



Incidence of 49 elements in the blood and scute tissues of nesting hawksbill turtles (*Eretmochelys imbricata*) in Holbox Island

Maribel Escobedo Mondragón^a, Octavio P. Luzardo^{b,c,*}, Manuel Zumbado^{b,c}, Ángel Rodríguez-Hernández^b, Cristian Rial Berriel^b, Héctor Vicente Ramírez-Gomez^d, Carlos González-Rebeles Islas^a, Roberto F. Aguilar Fisher^a, J. Rene Rosiles Martínez^e

^a Department of Ethology, Wildlife, and Laboratory Animals, Veterinary Medicine and Zootechnic Faculty, National Autonomous University of Mexico, University City 3000, 04510 Mexico City, Mexico

^b Toxicology Unit, Department of Clinical Sciences, Health Sciences Faculty, University of Las Palmas de Gran Canaria, Paseo Blas Cabrera Felipe s/n, 35016 Las Palmas de Gran Canaria, Spain

^c Spanish Biomedical Research Centre in Physiopathology of Obesity and Nutrition (CIBERObn), Paseo Blas Cabrera Felipe s/n, 35016 Las Palmas, Spain

^d Department of Developmental Genetics and Molecular Physiology, Institute of Biotechnology, National Autonomous University of Mexico, Av. Universidad 2001, 62210 Cuernavaca, Morelos, Mexico

^e Toxicology Laboratory, Department of Animal Nutrition, and Bromatology, Veterinary Medicine and Zootechnic Faculty, National Autonomous University of Mexico, University City 3000, 04510 Mexico City, Mexico

ARTICLE INFO

Article history:

Received 17 February 2020

Received in revised form 22 November 2020

Accepted 23 November 2020

Available online 4 December 2020

Keywords:

Hawksbill

Sea turtle

Rare earth elements

Caribbean Region

Inorganic emergent pollutants

Elements

ABSTRACT

Due to progressive urban development along the Mexican Caribbean coastline, it is crucial to gauge the impact of anthropogenic contamination of marine ecosystems through biomonitoring procedures. In the current study, we quantified the concentration of 49 inorganic elements in the blood and scute tissues of clinically healthy nesting hawksbill sea turtles (*Eretmochelys imbricata*). The elements were classified into four groups: Group A: essential mineral elements with toxic potential; Group B: non-essential elements with high toxicity; Group C: toxic non-essential minority elements and Trace Elements (TE); and Group D: rare-earth elements (REE) and other TE. Almost all the samples in both tissues showed perceptible levels of all the quantified elements. The only element identified with a correlation between blood and scute was arsenic (As), which could indicate a fast excretion through this type of keratinized tissue. The bio-accumulation of inorganic elements is a complex process, requiring the simultaneous examination of different tissues to evaluate the exposure. Our study reinforces the usefulness of scute tissue as a non-invasive sampling technique for the evaluation of persistent pollutants in marine turtles.

© 2020 Elsevier B.V. All rights reserved.

1. Introduction

Biomonitoring inorganic elements as persistent pollutants has become an important tool to provide baseline measurements for further studies on the health status of marine life. Being aware of the contamination levels in developed areas enables timely decision-making for wildlife management and conservation (Patino-Martinez et al., 2014).

Hawksbill turtles are selective feeders. Sponges comprise 95.3% of their diet throughout the Caribbean Region, while the rest consists of jellyfish, mollusks, fish, marine algae, crustaceans, and other sea plants and benthic invertebrates. They forage in benthic

habitats, over coral reefs, rock outcroppings, seagrass pastures, and mangrove-fringed bays (Bjorndal, 1996). These coastal habitats are often in close proximity to sources of persistent inorganic pollutants, which make their way into the marine environment from industrial, domestic and agricultural sources (Ehsanpour et al., 2014).

No studies on the biomonitoring of sea turtles to date have included rare-earth elements (REE). There is growing concern regarding environmental pollution produced by REE and other trace elements (TE), which are extensively and increasingly employed in the manufacture of consumer electronics and new technologies, so some authors consider these REE as emerging pollutants to be considered in biomonitoring studies (Goode-nough et al., 2017; Deetman et al., 2018). Although they have not been classified as toxic or priority pollutants for the marine ecosystem, some studies have reported REE with regard to health effects, toxicity and concentration in different species and tissues

* Corresponding author at: Toxicology Unit, Department of Clinical Sciences, Health Sciences Faculty, University of Las Palmas de Gran Canaria, Paseo Blas Cabrera Felipe s/n, 35016 Las Palmas de Gran Canaria, Spain.

E-mail address: octavio.perez@ulpgc.es (O.P. Luzardo).

(Pagano et al., 2015a,b), providing evidence of adverse effects, including inflammation, oxidative stress and tissue damage in liver, lungs and kidneys in medium-term exposure (Pagano et al., 2012).

In the current study we evaluate the concentrations of 49 environmentally relevant inorganic elements of anthropogenic origin, including REE and TE in nesting hawksbill turtles (*Eretmochelys imbricata*) using blood and scute tissues to provide the baseline data for future studies of monitoring and contamination assessment risk programs in the Caribbean Region.

2. Materials and methods

Between May through June 2018, we collected 19 blood and scute samples of clinically healthy nesting hawksbill sea turtles (*Eretmochelys imbricata*) from the beaches of Holbox Island, Quintana Roo State, an important hawksbill nesting area. The study site is located along the coast between the coordinates 21°33'42.3"N; 87°20'13.8"W and 21°35'35.8"N; 87°07'11.8"W, covering a total length of 24 km. The sampling permits (Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) were issued by the Secretary for Natural Resources Management in Mexico. The research methods and animal welfare measures were evaluated and approved by our local institutional Ethical Committee, at the Veterinary Medicine and Zootechnic Faculty (FMVZ), National Autonomous University of Mexico (UNAM). To avoid double sampling by recapture, individual turtles were marked with flipper tags and series numbers were registered.

