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RESEARCH ARTICLE

Essential and trace metals in a post-nesting olive ridley turtles (*Lepidochelys olivacea*) in Ceuta beach, Sinaloa, Mexico

Vicente Olimón-Andalón[1] · Jorge Valdés-Flores[1] · Cesar Paul Ley-Quiñonez[2,3] · Alan A. Zavala-Norzagaray[2,3] · A. Alonso Aguirre[4] · Nidia León-Sicairos[5] · Jorge Velázquez-Román[5] · Hector Flores-Villaseñor[5,6] · Erika Acosta-Smith[5] · Igmar Sosa-Cornejo[1] · Marco Valdez-Flores[5] · Catherine Edwina Hart[3] · Adrian Canizalez-Román[5,7]

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Abstract
Trace metals have been found in sea turtle blood and tissues and may represent a threat to these endangered species. Essential trace metal (Cu, Zn Cd, Pb, As, and Hg) concentrations were determined in blood of adult female, post-nesting olive ridley turtles *Lepidochelys olivacea* (n = 35) on Ceuta beach, Sinaloa, Mexico. Essential metals (Zn and Cu) analyzed were found in higher concentrations than toxic metals (Cd and Pb), while As and Hg concentrations were below the limits of detection (0.01 μg g^{-1}). Low Pb concentrations (0.09 μg g^{-1}) were previously observed in sea turtles in the Gulf of California. There were no significant correlations found between curved carapace length (61.00–71.00 ± 2.29) vs metal concentrations ($p > 0.05$). Cd levels were relatively high when compared to other species and populations of sea turtles worldwide and Cd may represent the greatest risk for sea turtles in the Mexican Pacific. Such concentrations of Cd may pose a further risk to sea turtles through bioaccumulation from the nesting female to offspring which may affect embryo development.

Keywords Cadmium, · Ecotoxicology, · Blood, · Sea turtle, · Bioaccumulation, · Toxicity

Introduction

Ocean pollution is a global problem (Wilcox and Aguirre 2004). One major group of environmental contaminants are metals and metalloids; both documented to be a threat to humans, domestic animals, wildlife, and ecosystems (Ross et al. 2017). Multiple studies have detected trace metals in marine sediments (Páez-Osuna et al. 2017), aquaculture products (Delgado-Alvarez et al. 2015a, 2015b), and fish (Soto-Jimenez et al. 2003, 2011; Quintero-Alvarez et al. 2012) in the Gulf of California.

The trace metals most commonly studied in ecotoxicology are Hg, Pb, As, and Cd, which are considered toxic and a threat to marine vertebrates (Godley et al. 1999a; Yokel et al. 2006; Eisler 2010; Jerez et al. 2010). Studies also focus on Zn and Cu which are essential for the development and functioning of physiological processes and are two of the most abundant elements in organisms (Ley-Quinonez et al. 2013; da Silva et al. 2014; Ley-Quinonez et al. 2017); however, at high concentrations, both have toxic effects (ATSDR 2004, 2005). Such pollutants are transferred along the food chain resulting in bioconcentration and bioaccumulation,

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✉ Adrian Canizalez-Román
canizalez@uas.edu.mx

1 Programa Doctorado en Ciencias Biológicas, Facultad de Biología, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, Mexico

2 Instituto Politécnico Nacional, CIIDIR-SINALOA, Guasave, Sinaloa, Mexico

3 Investigación, Capacitación y Soluciones Ambientales y Sociales A.C. (ICSAS), 63160 Tepic, Mexico

4 Department of Environmental Science and Policy, George Mason University, Fairfax, VA, USA

5 CIASaP, School of Medicine, Autonomous University of Sinaloa, 80246 Culiacan, Sinaloa, Mexico

6 Secretariat of Health, The Sinaloa State Public Health Laboratory, 80020 Culiacan, Sinaloa, Mexico

7 Secretariat of Health, The Women's Hospital, 80127 Culiacan, Mexico

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representing a health risk to higher-level organisms such as endangered sea turtles (Godley et al. 1999a, b; Storelli and Marcotrigiano 2000; Aguirre et al. 2006; Frías-Espericueta et al. 2006; Gardner et al. 2006; Cortés-Gómez et al. 2017; Ley-Quinonez et al. 2017).

