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Inorganic elements in blood, eggs, and embryos of olive ridley sea turtle (*Lepidochelys olivacea*) from Sanquianga Natural National Park, Colombia

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ABSTRACT

The concentration of eight inorganic elements (As, Cd, Cr, Cu, Hg, Pb, Se and Zn) was analysed for the first time in the blood (adults), eggs, embryos, embryo carapaces, and sand from nests of olive ridley turtles (*Lepidochelys olivacea*) of the Sanquianga National Natural Park (Colombian Pacific coast). Zinc was the element that showed the highest concentration, followed by Cr and Se. Sand and embryo carapaces were the samples that showed the highest concentrations. Significant correlations were identified between the elements, being most of them reported by first time in this species. Molar ratio Se:Hg was greater than 1 in all the samples, indicating that there is sufficient Se to bind to Hg, and therefore, counteracting its potential toxicity to health. Likewise, five correlations were associated with the carapace of the embryos and none with sand, suggesting maternal transfer contamination. The results obtained provide novel information about exposure to inorganic elements in nesting sea turtles in the eastern tropical Pacific.

1. Introduction

Marine pollution, product of anthropogenic activities, is a problem that affects worldwide marine ecosystems (Cortés-Gómez et al., 2018a), impacting both wild and commercial species (Wilcox and Aguirre, 2004; Olimón-Andalón et al., 2021; de Farias et al., 2022). Inorganic elements are part of the main pollutants in marine environments given their high level of dispersion, being found in sediments (Páez-Osuna et al., 2017), waters (Mohod and Dhote, 2013; Shanbehzadeh et al., 2014), plants (Asati et al., 2016; Rehman et al., 2021) and biota (Rehman et al., 2021; Sun et al., 2022). Some of these elements also have a high bioaccumulation and biomagnification potential, depending on their biological, ecological and environmental characteristics and those of the organisms (Szynkowska et al., 2018). Thus, it is possible to identify them in high concentrations in species at high trophic levels (Storelli et al., 2005; Lawson et al., 2020) and long-lived species such as sea turtles, where adverse health effects have been reported (Anan et al., 2001; Miguel et al., 2022).

According to the Red List of the International Union for Conservation of Nature (IUCN), six of the seven species of sea turtles that exist worldwide are in some risk category (IUCN, 2022). Among these, olive ridley turtle (*Lepidochelys olivacea*) is the most abundant species globally (Marcovaldi, 1999; Abreu-Grobois and Plotkin, 2014). It is also the most numerous nesting species of the five that have been reported in the Colombia- Eastern Tropical Pacific (C-ETP) (Barrientos-Muñoz et al., 2015), with the most important nesting areas in South America localized at Northern (Chocó) and Southern (Nariño) zones of the C-ETP (Martínez and Páez, 2000; Hinestroza and Páez, 2001; Barrientos-Muñoz et al., 2014).

In the Pacific zone of Colombia, Chocó and, to a lesser extent, Nariño,

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are the regions where the highest levels of industrial and artisanal gold extraction have been reported (UPME, 2018). Therefore, the sandy beach areas of these regions are expected to have suffered greater impact by the polluting substances used for these activities, such as mercury (Hg). Furthermore, the process to obtain cocaine hydrochloride, which is carried out in these regions, also generates the dumping of large quantities of chemical precursors into water sources such as: vanadium (V), palladium (Pd), nickel (Ni), manganese (Mn), chromium (Cr) and lead (Pb), which are part of the solvents and precursors used in the extraction and purification of this alkaloid (Ávila Hernández, 2014). Considering the high levels of production of cocaine hydrochloride in the region, this activity is considered one of the largest contributors to environmental pollution by inorganic elements in the marine-coastal environments of the C-ETP (UNODC-SIMCI, 2021), and probably increasing the risk of exposure to inorganic contaminants of the nesting beaches present in these regions of the C-ETP.

Although some inorganic elements (e.g. copper (Cu), selenium (Se), zinc (Zn), and Cr) are essential and play an important role in the growth and metabolism of organisms (Camacho et al., 2013), exposure to higher concentrations may cause negative effects on sea turtles. On the other hand, non-essential inorganic elements (e.g. arsenic (As), cadmium (Cd), Pb and Hg), may cause these negative effects even at low concentrations (Huang et al., 2015). Some of these effects include alterations in the endocrine and immune system (Camacho et al., 2013), development of malformations (Martín-del-Campo et al., 2019; Choi et al., 2020), variation in the number of carapace's scutes and pigmentation disorders, which have been identified with high prevalence in *L. olivacea* (Cortés-Gómez et al., 2018b; Martín-del-Campo et al., 2021), and even, high rates of infertility and embryonic mortality (Hopkins et al., 2013; Savoca et al., 2022).

In this study, we evaluated the concentration of some inorganic elements (As, Cd, Cr, Cu, Hg, Pb, Se, and Zn) and the molar ratio selenium: mercury (Se:Hg) in blood of breeding females, unhatched eggs, dead embryos and sand of the nests of *L. olivacea* present on the nesting beaches of the Sanquianga Natural National Park (SNNP), in the Southern region of C-ETP, by addressing the following questions: (1) What is the exposure to essential and non-essential inorganic elements in females, eggs and embryos in the nesting zone of *L. olivacea* in the SNNP?, and (2) what is their risk of Hg toxicity based on the molar ratio Se:Hg? We hypothesized that the concentrations of essential and non-essential inorganic elements would be higher in the females, eggs, and embryos of *L. olivacea* from nesting beaches with higher concentrations of inorganic elements due to gold extraction and cocaine hydrochloride processing present on the nesting beaches of SNNP. This information will fill the gap of knowledge about the toxicological status of *L. olivacea* in SNNP and will allow to obtain reference values regarding the exposure to inorganic elements of this population in the C-ETP.