2.1. Blood sampling

Whole blood samples (5 ml) were collected from the cervical sinus using single-use needles (21 gauge), plastic syringes, and blood collection tubes containing lithium heparin to avoid clotting (Campbell, 2012). The dorsal neck region was previously wiped clean and disinfected (gauze with ethanol 70° and neck with povidone-iodine 3%) to reduce sample contamination and health risk in the specimens. The samples were collected when the turtles returned to the sea after laying eggs to minimize any disturbance to nesting. In addition, a complete visual physical examination was performed, and the size of the turtles was evaluated based on the curve carapace length and width. During fieldwork and transportation to the processing site, the samples were kept with a coolant gel (−4 °C) in ice chests in the Toxicology Laboratory at the FMVZ-UNAM.

2.2. Scute sampling

According to modified protocols (Bjorndal et al., 2010), supra-caudal scute samples (<1 g) were collected after successfully blood sampling the specimens and cleaning the carapace. The region was previously wiped clean and disinfected with a sequence of gauze with ethanol 70° and gauze with povidone-iodine 3% to reduce sample contamination or health risk to the specimens. This is a proven non-invasive procedure, which does not affect the health or physical condition of the specimen (Bjorndal et al., 2010; Komoroske et al., 2011, 2012).

2.3. Analysis of elements

The whole blood and scute samples were frozen at −20 °C until analysis. In the Toxicology Laboratory at the FMVZ-UNAM, the blood samples were homogenized by manual shaking oscillation and the scute samples were washed with deionized water and a brush with plastic bristles to eliminate any superficial material

from the environment. A 1 g fraction of whole blood and 0.5 g of scute were used for the quantification of the elements. These samples were digested by the humid digestion process in 2 ml of nitric acid at 60% and 0.5 ml of hydrogen peroxide at 10% following the NOM-117-SSA1-1994 methodological standards. Once completely digested, the processed samples were filtered with Whatman No. 2 filter paper and diluted. The digested samples were sent to the Clinical and Analytical Toxicology Laboratory of the Las Palmas de Gran Canaria University, where they were stored at −4 °C till their analysis. All the samples were received in perfect condition and correctly identified.

We determined the concentration levels of 49 elements, which were classified according to their biological and toxicological importance (Goyer and Clarkson, 2001) into four groups: Group A: essential mineral elements with toxic potential: Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), Mn (manganese), Mo (molybdenum), Ni (nickel), Se (selenium) and Zn (zinc). Group B: non-essential elements with high toxicity: Al (aluminum), As (arsenic), Be (beryllium), Cd (cadmium), Pb (lead) and Hg (mercury). Group C, toxic non-essential minority elements: Ag (silver), Au (gold), Ba (barium), Bi (bismuth), Ga (gallium), Pd (palladium), Pt (platinum), Sb (antimony), Sn (tin), Sr (strontium), Th (thorium), Ti (titanium), Tl (thallium), U (uranium) and V (vanadium). Group D: rare-earth elements (REE) and other trace elements (TE): Ce (cerium), Dy (dysprosium), Eu (europium), Er (erbium), Gd (gadolinium), Ho (holmium), In (indium), La (lanthanum), Lu (lutetium), Nb (niobium), Nd (neodymium), Os (osmium), Pr (praseodymium), Ru (ruthenium), Sm (samarium), Ta (tantalum), Tb (terbium), Tm (thulium), Y (yttrium) and Yb (ytterbium).

For the element analyses, we employed an Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan) equipped with standard nickel cones, Ultra High Matrix Introduction (UHMI) system, and a Cross-Flow Nebulizer with a make-up gas port (X400 Nebulizer, Saville Corporation, Eden Prairie, MN, USA). We followed the previously validated procedure in our laboratory, using certified reference materials (González-Antuña et al., 2017). Two standard curves (twelve points, 100–0.005 ng/mL) were made to avoid interferences between elements: a) one using a commercial multi-element mixture (CPA Chem Catalog number E5B8.K1.5N.L1, 21 elements, 100 mg/L, 5% HNO₃) containing for all the inorganic elements (metals, metalloids, and non-metals) b) multi-element mixture tailor-made in our laboratory, which contained the REE and TE most frequently employed in the high-tech industry. The concentrations of elements below the limit of detection (LOD) were assigned a zero (0) value and all the metal concentrations were expressed as micrograms per gram of wet weight (μg/g^{−1} of w.w.).

2.4. Statistical analysis

Database management and statistical analysis were performed using R software (R-3.5.2 version). The mean, standard deviation, median, and range were determined for each parameter. Because the data were not normally distributed, the statistical analyses involved the use of non-parametric tests. The differences among the tissues were tested through Wilcoxon signed-rank test in the paired analysis. In addition, continuous variables were analyzed by the Spearman correlation test. The *P* values of less than 0.05 (two-tail) were considered statistically significant.

3. Results and discussion

The complete profile and *P* values of all inorganic elements in blood and scute is shown in Fig. 1, the distribution of blood and scute concentrations is listed in the order of highest to lowest concentration of the element between both tissues.

Group A, corresponding to essential elements, showed the highest concentrations. For the great majority of elements, the scute concentrations were higher than those found in the blood, except for Fe, Se, Be, Hg, Ag, Ce, and Os. These findings support previous reports, suggesting that scute in sea turtles acts as a repository where metals are stored after they have been metabolized and excreted into a keratinized tissue (Komoroske et al., 2011, 2012; Bezerra et al., 2013). Moreover, it is also indicative of longer-term exposure and useful for monitoring concentrations of inorganic elements (Jakimska et al., 2011a,b). These findings are consistent with those reported in other studies (Komoroske et al., 2011, 2012; Bezerra et al., 2013).

Essential minerals are required for physiological processes in the body and are commonly found in high concentrations in different tissues (Cortés-Gómez et al., 2017; Jakimska et al., 2011a,b). To date, there have been no known studies in nesting hawksbill turtles that have used scute as a biomonitoring tissue, and to our knowledge, ours is the first study to analyze inorganic mineral concentrations of scute in hawksbill turtles. Table 1 summarizes the concentrations of the elements found in blood and scute tissues.