The Gulf of California ecoregion is home to five species of sea turtles including the leatherback *(Dermochelys coriacea)*, green (*Chelonia mydas*), loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), and olive ridley (*Lepidochelys olivacea)* turtles (Briséño 2006; Zavala-Norzagaray et al. 2007a) (Lemus and López 2002; Zavala-Norzagaray et al. 2007b). Of these species, olive ridleys are the most abundant, using extensive foraging areas and nesting beaches within the state (Briséño 2006; Zavala-Norzagaray et al. 2017), like Ceuta beach, located in the state of Sinaloa, Mexico, which is catalogued as a sea turtle sanctuary and priority beach for *L. olivacea turtle* nesting, with a mean of 621 nests laid annually over an extension of 35 km of beach (CONANP 2009; Sosa-Cornejo et al. 2016).

Olive ridley turtles are classified as endangered (SEMARNAT 2010) in Mexico; whereas internationally, they are listed as vulnerable (IUCN 2014); however, in coastal areas of the Gulf of California, the illegal consumption of sea turtle meat and eggs is extensive to date (Mancini and Koch 2009; Senko et al. 2009; Mancini et al. 2011).

On the other hand, previous studies in Sinaloa have documented metal accumulation in both olive ridleys, stranded dead on Ceuta nesting beach (Frías-Espericueta et al. 2006) and captured live, in foraging areas (Zavala-Norzagaray et al. 2014). However, further studies are needed to provide information on the possible bioaccumulation of trace metals in nesting females and the risk associated with the transfer of these contaminants to their offspring (Páez-Osuna et al. 2010a, b, 2011).

The objective of this study was to determine the concentration of trace metals in the blood of a post-nesting olive ridley turtle population on Ceuta beach, Sinaloa, Mexico.

Materials and methods

Between August and September 2018, 35 blood samples were taken from post-nesting olive ridley turtles nesting at Ceuta beach, Sinaloa, Mexico (length: 35 km, 23° 58' 54" N 107° 03' 00" W and 23° 43' 00" N 106° 50' 00" W) (Fig. 1). A total of 5 ml of blood was collected from the dorsal cervical sinus of each turtle using a 21 gauge needle and 10-ml syringe and then transferred into a tube with EDTA as anticoagulant (Owens 1999; Ley-Quinonez et al. 2017). Samples were stored in refrigeration at 4 °C until laboratory processing. Curved carapace length (CCL) and width (CCW) were measured for each turtle after nesting was concluded and were used to determine age (Gardner et al. 2006; Páez-Osuna et al. 2010a, b; Cortés-Gómez et al. 2014). Individual turtles

