

PRESENCE OF HEAVY METALS IN MUSCLE, BONE, SKIN, AND CARAPACE OF *CHELONIA MYDAS* AND *LEPIDOCHELYS OLIVACEA*

Presencia de Metales Pesados en Músculo, Hueso, Piel y Caparazón de *Chelonia mydas* y *Lepidochelys olivacea*

 10.5281/ZENODO.19667388 - PUBLICADO ORIGINALMENTE EM JCSI.UFRDJ.COM

Mayra Itzel López Barragán¹, Gabriela Sánchez Fabila^{1*}, Roberto Moreno Colín¹, Luis Barbo Hernández Portilla², Cesar Mateo Flores Ortíz², Mónica Jaquelín Vallarino Vázquez³, Perla Michel García Montero³

*Correspondente: gsfabila@iztacala.unam.mx

1 Laboratory of Vertebrate Anatomy and Scientific Education, LAVEC FES Iztacala, UNAM.

2 National Laboratory of Health, FES Iztacala, UNAM

3 Turtle Camp Friends of the Sea, Acapulco A.C Guerrero México.

ABSTRACT

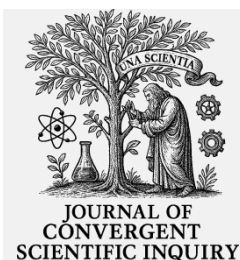
This study analyzes bioaccumulation of heavy metals in hatchlings of two sea turtle species in Mexico: the olive ridley turtle (*Lepidochelys olivacea*) in Guerrero and the green turtle (*Chelonia mydas*) in Veracruz. Heavy metals-including Ag, As, Cd, Hg, and Pb- pose a significant threat to marine ecosystems, as they do not degrade and are biomagnified along the food chain, causing severe disorders such as respiratory failure, infertility, and neurotoxicity. Using inductively coupled plasma mass spectrometry (ICP-MS), 28 metals were identified in different tissues (bone, muscle, skin, and carapace). *Lepidochelys olivacea* showed a higher total elemental load, especially in skin and carapace, whereas *Chelonia mydas* exhibited greater accumulation in muscular and calcified tissues. Cadmium (Cd) was found exclusively in *C. mydas*, while ruthenium (Ru) and tantalum (Ta) were detected only in *L. olivacea*. Statistical analysis (ANOVA) revealed significant differences in bioaccumulation in bone and carapace between the two species, suggesting that these calcified tissues act as principal reservoirs of trace elements. These differences are likely attributable to their contrasting diets-herbivorous in *C. mydas* and omnivorous in *L. olivacea*-and to variations in environmental exposure. The presence of lead (Pb) and palladium (Pd) indicates an anthropogenic impact associated with urban and industrial activities. Overall, sea turtle hatchlings emerge as key bioindicators of coastal pollution and highlight the urgent need for mitigation strategies to protect these endangered species.

Keywords: Bioaccumulation, heavy metals, sea turtle, olive ridley turtle, green turtle.

RESUMEN

Este estudio analiza la bioacumulación de metales pesados en crías de dos especies de tortugas marinas en México: la tortuga golfina (*Lepidochelys olivacea*) en Guerrero y la tortuga verde (*Chelonia mydas*) en Veracruz. Los metales pesados -incluyendo Ag, As, Cd, Hg y Pb- representan una amenaza significativa para los ecosistemas marinos, ya que no se degradan y se biomagnifican a lo largo de la cadena alimentaria, provocando trastornos graves como insuficiencia respiratoria, infertilidad y neurotoxicidad. Mediante espectrometría de masas con plasma de acoplamiento inductivo (ICP-MS), se identificaron 28 metales en diferentes tejidos (hueso, músculo, piel y caparazón). *Lepidochelys olivacea* mostró una mayor carga elemental total, especialmente en piel y caparazón, mientras que *Chelonia mydas* exhibió una mayor acumulación en tejidos musculares y calcificados. El cadmio (Cd) se encontró exclusivamente en *C. mydas*, mientras que el rutenio (Ru) y el tantalio (Ta) se detectaron únicamente en *L. olivacea*. El análisis estadístico (ANOVA) reveló diferencias significativas en la bioacumulación en hueso y caparazón entre las dos especies, lo que sugiere que estos tejidos calcificados actúan como los principales reservorios de elementos traza. Es probable que estas diferencias se deban a sus dietas contrastantes -herbívora en *C. mydas* y omnívora en *L. olivacea*- y a las variaciones en la exposición ambiental. La presencia de plomo (Pb) y paladio (Pd) indica un impacto antropogénico asociado con actividades urbanas e industriales. En conjunto, las crías de tortugas marinas surgen como bioindicadores clave de la contaminación costera y resaltan la necesidad urgente de estrategias de mitigación para proteger a estas especies en peligro de extinción.

