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Chemical Element Concentrations in the Blood of Green Turtles (Chelonia Mydas) Captured at Fernando De Noronha Marine National Park, Brazil

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Abstract

Green turtles may be used as biomonitors of marine environments because of their longevity and feeding habits (omnivorous during the first years of life and herbivorous during the juvenile and adult stages), which can indicate the degree of ocean contamination in the areas where they feed. Studies of metal and chemical element concentrations in the blood of green turtles are still rare; therefore, the results are difficult to interpret, although it appears that the serum levels of certain elements found in green turtles are much higher than the minimal risk levels indicated for human health. The objective of this study was to measure the concentrations of the essential elements Calcium (Ca), Selenium (Se), Zinc (Zn), Manganese (Mn), Cobalt (Co), Copper (Cu) and Molybdenum (Mo) and the metals Aluminum (Al), Arsenic (As), Lead (Pb), Cadmium (Cd), Lithium (Li), Cesium (Cs), Barium (Ba), Rubidium (Rb), Uranium (U), Thallium (Tl), Beryllium (Be), Antimony (Sb) and Tellurium (Te) in the blood of 31 juvenile green turtles captured in Fernando de Noronha Marine National Park (Parque Nacional Marinho de Fernando de Noronha), Brazil, and to correlate these concentrations with the curved carapace length to identify possible cumulative effects. Furthermore, because the basal levels for these elements have not yet been established for green turtles, the effects of these chemicals on the health of the species are still unknown. Thus, most of these contaminants should be described as "alarming" until further clarification.

Keywords: Biomonitors; Marine enviroments; Pollution; Elemental analysis; Mass spectometry; Sea turtles

Introduction

Chelonia mydas is a sea turtle with a worldwide distribution in tropical and subtropical seas between 40°N and 40°S latitude. This species has the most coastal habit of sea turtles and is capable of entering river and lake estuaries [1]. In these neritic zones (waters close to the shore), green turtles primarily feed on seagrasses and algae [2]. In addition, their feeding habit varies from omnivorous during the first years of life [3] to herbivorous following the post-pelagic phase [4]. This species is considered threatened by the International Union for Conservation of Nature (IUCN) and vulnerable by the List of Endangered Species of the Chico Mendes Biodiversity Institute (Instituto Chico Mendes de Biodiversidade - ICMBio). The decrease in green turtle populations has been attributed to coastal development, accidental capture in fishing gear, human consumption, climate changes, pollution and pathogens [5]. A number of studies conducted on sea turtles in different parts of the world have focused on the detection and measurement of toxic metals and essential elements [6-13], which likely play relevant roles in the decrease of sea turtle populations [14]. Despite the importance of the Brazilian coast for the development of five of the seven sea turtle species, the only study to detect chemical elements in turtles in Brazil was published in 2009 by Barbieri.

Certain chemical elements are essential to life; however, many chemicals can be metabolized or bioaccumulated and become toxic for organisms. Vertebrates exposed to high concentrations of certain chemical elements or small concentrations for long periods of time may exhibit neurological, reproductive, gastrointestinal, respiratory, hepatic, immunological, renal or dermatological symptoms [15].

The objective of this study was to measure the concentrations of the essential elements Calcium (Ca), Selenium (Se), Zinc (Zn), Manganese (Mn), Cobalt (Co), Copper (Cu) and Molybdenum (Mo) and the metals Aluminum (Al), Arsenic (As), Lead (Pb), Cadmium (Cd), Lithium (Li), Cesium (Cs), Barium (Ba), Rubidium (Rb), Uranium (U), Thallium (Tl), Beryllium (Be), Antimony (Sb) and Tellurium (Te) in the blood of 31 juvenile green turtles captured in Fernando de Noronha Marine National Park (Parque Nacional Marinho de Fernando de Noronha), Brazil, and to correlate these concentrations with the curved carapace length to identify possible cumulative effects.

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Materials and Methods

Blood collection and specimen identification

Thirty-one juvenile green turtles were intentionally captured at Sueste Beach (Praia do Sueste; 3°86'68"S; 32°42'60"W), Fernando de Noronha Marine National Park, Brazil between 26 April and 4 May 2013 (Figure 1). The specimens were identified using the tag number supplied by the Tamar/ICMBio project. Measurements of the curved carapace length (CCL) [16], a parameter that is used to determine the life stage of the animal, were performed. Because sex cannot be determined in juveniles using phenotypic parameters, sex determination was not performed. One milliliter of blood was collected from the cervical venous sinus using the occipital bone as a reference. The turtles were released immediately following blood collection, and the blood samples were placed in Vacuette® Trace Elements Sodium Heparin (Greiner Bio-one, Americana, SP, Brazil) tubes and kept in ice until they were stored at -20°C for further analysis.

