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## Baseline

Trace elements distribution in hawksbill turtle (*Eretmochelys imbricata*) and green turtle (*Chelonia mydas*) tissues on the northern coast of Bahia, BrazilGustavo R. de Macêdo<sup>a</sup>, Taiana B. Tarantino<sup>b</sup>, Isa S. Barbosa<sup>b</sup>, Thaís T. Pires<sup>c</sup>, Gonzalo Rostan<sup>c</sup>, Daphne W. Goldberg<sup>c,d</sup>, Luis Fernando B. Pinto<sup>a</sup>, Maria Graças A. Korn<sup>b,\*</sup>, Carlos Roberto Franke<sup>a</sup><sup>a</sup> Department of Animal Production, School of Veterinary Medicine and Zoo-technique, Federal University of Bahia (UFBA), 40170-110 Salvador, Bahia, Brazil<sup>b</sup> Institute of Chemistry, Federal University of Bahia (UFBA), Ondina, Campus UFBA, 40170-115 Salvador, Bahia, Brazil<sup>c</sup> Brazilian Center for the Protection and Research of Sea Turtles Foundation (Pro-Tamar Foundation), Salvador, Bahia, Brazil<sup>d</sup> Department of Biochemistry, State University of Rio de Janeiro, 20551-030 Rio de Janeiro, RJ, Brazil

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## ABSTRACT

Concentrations of elements (As, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, V, Zn) were determined in liver, kidneys and bones of *Eretmochelys imbricata* and *Chelonia mydas* specimens found stranded along the northern coast of Bahia, Brazil. Results showed that the concentrations of Cd, Cu, Ni and Zn in the liver and kidneys of juvenile *C. mydas* were the highest found in Brazil. We also observed a significant difference ( $p < 0.05$ ) on the bioaccumulation of trace elements between the two species: Al, Co, Mo, Na and Se in the liver; Al, Cr, Cu, K, Mo, Ni, Pb, Sr and V in the kidneys; and Al, Ba, Ca, Cd, Mn, Ni, Pb, Se, Sr and V in the bones. This study represents the first report on the distribution and concentration of trace elements in *E. imbricata* in the Brazilian coast.

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Environmental contaminants, such as trace elements and pesticides, may bioaccumulate in marine food chains and reach especially high concentrations in species, such as sea turtles, that are long-lived and occupy high trophic levels (Anan et al., 2001). Toxic elements exposure has been linked to serious diseases and developmental disorders, such as, reproductive impairment, immune-system diseases, neurological disorders as well as carcinogenic effects. The problem not only affects the terrestrial species, but also a variety of marine species, such as sea birds and marine mammals (De Guise et al., 2003; Franson, 1996; Law, 1996; Peakall, 1996; Reijnders, 2003).

In Brazil, sea turtles are fully protected by law and are included in the Brazilian government's official list of endangered fauna (Marcovaldi et al., 2011; Almeida et al., 2011). Hawksbills (*Eretmochelys imbricata*) are currently classified as Critically Endangered and green turtles (*Chelonia mydas*) as Endangered by the International Union for Conservation of Nature (IUCN, 2011). Sea turtles are clearly under threat of extinction due to human activities. Commercial fishing, marine pollution and loss of nesting habitat are among the human-caused threats pushing sea turtles toward extinction (Hamann et al., 2010). However, information

regarding populations of sea turtles from Brazil is scarce. For example, although the recognized importance of Brazilian waters for the development and reproduction of several species of sea turtles, only three studies performed on sea turtles found in these waters were published recently (Barbieri, 2009a; Bezerra et al., 2012; Silva et al., 2014).

The Areembepe beach, located in the district of Camaçari, on the northern coast of Bahia, is one of the most important nesting grounds in Brazil (Marcovaldi and Marcovaldi, 1999). Recent studies also characterize the area as a potential foraging ground for juvenile *C. mydas* and *E. imbricata* (Macedo et al., 2011). The northern coast of Bahia, which hosts a great number of juvenile and adult turtles, also has an enormous industrial concentration, which poses a major threat to the sea turtles' survival. According to Bjørndal (2000) studies on foraging grounds are of great importance to determine priority conservation areas for sea turtle populations and to ensure ecological integrity.

Trace elements distribution and concentration have already been described in sea turtles at different stages of development. However, these data rarely exist for *E. imbricata*, especially along the Brazilian coast. This study aims to investigate the distribution and concentration of trace elements in tissues (liver, kidneys and bones) of two sea turtle species (*E. imbricata* and *C. mydas*) and to establish a comparison between the two species.

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Twenty-six necropsies were conducted on juvenile individuals of *E. imbricata* ( $n = 16$ ) and *C. mydas* ( $n = 10$ ), between January 2010 and July 2011. The animals were found stranded on Arembepe beach ( $-12,97786$ ;  $-38,4216$  at  $-12,70848$ ;  $-38,12327$ ), in Municipal District of Camaçari, Bahia, Brazil (Fig. 1). Most of the turtles apparently were alive when they beached, or had died very recently. Some of them died while being rehabilitated at Tamar Project rehabilitation facility, located in Praia do Forte, Mata de São João Municipal District, in the State of Bahia.