2. Materials and methods

2.1. Sample collection

Samples were collected from the Mulatos and La Vigía beaches of the SNNP, located in the department of Nariño, in the southern Colombian eastern tropical pacific (2° 39' 17'' north latitude and 78° 16' 53'' west longitude) (2° 39' 28.7'' north latitude and 78° 18' 14.8'' west longitude), respectively (Fig. 1).

This natural area belongs to the Sanquianga system, a deltaic complex in which the Patía, Sanquianga, La Tola, Aguacatal, Tapaje Viejo and numerous islands converge (Parques Nacionales Naturales de Colombia, 2018). There, the average monthly rainfall regime is between 1032 mm and 2790 mm with a bimodal distribution, but with permanence of rain throughout the year. The multi-annual average temperature ranges between 21.71 °C and 26.09 °C (Mejía, 2011) and the tidal activity is semidiurnal (two high tides and low tides of 12.25 hours), so the representative ecosystem is the tropical humid forest (Parques Nacionales Naturales de Colombia, 2018).

During the 2022 nesting season, the beaches were monitored between October 30th and November 7th, as part of the SNNP sea turtle monitoring program. In these days, samples were taken from individuals located on the beaches in workdays of 6–7 hours per day with different schedules a day, according to the behaviour of tidal activity for the area. Also, on November 1th and 6th, samples of unhatched eggs were collected from the nests present in the turtle farm in the natural area.

Through the Marco permit for the collection of specimens 1070 of August 28th of 2015 given by the National Environmental Licensing Authority of the Republic of Colombia, blood samples were taken from nesting females (n = 4) from the dorsal cervical sinus with a 32-gauge

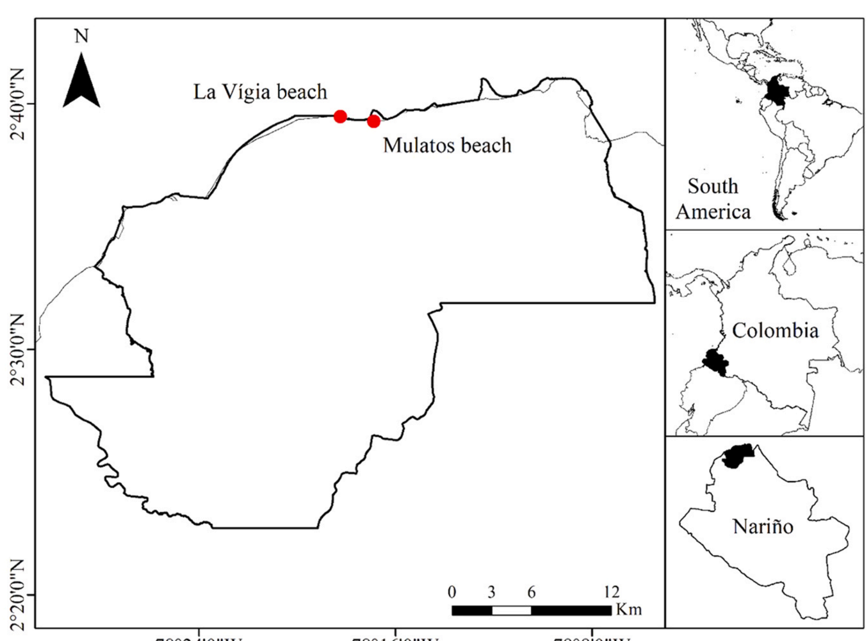

Fig. 1. Location of the study area, Mulatos beach and La Vigía beach, within the Sanquianga National Natural Park, located north of the department of Nariño, Colombia.

hypodermic needle and a 10 mL syringe in 10 mL Vacutainer. Before each extraction, the neck region was disinfected with 70 % antiseptic alcohol. Likewise, from nine nesting females, two eggs were randomly collected from each at the time of oviposition before touching the sand, which were later divided into yolk, albumin, and eggshells. After hatching, 24 unhatched eggs and sand (100 g each, in triplicate) were collected from 16 nests. Content of unhatched eggs were classified (Fig. 2) as developmental stage 1 (S1): eggs without embryo formation and without red or pink pigmentation (n = 12); developmental stage 2 (S2): eggs with embryo formation with carapace and presence of yolk larger than the embryo (n = 6); and developmental stage 3 (S3): eggs with complete embryo formation, larger than stage 2, and yolk smaller than the embryo (n = 6). Because S3 embryos showed developed carapaces, in 3 S3 samples, those were separated from the embryo to be analysed separately, while in the rest, the whole embryo with the carapace we analysed. Detailed information about the samples is shown in Fig. 3.

All samples from nesting females and nests were kept refrigerated (4 °C) until they were transported to the laboratory of the Animal Ecology Research Group of the Universidad del Valle where they were kept frozen until processing.