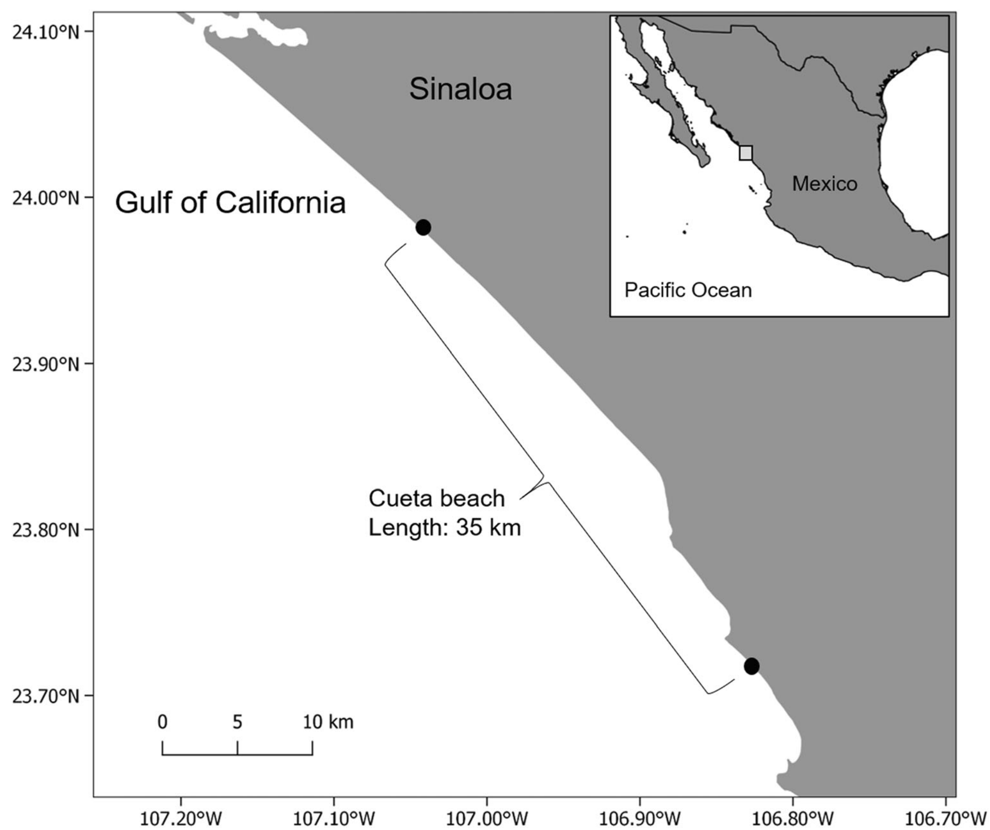

Fig. 1 Ceuta Beach, Sinaloa, Mexico. Nesting area of sea turtle *Lepidochelys olivacea*

were subjected to a detailed physical examination to document any skin damage, tumors, flipper amputations, physical anomalies, emaciation, weakness, and epibiotic load (Deem et al. 2009; Espinoza-Romo et al. 2018). Once this procedure was completed, the turtles were released unharmed to continue their return crawl to the sea.

In the laboratory, 0.5 g of blood (wet weight) was processed using an acid mixture of 5 mL of HNO_3, HCI, and H_2O_2 (proportion 2:2:1) in a microwave system (*MARS Xpress,* CEM) during 35 min (Ley-Quinonez et al. 2011, 2013, 2017; Zavala-Norzagaray et al. 2014). During the digestion process, two replicates of the reference material (RM) TORT-3 (National Research Council of Canada, Ottawa) were used to identify the evaporation and recovery percentage, and two blood samples were added with a standard multielement SIGMA 6000 (PerkinElmer) (0.06 μL) to identify equipment efficiency and possible interference generated by the analysis tissue. Four targets were placed (deionized water), to identify possible contamination and ensure analysis accuracy.

Trace metal concentrations (based on wet weight) were determined with an optical emission inductively coupled plasma atomic spectrophotometer (ICP-AES) model OPTIMA 4300TM DV (PerkinElmer). Calibration curves were made for the spectrophotometer using a multielement standard SIGMA 6000 (PerkinElmer). Limits of detection (LOD) were established using the wavelength (mm) recommended by the distributor (0.01 μg g^{-1} for Cd, As, and Hg, for Ni and Pb, and 0.04 μg g^{-1} for Zn, Cu, Mn, and Se). Recovery of RM (TORT-3) and standard repetitions added were between 84 and 98%.

Mean, range ± standard deviation (SD), and minimum and maximum concentrations (min-max) in micrograms per gram (μg g^{-1}) were calculated. Kolmogorov-Smirnov test was used as a normality test. The Pearson's correlation coefficient test was used to establish relationships among metal concentrations or metals vs turtle morphometry; and p *values* < 0.05 were considered statistically significant. Statistical analyses were performed using Minitab® 17.1.0 (Minitab Inc., State College, PA, USA).

Results and discussion

During this study, all turtles were healthy with no injuries or deformities and zero to low epibiotic load. Turtles had a mean CCL of 65.52 cm (61.0–71.0 cm ± 2.29) and CCW of 70.08 cm (63.0–74.0 cm ± 3.10).

Essential trace elements (Zn and Cu) were found in higher concentrations (9.43 μg g^{-1} ± 6.56 and 2.11 μg g^{-1} ± 0.94, respectively) than toxic metals (Cd 0.61 μg g^{-1} ± 0.51 and Pb 0.099 μg g^{-1} ± 0.063) (Table 1). The concentration of metals in blood of post-nesting sea turtles decreased in the following order Zn > Cu > Cd > Pb. As and Hg concentrations were below of limit detection (BLD). No relationships among metals or metals concentration vs turtle size were observed ($p > 0.05$) (Table 2).