After determining the turtles' species, the animals were measured – curved carapace length (CCL) and curved carapace width (CCW) as suggested by Bolten (2000), had their sex determined by observation of the gonads and were assigned to one of four condition categories: good, fair, bad or emaciated. Tissue samples (liver, kidneys and bones) were collected during necropsy, placed in Ziploc bags and frozen at  $-20^{\circ}\text{C}$  for subsequent analysis. Additionally, we examined the occurrence of marine debris in the turtles' gastrointestinal tract.

Tissue samples were dried for 24 h in lyophilizer. Tissue fragments were then ground and weighed. Occasionally, insufficient tissue was obtained, specifically from *E. imbricata*, after the

samples were weighed. For this reason, these samples were randomly paired and homogenized, generating a total of eight samples for *E. imbricata* and ten samples for *C. mydas* turtles.

The acid digestion of the samples was performed using a commercial high-pressure laboratory microwave oven (Milestone Ethos 1600 Microwave Labstation, Sorisole, Italy) operating at a frequency of 2450 Hz with an energy output of 900 W. Approximately 0.25 g of the tissue samples were placed in Teflon® vessels with a mixture of  $\text{HNO}_3$  (3.5 mL), ultrapure water (3.5 mL) and  $\text{H}_2\text{O}_2$  (1.0 mL). The heating programme was performed in four successive steps. In the first step the temperature was linearly increased up to  $90^{\circ}\text{C}$  in 6 min. In the second step, the temperature was kept at  $90^{\circ}\text{C}$  for 4 min. In the third step, the temperature was linearly increased up to  $180^{\circ}\text{C}$  in 8 min and, in the fourth step the temperature was kept at  $180^{\circ}\text{C}$  for 15 min. After cooling, the digest was diluted to 20 mL with ultrapure water. Three replicates of each sample were analyzed. Blank assays were carried out.

Twenty-two trace element concentrations (As, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, V, Zn) were determined using inductively coupled plasma optical emission spectrometry (ICP OES) (Vista Pro, Varian, Australia) and

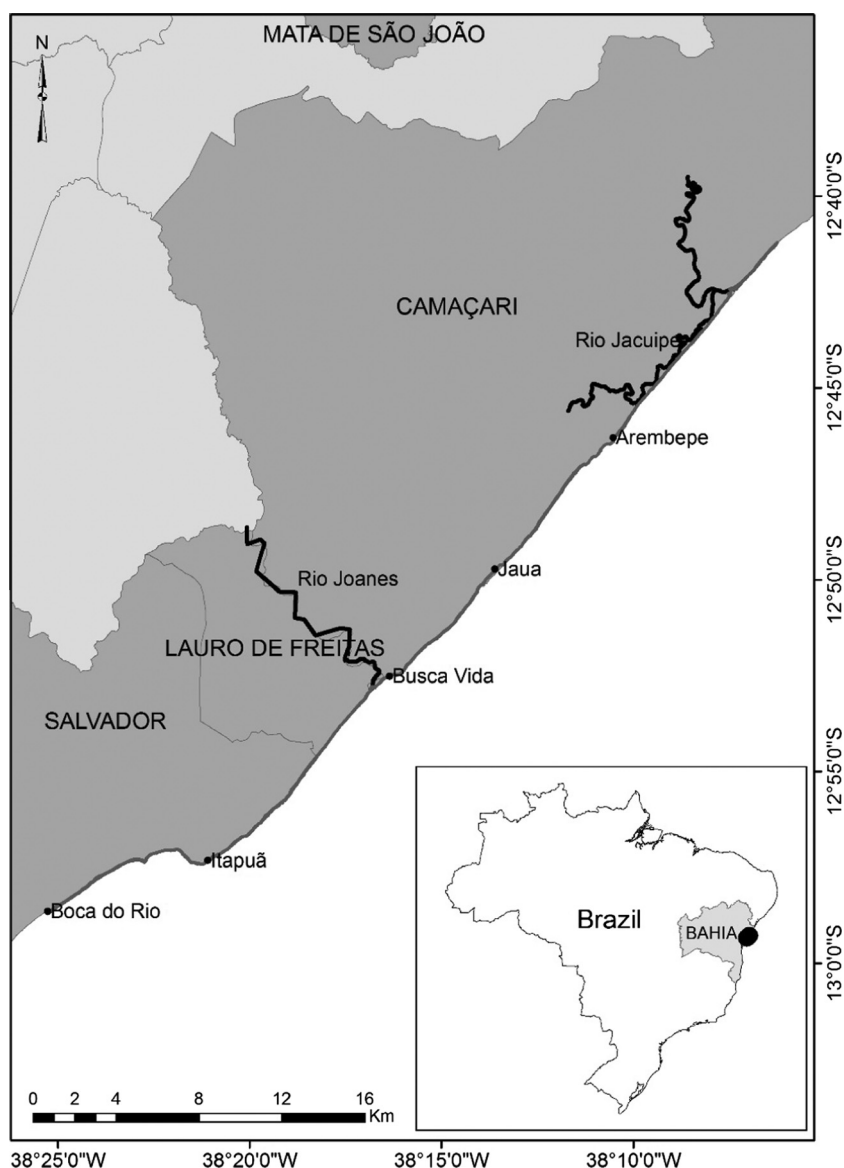


Fig. 1. Collection station in the Arembepe beach, Bahia State, Brazil.

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