ECOTOX BRASIL
SOCIEDADE BRASILEIRA DE ECOTOXICOLOGIA

Ecotoxicol. Environ. Contam., v. 14, n. 1, 2019, 65-72
doi: 10.5132/eec.2019.01.08

EEC

Heavy metals in blood and in nests affect reproduction parameters in *Eretmochelys imbricata*, Linnaeus, 1766 (Testudines: cryptodira)

Simões, T.N[1]; Silva, A.C[2]; Santos, A.M.M.S.[1] & Chagas, C.A[1]

[1]Programa de Pós–graduação em Saúde Humana e Meio Ambiente, Universidade Federal de Pernambuco. Vitória de Santo Antão, Brazil

[2]Ecoassociados NGO, Conservação de tartarugas marinhas, baobás e recifes de corais. Ipojuca, Brazil

Received May 22, 2019; Accept November 05, 2019

Abstract

In this study, blood, eggshells, and nest sediments of sea turtles *Eretmochelys imbricata*, collected during their reproductive period, were analyzed by X-Ray fluorescence to determine the presence and concentrations of Ni, Co, Cu, Pb and Cr. Moreover, we analyzed the possible interference of such metals in some reproductive data. All elements were detected in low concentrations. Ni was the element found in the highest concentrations in the blood, eggshells, and sediment. Our results demonstrated that females with the highest concentrations of Ni in their blood presented fewer eggs in their nests; and that when the concentration of Cu in their blood is higher, the number of eggs increases. Cu has a different effect when in the sediments covering the nests; Cu concentrations in the sediment appear to be related to an increase in dead embryos. Hence, our results demonstrate that sea turtles are exposed and contaminated by heavy metals, and that such metals may influence the reproduction of hawksbill turtles *E. imbricata*, and probably other species of sea turtles.

Keywords: conservation; hawksbill turtle; marine pollution; trace elements.

INTRODUCTION

Anthropogenic action has been spewing large amounts of heavy metals in seas and oceans around the globe (Nriagu & Pacyna, 1988; Çelik *et al.* 2006); such pollutants influence the quality of the ecosystems and may cause a decline in populations and genetic storage of marine organisms, such as sea turtles. Although the decrease in populations of sea turtles around the world is mainly related to incidental capture in fisheries, degradation of nesting areas, photo–pollution and marine pollution (Anan *et al.* 2001; Hamann *et al.* 2010; Marcovaldi *et al.* 2011), several studies have reported that heavy metal contamination may cause decline in sea turtle populations (Godley *et al.* 1999; Sakai *et al.* 2000). The presence of such compounds in female turtle tissues may decrease reproduction success by different mechanisms, such as interfering in the number of eggs or in the development of the embryo. Thus, the analysis of tissues and eggshells may provide information about heavy metal contamination in such organisms, as well as insights about the influence that those compounds have in reproductive success of sea turtles.

The presence of heavy metals in sea turtle tissues has been revealed by some studies that examined organs of turtles found dead; however blood samples and eggshells found in nests seem optimal sample matrices because they can reveal the presence of heavy metals in live animals (Páez–Osuna *et al*. 2010a; Macêdo *et al.* 2015). Through these samples, it is possible to obtain information and make inferences about the effects of metals in females turtles, as well as about maternal transference, contamination of embryos, and hatching success (Lam *et al*. 2006; Guirlet *et al.* 2008; Páez–Osuna *et al*. 2010a; Ley–Quinõénez *et al*. 2013). In addition, the analysis of the sediment that cover the nests may provide information about environmental quality, as well as about embryos exposition to heavy metals (Guirlet *et al*. 2008).

Sea turtles are considered bioindicators of heavy metal contamination in marine ecosystem because they feed on different trophic levels, live for many years and have a

*Corresponding author: Thyara Noely Simões; e-mail: thyara.noely@gmail.com

broad geographical distribution (Lam *et al*. 2004). Such characteristics increase the chances of metal bioaccumulation through feeding, and also increase the chances of contamination by exposure to the pollutants in water or sediment (Anan *et al.* 2001; Kampalath *et al.* 2006; Barbieri, 2009; Páez–Osuna *et al.* 2010a). *Eretmochelys imbricata* (Linnaeus, 1766) is the most common nesting species along the southern coast of Pernambuco (Moura *et al.* 2012; Simões *et al.* 2014) and is a good study model to analyze the presence and the influence of heavy metals on the marine environment along the Brazilian northeastern coast. Currently, the species is classified as "critically endangered" of extinction by the International Union for Conservation of Nature (IUCN, 2008). The presence of heavy metals in blood eggshells, and nests of *E. imbricata*, can indicate the pollution status in the environment; also, the interference of such metals in reproduction of sea turtles can provide information about how those metals may influence their populations. In this study, we evaluated the contents of nickel (Ni), cobalt (Co), copper (Cu), lead (Pb) and chromium (Cr) in blood, eggshells and nests sediments of *E. imbricata*; the possible effects of these metals on reproductive parameters in *E. imbricata* were also analyzed.

METHODS

Eighteen females of *E. imbricata* and their respective nests were randomly chosen for the study. Blood samples and biometric data were collected from each animal immediately after eggs post. Each nest was monitored until hatching, when eggshells and sediment were collected. Blood, eggshells, and sediment samples were analyzed to determine concentrations of Ni, Co, Cu, Pb, and Cr by X- Ray fluorescence. The study was authorized under the license from SISBIO/ICMBIO Nº 45623–1 and was conducted according to the regulations of the Ethics Committee on Animal Use Nº 23076.054310/2014–70 from January 2015 to July 2015.