Concentrations within Group A (essential mineral elements with toxic potential) are summarized in Table 1, where no elements were found below LOD. Median elemental values in the blood were ranked as: Fe > Zn > Se > Cu > Ni > Mn > Mo > Cr > Co, while those in scute were ranked as: Fe > Zn > Ni > Cu > Cr > Mn > Se > Mo > Co. Elements found to be in the greatest concentrations in blood were metals preferentially associated with red blood cells (Fig. 1) relative to whole blood (Goyer and Clarkson, 2001). In other studies of different species of sea turtles (Camacho et al., 2014; Jerez et al., 2010; Ley-Quiniñonez et al., 2011), Zn has been reported to be the most abundant element present in different tissues.

Hawksbill sea turtles are one of the least-studied species regarding their contamination status, as only the levels of inorganic elements in the Pacific (Anan et al., 2001; Suzuki et al., 2017), Persian Gulf (Ehsanpour et al., 2014), East Wider Caribbean Region (Dyc et al., 2015) and Atlantic (Camacho et al., 2014) populations have been reported. A study on nesting hawksbill turtles (Ehsanpour et al., 2014) had reported Cu, Zn and TE elements in whole blood. However, the results were originally published in dry weight and no moisture content was reported by the authors. Compared to other studies, the concentrations of Cr, Cu, Mn, Ni, Se and Zn in whole blood of juvenile hawksbills were found at a rate of 4.4 to 4.7 times lower (Camacho et al., 2014) than those reported in the present study, while Ni and Se presented a greater difference, with 37 and 12.3 times lower respectively.

These differences could be related to geographic variations in the diet, age, and physiological status. Juvenile hawksbills typically consume what is available at the pelagic zone, whereas adults, who feed in coastal waters, subsist on a diet comprised primarily of sponges. Some studies have indicated that bio-accumulation of Se by sponges could be due to the high dependence of the species on the element for growth (Müller et al., 2005). As these sponges represent a significant portion of adult hawksbills' diet, this relationship probably accounts for the high levels of Se found in their tissues.

Some authors like Ley-Quiniñonez et al. (2011) mention the difficulty in establishing whether the high concentrations of Se in sea turtles can be considered a toxic factor; however, selenomethionine is considered to be the primary form of organic selenium relevant for bio-accumulation and toxicity in wildlife (Dyc et al., 2015, 2016). It has been suggested that the presence of Se in sea turtle eggs could be toxic for the embryo (Lam et al., 2006), and a mother-embryo metal transfer has been demonstrated in sea turtles (Paez-Osuna et al., 2010a,b, 2011; Dyc et al., 2015). The

registration of high concentration should not be underestimated, as much remains to be learned regarding Se toxicity for reptiles and marine wildlife. The essential elements (Cu, Cr, Mn, Ni, Se, and Zn) play vital roles in tissue metabolism and growth. However, the negative effects of some of these elements on sea turtles are not well known.

Concentrations within Group B (non-essential elements with high toxicity) are summarized in Table 1, where only beryllium (Be) was present above LOD in 56% of blood samples and below LOD in all scute samples. All other elements were present in both tissues. Most of the values in this group were similar to others published for different species (Cortés-Gómez et al., 2017). The median elemental values in the blood and scute were ranked as: Al > As > Pb > Cd > Hg > Be.

It is noteworthy that the levels of As in scute and blood were not significantly different ($P = 0.7019$), which could indicate a fast excretion through this type of keratinized tissues such as scute (Goyer and Clarkson, 2001). The arsenic concentrations in blood (median $0.82 \mu\text{g/g}^{-1}$ in w.w.) were found at double the levels reported in juvenile hawksbills (Camacho et al., 2014). The arsenic compounds in seawater are mainly comprised of arsenates including arsenite, methylarsonic acid, and dimethylarsinic acid (DMA) (Saeki et al., 2000), where DMA tended to be the most common arsenic compound found in sea turtles and in higher concentrations in hawksbill turtles (Saeki et al., 2000). These arsenic compounds bio-accumulate more intensively in sea turtles than in other marine animals, and the explanation for it could be that their preys contain high levels of arsenic or that the metabolic processes are different for sea turtles (Jakimska et al., 2011a). Although DMA is less toxic, it may be necessary to investigate the influences of DMA on different tissues of sea turtles due to the DNA damage induced by free radicals of DMA metabolites, dimethylarsine and molecular oxygen in other species (Okada and Yamanaka, 1994).

The concentrations within Group C (toxic non-essential minority elements TE) are summarized in Table 1, where no elements were found below LOD. The median elemental values in the blood were ranked as: Sr > Ba > Sn > V > Ti > Sb > U > Ag > Bi > Pd > Tl > Th > Pt > Ga > Au; while those in scute were ranked as: Sr > Ba > Sn > Ti > V > Sb > Pd > U > Bi > Pt > Ag > Ga > Tl > Th > Au. The scute concentrations of these elements were higher than those found in the blood except for Ag.

The most studied TE in sea turtles to date are Ag, Ba, Sb, Sn, Sr, Ti, Tl, and V (Komoroske et al., 2011; Labrada-Martagón et al., 2011; McFadden et al., 2014; Carneiro da Silva et al., 2016; Villa et al., 2017). These concentrations were lower than those in our study. The mechanism of action underlying the adverse effects of TE is largely unknown in many cases.

The concentrations within Group D (REE and other TE) are summarized in Table 1, where only osmium (Os) was found below LOD in half of the samples of blood, and 44% of scute samples. The median elemental values in the blood were ranked as: Ce > La > Nd > Y > In > Gd > Pr > Nb > Sm > Dy > Yb > Eu > Er > Ho > Tb > Lu > Tm > Os > Ta; while those in scute ranked as: Ce > La > Nd > Y > In > Nb > Pr > Sm > Gd > Dy > Yb > Er > Eu > Ho > Tb > Ta > Lu > Tm > Os. The scute concentrations of these elements were higher than those found in the blood, except for Ce and Os. Most of the REE studied to date in other species are mainly Ce, La, Pr, Nd, Ho, and Tb; these elements have been shown to produce cytogenetic abnormalities, induce a differential expression of the genes involved in immune response to inflammation, apoptosis, oxidative stress, and tissue damage on the kidneys, liver, and lungs (Cheng et al., 2014; Pagano et al., 2015a,b).