2.2. Laboratory processing

Once in the laboratory, the samples were dried until constant weight by oven (80 °C - 100 °C) except for the eggshells, which were dried at room temperature for 2–3 months. Subsequently, each sample was homogenized in porcelain mortars, previously washed, and transported through the CITES export permit 47784, issued by the Ministry of Environment and Sustainable Development of Colombia to the Laboratory of Toxicology of the University of Murcia (Spain).

There, the samples were analysed according to a slight modification of the method described by Martínez-López et al. (2019) for the identification and quantification of inorganic elements through inductively coupled plasma optical emission spectrometry (Agilent Technologies ICP-MS. Model 7900). The samples (0.1 g dry weight (dw)) were digested with 4.5 mL of concentrated nitric acid and 1 mL of hydrogen peroxide (69 %, 30 %, respectively) in a closed Teflon PFA container in microwaves (Milestone, Ultrawave Model) for 20 min at 220° C in five times and finally diluted with 10 mL of ultrapure water (18.2 MΩ) from a Merck Milipore Mili-Q Rerefence A+ water purification system. The calibration curve was prepared from a multi-element standard solution (CertiPUR, Merck). Two “blanks” (reagents only) were analysed, and accuracy was verified with Standard Reference Material analysis (Merck ICP IV Certipur Multi-Element Standard Solution, Merck ICP Mercurio Certipur Standard, and Agilent Technologies Initial Calibration Verification Standard) to check reagent purity and contamination. The recoveries ranged from 96.2 % to 104.8 % using fortified continental water and 86.9–107.1 % using a reference biological matrix (mussel tissue, ERM®, certified reference material). The detection limits were 0.000051 μg g^{-1} for As, 0.000025 μg g^{-1} for Cd, 0.000025 μg g^{-1} for Cr, 0.000034 μg g^{-1} for Cu, 0.000007 μg g^{-1} for Hg, 0.000020 μg g^{-1} for Pb, 0.001331 μg g^{-1} for Se, 0.000159 μg g^{-1} for Zn. All identified inorganic element concentrations were expressed in μg g^{-1} dw. For comparisons with other studies, values were converted to wet weight (ww), according to the percentage of moisture found for each sample and reported in Supplementary material (Tables S1 and S2).

2.3. Data analysis

For data analysis, a descriptive statistic (arithmetic means, standard deviations, medians, and ranges) of the concentrations of the inorganic elements was performed. Normality was checked using the Shapiro-Wilk test and since these did not present a normal distribution, the non-parametric Kruskal-Wallis test was used to identify differences between the data sets, accompanied by a Mann-Whitney test with Bonferroni correction to find these differences. Spearman’s test was used to find correlations between elements and between elements and matrices. All analyses were performed using R Studio software version 2022.02.0–443. Results were considered significant when the p value was < 0.05.

In addition, we evaluated the Hg detoxification process through the calculation of the Se:Hg molar ratio described by Mendez-Fernández et al. (2014). This ratio was calculated as Se:Hg (Se (μg g^{-1} dw)/Hg (μg g^{-1} dw))(78.96 (g mol^{-1})/200.59 (g mol^{-1})),where 78.96 g mol^{-1} and 200.59 g mol^{-1} are the atomic masses of Se and Hg, respectively.

3. Results

3.1. Concentrations of inorganic elements

The concentrations of As, Cd, Cr, Cu, Hg, Pb, Se and Zn, and the molar ratio Se:Hg are presented in Tables 1 and 2, and Supplementary material (Table S1 and S2). All the samples presented quantifiable inorganic elements. Overall, clearly Zn, Cr and Se were the elements with the highest mean concentrations, while Hg was the element with the lowest concentration. The essential elements with toxic potential were ranked in the following order: Zn> Cr> Se> Cu (Table 1) and the non-essential elements with high toxicity, in the following order: As> Pb> Cd> Hg (Table 2).

Regarding the samples, carapace of the S3 embryos and blood were the tissues with the highest concentrations of essential and non-essential elements most frequently present, respectively. Sand presented the highest concentrations of Zn, Cr, As and Pb (Tables 1 and 2 and Supplementary material, Table S1 and S2).

Significant differences were found between the matrices (Kruskal-Wallis, $p < 0.05$), being the concentrations of As, Cd, Cu, Hg, Se and Zn in the eggshell significantly different than those in yolk and albumen, and in embryo S1 (Mann-Whitney, $p < 0.05$).

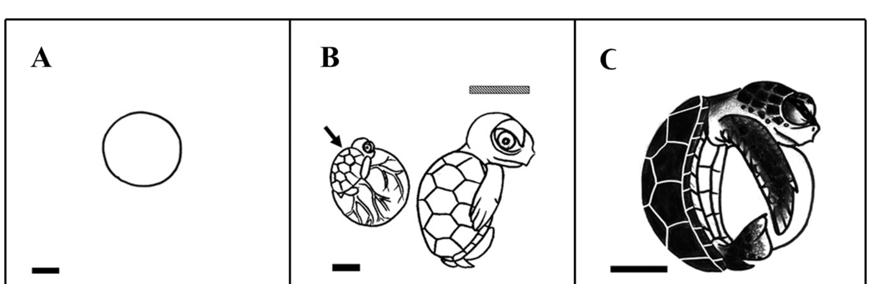

Fig. 2. Different embryonic stages established in the samples of exhumation nests. A) Embryo S1; B) Embryo S2; C) Embryo S3. Taken and modified from Booth et al., (2020).