Elemental analysis

The analyses were conducted with an inductively coupled plasma mass spectrometer (ICP-MS) operating with high-purity argon (99.999%, Praxaair, Brazil). The sample introduction system was composed of a quartz cyclonic spray chamber and a Meinhard® nebulizer connected by Tygon® tubes to the ICP-MS's peristaltic pump (set at 20 rpm). The ICP-MS was operated with a Pt sampler and skimmer cones purchased from PerkinElmer (Shelton, CT, USA). High purity de-ionized water (resistivity 18.2 MΩ cm^{-1}) used for preparation of samples and solutions was obtained using a Milli-Q water purification system (Millipore RiOs-DI™, Bedford, MA, USA). All of the reagents were of analytical-reagent grade except for HNO_3 which was previously purified in a quartz sub-boiling still (Kürner Analysentechnik) before use. A clean laboratory and laminar-flow hood capable of producing class 100 air flow were used for preparing the solutions. Rhodium (1000 mg L^{-1}) and multi-element solution (10 mg L^{-1}) were obtained from PerkinElmer. Triton® X-100 was purchased from Sigma–Aldrich (St. Louis, MO, USA). Plastic bottles and cryogenic vials were cleaned by soaking in 15% (v/v) HNO_3 for 24 h, rinsing five times with Milli-Q water and drying in a class 100 laminar flow hood before use. Sample preparation and analysis were performed in a class 1000 clean room. A base blood was derived from sheep (undosed animals) for the matrix-matching calibration. The samples and calibration curves were diluted 1:50 into a 15-mL polypropylene Falcon® tube (Becton Dickinson) with a solution containing 0.01% (v/v) Triton® X-100, 0.5% (v/v) nitric acid and 10 µg L^{-1} of the internal standards. To verify the accuracy and precision of the blood samples, human blood reference materials (RM) QMEQAS07B06 and QME- QAS07B03 from the L'Institut National de Santé Publique du Quebec (Canada) were analyzed [17].

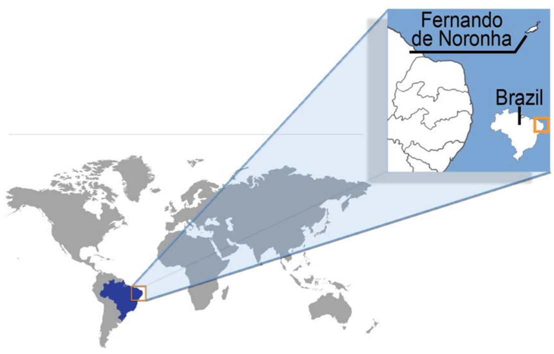

Figure 1: Map of Fernando de Noronha, Brazil.

Data presentation and statistical analysis

Data handling and statistical analyses were performed using the software R (R Development Core Team, 2013).

Correlations between the concentrations of the 20 quantified elements and the CCL were analyzed using the Spearman's rank correlation coefficient because the CCL data were not normally distributed.

Significant correlations between the elements and CCL were tested using Spearman's correlation test (ρ) or Pearson's correlation test (r) depending on whether both concentrations exhibited a normal distribution. P values lower than 0.05 (two-tailed) were considered significant.

Results

The median, first quartile and third quartile for the CCL and quantified elements are presented in Table 1. Among the 20 analyzed elements, the Spearman's correlation test revealed that CCL was significantly negatively correlated with Se (ρ=-0.48, p<0.01) and significantly positively correlated with Zn (ρ=0.42, ρ=0.02), Pb (ρ=0.41, ρ=0.02) and Cs (ρ=0.35, ρ=0.05) (p<0.05).

When both elements exhibited a normal distribution, the correlations were quantified using Pearson's correlation test, and when both elements exhibited a non-normal distribution, the correlations were quantified using Spearman's correlation test. The significant correlations observed between the elemental concentrations are presented in Table 2.

Discussion

The concentrations of chemical elements in the blood of the juvenile green turtles appeared to vary greatly from previously reported values for other locations. This result is shown in Table 3, which presents a comparison between our results and the results of studies conducted in different locations worldwide.

