70	1.80
Globulin (g/dL)*	2.36	0.48	1.00	3.50	1.90	0.42	1.00	2.80
ALT (U/L)**	2.57	1.32	1.10	8.30	4.07	1.82	1.20	9.00
AST (U/L)**	163	94.1	64.0	685	227	128	90.6	662
Alkaline phosphatase (U/L)*	10.0	2.88	4.00	17.0	8.30	2.37	4.00	12.0
Serum iron (µg/dL)	59.0	25.9	21.0	122	51.4	26.5	14.0	118
CPK (U/L)	378	253	75.0	1254	384	325	57.0	1553
Magnesium (mg/dL)	5.25	0.97	2.10	8.01	5.34	1.04	3.33	8.64

* Significant difference by Student t test ($p<0.05$). **Significant difference by Mann-Whitney U test ($p<0.05$)*CCL* curved carapace length, *CCW* curved carapace width, *BCI* body condition index, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CPK* creatine phosphokinase.

4. Discussion

Most studies about ecotoxicology and health monitoring in sea turtles have been made in areas that are pristine or receive some level of contaminants. This is the first study to assess the health of loggerhead sea turtles from a nesting area affected by the tailings from a collapsed dam. Additionally, this is the first paper reporting levels of heavy metals in the blood of loggerhead turtles from South America.

4.1. Morphometric and health assessment

Most of the turtles sampled in Praia do Forte had good body condition, while only half of the loggerhead turtles from Povoação had this score. Additionally, PV turtles had significantly more epibionts than PF turtles and epibionts gradually increased as body condition deteriorated, which corroborates the hypothesis of some authors that considered epibiont load as an indicator of physically compromised turtles [28,29]. The higher incidence of ectoparasites in PV animals may represents a health hazard to sea turtles, since parasitic leeches can cause direct damage and/or be a vector for infectious agents (e.g., fibropapillomatosis). In fact, in the affected area two individuals had suggestive fibropapillomatosis tumors (Fig. S1), and six showed eye lesions.

Macroscopically, these animals had intense conjunctival hyperemia and eyelid edema (Fig. S2), associated or not with corneal lesions (Fig. S3), suggesting a blepharoconjunctivitis or keratoconjunctivitis. This condition had already been reported in the 2017/2018 nesting season by our team (Personal communication), without previous reports. Although results from microbiological examination showed opportunistic bacteria and fungi (*Myroides odonatum, Staphylococcus aureus, Bacillus subtilis, Aspergillus terreus, Cryptococcus albidus*) with low pathogenicity, they still can contribute to diseases in immunosuppressed animals. It is not known whether these microorganisms can be causative or are secondary to the lesions. Therefore, it was not possible to determine a precise etiological diagnosis of the presented condition. Molecular methodologies for viral diagnosis, as well as histopathological examination (biopsy) of the eyelid, will be necessary to assess if the disease is related to immunosuppression and if it is correlated with the exposure to mining waste in that area.

Eye diseases reported in sea turtles include keratoconjunctivitis, parasitic conjunctivitis, blepharitis, fibropapilloma, cataract, heterophilic scleritis, corneal ulceration, and keratitis, and they can affect the pri-

Table 3
Heavy metal levels in the blood and carapace of loggerhead sea turtles from Povoação and Praia do Forte, Brazil.