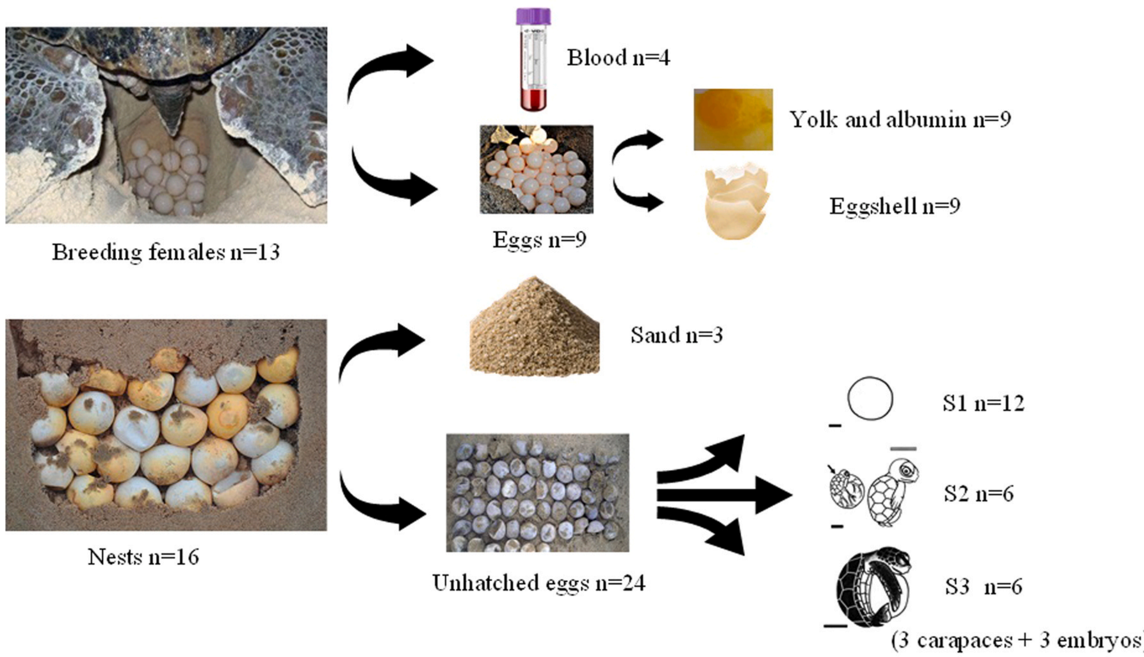

Fig. 3. Classification of samples analysed according to their origin. Image of nesting females: Diario Última Hora. Tortugas marinas, 2019. Image of blood: MedicalExpo,. Image of eggs: Devdmmp, 2020. Image yolk and albumin: L.N.Hurtado-Sierra. Image of nests: iStock Image of unhatched eggs: Sánchez-Laso, 2013 Other images: .Canva, 2024

Table 1
Concentrations of essential elements with toxic potential (μg g^{-1}, dw) in blood, egg content (yolk and albumen), eggshell, embryos in development stage 1 (S1), embryos in development stage 2 (S2), embryos in development stage 3 (S3) and carapaces of embryos in development stage 3 (Carapace S3) of *L. olivacea* and sand present from the SNNP.

Essential elements with toxic potential					
Type of sample	**Statistics**	**Cr**	**Cu**	**Se**	**Zn**
Blood (n = 4)	Mean ± SD	0.23 ± 0.17	3.44 ± 0.17	40.56 ± 14.31	61.86 ± 10.12
	Median	0.21	3.45	39.63	63.58
	Range	0.06–0.45	3.23–3.62	25.82–57.16	48.12–72.16
Yolk and Albumen (n = 9)	Mean ± SD	0.09 ± 0.02	3.69 ± 0.50	8.30 ± 1.90	109.48 ± 18.01
	Median	0.08	3.46	8.83	108.12
	Range	0.06–0.13	3.07–4.55	5.92–11.74	90.55–140.91
Eggshell (n = 9)	Mean ± SD	0.21 ± 0.20	9.88 ± 1.17	2.99 ± 1.85	4.67 ± 1.36
	Median	0.13	9.70	2.66	4.47
	Range	0.07–0.72	8.43–12.21	1.59–7.82	2.85–7.24
Embryo S1 (n = 12)	Mean ± SD	0.57 ± 1.63	4.34 ± 0.46	6.69 ± 1.34	96.44 ± 19.03
	Median	0.09	4.10	6.35	100.56
	Range	0.06–5.74	3.69–4.94	4.87–9.69	73.07–132.90
Embryo S2 (n = 6)	Mean ± SD	0.39 ± 0.74	4.42 ± 0.91	2.52 ± 3.51	136.77 ± 31.32
	Median	0.08	4.32	0.32	139.81
	Range	0.06–1.91	3.46–5.97	0.17–7.12	90.77–166.31
Embryo S3 (n = 3)	Mean ± SD	2.02 ± 1.63	9.61 ± 3.04	11.15 ± 2.74	101.97 ± 5.58
	Median	1.95	10.20	11.13	103.14
	Range	0.43–3.68	6.32–12.30	8.42–13.90	95.90–106.88
Carapace (S3) (n = 3)	Mean ± SD	7.62 ± 5.01	3.42 ± 0.31	8.35 ± 2.56	163.47 ± 27.69
	Median	7.31	3.58	7.78	175.33
	Range	2.77–12.78	3.07–3.62	6.12–11.14	131.83–183.25
Sand (n = 3)	Mean ± SD	93.34 ± 41.01	10.99 ± 2.15	0.85 ± 0.09	96.86 ± 31.62
	Median	97.88	9.76	0.85	111.49
	Range	50.25–131.90	9.73–13.74	0.77–0.94	60.57–118.53