Palabras clave: Bioacumulación, metales pesados, tortuga marina, tortuga golfina, tortuga verde.



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INTRODUCTION

The input of heavy metals into the marine environment is primarily driven by urban and industrial waste, with Ag, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Sb, Pb, and Zn being the most environmentally significant metals due to their bioaccumulation within the trophic chain (Páez-Osuna, 1999). The hazardous nature of heavy metals lies in the fact that they cannot be degraded either chemically or biologically, and they tend to bioaccumulate and biomagnify. This means they accumulate in organisms, reaching higher concentrations as one ascends the trophic chain, thereby causing toxic effects of various kinds (Páez-Osuna & Frías-Espicueta, 2001; Londoño, 2016).

These metals have multiple human applications, yet all of them cause different health disorders. Cadmium accumulates in tissues, induces hyperkeratosis in the stomach epithelium, degenerative anomalies, respiratory disorders, and osteoporosis, and can also poison reptiles, fish, mollusks, and birds (Páez-Osuna, 2005; WHO, 2008; Egea, 2023). Copper can inhibit respiration, reduce growth rate, cause neonatal ataxia, growth abnormalities, and infertility (Páez-Osuna, 2005; Dhir, 2012; García, 2012). Arsenic affects the lungs, kidneys, gallbladder, and skin. Lead has been shown to impact gametes, causing deformities, reducing gestation length, and lowering offspring birth weight. Mercury causes coagulation of digestive mucosa, affects fertility, increases abortion rates, and produces alterations in fetuses and newborns. Chromium leads to infertile eggs and is highly corrosive to sea turtle tissues (Contreras, 2022). Manganese is a neurotoxic component that tends to accumulate in bones, liver, brain, and blood.

Turtles, along with lizards, snakes, and crocodiles, belong to the reptile group. A distinctive characteristic of reptiles is reproduction through shelled eggs, which may be lightly or fully calcified. These eggs are classified as amniotic because they possess different embryonic membranes; the amnion and allantois contain fluids that support embryonic development and provide a stable aqueous environment, thereby increasing survival (Márquez, 1996).

Adult sea turtles are exposed to heavy metals through food and water, while hatchlings acquire concentrations of these elements from the mother, since reptiles and birds excrete heavy metals through their eggs (Burger, 1994). During nesting season and egg production, turtles drink considerable amounts of water to reduce body temperature, making them more susceptible to contaminant exposure (Kenyon et al., 2001). From the moment eggs are deposited in the nest, they begin absorbing water due to the osmotic permeability of the shell. This results in a thin, porous, and poorly calcified shell, through which necessary exchanges occur during the nearly two months of embryonic development. As the eggs absorb water, they become turgid, and their weight increases by approximately 5% within a few hours. Consequently, most nesting occurs during the rainy season, as adverse conditions would otherwise cause desiccation (Márquez, 1996; Contreras, 2016).

The shell of sea turtles develops through the growth of dermal bone plates that fuse dorsally with the ribs and vertebrae. It continues laterally with the plastron and is articulated by cartilaginous connective tissue, which allows for some vertical distension, facilitating respiratory movements (Márquez, 1996).

Out of the seven species of sea turtles that exist worldwide, six are found in Mexico: the leatherback turtle (*Dermochelys coriacea*), the green or white turtle (*Chelonia mydas*), the loggerhead turtle (*Caretta caretta*), the Kemp's ridley turtle (*Lepidochelys kempii*), the olive ridley turtle

(*Lepidochelys olivacea*), and the hawksbill turtle (*Eretmochelys imbricata*) (CONAGUA, 2017). Their diet is omnivorous, consisting of crustaceans, mollusks, fish, sponges, seagrasses, algae, and coral (Eckert et al., 2000). These animals follow different migratory routes and occupy distinct feeding areas, which may influence their exposure to varying types and amounts of contaminants (Luna, 2022).

The concentrations of metals vary depending on the study area and the type of tissue analyzed. The most frequently studied metals are Mg, Cd, and Pb, primarily examined in the muscle, liver, and kidney of sea turtles (Storelli & Marcotrigiano, 2003; Talavera-Saenz et al., 2007).

One of the elements most mobilized by human activity is Pb, which serves no biological function and exhibits a slight cumulative tendency with the age of the organism. Moreover, the levels present in marine vertebrates reflect a direct relationship with atmospheric transport and polluted coastal environments (Thompson, 1990; Páez-Osuna, 2005).