In both tissues, cerium (Ce) was the element of this group found at the highest concentrations. It is relevant to mention that this element has been described to be toxic in Sprague-Dawley

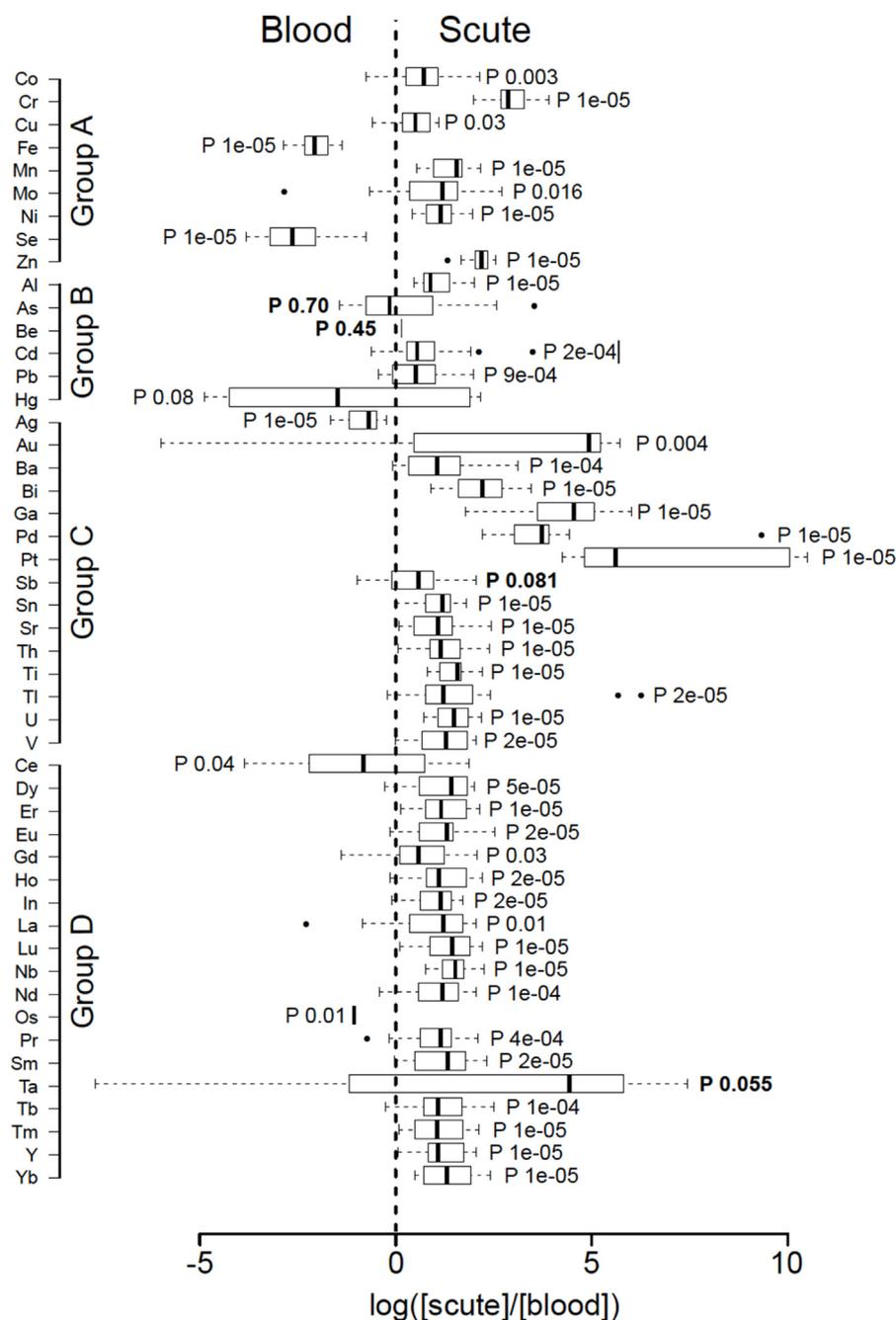


Fig. 1. Distribution of blood and scute concentrations in groups A, B, C, and D. Statistical significance (*P* values) is indicated between blood and scute values in the paired analysis. The Wilcoxon signed-rank test was performed for each element. The central line in each box plot represents the median value; the box denotes the data spread from 25% to 75%, and the whiskers reflect 10%–90%, upper and lower whiskers (largest and smaller data numbers) are noted with dotted line in each box plot. The outliers for each distribution are plotted. Box plots are side orientated (left or right) in the order of highest to lowest concentration of the element between blood (left) and scute (right). In this figure we can observe that for the great majority of the elements analyzed scute concentration were higher than those found in the blood.

rats, causing liver tissue damage (hydropic degeneration of the hepatocytes, dilation of the sinusoids, portal inflammation, and fibrosis of the liver) (Nalabotu et al., 2011). The second most abundant element was lanthanum (La), for which toxicity has also been demonstrated. Thus, some studies have demonstrated that La causes nephrotoxicity (histopathological changes in the kidneys, changes in lipid peroxidation levels, increased activity of oxidative stress, decreased superoxide dismutase activity) in mice (Zhao et al., 2013), and neurotoxicological consequence of long-term exposure, disturbance of the homeostasis of trace elements, enzymes, and neuro-transmitter systems in the brain of rats (Feng et al., 2006).

Some authors like Wang and Yamada (2006) have reported that REE are present at low concentrations in marine systems in a normal way. Considerable field observations and laboratory experiments have been conducted to study the marine cycling of REE (Wang and Yamada, 2006; Zheng et al., 2016). Despite these efforts, substantial uncertainties remain about the processes that control the distribution in the ocean, the ability to interpret patterns, the influence of physical transport, the bio-geochemical processes, and the different sources responsible for additional plumes, like those reported in 2016 by Zheng et al. in the tropical South Atlantic current about the abnormal increase of Ce from

Table 1Inorganic elements concentrations ($\mu\text{g/g}$ -1 en w.w.) in blood and scute tissues of nesting hawksbill turtles of the Yum-Balam Biosphere Reserve, Holbox, Mexico.