3.2. Relationships among inorganic elements

Ten significant correlations were identified among the elements analysed, of which seven were positive: four of these between As and Cd (*Rho* = 0.607, $p = 0.003$), Se (*Rho* = 0.449, $p = 0.001$), Hg (*Rho* = 0.443, $p = 0.001$) and Pb (*Rho* = 0.421, $p = 0.002$); two between Hg and Cd (*Rho* = 0.606, $p = 0.038$) and Se (*Rho* = 0.517, $p = 0.0001$), the latter being highly significant; and one between Cr and Pb (*Rho* = 0.597, $p = 0.005$). The three negative correlations were found between Cu and Hg (*Rho* = −0.415, $p = 0.003$), Se (*Rho* = −0.472, $p = 0.0006$) and Cd (*Rho* = −0.482, $p = 0.0004$), of which the last two were highly significant.

On the other hand, the mean Se:Hg molar ratio was greater than 1 in all the samples. The mean highest molar ratio was 1163.02 while the lowest was 238.67.

Table 2
Concentrations of non-essential elements with high toxicity and the molar ratio Se:Hg (μg g^{-1}, dw) in blood, egg content (yolk and albumen), eggshell, embryos in development stage 1 (S1), embryos in stage of development 2 (S2), embryos in development stage 3 (S3) and carapace of embryos in development stage 3 (Carapace S3) of *L. olivacea* and sand present from the SNNP.

Non-essential elements with high toxicity						
Type of sample	**Statistics**	**As**	**Cd**	**Hg**	**Pb**	**Ratio Se:Hg**
Blood (n = 4)	Mean ± SD	2.96 ± 2.59	0.67 ± 0.31	0.12 ± 0.07	0.32 ± 0.05	962.62 ± 449.98
	Median	2.80	0.69	0.10	0.33	884.71
	Range	2.37–3.89	0.34–0.94	0.07–0.22	0.24–0.36	548.55–1532.51
Yolk and Albumen (n = 9)	Mean ± SD	0.48 ± 0.13	0.40 ± 0.18	0.03 ± 0.01	0.14 ± 0.02	776.12 ± 233.24
	Median	0.43	0.31	0.02	0.14	815.17
	Range	0.33–0.68	0.15–0.69	0.02–0.05	0.11–0.18	346.06–1024.99
Eggshell (n = 9)	Mean ± SD	0.01 ± 0.01	0.02 ± 0.04	0.01 ± 0.002	0.22 ± 0.12	1163.02 ± 1195.70
	Median	0.01	0.01	0.01	0.16	733.21
	Range	0.01–0.02	0.01–0.12	0.01–0.01	0.13–0.46	618.81–4325.82
Embryo S1 (n = 12)	Mean ± SD	0.35 ± 0.10	0.21 ± 0.05	0.03 ± 0.01	0.16 ± 0.06	528.68 ± 114.58
	Median	0.33	0.22	0.04	0.15	532.89
	Range	0.25–0.64	0.15–0.30	0.02–0.04	0.10–0.31	378.34–701.25
Embryo S2 (n = 6)	Mean ± SD	0.30 ± 0.10	0.21 ± 0.11	0.04 ± 0.02	0.17 ± 0.06	244.97 ± 393.90
	Median	0.29	0.20	0.04	0.15	22.19
	Range	0.19–0.47	0.05–0.38	0.02–0.06	0.11–0.28	6.68–971.93
Embryo S3 (n = 3)	Mean ± SD	0.59 ± 0.13	0.81 ± 0.33	0.09 ± 0.02	0.21 ± 0.02	304.41 ± 26.14
	Median	0.56	0.81	0.10	0.21	299.58
	Range	0.48–0.73	0.48–1.14	0.07–0.11	0.20–0.24	281.02–332.63
Carapace (S3) (n = 3)	Mean ± SD	0.58 ± 0.20	0.24 ± 0.04	0.04 ± 0.03	0.35 ± 0.05	660.24 ± 308.10
	Median	0.60	0.26	0.03	0.33	621.45
	Range	0.37–0.76	0.21–0.27	0.02–0.08	0.31–0.40	373.38–985.91
Sand (n = 3)	Mean ± SD	4.96 ± 2.59	0.03 ± 0.00	0.01 ± 0.01	2.48 ± 0.29	238.67 ± 160.92
	Median	3.56	0.03	0.01	2.35	261.42
	Range	3.37–7.95	0.03–0.03	0.01–0.03	2.29–2.82	67.58–387.01

3.3. Relationships of elements among type of samples

3.3.1. Essential elements with toxic potential

Two significant correlations were identified in this group, which were negative and strong. In this way, one correlation was found for Cu concentrations between carapace and the eggshell ($Rho = -1.000$, $p = 0.014$) and for Se concentrations between the carapace and yolk and albumen. ($Rho = -1.000$, $p = 0.012$) (Table 3).