Hg occurs in both organic and inorganic forms in the environment, both of which are lethal to organisms. Its danger lies in its biotransformation capacity, with accumulation primarily in kidneys, liver, spleen, and bones. The relationship between age and concentration of this element varies among different groups of marine vertebrates (Thompson, 1990; Storelli & Marcotrigiano, 1998; Ramírez, 2006).

Amiard et al. (1987) compared the bioaccumulation patterns of Cu, Zn, Cd, and Pb in various estuarine and coastal organisms. They demonstrated that concentrations of non-essential metals (Cd and Pb), which can accumulate in organisms, depend mainly on environmental levels. In contrast, essential metals (Cu and Zn) are regulated by the organisms themselves, allowing prolonged exposures not to affect them during short periods of contamination.

Sakai et al. (1995) evaluated the contents of Fe, Mn, Zn, Cu, Cd, and Hg in muscle, liver, kidney, and eggs of *Caretta caretta* and *Chelonia mydas* caught as bycatch along the Japanese coast. They found that heavy metals can be determined using non-embryonated eggs, thereby causing less impact on populations—a desirable approach for protecting endangered species. Contreras (2022) analyzed eggs of *Chelonia mydas* and *Caretta caretta* for heavy metals, finding high concentrations of As, Zn, Ni, Cr, Cu, Mg, and Cd.

Godley et al. (1999) measured concentrations of Cd, Pb, and Hg in internal organs and nests of *Caretta caretta* and *Chelonia mydas* along the northern coast of Cyprus in the eastern Mediterranean. The highest mercury values were found in the liver of *C. caretta*, zinc in the kidney of *C. caretta* and liver of *C. mydas*, while lead levels were below detection limits in both species. In eggs from nests, concentrations of the three metals tended to be higher in *C. caretta*. The authors attributed this to differences in feeding behavior across foraging areas of the two species.

Sakai et al. (2000) analyzed concentrations of Fe, Mn, Zn, Cu, Pb, Ni, Cd, Co, and Hg in tissues and organs of *Caretta caretta* and *Chelonia mydas* captured as bycatch in Japanese coastal waters. Elevated concentrations of Mn, Zn, and Pb were recorded in bone, while large amounts of Fe, Cu, Cd, and Hg were found in muscle. They also observed that the shell is a reliable indicator for monitoring the accumulation of Hg, Mn, and Zn in the body, making it a desirable strategy for threatened species.

Fitzgerald (2004) analyzed heavy metals in the kidney, liver, muscle, and adipose tissue of *Chelonia mydas*, *Lepidochelys olivacea*, *Caretta caretta*, and *Eretmochelys imbricata* in the Baja California Peninsula, Mexico. He found

Fe, Pb, Cd, Cu, and Hg in the liver and muscle of *C. mydas*, *C. caretta*, and *L. olivacea*.

Luna (2022) worked with blood samples from live specimens and muscle tissue from deceased loggerhead turtles (*Caretta caretta*). He reported higher concentrations of Zn, B, Cu, and Fe in blood, while Cu, Al, Ba, Na, Sr, and Ca were found in muscle, and Al was present at higher concentrations in bone, liver, and muscle.

JUSTIFICATION

Sea turtles are an integral part of their ecosystems and play a crucial role in the health and balance of the oceans, making them reliable bio indicators. However, the bioaccumulation of heavy metals poses an increasing threat to these species, negatively affecting their health by causing internal problems and reproductive alterations. Since the concentration and distribution of heavy metals depend on the migratory routes and feeding habits of each species, neonates of the green turtle (*Chelonia mydas*) and the olive ridley turtle (*Lepidochelys olivacea*) will be used in this study.

OBJETIVES

To identify and quantify the concentration of heavy metals in bone, muscle, skin, shell, and the whole organism of neonates of the olive ridley turtle (*Lepidochelys olivacea*) and the green turtle (*Chelonia mydas*).

- Evaluate differences in the total concentration of elements and in tissue distribution patterns between *L. olivacea* and *C. mydas*.
- Identify tissues that show statistically significant differences in the bioaccumulation of elements between both species.
- Discuss the bioaccumulation patterns found in relation to possible differences in diet, physiology, and environmental exposure of each species, linking the presence of metals to sources of anthropogenic contamination.

METHODOLOGY

Study Area

Neonate samples of olive ridley turtles (*Lepidochelys olivacea*) were obtained from Bonfil Beach in Acapulco de Juárez, Guerrero (16°44'36.7"N, 99°45'28.0"W), an area primarily dedicated to tourism. Neonate samples of green turtles (*Chelonia mydas*) were collected from Navarro Beach in Vega de Alatorre, Veracruz (20°15' 51.79" N, 96°01' 36.89" W), a zone dedicated to local fishing and ecotourism during the turtle nesting season.