Study area

The study area is located in Ipojuca, state of Pernambuco, at -8.401667°S; -35.062500°W, and the nests are spread over five beaches: Muro Alto (2.5 km long), Cupe (2.4 km long), Merepe (3.5 km long), Porto de Galinhas (1.5 km long), and Maracaípe (3,1 km long), totalizing 13 km (figure 1). These beaches feature sandstone reefs parallel to the shore; such structures support the development and growth of corals, algae and small marine animals, and is used as foraging area by sea turtles (Moura *et al.* 2012). The area is monitored by NGO Ecoassociados, an institution working in the monitoring, and conservation of sea turtles, and in helping identification of animals and nests.

Collection of samples in the field

Initially, the beaches were inspected during the night to identify the time when each one of the 18 females left the sea to nest. During nesting, biometric data on the curved carapace length (CCL) and curved carapace width (CCW) were collected with a tape measure. After egg posting, 10 mL of blood ware collected from the dorsal cervical sinus (Owens & Ruiz, 1980) and stored in a vacutainer tube containing heparin. Then, the properly identified samples were kept at 4°C and transported to the laboratory, where they were stored at -20°C until processing.

The nests were isolated with tapes and wood stacks for protection and referenced using a GPS; all nests were then monitored daily. After the period of hatching (45–60 days), each nest was cleaned, and the number of eggs, the number of live hatchlings, newborn mortality, and the number of unhatched eggs were recorded in spreadsheets. Then, 20 eggshells, randomly selected, and the sediment where they were incubated (bottom of the nests), were collected. Each sample was packed in 2 L plastic bags, transported to the laboratory, and stored at room temperature.

Laboratory analyses of heavy metals

Blood samples were centrifuged at 3,000 rpm for 15 minutes to separate blood cells and particles suspended in the plasma. Thereafter, 500 µL of plasma were separated and diluted in 1,500 µL of ultrapure water (Milli–Q water); then, 1 mL of the dilution was stored in Eppendorf® tubes.

Eggshells from each nest were dried at 50°C, crushed, weighed and placed in a glass container, to which 8 mL of concentrated nitric acid (HNO_3) were added. Each solution formed with the samples was placed in a digester exhaust camera for digestion at 95°C, for approximately 3 h. Then, each solution received 2 mL of distilled water and 10 mL of hydrogen peroxide (H_2O_2). This mixture was again heated for approximately 2 h. After cooling, 1 mL of the final product was transferred to Eppendorf® tubes, which were subsequently stored.

Sediment samples were dried at 50°C (± 2°C) and subsequently crushed and separated into 2 g subsamples for digestion following the method 3050B of USEPA (United States Environmental Protection Agency). After digestion, 1 mL of the obtained products was transferred to an Eppendorf® tube and then stored.

The chemical analyses were performed by X–ray fluorescence (XRF). For this process, each sediment, blood and eggshell sample received 100 µL of Gallium (Ga) solution (102.5 mg L^{-1}), used as a standard solution. Then, the mixtures were stirred for homogenization and 5 µL of each sample were pipetted on an acrylic reflector (in triplicate) and dried in an oven at approximately 60ºC.

After drying, all measurements were performed at the XRF beamline of the Brazilian Synchrotron Light Laboratory, in Campinas (Brazil). A 13.5 keV monochromatic beam of 0.1 mm high by 5 mm wide was used to excite the samples under total reflection conditions. Samples were measured for 500 s using a silicon drift detector (KETEK, GmbH) with an energy resolution of 140 eV at 5.9 keV and the collected X–

ray fluorescence spectra were evaluated using the PyMCA software (Solé *et al.* 2007). All analyses were performed in an air atmosphere. The results obtained for each item sampled were expressed in mg Kg^{-1}.

Statistical analyses

General Linear Models (multi–way regression analysis) (Mccullagh & Nelder, 1989) were performed to estimate whether the concentration of five heavy metals (Ni, Co, Cu, Pb and Cr) in each substrate (blood and eggshells of live hatchlings, and sediments of nests), considered in this study as predictor variables, affects each of the dependent variables (total eggs, unhatched eggs, stillbirths turtles, live hatchlings and hatching success). Furthermore, a multiple linear regression analysis was performed to verify to what extent the concentration of heavy metals in the blood of females can predict its size as measured by CCW. Before "running" each model, we used the "Variance Inflation Index" to analyze whether there was an autocorrelation between predictor variables. All heavy metals which showed autocorrelations with index values above 3 were excluded from the analyses. After running the models, we performed a residual analysis (raw residual normality and Cook's Distance) to verify whether the data used were well–adjusted to the test assumptions. We also verified a possible correlation between the concentrations of each metal in the sediments and the concentrations of the metals in eggshells. All tests were performed using the software Statistica 7.0 (StatSoft, 2004). The analyses were performed with a significance level considering $p<0.05$.