3.3.2. Non-essential elements with high toxicity

Three significant and strong correlations were identified in this group, of which two were negative and one was positive. Thus, negative correlations were found for As concentrations between carapace samples and blood ($Rho = -0.999$, $p = 0.023$) and for Se:Hg ratio with carapace samples and eggshell ($Rho = -1.000$, $p = 0.003$). The positive correlation occurred for Pb concentrations between the carapace and the yolk and albumen ($Rho = 0.997$, $p = 0.049$) (Table 3).

4. Discussion

All inorganic elements were detected in all types of samples analysed. However, the concentration pattern varied according to the chemical nature of the element, as expected, with higher concentrations of essential elements. This tendency normally occurs in nature, because essential elements participate in physiological processes and embryonic development, which can lead to high requirements for these elements in organisms (Esposito et al., 2023).

Table 3
Essential elements with toxic potential and non-essential elements with high toxicity with significant correlations by *L. olivacea* tissue.

Tissue	Elements with positive correlation	Elements with negative correlation
Blood		Se, As
Yolk and Albumen	Pb	
Eggshell		Cu, Se:Hg
Carapace (S3)	Pb	Cu, Se, As, Se:Hg

4.1. Risk assessment of essential elements with toxic potential

In this group, Cr and Cu presented the highest concentrations in the sand (93.34 μg g^{-1}; 10.99 μg g^{-1} dw, respectively), approximately 9 and 5 times higher than those reported in other nesting beaches in Turkey (Yalcın-Oezdilek et al., 2006; Candan et al., 2021), Saudi Arabia (Tanabe et al., 2022) and China (Jian et al., 2021) (see Table S3 for details). This could be given by mineral refining activities (Choppala et al., 2013), such as gold (Telmer and Veiga, 2009; UNEP, 2013); and fossil fuel burning (Choppala et al., 2013), such as coal for cocaine production occurring in the study area (Parques Nacionales Naturales de Colombia, 2018). According to Yalcin-Oezdilek et al. (2006), Cr concentrations equal to and greater than 5.40 μg g^{-1} dw in sand have a negative effect on the hatching success of *Chelonia mydas* hatchlings. In our case, although the concentration of Cr in sand was 14 times higher than that reported by these authors, we cannot attribute this relationship due to the lack of information we have on hatching rates in the study area. On the contrary, Cu has not been related to hatching rate (Jian et al., 2021). However, coinciding with our study, Cu concentrations were higher in the eggshell than in the content of *L. olivacea* reported in Mexico (Páez-Osuna et al., 2010, table S3), probably due to a higher affinity of Cu with the eggshell.

Meanwhile, Se presented the highest concentrations in blood (40.56 μg g^{-1} dw), a value more than 2 times higher than that reported for *Eretmochelys imbricata*, *Caretta caretta* (Mondragón et al., 2023) and *L. olivacea* (Cortés-Gómez et al., 2014; 2018b) (Table S3), Differences in feeding habits of each species is also an important factor that can influence bioaccumulation and biomagnification of contaminants (Gardner et al., 2006). However, both *E. imbricata* and *L. olivacea* have omnivorous habits (Stringell et al., 2016; Montenegro and Bernal, 1982) and *C. caretta* is a carnivorous species (Tomás et al., 2001). Hence, the differences found with other studies indicate that the prey ingested by females at feeding sites may be more contaminated by Se (Tanabe et al., 2022). We hypothesize that this contamination of dams is given by the waste produced in gold and silver mining (Lemly, 2004) carried out in Ecuador (Carling et al., 2013), a potential feeding area for the population of *L. olivacea* nesting in the SNNP. The potential toxicity is not clear, because it has been suggested that they can develop mechanisms to regulate Se concentrations (together with Zn and Cu) as a prevention strategy against toxic effects (Maffucci et al., 2005).

Zinc presented the highest concentrations in the carapace (163.47 μg g^{-1} dw), a value that could not be compared with other studies, because there is no information available for this tissue at this stage of development. However, considering the published information from carapaces of adult individuals, we presume that our concentration is considerably higher than in *C. mydas* (Agostinho et al., 2020), *E. imbricata* (Mondragón et al., 2021) and *C. caretta* (Miguel et al., 2022) (Table S3). We attribute the presence of Zn in the carapace to a possible maternal transfer due to the ingestion of contaminated prey by nesting females and/or to the existence of this element in the sand of the nest, because there are previous studies that have found positive correlations between the concentrations present in the blood of nesting females and eggs of *L. olivacea* and *C. mydas* (Páez-Osuna et al., 2010; Sinaei and Bolouki, 2017) and between the concentrations present in the sand from the nests and eggs of *C. mydas* (Jian et al., 2021).

According to our results, Zn was the most abundant essential element in the analysed samples, which coincides with previous studies (van de Merwe et al., 2009; Joseph et al., 2014; Esposito et al., 2023; Table S3), probably reflecting its importance as a necessary element in physiological processes (Esposito et al., 2023). The high concentration of Zn in the carapace of embryos demonstrates its vital participation in growth (Huang et al., 2010) and embryonic development (Esposito et al., 2023) and in keratin synthesis (Prasad, 2006). Thus, in sea turtle embryos it can be found up to 10 times higher Zn than in the rest of marine fauna (Sakai et al., 2000a) and present, usually, in higher concentrations than other elements (Sinaei and Bolouki, 2017). However, there could be a risk for *L. olivacea* embryos given that this Zn concentration found in the carapace of embryos is significantly higher than that reported in adult carapaces, and concentrations equal to 6.33 μg g^{-1} dw of Zn in bird eggshells are positively correlated with their thickness (Rodríguez-Navarro et al., 2002), which may represent a threat to reproductive success.