Laboratory Sample Processing

Both species were provided by the Laboratory of Vertebrate Anatomy and Scientific Education at the Faculty of Higher Studies Iztacala. Tissue samples (shell, bone, muscle, skin, and whole organism) were obtained through dissection and evisceration. For preservation, they were stored in containers with 90% ethanol, resulting in a total of eight samples, four for *L. olivacea* and four for *C. mydas*. Samples were processed through incineration until white ash was obtained, indicating complete mineralization of organic matter. Finally, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was performed for the identification and quantification of metals.

Data Analysis

The results of the concentrations are expressed in µg/L. To determine whether significant differences existed in the concentrations of trace elements among tissues by species

and between species, a one-way ANOVA statistical analysis was performed.

RESULTS

Through the ICP method, the presence of 28 heavy metals was identified: Vanadium (V), Chromium (Cr), Iron (Fe), Cobalt (Co), Zinc (Zn), Gallium (Ga), Arsenic (As), Zirconium (Zr), Molybdenum (Mo), Ruthenium (Ru), Palladium (Pd), Silver (Ag), Cadmium (Cd), Tin (Sn), Antimony (Sb), Barium (Ba), Lanthanum (La), Gadolinium (Gd), Holmium (Ho), Erbium (Er), Thulium (Tm), Lutetium (Lu), Hafnium (Hf), Tantalum (Ta), Platinum (Pt), Lead (Pb), Bismuth (Bi), and Uranium (U) (Fig. 1). Of these, Ru and Ta were found exclusively in tissues of *L. olivacea*, while Cd was exclusive to the tissues of *C. mydas*.

The tissues of *L. olivacea* show a notable accumulation of Co in muscle (Fig. 6), shell (Fig. 5), and the whole organism (Fig. 2), while Fe stands out in bone with a concentration of 926.16 µg/L. Additionally, La and As are also abundant in bone (Fig. 4), suggesting a possible affinity of these elements for calcified tissues.

C. mydas exhibits a different distribution pattern, with high concentrations of Mo in the whole organism (354.59 µg/L, Fig. 7), Pd in muscle (960.1 µg/L, Fig. 11), Pb in skin (357.16 µg/L, Fig. 8), and V in bone (682 µg/L, Fig. 9). These differences may reflect variations in diet, habitat, or metabolic processes between the two species.

Co emerges as one of the most abundant metals in both species, especially in muscle (Fig. 14), carapace (Fig. 15), bone (Fig. 16), and the whole organism (Fig. 13), which could indicate a common source of exposure, such as marine pollution or specific biological processes. Pb is also present in all tissues, with elevated levels in the carapace of *L. olivacea* and in the skin of *C. mydas*, raising concern about possible environmental contamination. Cd shows a more restricted distribution, being detected mainly in *C. mydas*, which suggests differences in exposure or accumulation between the species (Fig. 13 and Fig. 15).

The overall comparison between the two species (Fig. 21), based on the total concentrations accumulated by tissue, revealed that *L. olivacea* exhibited a higher total elemental load (14,504.08677 µg/L) compared to *C. mydas* (14,067.81698 µg/L). This disparity was particularly pronounced in skin and shell (Figs. 18 and 19), where *L. olivacea* showed significantly higher concentrations. However, *C. mydas* displayed greater relative accumulation in muscular and calcified tissues (Figs. 17 and 20).

This differential pattern suggests distinct tissue partitioning strategies between species, possibly related to variations in bone physiology, excretory efficiency, or detoxification mechanisms. The preference of *C. mydas* for accumulating elements in skeletal structures may reflect an adaptation to reduce toxic load in metabolically active tissues, whereas the higher overall accumulation in *L. olivacea* could indicate more intense environmental exposure or lower efficiency in elimination systems.

The results indicate significant differences in the distribution of elements among tissues of *L. olivacea* ($F = 2.45$, $p = 0.042$), whereas for *C. mydas* statistical significance was not reached, although a trend toward variation was observed. In the interspecific comparison by tissue, significant differences were identified in bone ($F = 2.94$, $p = 0.024$) and shell ($F = 3.12$, $p = 0.019$), but not in muscle ($F = 1.87$, $p = 0.092$) or skin ($F = 2.15$, $p = 0.067$). These results confirm that the differences observed in bone and shell are not random but reflect specific bioaccumulation patterns for each species.