Group A: essential mineral elements with toxic potential concentrations					
	Blood		Scute		Bio-magnification ^a
	Median \pm SD	Mean (Range)	Median \pm SD	Mean (Range)	
Co	0.01 \pm 0.01	0.01 (n.d.-0.03)	0.02 \pm 0.01	0.02 (0.01-0.4)	2
Cr	0.04 \pm 0.04	0.05 (0.03-0.18)	0.94 \pm 0.32	0.96 (0.5-1.54)	17.5
Cu	0.79 \pm 0.88	1.10 (0.49-3.69)	1.45 \pm 0.47	1.48 (0.77-2.40)	1.7
Fe	180.07 \pm 36.39	181.99 (108.21-250.41)	22.75 \pm 10.25	24.74 (11.33-49.55)	0.1
Mn	0.14 \pm 0.05	0.14 (0.08-0.26)	0.57 \pm 0.20	0.59 (0.24-0.91)	4.5
Mo	0.08 \pm 0.47	0.20 (0.02-2.07)	0.18 \pm 0.09	0.21 (0.11-0.44)	3.3
Ni	0.74 \pm 0.47	0.88 (0.35-2.39)	2.40 \pm 0.69	2.57 (1.68-4.08)	3.2
Se	7.25 \pm 6.31	8.55 (1.16-23.54)	0.50 \pm 0.23	0.50 (0.15-1.11)	0.1
Zn	9.64 \pm 1.68	9.87 (7.67-13.28)	92.9 \pm 23.46	85.52 (39.80-124.14)	8.8
Group B: non-essential elements with high toxicity concentrations					
Al	1.81 \pm 0.85	1.90 (0.90-3.58)	4.46 \pm 2.22	5.20 (2.42-11.30)	2.4
As	0.82 \pm 0.69	0.81 (0.05-2.82)	0.68 \pm 0.68	0.80 (0.34-3.40)	0.9
Be	0.02 \pm 0.01	0.02 (n.d. - 0.05)	0.02 \pm 0.01	0.02 (n.d.-0.04)	0
Cd	0.01 \pm 0.01	0.01 (n.d.-0.02)	0.01 \pm 0.01	0.02(n.d.-0.04)	1.7
Pb	0.12 \pm 0.10	0.16 (0.06-0.46)	0.22 \pm 0.09	0.24 (0.11-0.47)	1.7
Hg	0.01 \pm 0.005	0.005 (n.d.-0.02)	0.01 \pm 0.02	0.01 (n.d.-0.07)	0.9
Group C: toxic non-essential minority elements, TE concentrations					
Ag	0.01 \pm 0.01	0.02 (0.01-0.04)	0.01 \pm 0.005	0.01 (n.d.-0.02)	0.5
Au	0.005 \pm 0.005	0.005 (n.d.-0.01)	0.01 \pm 0.01	0.01 (n.d.-0.02)	138.8
Ba	0.63 \pm 0.41	0.78 (0.27-1.93)	1.71 \pm 1.71	2.49 (0.90-6.12)	2.9
Bi	0.005 \pm 0.003	0.005 (n.d.-0.01)	0.02 \pm 0.01	0.02 (0.01-0.1)	9.2
Ga	0.005 \pm 0.003	0.005 (n.d.-0.02)	0.005 \pm 0.01	0.01 (n.d.-0.02)	95.3
Pd	0.005 \pm 0.004	0.005 (n.d.-0.008)	0.03 \pm 0.02	0.03 (0.01-0.07)	41.7
Pt	0.005 \pm 0.005	0.0 (n.d.-0.005)	0.02 \pm 0.01	0.02 (0.01-0.03)	270.7
Sb	0.02 \pm 0.02	0.3 (0.01-0.08)	0.04 \pm 0.02	0.04 (0.01-0.09)	1.8
Sn	0.23 \pm 0.06	0.23 (0.13-0.35)	0.8 \pm 0.36	0.74 (0.29-1.37)	3.3
Sr	1.44 \pm 0.44	1.48 (0.77-2.53)	3.67 \pm 3.28	4.83 (1.62-15.49)	3
Th	0.005 \pm 0.005	0.0 (n.d.-0.005)	0.01 \pm 0.005	0.0 (n.d.-0.01)	3.2
Ti	0.04 \pm 0.01	0.05 (0.03-0.07)	0.19 \pm 0.08	0.21 (0.08-0.37)	4.8
Tl	0.005 \pm 0.005	0.0 (n.d.-0.005)	0.01 \pm 0.005	0.01 (n.d.-0.01)	3.4
U	0.01 \pm 0.002	0.01 (0.005-0.01)	0.03 \pm 0.01	0.03 (0.01-0.05)	4.4
V	0.04 \pm 0.02	0.05 (0.02-0.09)	0.12 \pm 0.08	0.15 (0.07-0.35)	3.6
Group D: REE and other TE concentrations					
Ce	0.04 \pm 0.08	0.06 (0.003-0.31)	0.01 \pm 0.01	0.02 (0.01-0.07)	0.4
Dy	0.005 \pm 0.005	0.0 (n.d.-0.01)	0.005 \pm 0.03	0.01 (n.d.-0.02)	4.1
Er	0.005 \pm 0.003	0.0 (n.d.-0.01)	0.08 \pm 0.005	0.01 (n.d.-0.03)	3.2
Eu	0.005 \pm 0.005	0.0 (n.d.-0.02)	0.005 \pm 0.03	0.01 (n.d.-0.03)	3.7
Gd	0.01 \pm 0.01	0.01 (n.d.-0.02)	0.02 \pm 0.01	0.01 (n.d.-0.03)	1.8
Ho	0.005 \pm 0.003	0.0 (n.d.-0.01)	0.08 \pm 0.005	0.01 (n.d.-0.03)	3
In	0.001 \pm 0.0003	0.001(0.001-0.002)	0.003 \pm 0.002	0.003(0.001-0.01)	3.1
La	0.005 \pm 0.003	0.0 (n.d.-0.01)	0.08 \pm 0.005	0.01 (n.d.-0.03)	3.4
Lu	0.01 \pm 0.002	0.01 (0.005-0.01)	0.03 \pm 0.01	0.03 (0.01-0.05)	4.3
Nb	0.005 \pm 0.005	0.0 (n.d.-0.005)	0.01 \pm 0.005	0.0 (n.d.-0.01)	4.6
Nd	0.005 \pm 0.003	0.0 (n.d.-0.01)	0.08 \pm 0.005	0.01 (n.d.-0.03)	3.3
Os	0.02 \pm 0.01	0.02 (n.d. - 0.05)	0.02 \pm 0.01	0.02 (n.d.-0.04)	0.4
Pr	0.005 \pm 0.005	0.0 (n.d.-0.005)	0.01 \pm 0.005	0.0 (n.d.-0.01)	3.2
Sm	0.005 \pm 0.005	0.0 (n.d.-0.005)	0.01 \pm 0.005	0.0 (n.d.-0.01)	3.8
Ta	0.005 \pm 0.003	0.005 (n.d.-0.02)	0.005 \pm 0.01	0.01 (n.d.-0.02)	83.4
Tb	0.005 \pm 0.003	0.0 (n.d.-0.01)	0.08 \pm 0.005	0.01 (n.d.-0.03)	3
Tm	0.006 \pm 0.004	0.0 (n.d.-0.02)	0.07 \pm 0.006	0.01 (n.d.-0.04)	2.9
Y	0.02 \pm 0.005	0.005 (n.d.-0.005)	0.06 \pm 0.006	0.009 (n.d.-0.1)	2.9
Yb	0.005 \pm 0.003	0.0 (n.d.-0.01)	0.08 \pm 0.005	0.01 (n.d.-0.03)	3.7