The geographical position and urban/industrial development of the areas in the compared studies are listed as follows: Gaus et al. [18] studied green turtles in Gladstone, Australia, an area with large-scale industrial development, varied industries and a seaport; Komoroske et al. [19] measured the element concentrations in green turtles in San Diego Bay, United States, an impacted navigation area; Van de Merwe et al. [13] studied recently deceased green turtles that were under rehabilitation at the Center for Marine Animals in Gold Coast, Australia; and Labrada et al. [20] studied turtles in two coastal lagoons in Baja California, Mexico, an area with urban development and waste discharge. The Fernando de Noronha Marine National Park is considered a clean area because it is an oceanic island without industrial development and with low environmental impacts relative to the other four locations.

We observed a negative correlation between CCL and Se serum levels. This finding is consistent with that of Komoroske et al. [19], who also observed a correlation between CCL and As, Cu, Cd and Hg. The Se levels observed in the present study in the sea turtles from Fernando de Noronha National Park were much lower than those previously reported and almost half the values observed by Komoroske et al. [19]. According to the Agency for Toxic Substances and Disease Registry (ATSDR), exposure to high levels of Se or prolonged exposure

Analysis	First quartile	Median	Third quartile
CCL (m)	0.68	0.72	0.77
Al (μg/L)	60.41	80.31	105.60
As (μg/L)	166.45	204.84	462.84
Ba (μg/L)	2.65	3.29	4.11
Be (μg/L)	0.74	1.07	1.44
Ca (μg/L)	59.49	71.98	89.62
Cd (μg/L)	4.66	12.58	12.58
Co (μg/L)	27.48	40.24	57.04
Cs (μg/L)	0.67	0.83	0.91
Cu (μg/L)	626.26	787.30	861.71
Li (μg/L)	28.68	32.16	35.85
Mn (μg/L)	30.42	40.76	57.87
Mo (μg/L)	8.77	10.16	12.62
Pb (μg/L)	14.24	25.29	36.47
Rb (μg/L)	954.40	1130.75	1300.05
Sb (μg/L)	4.13	4.96	6.37
Se (μg/L)	191.49	282.61	379.69
Te (μg/L)	0.23	0.30	0.42
Tl (μg/L)	0.02	0.08	0.22
U (μg/L)	0.22	0.29	0.42
Zn (μg/L)	11.03	12.18	13.81

Table 1: Median, first quartile and third quartile of the curved carapace length (CCL) and concentrations of 20 chemical elements in the blood of juvenile green turtles (*C. mydas*) captured at Fernando de Noronha Marine National Park, Brazil.

Elements tested	Correlation coefficient value (Pearson=*r* and Spearman=*p*)	P-value
Cd x As	*p*=0.38	0.03
Cd x Cu	*p*=0.68	<0.01
Cs x Cd	*p*=0.56	0.001
Cs x Cu	*r*=0.67	<0.001
Pb x Cd	*p*=0.64	<0.001
Pb x Cs	*r*=0.64	<0.001
Pb x Cu	*r*=0.64	<0.001
Se x As	*p*=0.86	<0.001
Zn x Cd	*p*=0.44	0.01
Zn x Cs	*p*=0.44	0.01
Zn x Cu	*p*=0.48	0.01
Zn x Pb	*p*=0.56	0.001

Table 2: Statistically significant correlations between elements (Pearson=*r* and Spearman=*p*).

results in increased serum Se concentrations over time [21]. However, decreasing Se levels are observed when the individual has a disease or is at an advanced age. The green turtles at Fernando de Noronha were in good physical conditions, which lead us to hypothesize that this feeding area has low Se concentrations because Se has a relatively short half-life that varies from hours to days. Studies with mammals have shown that Se is usually positively correlated with other metals, especially As, Hg and Cd, although this association was not observed in the present study. The metabolism of Se may be significantly altered by interactions with other metals, chemical products and psychochemical factors [22], which increase the difficulty of interpreting Se concentrations.

Compared with Se, the elements Zn, Pb and Cs were positively correlated with CCL. The mean Zn concentration observed in the present study was slightly higher than the concentration observed by Labrada et al. [20] in two sites in Mexico. Green turtles appear to typically exhibit high Zn serum levels [23-25]. The most important routes of exposure to Zn are feeding and contaminated soil/sediments and serum Zn levels increase rapidly following absorption [26]. Therefore, the archipelago may be the main source of contamination.