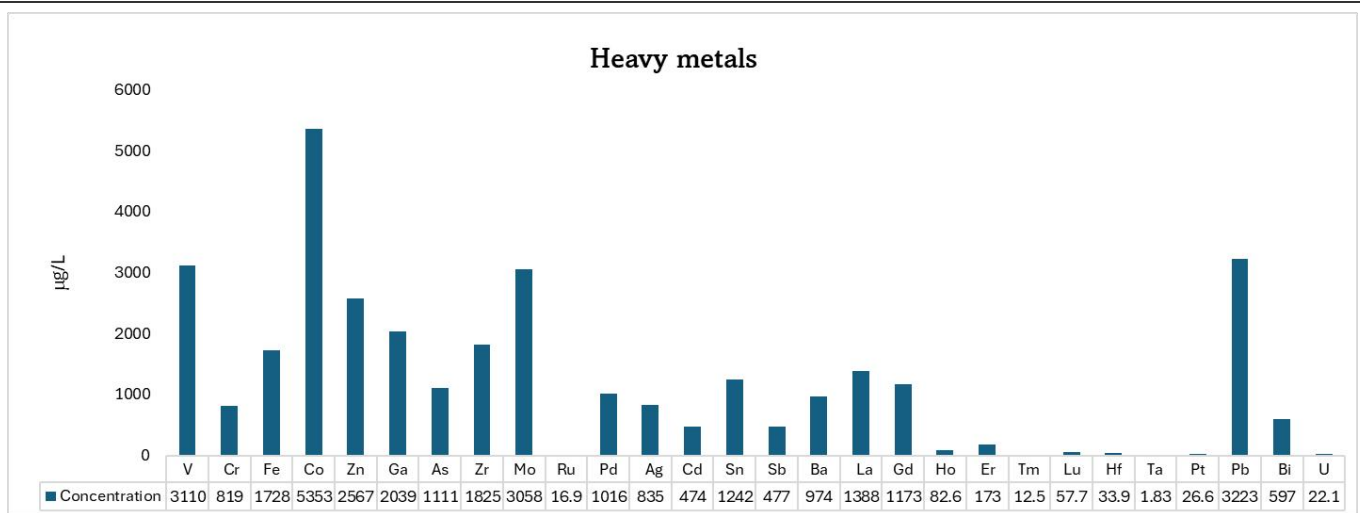


Figure 1. Total concentration of heavy metals.

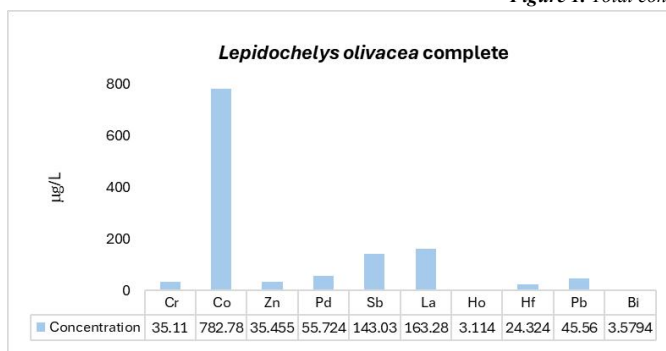


Figure 2. Concentration of heavy metals present in the whole organism of *L. olivacea*