RESULTS

Table 1 summarizes the results of reproductive parameters collected in the 18 monitored nests. The nests presented together 2,573 eggs (mean: 142.94) with 1,668 live hatchlings, ranging from 6 (nest 01) to 142 (nest 04). The nests 01, 06 and 18 did not presented stillbirths hatchlings, while the nests 02 and 12 presented 31 and 51 stillbirth hatchlings, respectively. A total number of 704 unhatched eggs were recorded, ranging from 5 (nest 02) to 106 (nest 13) unhatched eggs per nest. Hatching success (mean: 63.2% ± 8.19%) was lower in the nest 18 (18.33%), and higher in the nests 06 and 11 (86% and 85.71%, respectively).

Table 1 Characterization of *Eretmochelys imbricata* nests regarding the number of live hatchlings, stillbirth hatchlings, unhatched eggs, total eggs and hatching success (%).

Female/ Nest	Nº Total eggs	Nº Unhatched eggs	Hatching success (%)	Nº Stillbirth hatchlings	Nº Live hatchlings
01	16	10	37.5	00	06
02	165	05	78.18	31	129
03	123	43	64.22	01	79
04	194	47	73.19	05	142
05	121	38	66.94	02	81
06	152	23	84.86	00	129
07	146	32	75.34	04	110
08	131	46	59.54	07	78
09	159	29	64.15	28	102
10	163	18	73.61	25	120
11	105	10	85.71	05	90
12	184	06	69.02	51	127
13	157	106	24.2	13	38
14	184	39	65.76	24	121
15	133	25	80.45	01	107
16	170	74	54.7	03	93
17	150	55	62.67	01	94
18	120	98	18.33	00	22
Total	2,573	704	1,138.37	201	1,668

The X-Ray fluorescence analysis revealed that all the samples of blood, eggshells, and sediment presented some level of contamination by Ni, Co, Cu, Pb, and Cr. Ni was the element found in the highest concentration in the three matrices (mean: 1.71 mg Kg^{-1} in blood, 4.689 mg Kg^{-1} in eggshells, and 0.451 mg Kg^{-1} in sediment), while Cr was the lowest concentrated metal in blood (mean: 0.085 mg Kg^{-1}), Pb was the lowest concentrated metal in eggshells (mean: 0.041 mg Kg^{-1}), and Co was the lowest concentrated metal in sediment (mean: 0.029 mg Kg^{-1}) (figure 1).

General linear model showed that concentration of metals in the blood of females interfere in the number of eggs in their nests. When the concentration of Cu in the blood of turtles was higher, their nests presented more eggs ($p = 0.016$); when the concentration of Ni in the females' blood was higher, the number of eggs in the nest was lower ($p = 0.031$). Multiple linear regression showed that the number of stillbirths increases with the increase of Cu concentration in the sediment ($p = 0.031$). The metals presented in the sediment or eggshells had no influence on the number of eggs, and the concentration of metals in the sediment was not correlated to the concentration of those metals in eggshells. There was no evidence of maternal transfer of metals to the eggshells according to multiple linear regression ($p > 0.05$). Regarding the size of females, our results demonstrate that larger animals, measured by CCL, presented higher concentrations of Ni in their blood ($p = 0.048$).

DISCUSSION

Our study demonstrates that the population of *E. imbricata* which nests in the coast of Pernambuco presents varying concentrations of Ni, Co, Cu, Pb, and Cr in their blood and eggshells; furthermore, their nests are located in contaminated sediments. The contamination of sea turtles by heavy metals has been demonstrated over the past two decades. A study on dead juveniles, conducted by Macêdo *et al.* (2015) on the coast of the state of Bahia, related the presence of 22 heavy metals in the liver, kidney and bones of green turtles and hawksbill turtles. Anan *et al.* (2001) studied 18 elements in the liver, kidney and muscles of *E. imbricata* and *C. mydas* captured dead in Japan. Both studies found

