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Trophic behavior of inorganic elements in nesting sea turtles (*Chelonia mydas, Eretmochelys imbricata*, and *Caretta caretta*) in Quintana Roo: Biomagnification and biodilution effect in blood and scute tissues

Maribel Escobedo Mondragón [a], Octavio Pérez Luzardo [b,c,*], Luis Alberto Henríquez-Hernández [b,c], Ángel Rodríguez-Hernández [b], Manuel Zumbado [b,c], J. René Rosiles Martínez [d], Fernando González Farias [e], Gerardo Suzán [a], Carlos González-Rebeles Islas [a]

[a] Departamento de Etología, Fauna Silvestre y Animales de Laboratorio, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Av. Universidad 3000, C.U., Coyoacán, 04510 CDMX, Mexico
[b] Toxicology Unit, Department of Clinical Sciences, Universidad de 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] Laboratorio de Toxicología, Departamento de Nutrición Animal y Bromatología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Av. Universidad 3000, C.U., Coyoacán, 04510 CDMX, Mexico
[e] Laboratorio de Química Ambiental, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Cto. Exterior s/n, C.U., Coyoacán, 04510 CDMX, Mexico

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ABSTRACT

The biomagnification and biodilution of inorganic pollutants, have a close correlation on the structure and function of trophic change behavior; sea turtles represent an excellent bioindicator model to identify their impact in marine ecosystems. To understand pollution effects on marine ecosystems, we quantified the bioconcentration of 50 inorganic elements in the blood and scute tissues of three nesting species of sea turtles (*Chelonia mydas, Eretmochelys imbricata* and *Caretta caretta*), collected in Quintana Roo State from July 2017 to August 2018. As a general trend, essential mineral elements with toxic potential showed the highest concentrations in both tissues; significant increase concentration of arsenic, mercury, and cerium levels was observed with increasing trophic levels indicating its biomagnification while a significant decrease in manganese and bismuth showed a biodilution effect. We expect that our findings can be used as baseline data in future biomonitoring and contamination risk assessment programs in the region.

1. Introduction

Advancement of industrial, agricultural, and medical technologies, and its waste disposal into coastal waters have resulted in marine ecosystem changes. Inorganic elements as pollutant compounds are a global concern due to their potential toxic effect, persistence, and ability to bioaccumulate in aquatic ecosystems (Griboff et al., 2018; MacMillan et al., 2017; Pagano et al., 2015a); some of them have drawn more attention such as heavy metals, however the growing increase of Rare Earth Elements (REE), used for technological applications and clean energy industries have been relatively scaling up, stablishing evidence points of REE- aquatic bioaccumulation (Wang et al., 2021; Pagano et al., 2015b).

Quintana Roo State is located Northeast of the Yucatan Peninsula, Mexico. It's recognized for being a suitable habitat to support high marine biodiversity, and key area for sea turtle feeding, reproduction, and nesting (Zurita et al., 1993). Five of the seven species of sea turtles in the world are found in this region: green (*Chelonia mydas*), loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), kemp's ridley (*Lepidochelys kempii*), and leatherback (*Dermochelys coriacea*); being the

* Corresponding author at: Toxicology Unit, Department of Clinical Sciences, Universidad de 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. Pérez Luzardo).

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kemp's ridley turtle (Lk.) the only one that doesn't nest in the area (Patino-Martínez et al., 2014). Nevertheless, Quintana Roo pressures from economic development, tourism, and fast urbanization with consequences for water pollution, had impact negatively on marine ecosystems (Baker et al., 2020), requiring effective biomonitoring programs that allows long-term management in conservation for endangered species, such as sea turtles.

The use of blood and scute as reliable tissues to evaluate inorganic elements in sea turtles have increase in recent years, due to its ease application as non-invasive and non-destructive sampling techniques in wild populations, adding value to alternative approaches in ecotoxicology (Komoroske et al., 2011, 2012; Escobedo et al., 2021; Miguel et al., 2022). However, information regarding ecotoxicology of sea turtles is limited compared to other reptiles (lizards and snakes) (Hopkins, 2000). To our knowledge, only two studies have reported REE levels in sea turtles (Escobedo et al., 2021; Orós et al., 2021). Adult sea turtles are long-lived reptiles exposed to a broad chemical compound such as inorganic pollutants. Bioavailability, and bioaccumulation of these elements are dependent on multiple factors including trophic level, diet, life stage, species, physiological stage, and physicochemical characteristics of the exposed elements (Komoroske et al., 2012). Bioaccumulation of inorganic compounds from food intake is characterized by the processes by which these elements accumulate into tissues of living organisms, and its species-dependent to the mechanisms of metabolization. Therefore, we may find a variety of different species from the same environment or with similar diet having different concentrations of these compounds (Jakimska et al., 2011a). Bioaccumulation can vary across geographical areas; slide changes in foraging behaviors, and food resources among species in different regions bringing great challenges to the comprehension whether inorganic elements are trophically biomagnified or biodiluted through food webs (Sun et al., 2019).

To understand the trophic level behavior of inorganic elements between species, and identify their biomagnification and biodilution effect, we evaluated the concentrations of 50 environmentally relevant inorganic elements, including REE in nesting sea turtles (*Chelonia mydas, Eretmochelys imbricata,* and *Caretta caretta*).