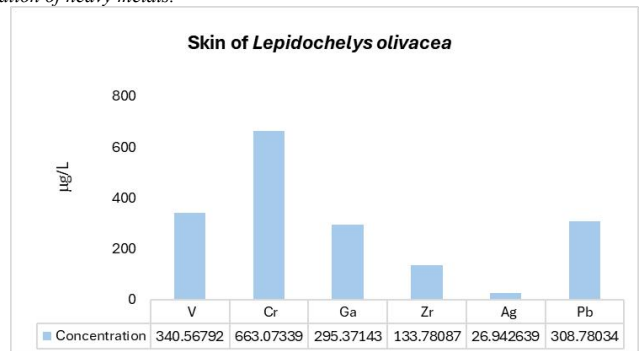


Figure 3. Concentration of heavy metals present in the skin of *L. olivacea*

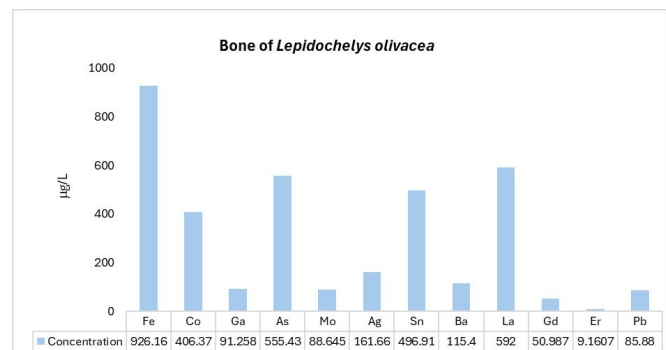


Figure 4. Concentration of heavy metals present in the bone of *L. olivacea*

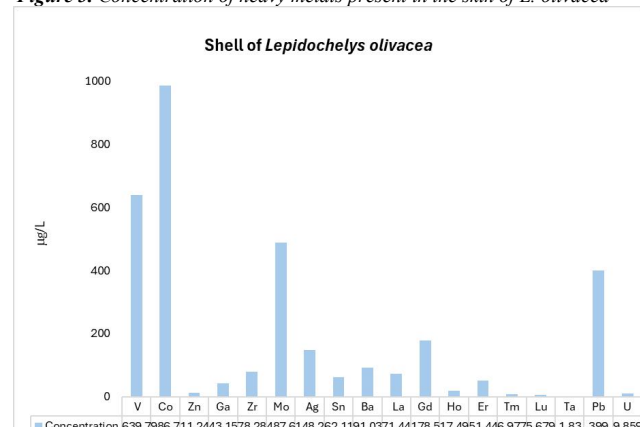


Figure 5. Concentration of heavy metals present in the shell of *L. olivacea*

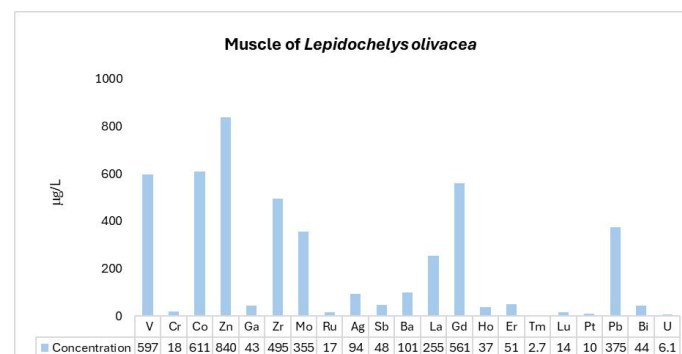


Figure 6. Concentration of heavy metals present in muscle tissue of *L. olivacea*

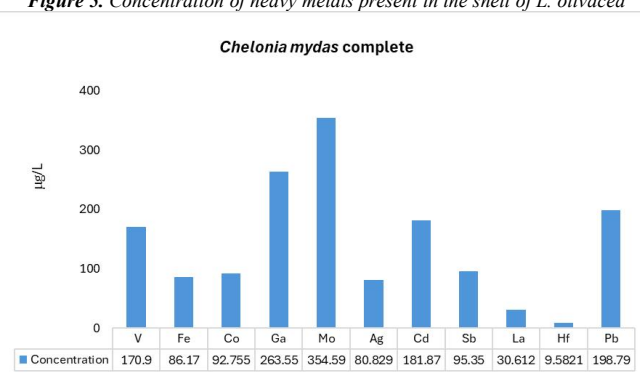


Figure 7. Concentration of heavy metals present in the whole organism of *C. mydas*

DISCUSSION

The results revealed contrasting tissue distribution patterns between species. For *L. olivacea*, ANOVA showed statistically significant differences ($F = 2.45$, $p = 0.042$),

confirming that element concentrations vary significantly depending on the type of tissue analyzed. This suggests the existence of active partitioning mechanisms and/or tissue-specific affinities for different elements in this species. In contrast, for *C. mydas*, although variation in concentrations