^aProportion of the biomagnified element in scute in relation to the amount recorded in blood, values ≤ 1 represent elements that were found in greater proportion in blood.
n.d. Non detected.

unknown sources, although all processes are recognized to be important for REE in marine systems.

These elements can bio-accumulate in marine invertebrates such as zooplankton and initiate a trophic chain transfer factor (TTF) (Palmer et al., 2006). Other studies in aquaculture on marine systems have reported REE concentrations in fish and bivalves (Squadron et al., 2016), some of them with very similar values (*Crassostrea gigas* Ce median, 0.02 $\mu\text{g/g}^{-1}$ in w.w.) to the scute concentrations of this study (see Table 1). Due to the lack of understanding regarding the toxicology of these emerging inorganic

elements, the potential effect of these concentrations should not be underestimated.

4. Conclusion

Owing to their unique suite of ecological and life-history attributes, sea turtles are excellent model organisms for contamination risk assessment programs and studies. With the advent of precise equipment capable of detecting pollutants at very low levels, inorganic elements in blood and scute can be accurately determined using non-invasive and non-destructive sampling techniques in wild populations.

Scute is a useful tissue for bio-monitoring concentrations of inorganic elements with an indication of longer-term exposure. However, the toxicokinetics are probably metal-specific and need future studies to facilitate the use of carapace tissues as non-lethal biomarkers of the bioaccumulation of inorganic elements. In order to properly measure the concentrations of Fe, Se, Be, Hg, Ag, Ce, and Os, it is recommended to use blood tissue instead of scute, while the rest of the elements are better identified through scute for the determination and bio-monitoring of inorganic pollutants.

The rapid development and widespread application of REE and TE technologies in industrialized countries necessitate additional information on the potential health effects derived from possible exposure to these compounds.

CRedit authorship contribution statement

Maribel Escobedo Mondragón: Conceptualization, Investigation, Data Curation, Formal analysis, Writing - original draft. **Octavio P. Luzardo:** Methodology, Software, Validation, Funding acquisition. **Manuel Zumbado:** Methodology, Software, Validation. **Ángel Rodríguez-Hernández:** Methodology, Software, Validation. **Cristian Rial Berriel:** Methodology, Software, Validation. **Héctor Vicente Ramírez-Gomez:** Software, Formal analysis. **Carlos González-Rebeles Islas:** Writing - review & editing, Visualization, Supervision. **Roberto F. Aguilar Fisher:** Writing - review & editing, Visualization, Supervision. **J. Rene Rosiles Martínez:** Methodology, Visualization, Supervision, Funding acquisition.

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.

Acknowledgments

We would like to acknowledge the non-governmental organization "Pronatura Península de Yucatan A.C." which provided valuable logistics support, with special recognition to the biologists Jalil Mezquita Ruíz and René Salinas Salazar, the local wildlife authorities CONANP, and the national environmental authorities SEMARNAT and DGVS. We are grateful to the people of Toxicology Unit of University of Las Palmas de Gran Canaria and National Autonomous University of Mexico (UNAM) for the funding and analyze of the samples, and the Georgia Sea Turtle Center for donate sampling materials for the research. Also thank the National Science and Technology Council for its funding support within the National Quality Postgraduate Program in UNAM.

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>.

Bezerra, M.F., Lacerda, L.D., Lima, E.H.S.M., Melo, M.T.D., 2013. Monitoring mercury in green sea turtles using keratinized carapace fragments (scutes). *Mar. Pollut. Bull.* 77, 424–427. <http://dx.doi.org/10.1016/j.marpolbul.2013.09.020>.

Bjorndal, K.A., 1996. Oraging ecology and nutrition of sea turtles. In: Lutz, P.L., Musick, J.A. (Eds.), *The Biology of Sea Turtles*. CRC Press, New York, pp. 199–232.

Bjorndal, K.A., Reich, K.J., Bolten, A.B., 2010. Effect of repeated tissue sampling on growth rates of juvenile loggerhead turtles *Caretta caretta*. *Dis. Aquat. Org.* 88, 271–273. <http://dx.doi.org/10.3354/dao02175>.

Camacho, M., Boada, L.D., Orós, J., López, P., Zumbado, M., Almeida-González, M., Luzardo, O.P., 2014. Monitoring organic and inorganic pollutants in juvenile live sea turtles: results from a study of *Chelonia mydas* and *Eretmochelys imbricata* in Cape Verde. *Sci. Total Environ.* 481, 303–310. <http://dx.doi.org/10.1016/j.scitotenv.2014.02.051>.

Campbell, T.W., 2012. Hematology of reptiles. In: Thrall, M.A., Weiser, G., Allison, R.W., Campbell, T.W. (Eds.), *Veterinary Hematology and Clinical Chemistry*. Wiley-Blackwell, UK, pp. 277–297.