2. Materials and methods

Between July 2017 to August 2018, we collected 160 blood and scute samples of clinically healthy nesting sea turtles: 60 greens (*C. mydas*), 19 hawksbills (*E. imbricata*), and 21 loggerheads (*C. caretta*), from nesting beaches of Holbox Island, Contoy Island National Park, Puerto Morelos Reef National Park, and X'Cacel-X'Cacelito sanctuary, located along the Northeast Coast of Quintana Roo State, Mexico. The sampling permits were issued by the Secretary for Natural Resources Management and General Direction of Wildlife in Mexico (SGPA/DGVS/05706/17, 000310/18, 003751/18). The research methods and animal welfare measures were evaluated and approved by our local institutional Ethics Committee, at Facultad de Medicina Veterinaria y Zootecnia (FMVZ) (MMVZ-2018/2–4), Universidad Nacional Autónoma de México (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 after nesting turtles had laying eggs to minimize any disturbance on nesting process. 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 in the Toxicology Laboratory at the FMVZ-UNAM, the samples were kept with a coolant gel (4 °C) in ice chests.

2.2. Scute sampling

According to modified protocols (Bjorndal et al., 2010), scute samples (0.5–1 g) were collected after successfully blood sampling. 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: nape region of green turtle (n1,n2), supracaudal region of hawksbill turtle (s1, s2), and lateral region of loggerhead turtle (l3, l4). 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 brushed with plastic bristles to eliminate any superficial material from the environment. A 1 g of whole blood and 0.5 g of scute of wet weight (w.w) were used for the quantification of the elements. These samples were digested by the humid digestion process in 1 g:2 ml of nitric acid (60 %), and 0.35 ml of hydrogen peroxide (10 %) following the methodological standards of the mandatory technical regulation NOM-117-SSA1-1994 (1995). Once 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 Universidad de Las Palmas de Gran Canaria (ULPGC, Spain), where they were stored at 4 °C till analysis.

We determined the concentration levels of 50 elements, which were classified according to their biological and toxicological importance into four groups according to Goyer and Clarkson (2001) classification and Agency for Toxic Substances and Disease Registry (ATSDR's) Substance Priority List (2022): 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: nonessential elements with high toxicity: Al (aluminum), As (arsenic), Be (beryllium), Cd (cadmium), Pb (lead) and Hg (mercury). Group C, toxic non-essential minority elements, included in ATSDR's Substance Priority List: 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), employed in the technology industry: 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 quantitative element analyses, we employed an Agilent 7900 ICPMSc (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, Savillex Corporation, Eden Prairie, MN, USA). We followed the previously validated procedure using certified reference materials (González-Antuña et al., 2017). The 4th generation Octopolar Reaction System (ORS4) was operated in helium mode (He2) to reduce polyatomic interferences and also discarding their mathematical correction. The recoveries obtained ranged from 83 to 116 % for the analytes included. Three aliquots of each digested sample were brought to a final concentration of 4 % (*v*/v) of nitric acid for introduction into the ICP-MS and taken in independent

autosampler vials to eliminate possible out-of-range values. A reagent blank was introduced every 12 vials of the batch to control the quality of the analysis. For this purpose, a commercial internal standard solution of (Sc (scandium), Ge (germanium), Rh (rhodium) and Ir (iridium) was added to the samples, blanks and calibrations to ensure the correct monitoring of the equipment.

Two standard curves (twelve points, 100–0.005 ng/ml) were made to avoid interferences between elements: a) one using a commercial multielement 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

Table 1
Inorganic elements concentrations (ng/g w.w.) in blood and scute tissues of nesting turtles of Quintana Roo State, Mexico.