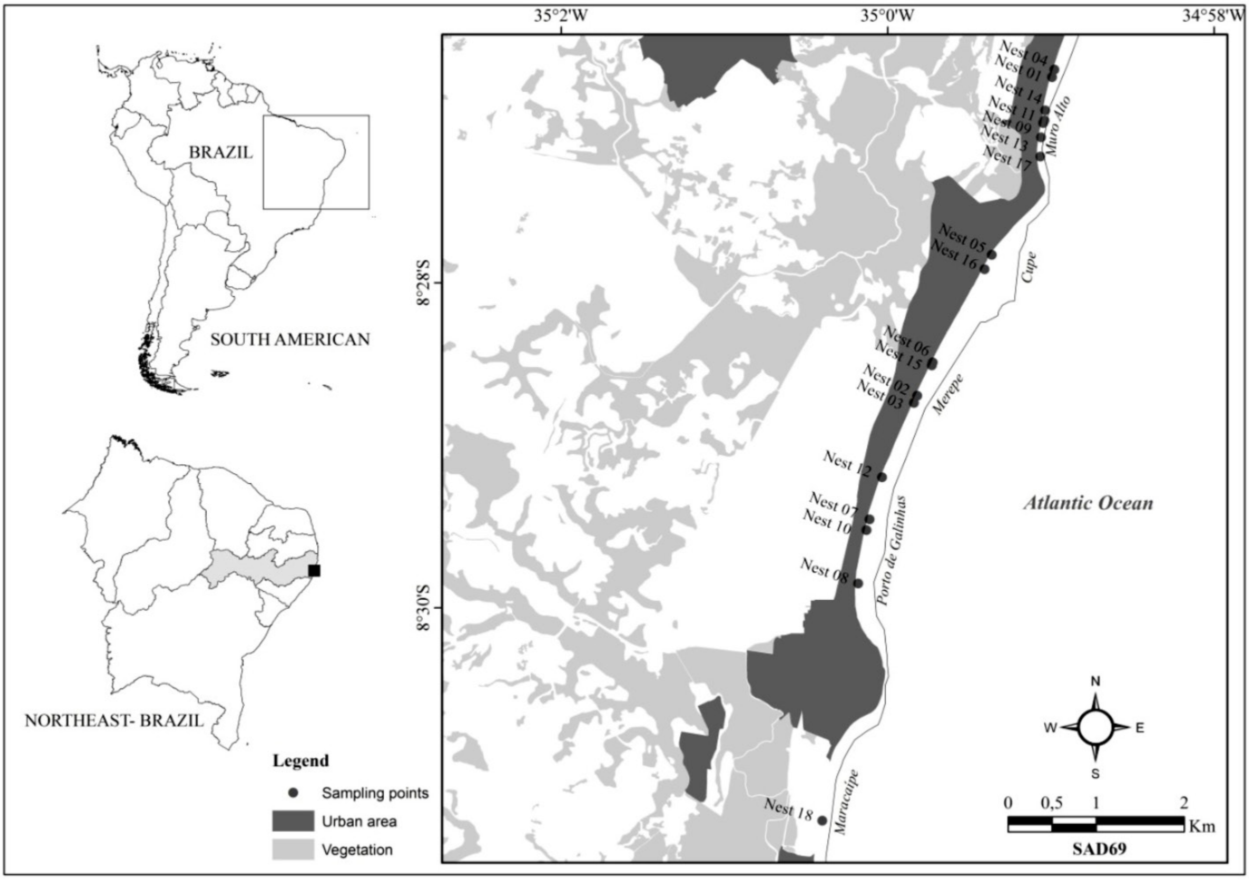

Figure 1 Location map of the study area and sampling sites (N = 18) in Muro Alto, Cupe, Merepe, Porto de Galinhas and Maracaípe beaches, in the municipality of Ipojuca, Pernambuco (PE) state, Brazil. The nest is presented according to identification in Table 1.

very low concentrations of the elements Ni, Co, Cu, Pb and Cr in hawksbill turtle tissues, something similar to the results obtained in this study; here, however, some of the blood and eggshell samples presented high concentrations of Ni. Table 2 summarizes and compares our results to some literature data. Studies conducted by Vazquez *et al.* (1997) and Guirlet *et al.* (2008) on *Dermochelys coriacea*, Kaska & Furness (2001) on *Caretta caretta*, and Lam *et al.* (2006) on *Chelonia mydas* pointed out that low concentrations of heavy metals in the blood and eggshells probably do not cause harm to sea turtles. However, the presence of heavy metals in the blood tissue and eggshells may indicate a recent exposure to toxic substances (Wolfe *et al.* 1998; Storelli & Marcotrigiano, 2003; van de Merwe, 2010).

Sea turtles can bioaccumulate heavy metals according to their dietary patterns (Bjorndal, 1997). Hawksbill turtles preferentially feed on marine sponges, and these may represent the source of heavy metals found in their body (Meylan, 1988; Macêdo *et al.* 2015). However, we do not know all the feeding sites of the turtles studied here, nor the pattern of metal contamination of the organisms that serve as food, which makes it difficult to interpret the results. Several studies report that sea turtles accumulate heavy metals mainly by food, and may store or eliminate them (Wang, 2002; Guirlet *et al.* 2008). Some authors report that reproduction (transfer to eggs) may represent a way to eliminate these elements from the organism of females (Sahoo *et al.* 1996; Godley *et al.* 1999). However, our results did not show any relationship between contamination in blood and contamination in eggshells. Results reported by Sakai *et al*. (1995), Storelli & Marcotrigiano (2003), Páez–Osuna *et al.* (2010b) and Páez–Osuna *et al*. (2011) were not able to find such a correlation. Statistical issues, as number of animals studied and the variation in concentration of the metals, may explain such lack of correlation. Guirlet *et al*. (2008) explain that some parameters, such as the characteristics of the species, the nature of the element and the level of contamination, may explain the non–transfer of heavy metals to eggs. More studies are necessary to provide a framework for understanding the patterns of maternal transfer.

Among the analyzed metals, Ni was that one found in the highest concentrations in blood and eggshells of *E. imbricata* (Figure 1), as Lam *et al.* (2006) also noted studying *C. mydas*. Our results demonstrate that the increase of Ni concentration in the blood of females is related with a decrease in the number of eggs in the nest. According to Forgacs *et al.* (2012), Ni has toxic effects on the ovaries, probably by oxidative stress, and reduce progesterone production and ovulation, what could explain our results. Furthermore, multiple linear regression demonstrated that