Table 1 Concentration of trace metals (μg g^{-1}) wet weight) in blood of nesting olive ridley turtles (*Lepidochelys olivacea*) collected in Ceuta beach, Sinaloa, Mexico, 2019

Variable	Mean ± SD	Min–max
Zn	9.430 ± 6.560	1.50–26.73
Cu	2.118 ± 0.945	0.56–3.83
Pb	0.099 ± 0.069 (10)	0.00–0.19
Cd	0.619 ± 0.518 (1)	0.03–1.70
As	LOD	-
Hg	LOD	-

LOD limit of detection

The data are presented as mean ± SD or range in parentheses. na, number of samples above the LOD in parenthesis if ≤ 16

Cu concentration was relatively high compared to other studies (Table 3), yet overall, there is little variation in levels between sea turtle species (Páez-Osuna et al. 2010a; van de Merwe et al. 2010; Camacho et al. 2013; Ley-Quinonez et al. 2013; Trocini 2013; Zavala-Norzagaray et al. 2014; Ley-Quinonez et al. 2017), except for leatherback turtles that have high levels of Cu perhaps related to their diet which is primarily based on gelatinous zooplankton (Guirlet et al. 2008; Xu et al. 2011; Ley-Quinonez et al. 2013). In general, Cu concentrations in blood are low for all sea turtle species, with all available studied reporting concentrations below 6.0 μg g^{-1} (Kenyon et al. 2001; Guirlet et al. 2008; Páez-Osuna et al. 2010a; van de Merwe et al. 2010; Ley-Quinonez et al. 2011; Ley-Quinonez et al. 2013; Zavala-Norzagaray et al. 2014). According to Ley-Quinonez et al. (2013) and Zavala-Norzagaray et al. (2014), these concentrations observed in post-nesting females at Ceuta beach can be considered "normal values" in sea turtle.

Zn is an essential element for normal growth and physiological processes within organisms and is therefore present in higher concentrations than other metals (Ruelas-Inzunza et al. 2005; Cornish et al. 2007; Elorriaga-Verplancken and Aurioles-Gamboa 2008; Griesel et al. 2008; Sinaei and

Table 2 Pearson' correlations among trace metals in blood of the olive ridley turtle (*Lepidochelys olivacea*)

	Zn	Cu	Pb	Cd	LCc (cm)
Cu	0.204				
Pb	−0.228	−0.212			
Cd	0.206	0.023	0.981	−0.107	
Acc (cm)	0.240	0.211	0.250	−0.124	0.731

Significant correlation at level 0.05

Table 3 Trace metals concentrations in sea turtle blood compared to levels found in the present study of nesting olive ridley turtles (*Lepidochelys olivacea*) at Ceuta beach, Sinaloa, Mexico, 2019

Species		Zn	Cu	Cd	Pb	Author
L. olivacea	Mexico	9.43 ± 6.56	2.11 ± 0.94	0.61 ± 0.51(1)	0.099 ± 0.06 (10)	*This study*
L. olivacea	Mexico	37.12 ± 3.67	1.02 ± 1.47	1.33 ± 0.20	BLD	Zavala-Norzagaray et al. (2014)
L. olivacea	Mexico	10.43 ± 4.12	0.60 ± 0.11	0.17 ± 0.10	0.02 ± 0.01	Cortés-Gómez et al. (2014)
L. olivacea	Mexico	58.4 ± 4.7	2.28 ± 0.40	0.45 ± 0.20	0.19 ± 0.03	Páez-Osuna et al. (2010a, 2010b)
C. mydas	Mexico	63.58 ± 17.06	1.71 ± 0.73	0.99 ± 0.35	BLD	Ley-Quinonez et al. (2013)
C. mydas	Mexico	13.92 ± 0.49	NA	0.06 ± 0.00	NA	Labrada-Martagón et al. (2011)
C. mydas	Iran	36.78 ± 3.20	2.01 ± 0.23	0.37 ± 0.02	0.77 ± 0.20	Sinaei and Bolouki (2017)
C. mydas	Australia	7.54 ± 063	0.97 ± 0.09	0.33 ± 0.009	NA	van de Merwe et al. (2010)
C. caretta	Mexico	44.81 ± 17.53	2.83 ± 0.62	1.80 ± 0.63	BLD	Ley-Quinonez et al. (2011)
C. caretta	Spain	4.97 ± 2.9	1.27 ± 8.46	0.29 ± 0.25	0.06 ± 0.02	Camacho et al. (2013)
C. caretta	Australia	11.54 ± 1.77	0.67 ± 0.13	0.30 ± 0.30	0.02 ± 0.01	Trocini (2013)
D. coriacea	French Guiana	44.4 ± 1.12	5.36 ± 1.12	0.32 ± 0.12	NA	Guirlet et al. (2008)
L. kempii	Mexico	7.50	0.52	NA	0.01	Kenyon et al. (2001)