Carneiro da Silva, C., Klein, R.D., Barcarolli, I.F., Bianchini, A., 2016. Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquat. Toxicol.* 170, 45–51. <http://dx.doi.org/10.1016/j.aquatox.2015.11.007>.

Cheng, J., Fei, M., Sang, X., Cheng, Z., Gui, S., Zhao, X., Sheng, L., Sun, Q., Hu, R., Wang, L., Hong, F., 2014. Gene expression profile in chronic mouse liver injury caused by long-term exposure to CeCl₃. *Environ. Toxicol.* 29, 837–846. <http://dx.doi.org/10.1002/tox.21826>.

Cortés-Gómez, A.A., Romero, D., Girondot, M., 2017. The current situation of inorganic elements in marine turtles: A general review and meta-analysis. *Environ. Pollut.* 229, 567–585. <http://dx.doi.org/10.1016/j.envpol.2017.06.077>.

Deetman, S., Pauliuk, S., Van Vuuren, D.P., Van der Voet, E., Tukker, A., 2018. Scenarios for demand growth of metals in electricity generation technologies, cars, and electronic appliances. *Environ. Sci. Technol.* 52 (8), 4950–4959. <http://dx.doi.org/10.1021/acs.est.7b05549>.

Dyc, C., Covaci, A., Debier, C., Leroy, C., Delcroix, E., Thomée, J.P., Das, K., 2015. Pollutant exposure in green and hawksbill marine turtles from the Caribbean Region. *Reg. Stud. Mar. Sci.* 2, 158–170. <http://dx.doi.org/10.1016/j.rsma.2015.09.004>.

Dyc, C., Far, J., Gandar, F., Poulipoulis, A., Greco, A., Eppe, G., Das, K., 2016. Toxicokinetics of selenium in the slider turtle, *Trachemys scripta*. *Ecotoxicology* 25, 727–744. <http://dx.doi.org/10.1007/s10646-016-1632-z>.

Ehsanpour, M., Afkhami, M., Khoshnood, R., Reich, K.J., 2014. Determination and maternal transfer of heavy metals (Cd, Cu, Zn, Pb and Hg) in the Hawksbill Sea Turtle (*eretmochelys imbricata*) from a nesting colony of Qeshm Island, Iran. *Bull. Environ. Contam. Toxicol.* 92, 667–673. <http://dx.doi.org/10.1007/s00128-014-1244-3>.

Feng, L., Xiao, H., He, X., Li, Z., Li, F., Liu, N., Zhao, Y., Huang, Y., Zhang, Z., Chai, Z., 2006. Neurotoxicological consequence of long-term exposure to lanthanum. *Toxicol. Lett.* 165, 112–120. <http://dx.doi.org/10.1016/j.toxlet.2006.02.003>.

González-Antuña, A., Camacho, M., Henríquez-Hernández, L.A., Boada, L.D., Almeida González, M., Zumbado, M., Luzardo, O.P., 2017. Simultaneous quantification of 49 elements associated to e-waste in human blood by ICP-MS for routine analysis. *Methods X* 4, 328–334. <http://dx.doi.org/10.1016/j.mex.2017.10.001>.

Goodenough, K.M., Wall, F., Merriman, D., 2017. The rare earth elements: Demand, global resources, and challenges for resourcing future generations. *J. Nat. Resour. Res.* 27, 201–216. <http://dx.doi.org/10.1007/s11053-017-9336-5>.

Goyer, R.A., Clarkson, T.W., 2001. Toxic effects of metals. In: Casarett, L.J., Klaassen, C.D., Amdur, M.O., Doull, J. (Eds.), *Casarett and Doull's Toxicology: The Basic Science of Poisons*. McGraw-Hill, New York, pp. 811–867.

Jakimska, A., Konieczka, P., Skora, K., Namiesnik, J., 2011a. Bioaccumulation of metals in tissues of marine animals, Part I: the role and impact of heavy metals on organisms. *Pol. J. Environ. Stud.* 20, 1117–1125.

Jakimska, A., Konieczka, P., Skora, K., Namiesnik, J., 2011b. Bioaccumulation of metals in tissues of marine animals, Part II: Metal Concentrations in Animal Tissues. *Pol. J. Environ. Stud.* 20, 1127–1146.

Jerez, S., Motas, M., Canovas, R.A., Talavera, J., Almela, R.M., Del Rio, A.B., 2010. Accumulation and tissue distribution of heavy metals and essential elements in loggerhead turtles (*Caretta caretta*) from Spanish Mediterranean coastline of Murcia. *Chemosphere* 78, 256–264. <http://dx.doi.org/10.1016/j.chemosphere.2009.10.062>.

Komoroske, L.M., Lewison, R.L., Seminoff, J.A., Deustchman, D.D., Deheyn, D.D., 2011. Pollutants and the health of green sea turtles resident to an urbanized estuary in San Diego, CA. *Chemosphere* 84, 544–552. <http://dx.doi.org/10.1016/j.chemosphere.2011.04.023>.

Komoroske, L.M., Lewison, R.L., Seminoff, J.A., Deustchman, D.D., Deheyn, D.D., 2012. Trace metals in an urbanized estuarine sea turtle food web in San Diego Bay, CA. *J. Sci. Total Environ.* 417, 108–116. <http://dx.doi.org/10.1016/j.scitotenv.2011.12.018>.

Labrada-Martagón, V., Tenorio Rodríguez, P.A., Méndez-Rodríguez, L.C., Zenteno-Savín, T., 2011. Oxidative stress indicators and chemical contaminants in East Pacific green turtles (*Chelonia mydas*) inhabiting two foraging coastal lagoons in the Baja California peninsula. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 154, 65–75. <http://dx.doi.org/10.1016/j.cbpc.2011.02.006>.

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.

Ley-Quinonez, C., Zavala-Norzagaray, A.A., Espinosa-Carreón, T.L., Peckham, H., Marquez-Herrera, C., Campos-Villegas, L., Aguirre, A.A., 2011. Baseline heavy metals and metalloid values in blood of loggerhead turtles (*Caretta caretta*) from Baja California Sur, Mexico. *Mar. Pollut. Bull.* 62, 1979–1983. <http://dx.doi.org/10.1016/j.marpolbul.2011.06.022>.