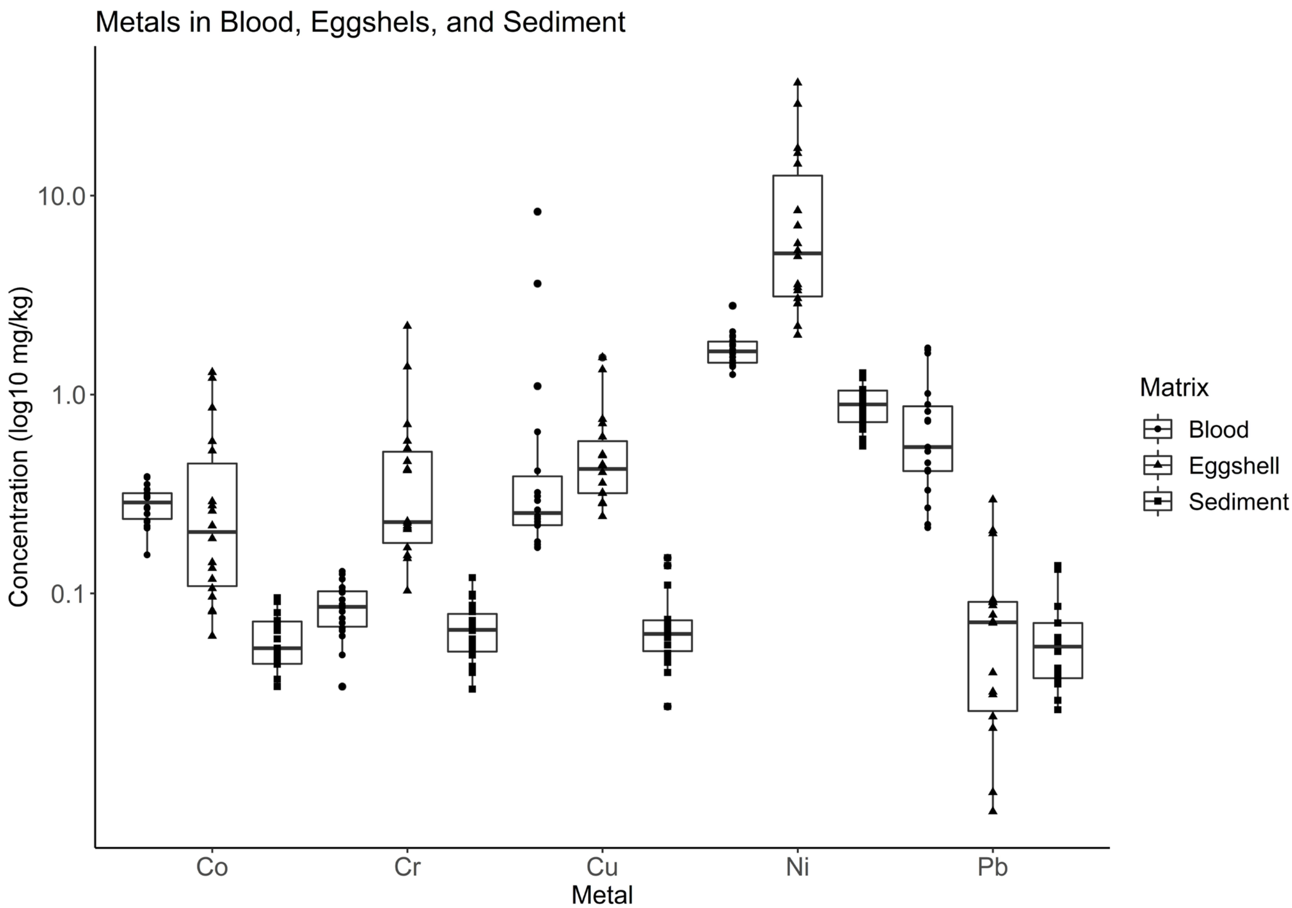

Figure 2 Concentrations of Ni, Co, Cu, Pb, and Cr in the blood, eggshells, and sediments covering the nests. The values of the concentrations were transformed to log10 in order to better represent the variation in the concentrations of the metals.

when the concentration of Cu in the blood of females is higher, their nests show more eggs. Some authors suggest that copper may influence the release of FSH and LH from the pituitary gland. Michaluk *et al.* (2006) suggest that copper binds to GnRH, released from the hypothalamus, and such a complex is more effective than the native GnRH in stimulating the release of FSH and LH from the pituitary gland. Thus, the increased amount of FSH and LH released into the bloodstream may eventually stimulate more oocytes in the ovary, increasing the number of eggs released.

When Cu is present in the nest sediment, however, its effects seem to be deleterious to the offspring; according to the multiple linear regression, with an increase in Cu concentration in sediment, there is an increase in the number of stillbirths in the nests. According to Canas & Anderson (2002), Marco *et al.* (2004) and Guirlet *et al.* (2008), the survival of hatchlings may be affected by contaminants from the nest sediment even during incubation. The metals present in the sediment probably reach the hatchling even at the final stage of development through the open pores of eggshells, and cause damage that can be fatal. The elements Cr, Ni, Cu and Pb, detected in the sediment of the nests (figure 1), were found at higher concentrations by Çelik *et al.* (2006) upon analyzing *C. mydas* nests in Turkey. These authors, as well as Shriadah (1999), argue that the presence of Cr can be explained by geological aspects and by the formation of rocks, while the presence of the other elements is likely to be due to anthropogenic activity (Kennish, 1997).

CONCLUSION

Our results demonstrate that the specimens of *E. imbricata* studied present different concentrations of the heavy metals Ni, Co, Cu, Pb, and Cr in their blood, evidencing the exposure of the population of sea turtles to contaminated environments. Eggshells and sediment were also contaminated by varying concentrations of those metals. Our statistical analyses do not show any correlation between the metals in the blood and the metals in the eggshells, nor any correlation between concentrations of metals in the eggshells and the concentrations of metals in the sediment. Probably, statistical issues and differences in how each metal pass from the dams' organism to the eggs make it difficult to find a pattern of maternal transfer of those metals. High concentrations of Ni in the blood of female turtles seem to cause a decrease in the number of eggs such female can post. On the other hand, high concentrations of Cu in the blood of dams cause an increase in the number of eggs in the nest. When in the sediment that cover the nest, Cu is toxic for embryos; our results demonstrate that an

Table 2 Concentration of heavy metals (mean) in blood, eggshells and sediments covering the nests in this study and in the literature (mg Kg^{-1}).