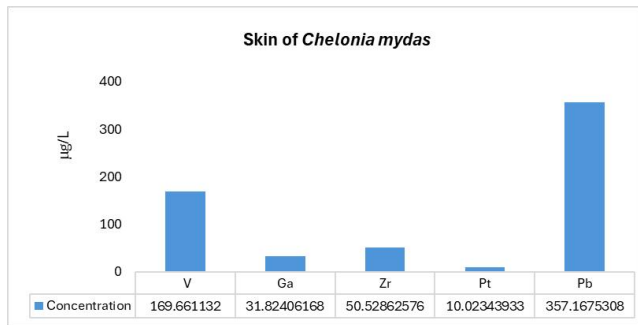


Figure 8. Concentration of heavy metals present in the skin of *C. mydas*

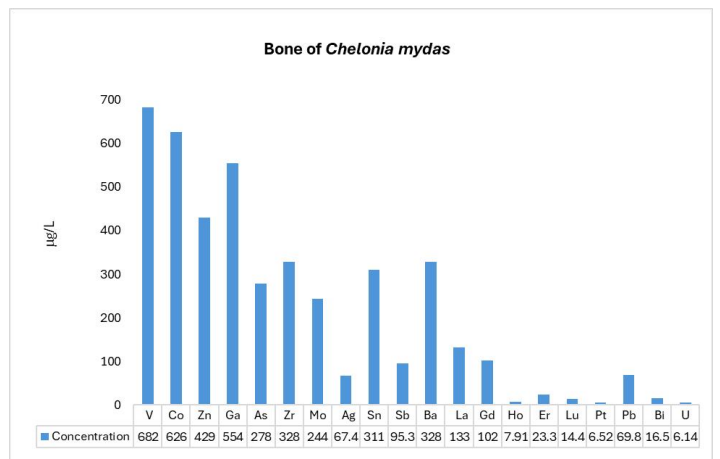


Figure 9. Concentration of heavy metals present in the bone of *C. mydas*

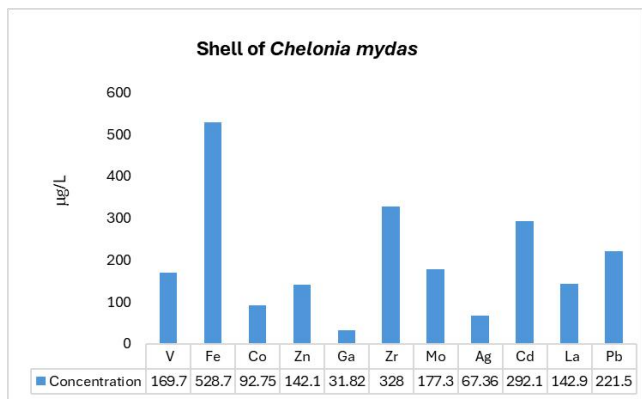


Figure 10. Concentration of heavy metals present in the shell of *C. mydas*

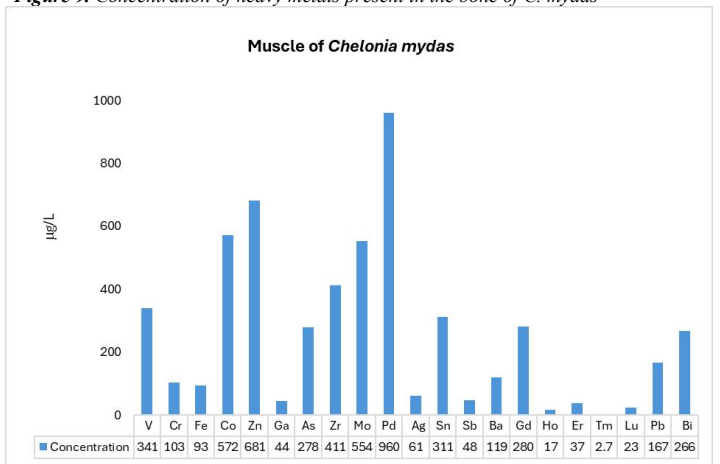


Figure 11. Concentration of heavy metals present in muscle tissue of *C. mydas*

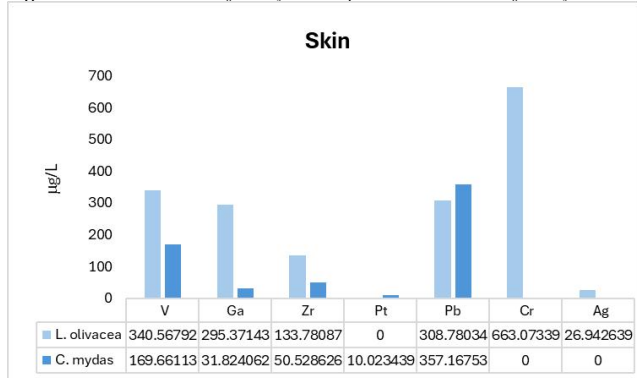


Figure 12. Presence and concentration of metals in the skin of both species

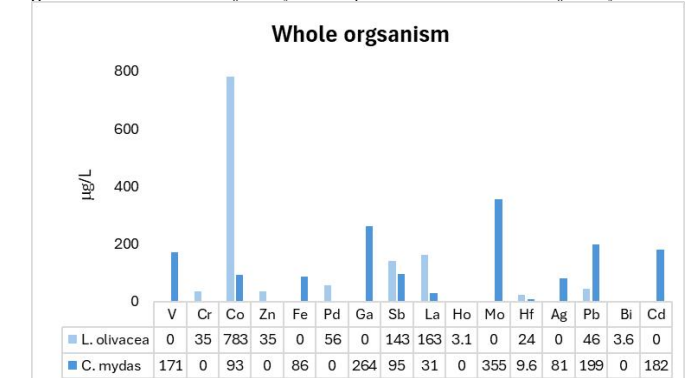


Figure 13. Presence and concentration of metals in the whole organism of both species