Parameter	Povoação (n = 66)				Praia do Forte (n = 37)			
	mean	SD	Min	Max	mean	SD	Min	Max
Blood (μg/g w.w)								
As[a]	1.29*	0.84	0.006	3.46	0.62	0.44	0.009	2.11
Cd[a]	0.15*	0.23	0.005	1.24	0.023*	0.03	0.15	0.30
Cr[a]	0.06*	0.09	0.0001	0.36	0.03*	0.06	0.00009	0.31
Cu[a]	2.45*	0.77	0.20	3.71	2.97*	0.76	0.70	4.22
Fe[a]	8.51*	4.75	2.43	27.5	6.90*	5.22	0.31	28.3
Hg	0.005*	0.012	0.000007	0.08	0.002	0.002	0.0002	0.013
Mn[a]	1.13*	0.09	0.85	13.7	1.30*	0.22	1.08	2.05
Pb[a]	0.016*	0.02	0.0003	0.12	0.014	0.011	0.0043	0.05
Zn[a]	10.48*	1.79	0.22	15.32	9.85*	1.84	4.08	13.6
Carapace (μg/g w.w)								
As	0.96	0.98	0.05	6.03	1.01	0.84	0.10	2.39
Cd	0.004	0.004	0.001	0.02	0.008	0.01	0.0002	0.05
Cr	0.50	0.90	0.01	3.27	0.39	0.46	0.01	1.81
Cu	0.73	0.41	0.11	2.49	0.99	0.74	0.21	3.20
Fe	358	411	29.9	2621	247	201	48.9	863
Hg[b]	0.01	0.02	0.0006	0.11	0.005	0.006	0.0007	0.03
Mn	8.44	5.21	2.62	24.8	7.16	4.91	2.08	18.4
Pb	0.05	0.08	0.000006	0.54	0.05	0.08	0.0004	0.34
Zn	33.7	16.3	5.90	86.9	34.1	25.7	3.59	97.9

* Significant differences in heavy metal levels between blood and carapace samples from the same area (p<0.05).
[a] Significant differences in heavy metal blood levels between samples from Povoação and Praia do Forte (p<0.05).
[b] Significant differences in heavy metal carapace levels between Povoação and Praia do Forte (p<0.05). *As* arsenic, *Cd* cadmium, *Cr* chromium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Pb* lead, *Zn* zinc.

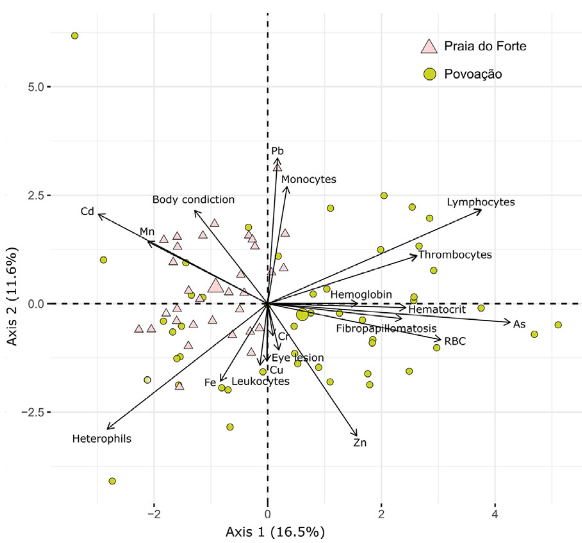

Fig. 2. Principal Component Analysis (PCA) plot showing the multivariate variation between heavy metals and some health parameters. Vectors indicate the direction and strength of each variable to the overall distribution. Green dots correspond to Povoação animals and light pink triangles correspond to Praia do Forte animals.

mary and accessory tear glands, the eyelids, and the eyeball [30]. Flint et al. [31] found mild to severe eye lesions in green sea turtles inhabiting areas impacted by agricultural and urban human activities. No specific etiology of the lesions was identified through histology or microbial culture and there was no indication of viral involvement. However, the authors claimed that this disease should be considered a potentially serious issue among coastal sea turtles of Northern Australia. In Brazil, the most common eye diseases are related to trauma and fibropapillomatosis in green turtles [32] or ocular spirorchiidiosis in green, loggerhead, and olive ridley turtles [33]. Eye lesions with a similar presentation in animals from the same nesting site raise doubts about the existence of another causal agent or predisposing factor in the environment that may be related to immunosuppression, which in turn could be related to environmental contaminants such as heavy metals, among other factors. Furthermore, there have never been reports of this disease in *C. caretta* from Praia do Forte, the largest nesting site of the species in Brazil, or from other areas monitored by Projeto TAMAR during the 40 years of beach monitoring.