Concentration in μg g^{-1} ; NA not analyzed, BLD below of limit detection

The data are presented as mean ± SD or range in parentheses. na, number of samples above the BLD in parenthesis if ≤ 16

Bolouki 2017). Zn concentrations obtained in this study are similar to those observed previously in nesting olive ridley turtles in the southern Mexican Pacific (10.43 μg g^{-1}) (Cortés-Gómez et al. 2014) and higher than levels found in others sea turtles species sampled on foraging grounds (Kenyon et al. 2001; van de Merwe et al. 2010; Camacho et al. 2013). However, this may be due to the geographical location and characteristics of the foraging area and appear not to represent a health risk to sea turtles (van de Merwe et al. 2009a; Páez-Osuna et al. 2010a; Zavala-Norzagaray et al. 2014).

Recently, an increasing trend in Cd, Pb, and Zn metal concentrations has been reported in the coastal areas of the Mexican Pacific, notably in the states of Sinaloa and Nayarit which may present a risk of toxic effects on the region's ecosystem health (Páez-Osuna 2014; Vazquez Botello et al. 2014). With regard to Gulf of California's food webs, biomagnification in organisms of Cd and Zn has been observed, while Cu shows a partial biomagnification (Jara Marini et al. 2014; Páez-Osuna 2014). However, Pb has been shown to biodilute through trophic levels (Szefer et al. 2006; Soto-Jiménez et al. 2008; Ruelas-Inzunza et al. 2010; Jara Marini et al. 2014). According to Soto-Jiménez et al. (2008), Pb concentrations in the Gulf of California were primarily linked to drilling activities for the production of gasoline in Mexico and the USA back in the 1980s. However, the current use of fuels low in Pb has resulted in a decrease of this metal in the ocean and its tropic chains (Páez-Osuna 2014).

Compared to other metals, low Pb concentrations have also been observed in sea turtle blood (Ley-Quinonez et al. 2011, 2013; Zavala-Norzagaray et al. 2014) and tissues (Gardner et al. 2006) in the Gulf of California. However, Pb levels found in this study (0.099 ± 0.063 μg g^{-1}) were higher than those reported by Cortés-Gómez et al. (2014) yet lower than concentrations reported by Páez-Osuna et al. (2010b) (Table 3); both of these studies were conducted on nesting olive ridley turtles in the Mexican Pacific. Páez-Osuna et al. (2010b) concluded that the Pb concentrations in sea turtles from Oaxaca (0.19 ± 0.03 μg g^{-1}) are a reflection of the low Pb concentrations in the area, and such levels do not represent a health risk for the nesting population of olive ridley turtles in this region.