	Green turtle (*Chelonia mydas*) $n = 60$		Hawksbill turtle (*Eretmochelys imbricata*) $n = 19$		Loggerhead turtle (*Caretta caretta*) $n = 21$		*P values*	
Elements				Median			[*]*P* Blood	[**]*P* Scute
	Blood	Scute	Blood	Scute	Blood	Scute		
Group A: essential mineral elements with toxic potential concentrations								
Co	3.58	22.15	9.38	18.80	5.03	16.65	0.00	0.40
Cr	66.61	1263.72	42.12	852.33	72.45	221.28	0.04	0.00
Cu	586.31	1962.53	789.45	1357.16	902.24	396.37	0.01	0.00
Fe	116,340.66	26,078.10	180,067.26	20,943.53	144,661.85	14,971.99	0.00	0.00
Mn	245.94	2215.69	136.46	541.02	145.19	586.13	0.00	0.00
Mo	47.83	314.98	77.34	167.76	128.37	278.31	0.01	0.00
Ni	283.62	3025.05	737.18	2397.41	1043.97	804.36	0.00	0.00
Se	50.39	80.82	7246.88	512.55	1128.68	995.67	0.00	0.00
Zn	6291.75	38,700.27	9636.74	92,900.37	11,075.18	287,244.73	0.00	0.00
Group B: non-essential elements with high toxicity concentrations								
Al	1165.43	6255.44	1813.43	4037.14	1693.39	6451.62	0.15	0.01
As	50.67	160.18	824.85	681.59	1246.40	948.10	0.00	0.00
Be	0.01	0.01	0.01	0.01	0.01	0.01	0.72	0.14
Cd	12.57	35.49	5.67	12.14	20.92	49.05	0.02	0.00
Pb	69.79	422.42	117.51	205.33	99.54	69.64	0.00	0.00
Hg	1.16	17.80	3.35	15.08	13.01	147.58	0.00	0.00
Group C: toxic non-essential minority elements, TE concentrations included in ATSDR's Substance Priority List								
Ag	3.92	10.35	14.77	6.32	10.43	8.18	0.00	0.00
Au	0.00	0.00	0.00	0.57	0.00	0.64	0.74	0.00
Ba	204.01	1033.73	631.89	1656.58	738.31	534.18	0.00	0.00
Bi	6.81	98.99	2.09	18.33	3.63	3.60	0.00	0.00
Ga	0.01	2.48	0.03	2.36	0.03	3.55	0.00	0.03
Pd	0.24	1.86	0.71	25.99	0.38	9.54	0.07	0.00
Pt	0.00	0.08	0.07	15.86	0.00	0.04	0.31	0.00
Sb	20.68	159.45	24.13	36.43	12.27	24.70	0.02	0.00
Sn	305.94	1493.24	231.28	657.95	367.60	42.42	0.05	0.00
Sr	1859.70	8385.66	1435.82	3582.12	1728.91	69,213.50	0.13	0.00
Th	0.08	0.62	0.16	0.53	0.17	0.82	0.00	0.00
Ti	33.21	247.38	41.22	180.66	48.46	230.78	0.01	0.04
Tl	0.00	0.45	0.22	0.66	0.00	10.61	0.00	0.00
U	5.53	43.73	6.59	24.67	6.92	188.95	0.44	0.00
V	20.70	141.35	42.03	110.14	49.04	884.15	0.00	0.00
Group D: REE and other TE concentrations employed in the technology industry								
Ce	3.03	20.74	38.23	11.25	22.63	22.97	0.00	0.00
Dy	0.09	1.01	0.20	0.65	0.22	3.49	0.00	0.00
Er	0.05	0.70	0.13	0.46	0.15	2.59	0.00	0.00
Eu	0.06	0.44	0.14	0.38	0.14	0.77	0.00	0.00
Gd	0.14	1.39	0.60	0.99	0.44	3.53	0.00	0.00
Ho	0.02	0.23	0.04	0.13	0.05	0.78	0.00	0.00
In	1.36	6.69	1.02	2.93	1.59	0.23	0.07	0.00
La	2.12	15.44	2.22	6.68	3.19	17.32	0.08	0.00
Lu	0.01	0.10	0.03	0.10	0.03	0.36	0.00	0.00
Nb	0.23	2.75	0.47	2.11	0.37	2.55	0.00	0.22
Nd	0.79	6.85	1.99	6.15	1.98	13.72	0.00	0.00
Os	0.01	0.00	0.01	0.00	0.01	0.00	0.17	0.00
Pr	0.21	1.89	0.50	1.46	0.51	3.12	0.00	0.00
Ru	0.00	0.00	0.00	0.00	0.00	0.00	0.90	0.00
Sm	0.11	1.25	0.32	1.08	0.36	3.11	0.00	0.00
Ta	0.00	0.09	0.00	0.11	0.00	0.06	0.44	0.16
Tb	0.02	0.20	0.04	0.13	0.04	0.55	0.00	0.00
Tm	0.01	0.10	0.02	0.06	0.02	0.36	0.00	0.00
Y	0.82	9.44	1.65	5.43	1.82	33.29	0.01	0.00
Yb	0.06	0.64	0.14	0.50	0.15	2.14	0.00	0.00

[*] Kruskal-Wallis test among bloods from the three species.
[**] Kruskal-Wallis test among scute from the three species, n: sampled size number, TE: trace elements, REE: rare earth elements.

frequently employed in the high-tech industry, c) linear calibration curves were found for all elements (regression coefficients >0.998).

Limits of detection (LOD) and quantification (LOQ) were calculated as the concentrations that respectively produced signals three and ten times higher than the averaged blanks instrumentally measured. Sample LOQ were calculated by multiplying the instrumental LOQ by the dilution factor (1:10 v:v). In general, the relative standard deviations (RSD) provided by the ICP-MS were lower than 5 % for all elements. The concentrations of elements below the limit of detection (LOD) were assigned a zero (0) value and all the metal concentrations were expressed as nanogram per gram of wet weight (ng/g of w.w.).

2.4. Trophic structure of sea turtles

To assign the trophic level of the sampled species of adult sea turtles, we used a five trophic level marine food web model in which categorized the species referenced to each diet (Godley et al., 1998; Hoopes et al., 2017) placing adult green turtle (*C. mydas*) as secondary consumer with a mainly herbivorous diet, adult hawksbill turtle (*E. imbricata*) as tertiary consumer with an omnivorous diet, and adult loggerhead turtle (*C. caretta*) as generalist predator with a carnivorous diet.

2.5. Statistical analysis

Database management and statistical analysis were performed using IBM SPSS software (v.27). 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, species and locations were tested through Kruskal-Wallis, or Mann-Whitney *U* test. In addition, continuous variables were analyzed by the Spearman rank correlation coefficient, and principal component analysis (PCA) using R software (R-3.5.2 version). The *P* values of <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 in the three species are shown in Table 1. Carapace size mean of sampled nesting turtles were 93.6 cm width and 104.2 cm length for nesting green turtles (*C. mydas*), 80.4 cm width and 86.9 cm length for nesting hawksbill turtles (*E. imbricata*), and 85.9 cm width and 96.4. cm length for nesting loggerhead turtles (*C. caretta*). No positive or negative correlations between carapace size and inorganic elements was found in the three species, except elements from group D of loggerhead turtles (*C. caretta*). Moderate positive correlations were observed between carapace size and lanthanum (La, r 0.58), cerium (Ce, r 0.54), neodymium (Nd, r 0.51), europium (Eu, r 0.57), gadolinium (Gd, r 0.52), tantalum (Ta, r 0.54). Loggerhead turtles have complex life histories with trans-oceanic migration, and slow growth that can take over 30 years to reach sexual maturity (Bolten, 2003). This finding suggests that loggerhead turtles (*C. caretta*) might have different incorporation mechanism of inorganic elements to their scutes, and carapace linked to their growth process.