4.2. Hematological and biochemical parameters

Several differences regarding hematological and biochemical parameters were found between animals from Povoação and Praia do Forte. PF turtles had significantly lower levels of Hct, Hb, RBC, leukocytes and lymphocytes count, calcium, potassium, protein, albumin, globulin, alkaline phosphatase, and uric acid compared to PV turtles. Previous studies have evaluated the changes in hematological and biochemical parameters in female sea turtles sampled over the entire nesting season. The authors found that the levels of total protein significantly decreased with the increasing number of nesting events [34–36], indicating that the sea turtles are relying on body lipids for nutrients. A potential explanation for this observed trend is decreased protein production or increased catabolism as the nesting season proceeds, nutritional stress, anorexia, and little or no food intake [34,37]. Perrault et al. [36] suggested that total protein levels could be used to assess whether sea turtles are early (1st to 4th clutches) or late (5th clutch or more) in their reproductive cycles.

Decreases in albumin levels have also been documented in nesting hawksbill (*Eretmochelys imbricata*) [38] and leatherback (*Dermochelys coriacea*) [34–36] and could result from its utilization in albumen production during amniotic egg formation [39], protein loss [37], and fasting [34,40,41]. Immune proteins also tend to decrease gradually during the nesting season [36] associated with a reduction in body condition [42], lower food intake [35], and the fact that γ-globulins are deposited into egg albumen [39].

Triglycerides and cholesterol levels are similar throughout the early and middle parts of the nesting season due to vitellogenesis [43] and the mobilization of lipid stores [42,44,45]. In the last clutches, there is

Table 4
Spearman correlations between blood heavy metals levels and hematological and biochemical parameters in loggerhead turtles from Povoação and Praia do Forte, Brazil.

Health parameter	Povoação						Praia do Forte	
	As	Cd	Cr	Cu	Hg	Pb	Cu	Hg
RBC	0.407**	-0.379**						
Hct	0.340**		-0.229*		0.469***			-0.428**
Hb						-0.259*		-0.423**
MCV	-0.255*	0.262*						
MCH	-0.349**	0.303**						-0.404*
MCHC						-0.236*		
Thrombocytes	0.358**	-0.346**						
Leukocytes	-0.233*							
Heterophils	-0.266*	0.324*						
Lymphocytes	-0.496***	-0.513***			-0.255*			
Eosinophils	-0.251*							
Monocytes						0.289*		
Albumin	-0.365**		-0.390**		0.394**			
Calcium	0.400***	-0.231*						
Cholesterol	0.364**				0.316*			
Iron	0.434***	-0.353**			0.442**			
Globulin	-0.342**	-0.280*	-0.346**		0.383**			
Protein	-0.367**	-0.267*	-0.436**		0.460**		0.473**	
Triglycerides	0.367**				0.326*	0.213*	0.613***	
Sodium		-0.217*						
AST			-0.276*					
Magnesium						0.315*		
Urea						0.213*		
Uric Acid				0.248*				

* $p < 0.01$
** $p < 0.001$
*** $p < 0.0001$Bold values indicate negative correlations between variables*RBC* red blood cell count, *Hct* hematocrit, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CPK* creatine phosphokinase

a shift to protein catabolism and triglycerides concentrations decreased [44]. These patterns of triglycerides concentrations could also be used to identify individuals that just arrived or are leaving the nesting area.

Decreases in calcium, phosphorous, sodium, and potassium have also been observed [34]. These decreasing trends may be associated with reduced food intake and mineral nutrient depletion as the nesting season proceeds [46]. Moreover, lower calcium levels are also a result of its allocation for eggshell formation [47]. Hct, RBC, and leukocyte count also tend to decrease as the nesting season progressed, indicating that females may become more stressed and fatigued, and with a worse body condition [34,35,42,48].