McFadden, K.W., Gómez, A., Sterling, E.J., Naro-Maciél, E., 2014. Potential impacts of historical disturbance on green turtle health in the unique & protected marine ecosystem of Palmyra Atoll (Central Pacific). *Mar. Pollut. Bull.* 89, 160–167. <http://dx.doi.org/10.1016/j.marpolbul.2014.10.012>.

- Müller, W.E.G., Borejko, A., Brandt, D., Osinga, R., Ushijima, H., Hamer, B., Krasko, A., Xupeng, C., Müller, I.M., Schröder, H.C., 2005. Selenium affects biosilica formation in the demosponge *Suberites domuncula*: Effect on gene expression and spicule formation. *FEBS J.* 272, 3838–3852. <http://dx.doi.org/10.1111/j.1742-4658.2005.04795.x>.
- Nalabotu, S.K., Kolli, M.B., Triest, W.E., Ma, J.Y., Manne, N.D., Katta, A., Addagarla, H.S., Rice, K.M., Blough, E.R., 2011. Intratracheal instillation of cerium oxide nanoparticles induces hepatic toxicity in male Sprague-Dawley rats. *Int. J. Nanomedicine* 6, 2327–2335. <http://dx.doi.org/10.2147/IJN.S25119>.
- Okada, S., Yamanaka, K., 1994. Induction of lung-specific DNA damage by methylarsenics via the production of free radicals. In: Nriagu, J.O. (Ed.), *Arsenic in the Environment, Part II: Human, Health and Ecosystem Effects*. John Wiley & Sons, Inc., New York, pp. 143–157.
- Paez-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. Contamin. Toxicol.* 59, 632–641. <http://dx.doi.org/10.1007/s00244-010-9516-3>.
- Paez-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>.
- Paez-Osuna, F., Calderon-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>.
- Pagano, G., Aliberti, F., Guida, M., Oral, R., Siciliano, A., Trifuoggi, M., Tommasi, F., 2015a. Rare earth elements in human and animal health: state of art and research priorities. *Environ. Res.* 142, 215–220. <http://dx.doi.org/10.1016/j.envres.2015.06.039>.
- Pagano, G., Guida, M., Tommasi, F., Oral, R., 2015b. Health effects and toxicity mechanisms of rare earth elements—knowledge gaps and research prospects. *Ecotoxicol. Environ. Saf.* 115, 40–48. <http://dx.doi.org/10.1016/j.ecoenv.2015.01.030>.
- Pagano, G., Tommasi, F., Guida, M., 2012. Comparative toxicity of cerium and of other rare earth elements (REEs) in plant and invertebrate test systems. In: Izyumov, A., Plaksin, G. (Eds.), *Cerium: Molecular Structure, Technological Applications and Health Effects*. Nova Science Publishers, New York, pp. 107–124.
- Palmer, A.S., Snape, I., Stark, J.S., Johnstone, G.J., Townsend, A.T., 2006. Baseline metal concentrations in *Paramoera walkeri* from East Antarctica. *Mar. Pollut. Bull.* 52, 1441–1449. <http://dx.doi.org/10.1016/j.marpolbul.2006.04.012>.
- Patino-Martínez, J., Vallarino, A., González Zuarth, C.A., 2014. Las especies bandera como bioindicadores ambientales: Las tortugas marinas (flag species as environmental bioindicators: The sea turtles). In: González Zuarth, C.A., Vallarino, A., Pérez Jiménez, J.C., Low Pfeng, A.M. (Eds.), *Bioindicadores: Guardianes de Nuestro Futuro Ambiental (Bioindicators: Guardians of Our Environmental Future)*, El Colegio de la Frontera sur (the South Border College). Instituto Nacional de Ecología y Cambio Climático (National Institute of Ecology and Climate Change, Mexico), pp. 441–450.
- Saeki, K., Sakakibae, H., Sakai, H., Kunito, T., Tanabe, S., 2000. Arsenic accumulation in three species of sea turtles. *Biomaterials* 13, 241–250. <http://dx.doi.org/10.1023/A:1009266520680>.
- Squadrone, S., Brizio, P., Stella, C., Prearo, M., Pastorino, P., Serracca, L., Ercolini, C., Abete, M.C., 2016. Presence of trace metals in aquaculture marine ecosystems of the Northwestern Mediterranean Sea (Italy). *Environ. Pollut.* 215, 77–83. <http://dx.doi.org/10.1016/j.envpol.2016.04.096>.
- Suzuki, K., Noda, J., Yanagisawa, M., Kameda, K., Sera, K., Nishi, Y., Shimamori, T., Tsukano, K., Morimoto, Y., Yokota, H., Asakawa, M., 2017. Plasma lead, silicon and titanium concentrations are much higher in green sea turtle from suburban coast than in those from rural coast in Okinawa, Japan. *J. Vet. Med. Sci.* 79, 2043–2047.
- Villa, C.A., Flint, M., Bell, I., Hof, C., Limpus, C.J., Gaus, C., 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. *Environ. Pollut.* 220 (Part B), 465–476. <http://dx.doi.org/10.1016/j.envpol.2016.10.085>.
- Wang, Z.-L., Yamada, M., 2006. Geochemistry of dissolved rare earth elements in the Equatorial Pacific Ocean. *Environ. Geol.* 52, 779–787. <http://dx.doi.org/10.1007/s00254-006-0515-7>.
- Zhao, H., Hong, J., Yu, X., Zhao, X., Sheng, L., Ze, Y., Sang, X., Gui, S., Sun, Q., Wang, L., Hong, F., 2013. Oxidative stress in the kidney injury of mice following exposure to lanthanides trichloride. *Chemosphere* 93, 875–884. <http://dx.doi.org/10.1016/j.chemosphere.2013.05.034>.
- Zheng, X.Y., Plancherel, Y., Saito, M.A., Scott, P.M., Henderson, G.M., 2016. Rare earth elements (REEs) in the tropical south atlantic and quantitative deconvolution of their non-conservative behavior. *Geochim. Cosmochim. Acta* 177, 217–237. <http://dx.doi.org/10.1016/j.gca.2016.01.018>.