Group A, corresponding to essential elements, showed the highest concentrations in all species. Essential minerals are commonly found at high concentrations due to its strong relation with physiological processes (Jakimska et al., 2011a, 2011b). Inorganic elements in scute presented concentration values from 0.1 to 8841.4 higher than blood samples. These findings are consistent with those reported in other studies witch propose that scute seems to be an appropriate tissue for biomonitoring long-term exposure and blood tissue for short-term exposure (Komoroske et al., 2012; Bezerra et al., 2013; Escobedo et al., 2021). To our knowledge, this is the first study to analyze rare earth elements (REE) in blood and scute tissues of green (*C. mydas*) and loggerhead (*C. caretta*) nesting turtles. Moderate positive correlation between scute and blood concentrations was found in the three species for chromium (Cr) (r 0.46 to 0.66); zinc (Zn, r 0.50), silver (Ag, r 0.52), titanium (Ti, r 0.52), niobium (Nb, r 0.66) in hawksbill turtles (*E. imbricata*); lead (Pb, r 0.55), platinum (Pt, r 0.65), cerium (Ce, r 0.57), indium (In, r 0.62), tantalum (Ta, r 0.72) in loggerhead turtles (*C. caretta*); and moderate negative correlation in thallium (Tl, r − 0.50), neodymium (Nd, r − 0.62), samarium (Sm, r-0.76) of loggerhead turtles (*C. caretta*). These findings suggest that these elements can be biomonitoring through keratinized tissues throughout non-invasive techniques like scute.

3.1. Bioconcentration

Concentrations found in Groups A, B, and C are in the usual environment concentrations reports around the globe (Miguel et al., 2022), however, some authors mention the difficulty in establishing whether the concentrations of some inorganic elements can be in toxic concentrations or relevant for bio-accumulation toxicity (Ley-Quiñonez et al., 2011). Due to the insufficiency of information regarding Group D elements in reptiles and marine organisms' concentrations found in this group should not be underestimated.

Concentrations within Group A (essential mineral elements with toxic potential) showed the highest concentrations, and no elements were found below LOD. Elements of this group that found to be in the greatest concentrations were elements associated with red blood cells, and most abundant elements present in different tissues of different species of sea turtles (Fe > Zn) (Camacho et al., 2014). Green turtle (*C. mydas*) showed 1.8 times higher concentration of manganese (Mn) respect the other two species. Studies in grass and seaweed indicate a high concentration of Mn due to its dependence as an enzymatic cofactor used as a mediator of oxidative stress (MnSOD) and its growth (Liu et al., 2016). As sea grass represent a significant portion of adult green turtle diet, this relationship probably accounts for the high levels of Mn found in their tissues.

The greatest concentrations within Group B (non-essential elements with high toxicity) were Al > As > Pb. Most of the values in this group were like others published for the same species (Camacho et al., 2013; Escobedo et al., 2021; Miguel et al., 2022), only beryllium (Be) was present below LOD in both tissues. Scute concentrations of these elements were higher than those found in the blood, except for arsenic (As) in blood tissue which was were 1.3 times higher than scute for hawksbill (*E. imbricata*) and loggerhead (*C. caretta*) turtles. It has been reported that arsenic compounds bio-accumulate more intensively in sea turtles with higher trophic level (Jakimska et al., 2011a; Miguel and Deus Santos, 2019), nevertheless due to the DNA damage induced by metabolites of arsenic compounds (Cao et al., 2019) these results should not be underestimated.

Concentrations within Group C (toxic non-essential minority elements) were higher in the three species than those reported in other studies using blood samples (Komoroske et al., 2011; Labrada-Martagón et al., 2011; McFadden et al., 2014; Carneiro da Silva et al., 2016; Villa et al., 2017) only platinum (Pt) was present below LOD in green (*C. mydas*) and loggerhead (*C. caretta*) samples. Scute concentrations of these elements presented concentration values from 1.5 to 8841.4 higher than blood samples. Except for silver (Ag) in hawksbill (*E. imbricata*), gold (Au) in green (*C. mydas*), and tin (Sn) in loggerhead (*C. caretta*). From an ecotoxicological view, Sn is a toxic cumulative element, mostly related to its ability as endocrine disruptor form organotin compounds (Marlatt et al., 2022), and its interference with the metabolism of essential elements: Zn, Cu, Ca, and Fe (Tomza-Marciniak et al., 2019). Nevertheless, the mechanism of toxicity underlying these concentrations, and adverse effects in sea turtles is unknown.

The concentrations within Group D (REE and other TE) showed the lowest concentrations of the four groups, except for cerium (Ce), which also present a higher concentration in blood rather than scute tissue for hawksbill (*E. imbricata*). The toxicodynamics and toxicokinetics

underlying Ce concentrations are largely unknown in marine organisms, despite being one of the most studied elements among REE (Blinova et al., 2020). Registration of higher concentration of Ce in blood rather than scute should not be underestimated. It has been reported in aquatic organisms' developmental anomalies, affected redox parameters, inhibition of mitotic activity, increase of aberrations in the offspring, among other xenobiotic alterations related to Ce-exposure (Huang et al., 2022; Pagano et al., 2016). Osmium (Os) and ruthenium (Ru) were present bellow LOD in both tissues in the three species. The rest of the elements corresponding to this group had higher scute concentrations than those found in the blood.