In this context, the differences found in hematological and biochemical parameters between PV and PF may be related to nesting status. Protein levels of PF turtles are slightly lower than PV turtles and triglycerides and cholesterol levels are similar, it suggests that the turtles sampled in PV are early in their reproductive cycles and only a clutch or two in difference of PF turtles.

Previous studies suggested that biochemical and hematological parameters may vary among geographic locations, habitats [49], and breeding status [50]. Thus, the results found were compared with nesting loggerhead turtles from Brazil to confirm that the levels found were within normal ranges and the differences observed could be attributed to the physiological changes that accompany nesting. Only two studies that analyzed the same parameters were found [51,52], one of them also sampled females at Praia do Forte (2004-2006) and the other at Rio de Janeiro, where the population is genetically equivalent to Povoação [53]. The values found for PV and PF *C. caretta* are amongst the previously reported ranges (Table S1), so they are likely to represent normal physiological conditions. It is important to note that a limitation of this study was that sampling only occurred in sea turtles that came to the beach to nest. It is possible that animals that did not approach the beach could have had abnormal hematology or biochemistry values, and thus were not fit to complete their reproductive cycle and nesting [54].

4.3. Heavy metals- blood

Significantly higher levels of As, Cd, Cr, Fe, Pb, and Zn were found in the blood of PV turtles compared to PF turtles. Nesting turtles are in a physiological condition in which they have migrated from the feeding area to the reproductive and nesting areas. As aforementioned, they rarely feed during this period and need to mobilize energy reserves for egg production. Therefore, metal levels in the blood are likely to be influenced by their mobilization from target tissues, such as the liver, kidneys, muscles, and fat, and by the consumption of contaminated water to ensure egg production [18,55].

The rank order of blood concentration of metals was consistent with the standard of essential metals being in higher concentrations than the toxic ones: Zn > Fe > Cu> Mn > As > Cr > Cd > Pb > Hg in PF turtles and Zn > Fe > Cu > As > Mn > Cd > Cr > Pb > Hg in PV turtles. This trend was expected as essential metals are involved in several important physiological and homeostatic processes and are therefore generally maintained within narrow concentration ranges by regulatory mechanisms [56]. On the contrary, non-essential metals are not actively controlled by turtles and could change according to the level of exposure [57].

Under the assumption that heavy metal levels in sea turtles' blood reflect recent contamination, which is dependent on local and regional sources of these contaminants, results comparisons should be made with other studies from the same or close region [58]. No study has been published in Brazil about heavy metals in the blood of *C. caretta*, thus the results were compared with those from loggerhead sea turtle from different regions of the world (Table S3). Analyzing other loggerhead sea turtles' populations, PV and PF turtles have lower levels of most metals evaluated, only Cu levels are very similar to Mexican loggerheads and Mn levels are higher than the other places. However, comparisons between the results from this study and previous studies are problematic and possibly inaccurate because of the species behavior, foraging areas, methods of collection and sample storage, handling, processing,

Table 5
Spearman correlations between heavy metals within blood and carapace scutes of the loggerhead turtles from Brazil.

	Blood								Carapace						
	As	Cd	Cr	Cu	Fe	Hg	Mn	Zn	As	Cd	Cr	Cu	Fe	Mn	Pb
CCL						0.241*									0.278*
Blood															
Cd	-0.666***														
Cu			-0.375**												
Hg	0.451***	-0.325**			0.310**										
Mn	-0.325**	0.331**				-0.246*									
Zn	0.326**					0.348**									
Carapace															
As	0.502***														
Cr			0.320*		-0.325*			-0.307*							
Fe	-0.332**					-0.546***	0.276*								
Hg	0.273*	-0.259*			0.286*	0.598***	-0.305*				-0.455**		-0.267*		
Mn						-0.324*						0.376**	0.645***		
Pb									0.272*						
Zn	-0.286*			0.286*					0.668***	0.273*	0.328*	0.306*	0.250*	0.305*	0.608***

* $p < 0.01$
** $p < 0.001$
*** $p < 0.0001$Bold values show negative correlation between the variables*CCL* curved carapace length, *As* arsenic, *Cd* cadmium, *Cr* chromium, *Cu* copper, *Hg* mercury, *Mn* manganese, *Pb* lead, *Zn* zinc

and differences among methods used to quantify metals [21]. Additionally, it is important to consider the mobility that some substances have in moribund, sick, or stranded turtles, which could be a source of variation between debilitated and healthy turtles.