Species	Sample	Ni	Co	Cu	Pb	Cr	Author
Eretmochelys imbricata	Blood	1.711	0.284	0.951	0.729	0.085	This study
	Eggshells	4.689	0.181	0.268	0.041	0.237	This study
	Sediment	0.451	0.029	0.035	0.032	0.034	This study
Caretta Caretta	Eggshells	-	-	5.29	0.633	-	Kaska e Furness (2001)
		1.59	-	2.83	-	-	Ley-Quinonez et al. (2011)
		-	-	5.57	-	-	Sakai et al. (2000)
Dermochelys coriacea	Blood	-	-	1.34	0.18	-	Guirlert et al. (2008)
	Eggshells	-	-	-	0.011	-	Vazquez et al. (1997)
Chelonia mydas	Eggshells	0.012	3.30	1.30	0.11	0.47	Lam et al. (2006)
		3.64	-	18.43	0.04	0.55	Çelik et al. (2006)
	Sediment	27.31	-	17.77	1.21	37.89	Çelik et al. (2006)
Lepidochelys olivacea	Blood	2.8	-	2.28	-	-	Paez-Osuna et al. (2010)
Chelonia mydas agasiizzi	Blood	1.03	-	1.71	-	-	Ley Quinõénez et al. (2013)

increase in Cu concentration in the sediment that cover the nest causes an increase in the number of stillbirths. These results demonstrate that heavy metals in the organisms of the females and in the sediment covering the nest influence the reproduction capacity of the dams and the survival of the embryos. Hence, the increase of heavy metals in the environment, including in the trophic chain, may contribute to a decrease in populations and genetic diversity of sea turtles; however, more studies are necessary to better understand the biological mechanisms by which heavy metals influence reproduction in sea turtles.

ACKNOWLEDGMENTS

The authors would like to thank the Laboratório Nacional de Luz Síncrotron do Centro Nacional de Pesquisa em Energia e Materiais (CNPEM) for financing the analyses, the NGO Ecoassociados for its contribution and support for the development of the research. We also thank the laboratory of Bromatology of the Federal University of Pernambuco, Academic Center of Vitória (UFPE/CAV), for the aid in the study, and the National Council for Scientific and Technological Development (CNPq). And to the Animal Ethics Committee of the Federal University of Pernambuco, to Ministério do Meio Ambiente – MMA, and Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio for the consent of the licenses for research.

REFERENCES

ANAN, Y., KUNITO, T., WATANABE, I., SAKAI, H. & TANABE, S. 2001. Trace element accumulation in hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*) from Yaeyama Islands, Japan. Environ. Toxicol. Chem., 20: 2802–2814. http://dx.doi.org/10.1002/etc.5620201220

BARBIERI, E. 2009. Concentration of heavy metals in tissues of green turtles (*Chelonia mydas*) sampled in the Cananéia estuary, Brazil. Braz. j. oceanogr., 57: 243–248. http://dx.doi.org/10.1590/S1679-87592009000300007

BJORNDAL, K.A. 1997. Foraging ecology and nutrition of sea turtles. In: Lutz, P.L., Musick, J.A. (ed), The Biology of Sea Turtles. CRC Press, Boca Raton, Flórida, pp. 199–231.

CANAS, J.E. & ANDERSON, T.A. 2002. Organochlorine contaminants in eggs: The influence of contaminated nest material. Chemosphere, 47: 585–589. http://dx.doi.org/10.1016/S0045-6535(02)00019-X

ÇELIK, A., KASKA, Y., BAĞ, H., AUREGGI, M., SEMIZ, G., KARTAL, A.A. & ELÇI, L. 2006. Heavy metal monitoring around the nesting environment of green sea turtles in Turkey. Water Air Soil Pollut., 169: 67–79. http://dx.doi.org/10.1007/s11270-006-1562-0

FORGACS, Z., MASSÁNY, P., LUKAC, N. & SOMOSY, Z. 2012. Reproductive toxicology of nickel – Review. J. Environ. Sci. Health. A., 47: 1249–1260.

GODLEY, B.J., THOMPSON, D.R. & FURNESS, R.W. 1999. Do heavy metal concentrations pose a threat to marine turtles from the Mediterranean Sea? Mar. Pollut. Bull., 38: 497–502. http://dx.doi.org/10.1016/S0025-326X(98)00184-2

GUIRLET, E., DAS, K. & GIRONDOT, M. 2008. Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana. Aquat. Toxicol., 88: 267–276. http://dx.doi.org/10.1016/j.aquatox.2008.05.004