Further studies are needed to understand REE toxicity in sea turtles, concentrations found in this study should not be underestimated. In marine ecosystems, REE levels are known to be low, and may only become available in small amounts into the sediments of continental shelf, ocean bottom, and the atmosphere, although, their increasing report of additional and abnormal increase from unknown sources indicate that REE are the new emerging contaminants (Zheng et al., 2016). Despite the possible potential toxicological effects in sea turtles, REE may affect coastal sediment, neritic and benthic habits consequently, bioaccumulate in a set of marine biota including higher trophic marine organism such as sea turtles. Moreover, as other inorganic elements may lead to other types of impacts, like climate change, ionizing radiation, eutrophication, and public health (Jin et al., 2009; Balaram, 2019; Kirchhübel and Fantke, 2019).

Comparisons between studies from different turtle species and geographical areas can be very useful to infer about the species that are more susceptible to bioconcentrate, biomagnificate, and biodiluted inorganic compounds. Although few recent studies, that express inorganic elements levels according to blood wet weight, are available.

3.2. Trophic behavior

Multiple statistical analyses, including Principal Component Analysis (PCA), were conducted to investigate the bioconcentration analogies between species. PCA results of inorganic elements in all groups, and *P* values of Mann-Whitney U test were in accordance, suggesting that hawksbill (*E. imbricata*) and loggerhead (*C. caretta*) turtles have similar bioconcentrations in the blood (*P* 0.47), this similitude could be due to the type of diet in both species (omnivorous and carnivorous consumers respectively) in contrast with green turtle (*C. mydas*), which mainly have an herbivorous diet (Fig. 1. A). This finding support that blood samples are a useful for biomonitoring short-term exposure closely linked to trophic transfer factors, and suitable tissue for bioaccumulation models.

Otherwise, bioconcentration analogies in scute between species showed different bioconcentrations for the three species (*P* 0.001), with an increase concentration of the elements at higher trophic positions of the studied species (Fig. 2. A). This finding support previous reports, suggesting that scute is an adequate tissue for long-term exposure and useful for biomagnification and biodilution effects studies (Komoroske et al., 2011; Bezerra et al., 2013; Escobedo et al., 2021).

Keratinized tissues, such as scute could act as a sequester of inorganic elements from inside the body, which will be expelled when the scute of the carapace is sloughed. Scute bioconcentration might have some detoxifying mechanism linked to this process, like the mechanism suggested with shed feathers or sloughed skins in amphibians, birds, and reptiles (Martín et al., 2021). Other authors have described that another route of detoxification could be represented by biodilution process along the food web (Campbell et al., 2005; Jakimska et al., 2011b), however, our findings suggest opposite results (Fig. 2. A).

These findings support the trophic level selected for our study, placing adult green turtle (*C. mydas*) as secondary consumer, adult hawksbill turtle (*E. imbricata*) as tertiary consumer, and adult loggerhead turtle (*C. caretta*) as generalist predator. Moreover, reinforced the classical definition of biomagnification where chemical concentration increases in higher trophic positions in the food web (Jakimska et al., 2011a, 2011b), and suggest that differences between both tissues in the three species are diet dependent.

Currently, there are substantial gaps in our understanding of the adverse effects and mechanism of excretion in inorganic elements including REE to marine organisms. There are no toxicity threshold values for inorganic elements in sea turtles, hindering establish whether the concentrations of inorganic elements found in these different species at different trophic levels can be in toxic concentrations.

The biplots show the PCA scores of the explanatory variables (inorganic elements) as vectors (red lines) and individuals (i.e., red, blue, and green ellipses). Ellipses sizes are determined by a 0.95-probability level, and show the observations grouped by mark class. The magnitude of the vectors (red lines) shows the strength of their contribution to each principal component. Individual vectors (ellipses) pointing in similar directions indicate positively correlated variables, vectors pointing in opposite directions indicate negatively correlated variables, and vectors at proximately right angles indicate low or no correlation. A. PCA scores in blood samples, individual vectors of hawksbill (*E. imbricata*) and loggerhead (*C. caretta*) showed similar directions indicate positively