4.4. Heavy metals- carapace

The only significant difference observed between areas in carapace heavy metal levels was for Hg, which concentration was higher in PV turtles. The rank order of heavy metals in the scutes was the same in the two areas Fe> Zn> Mn> As> Cu> Cr> Pb> Hg> Cd, except for Hg and Cd that were in inverse order in PF turtles (Cd> Hg). It suggests that there is a trend of metal accumulation in the carapace, which is related to eating habits. This corroborates the idea that although PV and PF loggerhead populations are genetically different [53] and nest in distinct areas, they might migrate and remain in the same feeding areas and then separated again in the breeding season. However, the localization of these feeding areas is unclear. Telemetry studies indicate that PF turtles migrate to foraging grounds in northeastern Brazil [59], whereas PV turtles travel to the north and south [60].

Regarding carapace fragments, only two studies sampled in Brazil and just analyzed Hg levels. Worldwide, few articles used scutes to analyze heavy metals in *C. caretta* (Table S4). Compared with other loggerhead sea turtles' populations, PV and PF turtles had higher As, Cu, Fe, and Mn levels. In contrast Cd, Hg, Pb, and Zn levels were lower. PF turtles from this study and those of Rodriguez et al. [61,62] were sampled in the same area but in different years and data showed huge differences in Hg levels. It is important to highlight that many factors are known to influence heavy metals concentrations in scutes, including layering (i.e., upper scute layers might exhibit older exposure than lower layers), migration, and diet [63,64]. Mattei et al. [65] observed that Mn, Pb, and Zn accumulated in the area of the carapace which ossifies first (central area), while Cd, Cr, Cu, Sb, and V are mainly located in the lateral areas. Additionally, superficial carapace scute scraping of about 2 mm includes only the keratinized layer, whereas a 14 mm deep tiny carapace samples collected using core drill also includes different layers as dermis and bone [66]. These facts, unfortunately, hamper direct comparisons to our results but may provide an overview of heavy metal distribution in the other populations of *C. caretta*. Thus, comparisons within a study are more valuable because animals are sampled and analyzed in the same way.

4.5. Heavy metals- blood x carapace

Sea turtles from Povoação had significantly higher As, Cd and Cu concentrations in the blood compared to scutes, suggesting more recent exposure to these elements, while Cr, Fe, Hg, Mn, Pb, and Zn were higher in scutes, indicating an older exposure to these elements. PF turtles had higher levels of Cu and Cd in the blood (recent exposure) and Cr, Fe, Mn, and Zn in the scutes (long-term exposure). Several relationships were found among heavy metals in the blood and carapace (Table 5), though explaining the nature of each correlation remains speculative. Several factors could contribute to the range and variability of the correlations, such as age, sex, and health of individuals [64]. Many studies have already found negative and positive correlations among various heavy metals in sea turtles [20,48,58,63,67-70]. These positive correlations probably exist for many reasons, among them: loggerhead sea turtles have simultaneous exposure to these elements; the elements have similar transport mechanisms and physiological pathways in the body (metalloids and heavy metals are bound to red blood cells); there are also antagonistic effects between the elements; they reflect bioaccumulation and/or they are binding with the keratin in the carapace [20,48,67,70]. Negative correlations may be due to the different toxicokinetics of each metal; maternal transfer; different sources of each element; or their exposure is less constant [58,71]. Lead was the only metal in the carapace with a positive correlation with CCL, which indicates that it accumulates with age. However, the absence of other correlations between most of the metals and animals' size could be because the study group of animals was relatively homogenous in size and age (adult nesting female). Mercury levels in the blood correlate with CCL and with carapace mercury loads, supporting similar findings of Day et al. [63] and Komoroske et al. [20]. Many heavy metals in the carapace (As, Cd, Cr, Cu, Fe, Mn, and Pb) positively correlated with zinc. It is known that zinc mediated the transcription of metallothionein in response to metal toxicity [72]. Metallothioneins are metal-binding proteins involved in the detoxification of toxic trace elements such as Cd, Hg, Ag, and Pb and the homeostasis of essential elements (e.g. Cu, Zn) [73]. A similar process in sea turtles might explain the strong correlations between Zn and other metals found in the present study.