HAMANN, M., GODFREY, M.H., SEMINOFF, J.A., ARTHUR, K., BARATA, P.C.R., BJORNDAL, K.A., BOLTEN, A.B., BRODERICK, A.C., CAMPBELL, L.M., CARRERAS, C., CASALE, P., CHALOUPKA, M., CHAN, S.K.F., COYNE, M.S., CROWDER, L.B., DIEZ, C.E., DUTTON, P.H., EPPERLY, S.P., FITZ, SIMMONS, N.N., FORMIA, A., GIRONDOT, M., HAYS, G.C., CHENG, I.J., KASKA, Y., LEWISON, R., MORTIMER, J.A., NICHOLS, W.J., REINA, R.D., SHANKER, K., SPOTILA, J.R., TOMÁS, J., WALLACE, B.P., WORK, T.M., ZBINDEN, J. & GODLEY, B.J. 2010. Global research priorities for sea turtles: Informing management and conservation in the 21st century. Endanger. Species Res., 11: 245–269. http://dx.doi.org/10.3354/esr00279

IUCN. 2017. International Union for Conservation of Nature – IUCN. Red List of Threatened Species. http://www.iucnredlist.org/apps/redlist/details/8005/0.

KAMPALATH, R., GARDNER, S.C., MÉNDEZ-RODRÍGUEZ, L. & JAY, J.A. 2006. Total and methylmercury in three species of sea turtles of Baja California Sur. Mar. Pollut. Bull., 52: 1816–1823. http://dx.doi.org/10.1016/j.marpolbul.2006.09.019

KASKA, Y. & FURNESS, R.W. 2001. Heavy metals in marine turtle eggs and hatchlings in the Mediterranean. Zool. Middle. East., 24: 127–132. http://dx.doi.org/10.1080/09397140.2001.10637891

KENNISH, J. M. 1997. Practical Hand book of Estuarine and Marine Pollution. CRC, NewYork, pp 524.

LAM, J.C.W., TANABE, S., CHAN, S.K.F., YUEN, E.K.W., LAM, M.H.W. & LAM, P.K.S. 2004. Trace element residues in tissues of green turtles (*Chelonia mydas*) from South China Waters. Mar. Pollut. Bull., 48: 174–182. http://dx.doi.org/10.1016/j.marpolbul.2003.09.003

LAM, J.C.W., TANABE, S., CHAN, S.K.F., LAM, M.H.W., MARTIN, M. & LAM, P.K.S. 2006. Levels of trace elements in green turtle eggs collected from Hong Kong: Evidence of risks due to selenium and nickel. Environ. Pollut., 144: 790–801. http://dx.doi.org/10.1016/j.envpol.2006.02.016

LEY-QUIÑÓNEZ, C.P., ZAVALA-NORZAGARAY, A.A., RÉNDON-MALDONADO, J.G., ESPINOSA-CARREÓN, T.L., CANIZALES-ROMÁN, A., ESCOBEDO-URÍAS, D.C., LEAL-ACOSTA, M.L., HART, C.E. & AGUIRRE, A.A. 2013. Selected heavy metals and selenium in the blood of black sea turtle (*Chelonia mydas agasiizzi*) from Sonora, Mexico. Bull. Environ. Contam. Toxicol., 91: 645–651. http://dx.doi.org/10.1007/s00128-013-1114-4

MACÊDO, G.R., TARANTINO, T.B., BARBOSA, I.S., PIRES, T.T., ROSTAN, G., GOLDBERG, D.W., PINTO, L.F.B., KORN, M.G.A. & FRANKE, C.R. 2015. Trace elements distribution in hawksbill turtle (*Eretmochelys imbricata*) and green turtle (*Chelonia mydas*) tissues on the northern coast of Bahia, Brazil. Mar. Pollut. Bull., 94: 284–289. http://dx.doi.org/10.1016/j.marpolbul.2015.02.033

MARCO, A., LÓPEZ–VICENTE, M. & PÉREZ–MELLADO, V. 2004. Arsenic uptake by reptile flexible–shelled eggs from contaminated nest substrates and toxic effect on embryos. Bull. Environ. Contam. Toxicol., 72:983–990.

MARCOVALDI, M., LOPEZ, G., SOARES, L., SANTOS, A., BELLINI, C., SANTOS, A. DOS. & LOPEZ, M. 2011. Avaliação do estado de conservação da tartaruga marinha *Eretmochelys imbricata* (Linnaeus, 1766) no Brasil. Biodiversidade Brasileira, 1: 20–27.

MCCULLAGH, P. & NELDER, J.Á. 1989. Generalized Linear Models. Chapman & Hall, London.

MEYLAN, A. 1988. Spongivory in Hawksbill Turtles: A Diet of Glass. Science, 239: 393–395. http://dx.doi.org/10.1126/science.239.4838.393

MICHALUK, A., BLITEK, A., GAJEWSKA, A., KACZMAREK, M., OSTROWSKA–HLIWA, K., KOCHMAN, H., ZIECIK, A. & KOCHMAN, K. 2006. LH release by Cu and Ni salts and metal GnRH complexes, in vitro. Neuro. Endocrinol. Lett., 27: 483-486.

MOURA, C.C. DE M., GUIMARÃES, E. DA S., MOURA, G.J.B. DE, AMARAL, G.J.A. DO. & SILVA, A.C. DA. 2012. Distribuição espaço-temporal e sucesso reprodutivo de *Eretmochelys imbricata* nas praias do Ipojuca, Pernambuco, Brasil. Iheringia Ser. Zool., 102: 254–260. http://dx.doi.org/10.1590/S0073-47212012005000003

NRIAGU, J.O. & PACYNA, J.M. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature, 333: 134–139. http://dx.doi.org/10.1038/333134a0

OWENS, D.W. & RUIZ, G.J. 1980. New Methods of Obtaining Blood and Cerebrospinal Fluid from Marine Turtles. Herpetologica, 36: 17–20.