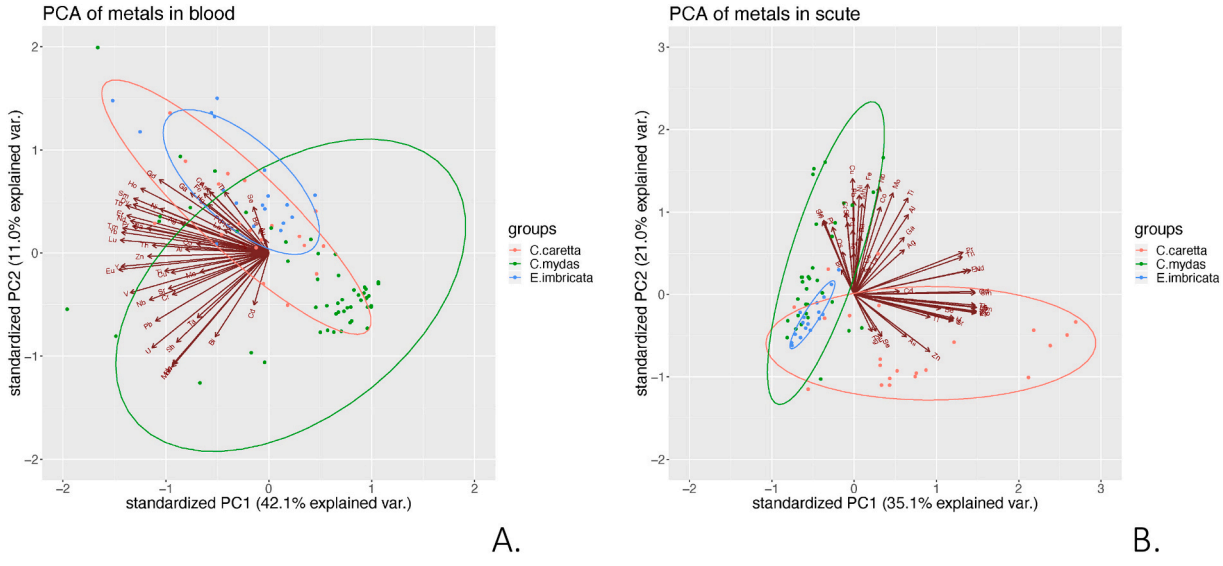

Fig. 1. Principal component analysis (PCA) biplot of sea turtles and inorganic elements (variables $n = 50$).

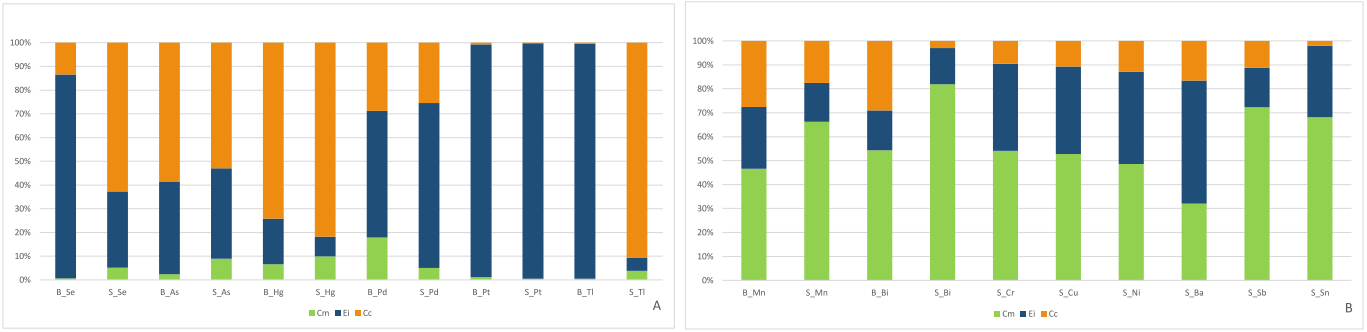

Fig. 2. Biomagnification and biodilution effects of inorganic elements in nesting sea turtles.

correlated variables; B. PCA scores in scute samples, individual vectors of the three species were pointing in different directions at right angles indicate negatively correlated variables, and low or no correlation between them.

3.3. Biomagnification and biodilution effect

An increase concentration of the elements at higher trophic positions were observed in 48 elements in blood and 39 elements in scute over the 50 studied inorganic elements, indicating its biomagnification effects. Since carapace size, physiological stage and life history patterns in sea turtles are strongly correlated (Bolten, 2003), it's important to mention that only loggerhead turtle (*C. caretta*) showed moderate positive correlations between carapace size and inorganic elements from group D. This finding suggest that not only trophic level might be a factor for biomagnification or biodilution effect, in some species such as loggerhead turtles (*C. caretta*) might be a growth pattern and metabolization mechanism linked to the incorporation of these inorganic elements to their scutes, and carapace. Future studies in other sea turtles species at the same or similar trophic level of loggerhead turtles (*C. caretta*) will be required to dilucidated this finding.

As keratinized tissue, scute showed to be the suitable tissue to observed biomagnification. Due to its ease to incorporate elements from the body over time (Komoroske et al., 2011). This could explain why scute concentrations in the three species are different, suggesting that the mechanism of excretion through scute could be diet dependent. A significant increase concentration of Se, As, Hg, Pd, Pt, and Tl levels were identified in both tissues of hawksbill (*E. imbricata*) and loggerhead (*C. caretta*) turtles (Fig. 2. A), respecting green turtle (*C.mydas*). Given the recognition of high arsenic (As) levels in marine organisms (Jakimska et al., 2011a), may be necessary to dilucidated arsenic toxic compounds concentration and effects on sea turtles with higher trophic level. Cerium (Ce) blood concentrations were found at a rate of 12.6 to 7.5 times higher in hawksbill (*E. imbricata*) and loggerhead (*C. caretta*) turtles respectively above green turtle (*C.mydas*) concentration. Elements who showed a decrease concentration with higher trophic levels, indicating its biodilution effect, were manganese (Mn) and bismuth (Bi) in both tissues, and Cr, Cu, Ni, Ba, Pt, Sb, and Sn in scute (Fig. 2. B) where green turtle (*C. mydas*) has greater concentrations (bioconcentration values from 1.7 to 35.2 higher) than hawksbill (*E. imbricta*) and loggerhead (*C. caretta*) turtles.These findings support that through non-lethal and non-invasive methods we can analyze biomagnification and biodilution effects, by studying three different species, with different diet, at different trophic levels.

Bioavailability of inorganic elements, specially REE and other trace inorganic elements in marine ecosystems depends on many different factors, such as the water hardness, alkalinity, pH, and dissolved organic carbon. All these features may be related with the bioaccumulation route, that subsequently impacts on its biomagnification effects in higher trophic levels (Arienzo et al., 2022). Considering that metal speciation is essential for improving our understanding of inorganic elements toxicity we can't ensure if this biomagnification and biodilution effects in nesting sea turtles are in toxic levels for their organism.