4.6. Heavy metals x Health parameters

Despite the physiological differences found between samples from each region, PV turtles had more significant correlations between health parameters and heavy metals concentration, indicating that these contaminants may have an impact on the physiological profile and, potentially, the health of loggerhead turtles that nest in the area affected by the tailings. Considering the large number of correlations found in this study, it is impractical and perhaps overly speculative to try to explain the nature of each correlation [67]. Some of them are described below.

Blood arsenic (As) levels of PV turtles had a significant negative correlation with leukocytes, heterophils, lymphocytes, eosinophils, albumin, globulin, and protein, suggesting a possible cumulative negative effect on the immune system. Register [74], Camacho et al. [58], and Perrault et al. [48] also found a negative correlation between arsenic vs albumin, arsenic vs WBC counts, and arsenic vs γ-globulins in loggerhead turtles. Thus, arsenic contamination may inhibit the immune responses against microorganisms, favoring infections [75].

It was observed a significant decrease in RBC, thrombocytes, lymphocytes, and total globulin correlated to increased blood cadmium (Cd) levels in PV turtles. Cd has also been linked to reduced γ-globulins and total globulins and increased A:G ratio in loggerhead turtles [48], decreased total globulin in green sea turtles [20], and reduced creatinine and glucose levels in olive ridley sea turtles [76]. García-Fernández et al. [77] studied stranded loggerhead turtles from the Mediterranean coastline and observed that the turtle with the highest renal Cd concentration died following a period of sickness due to a massive parasite's infestation. The authors concluded that high Cd levels might cause chronic problems in the immune system which have reduced its capacity to defend the organism against parasites. Camacho et al. [58] found a negative relationship between RBC vs Cd levels and assumed that this metal might cause anemia in loggerhead turtles that nest in Cape Verde.

Chromium (Cr) is also known to cause anemia, since it is distributed to and accumulated by erythrocytes. Once inside the cell, it is rapidly reduced and binds to hemoglobin and other ligands. Some studies have already shown that acute and chronic exposure to chromium reduced MCV, MCH, Hct, and Hb [78]. In PV turtles, chromium had a significant negative correlation with hematocrit. On the contrary, mercury had a positive association with hematocrit, which is consistent with the role of RBCs as the primary transport mechanism of Hg throughout the body [79].

Some studies with sea turtles showed that lead (Pb) negatively correlated with the A:G ratio, glucose, sodium, lymphocytes, albumin, α_2-globulins, and P-nitrophenyl acetate esterase activity, and positively correlated with globulin, total protein, uric acid, phosphorus, glucose, and AST [20,48,58,76]. In this study, higher levels of lead were associated with lower levels of Hb and MCHC and higher levels of monocytes, triglycerides, magnesium, and urea. It is known that lead inhibits the body's ability to synthesize hemoglobin by interfering with several enzymatic steps in the heme pathway and it also induces two types of anemia (connected with interfering heme biosynthesis or hemolytic anemia) [80]. Together these correlations provide a strong argument that the exposure to these metals may have an impact on the female sea turtle's health.

4.7. Effects over time

In chronically contaminated coastal systems like Povoação, recent chemical exposure may not be strong enough to elicit obvious response patterns in sea turtles. Over time, however, physiological acclimatization and associated energetic demands of chronic chemical biotransformation and elimination could result in altered biochemical and hematological profiles [20].