Cd is the metal that poses the greatest environmental health hazard to both humans and animals due to its toxicity. For that reason, it is of great interest in ecotoxicology (Storelli and Marcotrigiano 2003; Storelli et al. 2008), particularly in wildlife (Camacho et al. 2013; Cortés-Gómez et al. 2018) as the main source of exposure to Cd is through trophic chains (van de Merwe et al. 2009b; Ley-Quinonez et al. 2017; Ross et al. 2017). We found higher Cd concentrations than those reported in sea turtle blood from other regions (Kenyon et al. 2001; Guirlet et al. 2008; Páez-Osuna et al. 2010b; van de Merwe et al. 2010; Labrada-Martagón et al. 2011; Camacho et al. 2013; Trocini 2013; Cortés-Gómez et al. 2014; Sinaei and Bolouki 2017). Also, Cd was at lower levels than those previously reported in the same species and region but on foraging grounds (Zavala-Norzagaray et al. 2014). Cd levels were also lower than concentrations found in other sea turtle species from the same region (Ley-Quinonez et al. 2013; Ley-Quinonez et al. 2017) (Table 3). According to Storelli and

Marcotrigiano (2003), Cd accumulates in higher concentrations in carnivorous organisms, particularly those that feed on calcareous species such as crabs and mollusks (Storelli et al. 1998a; Ley-Quinonez et al. 2011). Other studies indicate that organisms that feed in semi-enclosed ecosystems tend to be more exposed to metal bioaccumulation processes (Sakai et al. 2000b; Storelli et al. 2008; Ley-Quinonez et al. 2013). The Gulf of California is an example of this, with previous studies showing Cd biomagnification through the regions trophic webs, with organisms at higher levels presenting greater concentrations of this metal (Jara Marini et al. 2014). This trend for high Cd concentrations has been documented in all sea turtle species found in the Gulf of California (Frías-Espericueta et al. 2006; Gardner et al. 2006; Kampalath et al. 2006; Ley-Quinonez et al. 2011; Zavala-Norzagaray et al. 2014). Also, these levels are the highest found in sea turtles globally; this is particularly evident in loggerhead turtles, a carnivorous species. Storelli et al. (2005) mentioned that Cd concentrations in sea turtles are a reflection of the contaminant load in foraging areas; however, Cd bioavailability in marine organisms is mainly due to global factors and does not necessarily reflect areas with high anthropogenic activity but also oceanic natural contributions. Organisms in the Pacific Ocean present a higher Cd concentrations than those in the Atlantic Ocean (Fraga et al. 2018). Cd concentrations in nesting populations of olive ridley turtles may represent a risk for turtle hatchlings after maternal transfer (Páez-Osuna et al. 2010a).

Hg and As are considered among the most important toxic elements in the environment (Yokel et al. 2006; Páez-Osuna 2014); however, in our study, As and Hg concentrations in blood were BLD. As concentrations in sea turtles show variation by species and food source (Storelli et al. 1998a; Storelli and Marcotrigiano 2000; Zavala-Norzagaray et al. 2014; Cortés-Gómez et al. 2017; Ley-Quinonez et al. 2017). Different studies have reported As levels on olive ridleys, representing low concentrations in blood and other tissues (1.19 to 3.34 $\mu g\ g^{-1}$) compared to other sea turtle species (Cortés-Gómez et al. 2014, 2017; Zavala-Norzagaray et al. 2014). According to Saeki et al. (2000), sea turtles that feed on algae and mollusks accumulate high levels of As, while omnivorous species, like olive ridleys (Márquez 1990; Páez-Osuna et al. 2010b), tend to accumulate lower levels of this metalloid (Agusa et al. 2008).

On the other hand, Hg concentrations in different sea turtles tissues tend to be $< 0.5\ \mu g\ g^{-1}$ (Storelli et al. 1998b; Sakai et al. 2000a; Anan et al. 2002; Maffuci et al. 2005; Kampalath et al. 2006; Innis et al. 2008; Day et al. 2010; Jerez et al. 2010; van de Merwe et al. 2010; Cortés-Gómez et al. 2017). Hg levels in sea turtle from the Gulf of California present concentrations BLD (Ley-Quinonez et al. 2011, 2013; Zavala-Norzagaray et al. 2014); however, even in low concentrations, this metal causes subtle negative impacts on sea turtle immune function depending on the type of Hg speciation like methylmercury (Day et al. 2007).