The histogram shows the biomagnification percentage of Se, As, Hg, Pd, Pt, Tl, and biodilution percentage of Mn, Bi, Cr, Cu, Ni, Ba, Sb, Sn. The concentrations of the elements are represented in the volume color of the bars of each tissue (b: blood, s: scute) and individuals (green: Cm, *Chelonia mydas*; blue: Ei, *Eretmochelys imbricata*; orange: Cc, *Caretta caretta*).

A. Biomagnification histogram of Se, As, Hg, Pd, Pt, and Tl, where hawksbill (*E. imbricta*) and loggerhead (*C. caretta*) tend to have the grates concentrations, meaning an increase concentration of the elements at higher trophic positions; B. Biodilution histogram of Mn, Bi, Cr, Cu, Ni, Ba, Pt, Sb, and Sn where green (*C. mydas*) has greater concentrations than hawksbill (*E. imbricta*) and loggerhead (*C. caretta*) turtles.

3.4. Sampling location trends in nesting green turtles (Chelonia mydas)

Due to the imparity between sampled individuals and their nesting areas for loggerhead turtle (*C. caretta*), and single sample location site for hawksbill turtles (*E. imbricata*) (Holbox Island). We only analyze differences between sampled locations for nesting green turtles (*C. mydas*): Puerto Morelos National Park ($n = 15$), X'cacel-X'cacelito sanctuary ($n = 20$), and Contoy Island National Park ($n = 17$).

Differences among locations were tested through Kruskal-Wallis test. We detected concentration values from 1 to 9.8 times higher of nesting green turtles (*C. mydas*) sampled from Puerto Morelos Reef National Park (Fig. 3. A, B). Significant difference was observed in blood concentrations for Cd and Ag with a rate of 9.8 and 5.6 times higher than nesting green turtles (*C. mydas*) sampled at Contoy Island National Park, which was the location with the lowest bioconcentrations.

Concerning scute bioconcentrations, a significant difference was observed for Au, Pd, Pt, Os, and Ta with a rate of 126.4, 116.0, 1765.7, 2127.6, 30.1 times higher compared to Contoy Island National Park, respectively (Fig. 3.B). These big differences between locations could be the consequence of bioaccumulation added from other locations during their migratory routes of these populations.

Following the presumption of blood been an appropriate tissue for biomonitoring short-term exposure of inorganic elements (Komoroske et al., 2011; Escobedo et al., 2021), Quintana Roo State coastal waters are an exposure source from inorganic elements bioconcentrations found in blood. With the implementation of flipper tagging efforts, tag-recapture data, and studies based on genetic markers (Encalada et al., 1999) for sea turtles research in Quintana Roo State, it can be assumed that these three locations are nesting beaches from three different populations. Seagrass meadows are critical for support marine ecosystems including food webs, sediment stabilization, carbon fixation, nutrient cycling, nursery habitat, and oxygen movement (Marba et al., 2006). It's a critical habitat for a large range of sensitive species, namely green turtles (*C. mydas*), and needs to be biomonitored to secure the health of this essential habitat. Chronic exposure to inorganic emergent