Although only three animals were recaptured in Povoação and statistical analyzes were not performed because of the small sample size, a decreasing trend in some hematological and biochemical parameters can be observed when the two sequential analyzes are contrasted (Table S2). If this decreasing trend remains to persist as the season progresses, some parameters may be below the normal ranges and the animals could manifest anemia and immunological effects at the end of the nesting season. One recaptured animal changed its body condition from good to average in 17 days. Two of them increased the epibiont and ectoparasite counts, and one developed an eye lesion.

Maternal health and energy stores of nesting sea turtles affect their reproductive output, since suboptimal health produces eggs with inadequate nutritional reserves, which may influence the survival of their offspring [35]. The occurrence of eye lesions, suggestive fibropapillomatosis tumors, worse body condition, higher ectoparasite count, greater epibiont loads, large number of correlations between health parameters and heavy metal levels, and higher levels of some metals in the blood of PV turtles (as PCA demonstrates) indicates that the tailings could exert a negative impact on the health and reproduction of these turtles. Continuous monitoring is very important to confirm if heavy metals from the mining dam rupture are influencing the physiological condition of loggerhead sea turtles that nest in this region.

5. Conclusions

This study provides relevant information about loggerhead sea turtles' health that nest in an area affected by the tailings of a collapsed dam in Brazil. Although the results do not identify definitive cause-effect relationships on the health of these animals, they provide ecologically relevant data to inform hypotheses warranting further investigation. The large number of correlations found in this study underscore the need for more studies that monitor these animals over the entire nesting season to verify the changes in the physiological and immunological condition since animals that were recaptured had higher metal levels in the blood in the second sampling and important changes in health parameters. Additionally, the data reported here are important for temporal and spatial comparative studies in the future and helps to complement and strengthen the management plans for these species and the ecosystems where they participate.

Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) [Finance Code 001 - Grant Number 88887.169245/2018-00]; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [grant number 307647/2016-1 and 312599/2020-0]

Ethical statements

The present research did not involve human experimentation.

Blood and scute samples were collected from sea turtles *in situ* and transported to the laboratory. No animals were taken from their natural habitat.

The study was performed under the licenses SISBIO 61063 and 60430 of the Chico Mendes Biodiversity Institute (ICMBIO).

This research was approved by the Ethic Committee in Animal Use.

All the procedures were made by specialists according to the Research and Management Techniques for the Conservation of Sea Turtles.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Camila Miguel: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Marcelo Renan de Deus Santos:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Adalto Bianchini:** Methodology, Resources, Writing – review & editing. **Monica Ryff Moreira Vianna:** Conceptualization, Writing – review & editing, Supervision.

Acknowledgements

We would like to thank the huge help of people from Projeto TAMAR-Povoação and Praia do Forte for all the field support and to allow the use of facilities, without whose assistance this work would not have been possible. To many people who helped with sample collection and laboratory analyses, especially the Instituto Marcos Daniel team, Paulo R. J. Filho, Nairana S. Fraga, Yhuri Nobrega, Ygor Machado, Débora Cristina Alves, Humberto Donateli Oliveira, and Phillipe Dangelo. To the laboratory assistants of FURG Dra. Patrícia Gomes Costa, Dra. Juliana Carriconde Hernandes and Dra. Liziane Cardoso Marube, for the metal analyzes. The present study was carried out as part of the Aquatic Biodiversity Monitoring Program, Ambiental Area I, established by the Technical-Scientific Agreement, DOU number 30/2018, between FEST and Renova Foundation.

Supplementary materials

Supplementary Fig. S1. Fibropapillomatosis in loggerhead sea turtle from Povoação, Espírito Santo state, Brazil.

Supplementary Fig. S2. Severe blepharoconjunctivitis in *Caretta caretta* from Povoação, Espírito Santo state, Brazil.

Supplementary Fig. S3. Keratoconjunctivitis in *Caretta caretta* from Povoação, Espírito Santo state, Brazil.

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jtemin.2022.100015.

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