In sea turtles, the curved carapace length (CCL) is used to determine age (Gardner et al. 2006; Páez-Osuna et al. 2010a, 2010b; Cortés-Gómez et al. 2014), and according to Hart et al. (2014), the CCL observed in the turtles studied (65.52 cm) are similar to the nesting olive ridley turtles in the Mexican Pacific (64 cm). Statistically, there was no significant correlation found between CCL vs metal concentrations ($p > 0.05$), possibly because there was little difference between the sizes of the studied organisms, since they were all adult female nesting turtles. However, these results are consistent with the previous studies in the same species in the Mexican Pacific (Frías-Espericueta et al. 2006; Páez-Osuna et al. 2010a, 2010b; Zavala-Norzagaray et al. 2014) and other sea turtles (Gardner et al. 2006; Ley-Quinonez et al. 2011; Ley-Quinonez et al. 2013, 2017). On the other hand, metal concentrations in blood of sea turtles in the Atlantic Ocean demonstrate positive correlation to size, particularly Pb (Kenyon et al. 2001; Wang 2005; Fussy et al. 2007; Camacho et al. 2012, 2013). This relationship may be due to oil drilling and extraction activity, being one of the main sources of contamination in the area. Oil contamination increases levels of toxic metals such as Ni, Cd, and Pb (Vazquez et al. 2002; Turner and Rabalais 2019) with potential risks for marine biota (Villanueva and Botello 1992; Botello et al. 2005; Ruiz-Fernández et al. 2019).

Conclusions

Blood has been successfully used to study trace metals found in sea turtles and, unlike other tissues, provides information on recent exposure to these environmental contaminants (Day et al. 2005; Ley-Quinonez et al. 2013, 2017; Bucchia et al. 2015). Metal concentration in blood is also correlated to that in other tissues, which further promotes its use in bioaccumulation studies (van de Merwe et al. 2010).

Our study suggests that toxic metals, including As and Pb, do not represent a risk to the health of post-nesting olive ridley turtles sampled at Ceuta beach as levels of these metals were low and were of similar concentrations to those found in other sea turtle species in the Gulf of California. Hg concentration was below LOD which corroborates the previous studies which found this element at low levels in sea turtles from the Mexican Pacific. However, it is important to consider that metal speciation occurs in Hg and this plays an important role in metal toxicity, particularly methylmercury, which even at low levels, can be harmful to organisms. Therefore, future research should consider the study on Hg speciation. In contrast, Cd levels were relatively high when compared to other species and populations of sea turtles worldwide and Cd may represent the greatest risk for sea turtles in the Mexican

Pacific. Such concentrations of Cd may pose a further risk to sea turtles through bioaccumulation from the nesting female to offspring which may affect embryo development.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Cesar Paul Ley-Quiñonez, Jorge Valdes-Flores, and Adrian Canizalez-Roman. The first draft of the manuscript was written by Vicente Olimon-Andalon and Cesar Paul Ley-Quiñonez, and all authors commented on the previous versions of the manuscript. All authors read and approved the final manuscript.

Conceptualization: Alfredo Alonso Aguirre, Cesar Paul Ley-Quiñonez, Jorge Valdes-Flores, and Adrian Canizalez-Roman

Methodology: Vicente Olimón-Andalón, Jorge Valdés-Flores, Hector Flores-Villaseñor, Erika Acosta-Smith, Marco Valdez-Flores and Igmar Sosa-Cornejo

Formal analysis and investigation: Nidia León-Sicairos, Catherine Edwina Hart and Alan Zavala-Norzagaray

Writing, original draft preparation: Vicente Olimón-Andalón, Jorge Valdés-Flores and Cesar Paul Ley-Quiñonez

Writing, review and editing: Alfredo Alonso Aguirre, Catherine Edwina Hart, Nidia Leon-Sicairos and Canizalez-Román

Supervision: Adrian Canizalez-Román

Data availability Most data collected in this study is presented in the current manuscript. Raw data is also available upon request.

Declarations

Ethical issues The research was approved by the Mexican Environment and Natural Resources Ministry (SEMARNAT); sampling, handling, and care of individuals were carried out under the proper research permits: SGPAC/DGVS/08562/17 and SGPA/DGVS/010518/18. Our study complied with all local, state, and national regulations. Meticulous efforts were made to assure that animals were subjected to the least suffering possible, as well as to reduce external stress, pain, and discomfort sources.

Conflict of interest The authors declare that they have no conflict of interest.

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