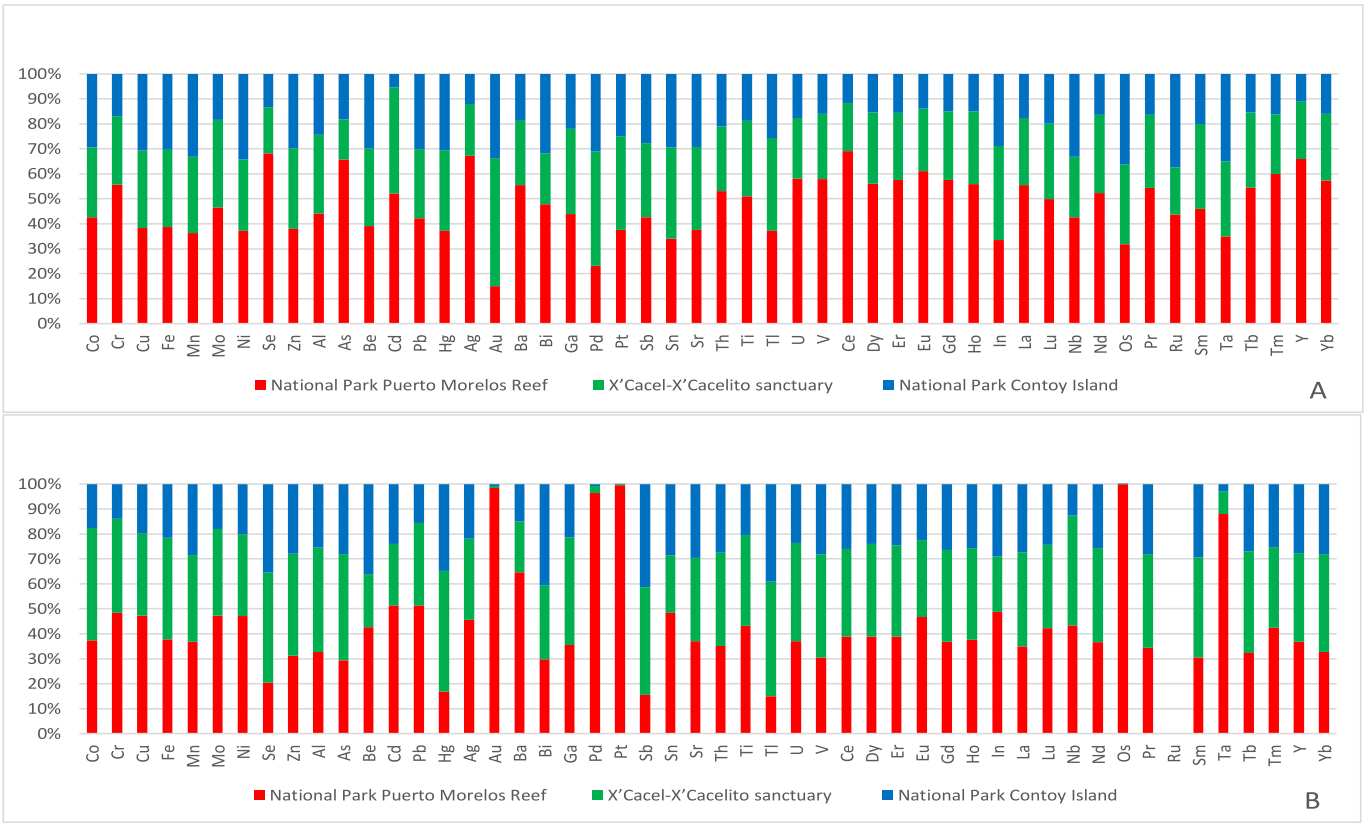

Fig. 3. Bioconcentration of inorganic elements in blood and scute tissues of nesting green turtles (*Chelonia mydas*) in three different sampling locations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pollutants in coastal waters may pose a chronic threat not just to sea turtle populations, it could also have impact into public health. However, there are considerable gaps between genetic structure, origin, connectivity between nesting and foraging areas, and bioavailability of inorganic pollutants in seagrass meadows that could lead into a better understanding of bioaccumulation factor in green turtles (*C. mydas*) and its exposure sources. Moreover, due to this lack of information, we can't assure that Puerto Morelos Reef National Park is an important exposure source of inorganic elements at Quintana Roo State, however, we can assert that their nesting green turtle (*C. mydas*) population had a higher exposure to these pollutants.

These results could indicate differences between polluted foraging areas during breeding season in Quintana Roo State, demonstrated the advantages of non-lethal and non-invasive methods for biomonitoring inorganic elements in protected species like sea turtles, and the usefulness of two different tissues that elucidated contrasting times of exposure.

The histograms show the bioconcentration percentage of the 50 analyzed inorganic elements in blood and scute tissues of nesting green turtles from three different nesting locations. The mean concentration of the elements is represented in the volume color of the bars of each location (red: Puerto Morelos Reef National Park, green: X'cacel-X'cacelito sanctuary; blue: Contoy Island National Park). A. Bioconcentration histogram of blood samples from nesting green turtles (*C. mydas*), the volume distribution of the bars showed Puerto Morelos Reef National Park as the location with higher concentrations. B. Bioconcentration histogram of scute samples from nesting green turtles (*C. mydas*), the volume distribution of the bars showed Puerto Morelos Reef National Park as the location with higher concentrations.

4. Conclusions

From a management perspective, to identify which species can bioconcentrate, biomagnify, and biodilute inorganic elements, allow us to have a better appreciation of the role of inorganic elements in marine ecosystems. With this study we have shown that direct monitoring of inorganic elements in sensitive organisms such as sea turtles with different types of diets can strengthen our understanding of trophic behavior of these emergent pollutants. Our study proposes a new approach to analyze biomagnification and biodilution effects in sea turtles using non-lethal and non-invasive techniques. Using precise equipment capable of detecting pollutants at very low concentrations, inorganic elements in blood and scute can be accurately determined in wild populations as an alternative approach to animal testing in ecotoxicology.

This study is limited to the analyze of bioconcentration, biomagnification and biodilution effects on three species of nesting sea turtles at Quintana Roo State. Future studies need to be done to understand the bioavailability of these emergent pollutants, and determinate the trophic transfer factor index for each species and population. The resulting anthropogenic input of REE into aquatic environment might create an environmental and public health concern time ahead, implement their quantification and bioavailability in biomonitoring studies is critical for future environmental safety assessment and regulations for these compounds.

CRediT authorship contribution statement

1. Maribel Escobedo Mondragón: Conceptualization, Investigation, Resources, Data Curation, Formal analysis, Writing - Original Draft, Project administration, Funding acquisition

2. Octavio Pérez Luzardo: Methodology, Software, Validation, Resources, Funding acquisition
3. Luis Alberto Henríquez-Hernández: Methodology, Software, Formal analysis
4. Ángel Rodríguez-Hernandez: Methodology, Investigation, Validation
5. J. René Rosiles Martínez: Methodology, Visualization, Supervision, Resources, Funding acquisition
6. Manuel Zumbado: Methodology, Investigation, Validation
7. Fernando González Farias: Writing - Review & Editing, Visualization, Supervision
8. Gerardo Suzán: Writing - Review & Editing, Visualization, Supervision
9. Carlos González-Rebeles Islas: Writing - Review & Editing, Visualization, Supervision

Term	Definition
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims
Methodology	Development or design of methodology; creation of models
Software	Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components
Validation	Verification, whether as a part of the activity or separate, of the overall replication/ reproducibility of results/ experiments and other research outputs
Formal analysis	Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection
Resources	Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools
Data Curation	Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse
Writing - Original Draft	Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation)
Writing - Review & Editing	Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre-or postpublication stages
Visualization	Preparation, creation and/or presentation of the published work, specifically visualization/ data presentation
Supervision	Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team
Project administration	Management and coordination responsibility for the research activity planning and execution
Funding acquisition	Acquisition of the financial support for the project leading to this publication

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Declaration of competing interest

There are no actual or potential conflicts of interest to declare for any author.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.114582.

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