4.2. Risk assessment of non-essential elements with high toxicity potential

In this group of elements, As presented the highest concentrations in the sand of the nests (4.96 μg g^{-1} dw). This concentration is 0.5 times higher than that those reported in a "moderately contaminated" nesting beach in Saudi Arabia (Tanabe et al., 2022) and lower, but very close to that reported in a nesting beach in China (Zhang et al., 2022) (Table S4). This high concentration has been attributed by some authors to gold mining activities (Eisler, 2003; Reis et al., 2007) such as those that occur near the study area (Parques Nacionales Naturales de Colombia, 2018). It is important to not underestimate the effects of this concentration on *L. olivacea* embryos nesting in this sand, because studies have shown that in substrates contaminated with As, reptile embryos have been found to accumulate considerable concentrations of this element (Marco et al., 2004). However, despite the lack of information on the presence of As in sea turtle embryos, there are studies that relate the presence of As with renal mineralization in *D. coriacea* embryos (Dennis et al., 2020) and deficiency in the transfer of Cu to the eggshell of *C. mydas* due to the exposure of this element from the nest sand (Morão et al., 2024), which could affect the hatching success of the embryos.

Cadmium presented the highest concentrations in S3 embryos (0.81 μg g^{-1} dw). However, due to the lack of studies on the presence of this element at this stage of development, comparisons were only performed with yolks and albumens. Hence, the concentration found in the yolk and albumen (0.24 μg g^{-1} ww) was 6 times higher than in those of *D. coriacea* eggs in South Africa (Du Preez et al., 2018), but lower than in eggs of the same species from Costa Rica (7 times; Roe et al., 2011), and of *C. mydas* and *E. imbricata* eggs in Indonesia (9 times; Tapilatu et al., 2020) (Table S4). According to Páez-Osuna et al. (2010), egg laying is not the main route of elimination of non-essential elements such as Cd in *L. olivacea*, but of essential elements such as Cu, Zn and Ni, due to the requirements for the offspring. The effects of Cd on sea turtles include haematological decreases (blood cells and globulin) with species differences (Komoroske et al., 2011; Perrault et al., 2017; Cortés-Gómez et al., 2018b), associated with anaemia (Camacho et al., 2013). Its exposure in *C. mydas* hatchlings has been associated with deviations in the sexual ratio (Barraza et al., 2023). However, the intensity of these effects in sea turtles is unclear since, as documented, Cd does not biomagnify nor bioaccumulate (Gray, 2002) and there is no tendency to find it in higher concentrations with increasing age in species such as *C. mydas* and *E. imbricata* (Pople et al., 1998; Sakai et al., 2000b; Anan et al., 2001), so its long-term effect on *L. olivacea* embryos nesting in the SNNP could not be certain.

On the other hand, Hg presented the highest concentrations in the blood of nesting females (0.12 μg g^{-1} dw), a value that was higher than that reported for *C. caretta* in Brazil (Miguel et al., 2022), and for *E. imbricata* and *C. mydas* in Mexico (Mondragón et al., 2023), although similar to *L. olivacea* in Mexico (Cortés-Gómez et al., 2021) (Table S4). Although Hg was the element with the lowest concentration in this study, it was 7 times higher than the concentration reported for *C. mydas* on a nesting beach in Brazil with the presence of iron mining (Miguel et al., 2022, Table S4). As mentioned above, feeding habits do not seem to be a cause of differences in the compared studies, except for *C. mydas*, an herbivorous turtle. Hence, similarly to Se, we attribute Hg high concentrations to the consumption of prey contaminated with this element by nesting females, due to the tendency of Hg to bioaccumulate and biomagnify through the food chain (Day et al., 2005; Komoroske et al., 2011; Miguel et al., 2022). Indeed, gold mining activities that make use of this element (Esdaile and Chalker, 2018) are known to be carried out near the natural area (Parques Nacionales Naturales de Colombia, 2018). Although Hg was the least abundant element in the samples analysed, the concentrations found should be considered with caution, because even at very low concentrations, it can cause high susceptibility to presenting infections and tumours in the sea turtles, as documented by Day et al. (2007). Taking into account the exposure that is currently occurring of this element in the study area, as indicated by its high concentration in the blood, it is important not to underestimate the effects if its presence is maintained over time, due to its bioaccumulative nature.