PÁEZ-OSUNA, F., CALDERÓN-CAMPUZANO, M.F., SOTO-JIMÉNEZ, M.F., RUELAS-INZUNZA, J.R. 2010a. Trace Metals (Cd, Cu, Ni, and Zn) in Blood and Eggs of the Sea Turtle *Lepidochelys olivacea* from a Nesting Colony of Oaxaca, Mexico. Arch. Environ. Con. On TOX., 59: 632–641. http://dx.doi.org/10.1007/s00244-010-9516-3

PÁEZ-OSUNA, F., CALDERÓN-CAMPUZANO, M.F., SOTO-JIMÉNEZ, M.F. & RUELAS-INZUNZA, J.R. 2010b. Lead in blood and eggs of the sea turtle, *Lepidochelys olivacea*, from the Eastern Pacific: Concentration, isotopic composition and maternal transfer. Mar. Pollut. Bull., 60: 433–439. http://dx.doi.org/10.1016/j.marpolbul.2009.10.004

PÁEZ-OSUNA, F., CALDERÓN-CAMPUZANO, M.F., SOTO-JIMÉNEZ, M.F. & RUELAS-INZUNZA, J. 2011. Mercury in blood and eggs of the sea turtle *Lepidochelys olivacea* from a nesting colony in Oaxaca, Mexico. Mar. Pollut. Bull., 62: 1320–1323. http://dx.doi.org/10.1016/j.marpolbul.2011.04.004

SAHOO, G., SAHOO, R.K. & MOHANTY-HEJMADI, P. 1996. Distribution of heavy metals in the eggs and hatchlings of olive ridley sea turtle, *Lepidochelys olivacea*, from Gahirmatha, Orissa. Indian. J. Mar. Sci., 25: 371–372.

SAKAI, H., ICHIHASHI, H., SUGANUMA, H. & TATSUKAWA, R. 1995. Heavy metal monitoring in sea turtles using eggs. Mar. Pollut. Bull., 30: 347–353. http://dx.doi.org/10.1016/0025-326X(94)00185-C

SAKAI, H., SAEKI, K., ICHIHASHI, H., SUGANUMA, H., TANABE, S. & TATSUKAWA, R. 2000. Species-specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. Mar. Pollut. Bull., 40: 701–709. http://dx.doi.org/10.1016/S0025-326X(00)00008-4

SHRIADAH, M.M.A. 1999. Heavy metals in mangrove sediments of the United Arab Emirates shoreline (Arabian gulf). Water. Air. Soil. Pollut., 116: 523–534. http://dx.doi.org/10.1023/A:1005139020681

SIMÕES, T., SILVA, S., SANTOS, E. & CHAGAS, C. 2014. Temperatura de incubação e razão sexual em filhotes recémeclodidos da tartaruga marinha *Eretmochelys imbricata* (Linnaeus, 1766) no município do Ipojuca, Pernambuco, Brasil. Pap. Avulsos Zool., 54: 363–374. http://dx.doi.org/10.1590/0031-1049.2014.54.25

SOLÉ, V.A., PAPILLON, E., COTTE, M., WALTER, P. & SUSINI, J. 2007. A multiplatform code for the analysis of energy-dispersive X-ray fluorescence spectra. Spectrochim. Acta. B, 62: 63–68. http://dx.doi.org/10.1016/j.sab.2006.12.002

STATSOFT, I.N.C. 2004. Statistica (data analysis software system), version 7. www.statsoft.com.

STORELLI, M.M. & MARCOTRIGIANO, G.O. 2003. Heavy metal residues in tissues of marine turtles. Mar. Pollut. Bull., 46: 397-400. http://dx.doi.org/10.1016/S0025-326X(02)00230-8

USEPA. 1996. United States Environmental Protection Agency. Acid digestion of sediments, sludges and soils. Method 3050b. Washington, D.C.: EPA. http://www.epa.gov/sw–846/pdfs/3050b.pdf.

VAN DE MERWE, J.P., HODGE, M., OLSZOWY, H.A., WHITTIER, J.M. & LEE, S.Y. 2010. Using blood samples to estimate persistent organic pollutants and metals in green sea turtles (*Chelonia mydas*). Mar. Pollut. Bull., 60: 579–588. http://dx.doi.org/10.1016/j.marpolbul.2009.11.006

VAZQUEZ, G.F., REYES, M.C., FERNANDEZ, G., AGUAYO, J.E.C. & SHARMA, V.K. 1997. Contamination in marine turtle (*Dermochelys coriacea*) egg shells of Playon de Mexiquillo, Michoacan, Mexico. Bull. Environ. Contam. Toxicol., 58: 326–333. http://dx.doi.org/10.1007/s001289900338

WANG, W. 2002. Interactions of trace metals and different marine food chains. Mar. Ecol. Prog. Ser., 243: 295–309. http://dx.doi.org/10.3354/meps243295

WOLFE, M.F., SCHWARZBACH, S. & SULAIMAN, R.A. 1998. Effects of mercury on wildlife: A comprehensive review. Environ. Toxicol. Chem., 17: 146-160. http://dx.doi.org/10.1897/1551-5028(1998)017<0146:EOMOWA>2.3.CO;2