Blood is considered the most common and accurate compartment for assessing recent exposure to lead [27,28]. The serum Pb concentrations observed in the present study were similar to those reported by Van de Merwe et al. [13]. The positive correlation between the serum Pb concentrations and CCL observed in our study indicates that the studied animals were exposed to Pb, thus increasing their contamination load. This finding is worrisome because minimal risk levels have not been established for this species. Nonetheless, the observed Pb concentrations appeared to be high relative to that of other vertebrates. In humans, serum Pb concentrations higher than 10 μg dL^{-1} result in the decreased biosynthesis of heme enzymes and increased blood pressure, and they promote neurologic effects in children [27].

Cs is found in the environment in its stable form (Cs^{133}) combined with other elements in the rocks, soil and dust. Many Cs compounds are dissolved in water. Plants and animals may present Cs in concentrations of 1-300 ppb [29]. The positive correlation between Cs and the CCL indicates that the animals remain in contact with this element.

No other correlations were observed between the quantified elements and CCL. In addition, a comparison of the serum levels of As, Cd, Mn, Co, Cu and Mo observed in the present study with that of previous reports indicated a wide variation. Although Komoroske et al. [19] and Gaus et al. [18] used the same elemental analysis methods (ICP-MS) as in the present study, only the Cu concentrations were similar. Determining baseline data for these elements in green turtles is important for their survival as well as for oceanic health because the concentration of chemical elements in sea turtle tissues are reflective of the concentrations in the environment they inhabit [30].

The highest correlation coefficient among element concentrations was observed between Se and As (p=0.86). The correlation between Se and As concentrations has been previously described for several animals, and the simultaneous exposure to these two elements is characterized by an antagonist interaction between the elements [31]. Strong positive linear correlations were observed between Pb and Cs, Pb and Cu, and Cs and Cu (r>0.60). Synergies and/or antagonisms

	Prioste et al. 2015 (n=31)	**Gaus et al. [18]** (n=40)	**van de Merwe et al. [13]** (n=16)	**Komoroske et al. [19]** (n=30)	**Labrada et al. [20] Bahia Magdalena** (n=14)	**Labrada et al. [20] - Punta Abreojos** (n=42)
Study site	Fernando de Noronha - Brazil	Gladstone - Australia	Gold Coast - Australia	San Diego Bay - USA	Baja California - Mexico	Baja California - Mexico
Method	ICP-MS	ICP-MS	ICP-MS	ICP-OES	AAS	AAS
Al	96	ND	NA	146	NA	NA
As	366	2300	4361	157	NA	NA
Cd	14	40*	35	13	30	60
Co	51	150	36	NA	NA	NA
Cu	757	780	1019	749	NA	NA
Mn	61	35	NA	463	NA	NA
Mo	19	11	NA	NA	NA	NA
Pb	27	18	22	1260	NA	NA
Se	424	1900	2447	776	1810	1590**
Zn	14050	8400	7924	NA	13580	13920

*n: 3; **n: 40; ND: Not detected; NA: Not analyzed; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry; AAS: Atomic Absorption Spectrometry

Table 3: Mean serum levels of the chemical elements detected in the juvenile green turtles at different study sites. The results are expressed in micrograms per liter (μg/L).

between inorganic and organic contaminants are complex and not well understood. Therefore, the data presented here are still preliminary and serve only as a basis for future studies. Further studies are required that include analyses of additional samples.

Conclusions

Green turtles may be used as biomonitors of marine environments because of their longevity and feeding habits (omnivorous during the first years of life and herbivorous during the juvenile and adult stages), which can indicate the degree of ocean contamination in the areas where they feed. Studies of metal and chemical element concentrations in the blood of green turtles are still rare; therefore, the results are difficult to interpret, although it appears that the serum levels of certain elements found in green turtles are much higher than the minimal risk levels indicated for human health.

The exact source of contamination could not be determined, and further studies on the anthropogenic pressure exerted on the marine environment are required because patterns were not observed in the results reported for different locations worldwide. Furthermore, because the basal levels for these elements have not yet been established for green turtles, the effects of these chemicals on the health of the species are still unknown. Thus, most of these contaminants should be described as "alarming" until further clarification.

The methods used here were efficient because blood collection is a minimally invasive and rapid procedure, and the animals were returned to the environment immediately following venipuncture. In addition, the analyses were performed in triplicate, and significant differences were not observed between replicates.

Similar studies using a larger number of animals are currently being conducted in other areas of the Brazilian coast, and the results may further clarify the relationship between inorganic elements and green turtle disease and survival. In addition, similar studies that include the analysis of stomach contents and algae collected from feeding areas will soon be performed to determine the interactions among the elements studied here.

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