Finally, Pb presented the highest concentrations in the nest sand (2.48 μg g^{-1} dw), higher than that reported in a nesting beach in Saudi Arabia (0.46 μg g^{-1} dw) (Tanabe et al., 2022), but 2 times lower than in a beach in Turkey (4.04 μg g^{-1} dw) (Candan et al., 2021) and 8 times lower than reported in China (16.68 μg g^{-1} dw) (Zhang et al., 2022) (Table S4). This concentration can probably be caused by the use of gasoline (Mao et al., 2009) during the cocaine hydrochloride production processes historically carried out in the study area (Parques Nacionales Naturales de Colombia, 2018). According to our results, Pb was an element with low concentrations, which could be explained by the moderate regulation through homeostatic processes of this element by *L. olivacea* (Maffuci et al., 2005; Páez-Osuna et al., 2010). However, given the ban on the use of leaded gasoline for more than a decade in Colombia (Decreto 1530), it is possible that Pb concentrations are currently decreasing and that the concentrations found for this element correspond to its accumulation produced in previous years in the sediment of the study area. Its effect on sea turtles at high concentrations includes reproductive, immunological, endocrine, and developmental alterations and the presence of cancer (Grillitsch and Schiesari, 2010), but these are not expected in this population due to Pb.

4.3. Relationships among inorganic elements

Similarly to Cortés-Gómez et al. (2021) the Se-As relationship was significant and positive in *L. olivacea*. However, the highly significant and positive Se-Hg correlation has not yet been reported for the species. According to Zwolak and Zaporowska (2012), the interactions between Se and As and Hg are given by the antagonistic role of Se on the pro-oxidant and genotoxic effects of As and Hg. In the case of Se:Hg molar ratio, which was greater than 1 in all samples, it would indicate that there is sufficient Se available to bind to Hg in the detoxification process (Martínez-López et al., 2019). Thus, Se concentrations may be counteracting the Hg concentrations and therefore potentially decreasing its toxicity and impact on the health of *L. olivacea* (Dietz et al., 2000; Perrault et al., 2011).

The significant and positive correlations between the non-essential elements with high toxicity (Hg-Pb, Hg-Cd, As-Cd) given in this research, suggest plausible similarities in the detoxification mechanisms of these elements (Jakimska et al., 2011a, 2011b). In accordance with what has been reported for the Hg-Cd relationship, this assessment has been evidenced in juvenile individuals of *C. caretta,* where significant hormonal changes accelerated metabolic activities and the parallel accumulation of Hg and Cd in the tissues (Storelli et al., 1998). Hence, we suggest that the tendency to find the Hg-Cd relationship in this study may be due to the metabolic increase in the formation of eggs and in the growth and development of neonates that may be increasing the parallel accumulation of these elements in the blood of nesting females and in S3 embryos. Similarly, the significant and positive correlation between Cr and Pb, also documented in *C. mydas* (Jian et al., 2021) could suggest similarities in the transport mechanisms of these two elements, as indicated by the high concentrations found in the sand of our research.

4.4. Relationships of elements among samples

We found that Cu, Se, As, Pb and the Se:Hg ratio were significantly and negatively correlated with the embryos' carapace, except for Pb, which was positive. However, we did not find any correlation with sand. In general, we suggest that the lack of correlation with sand could be due to the low number of sand samples or to the influence that local contamination could have on the concentrations of inorganic elements found in this *L. olivacea* population. This could be supported by the correlation found of these elements with the carapace, whose association would indicate that the concentrations in our study have maternal origin, through the transfer during the formation of the eggs. In this way, essential elements such as Cu, with a negative correlation for the carapace, are necessary elements for foetal growth and development (Wallace et al., 2005), which can be transmitted by the female to the embryo. Contrary to the correlation of Cu, the positive association of Pb with the carapace could not be related to the embryogenesis process. Probably due to the structural similarity with Ca, it would be transferred through the female's blood to the embryos (Fossette et al., 2007; Caut et al., 2006) for the formation of bones and carapace (Bilinski et al., 2001).

On the other hand, the negative correlation between Se and As with the S3 carapace and blood may suggest a possible maternal transfer of Se and As to the embryos, due to the exposure of these elements by mining activities (Reis et al., 2007) carried out at the feeding sites of nesting females.

5. Conclusion

This is the first study that reports the exposure to inorganic elements of a species of sea turtle in Colombia, filling the gap of knowledge for the population of *L. olivacea* present in the SNNP, thus presenting the beginning of a database for the area. According to our results, *L. olivacea* present in the SNNP show higher concentrations of inorganic elements than those reported previously worldwide. However, the concentrations tend to be higher in the sand of the nests and, to a lesser extent, in the blood of nesting females, probably because of the anthropogenic activities carried out near and within the study area.

In general terms, although the concentrations of most of the elements evaluated were higher than those of other zones and turtle species in the world, these comparisons may be inaccurate due to the low representativeness of some samples, the different behaviour of the species, the conditions of the feeding and nesting areas, as well as the variation in the methods of collecting and processing the samples. Likewise, although the non-essential elements were identified at low concentrations compared to the essential ones, the effects of contamination of feeding and nesting areas on the population of *L. olivacea* present in the SNNP are unknown due to the lack of information about toxic reference limits of these elements in sea turtles. It is considered important to monitor the population present in the SNNP, based on the results provided by this research.

CRediT authorship contribution statement

Emma Martínez-López: Writing – review & editing, Supervision, Funding acquisition. **Pilar Gómez-Ramírez:** Writing – review & editing, Supervision, Methodology. **Alan Giraldo:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Laura Nathalia Hurtado-Sierra:** Writing – original draft, Formal analysis, Data curation. **Adriana Azucena Cortés-Gómez:** Writing – review & editing, Supervision, Conceptualization. **Juan José Gallego-Zerrato:** Writing – review & editing, Formal analysis.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rsma.2024.103980.

Data availability

Data will be made available on request.

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