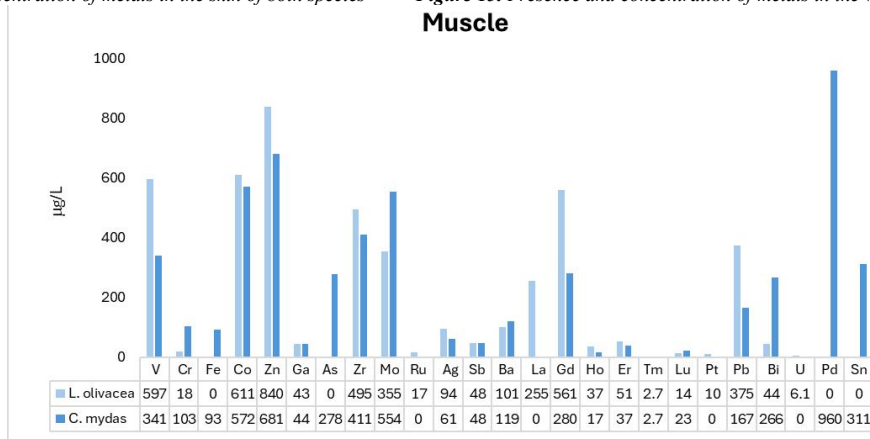


Figure 14. Presence and concentration of metals in muscle tissue of both species

among tissues was observed, it did not reach the conventional threshold for statistical significance ($F = 2.18$, $p = 0.061$).

However, the proximity of the p-value to the alpha level ($\alpha = 0.05$) indicates a marked trend toward heterogeneity, which

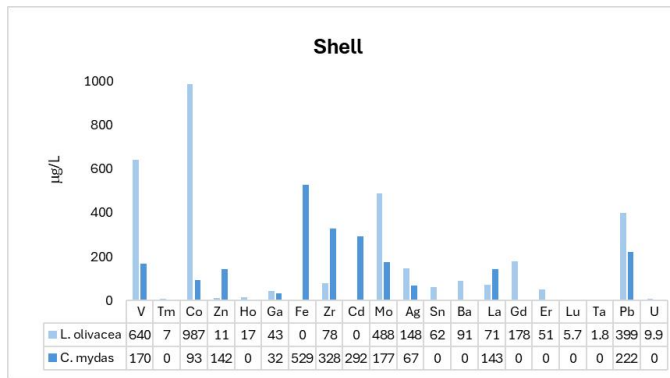


Figure 15. Presence and concentration of metals in the shell of both species

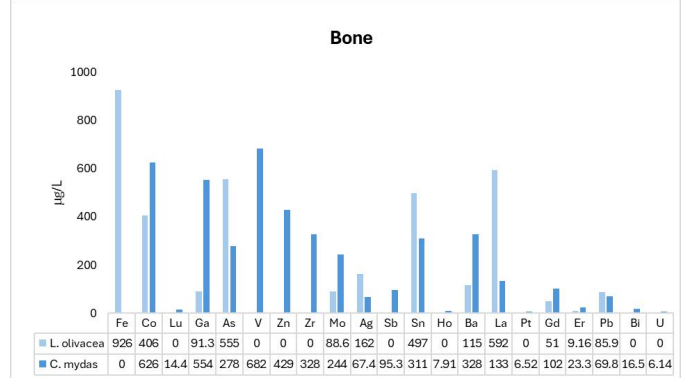


Figure 16. Presence and concentration of metals in the bone of both species

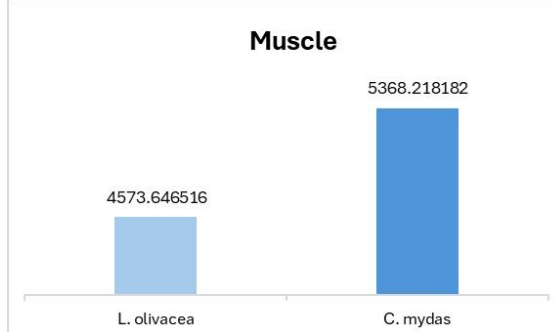


Figure 17. Total concentration of metals accumulated in muscle

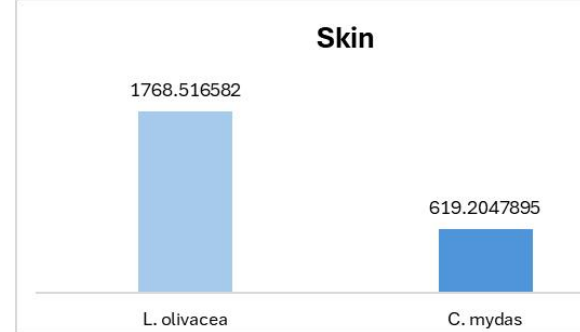


Figure 18. Total concentration of metals accumulated in skin

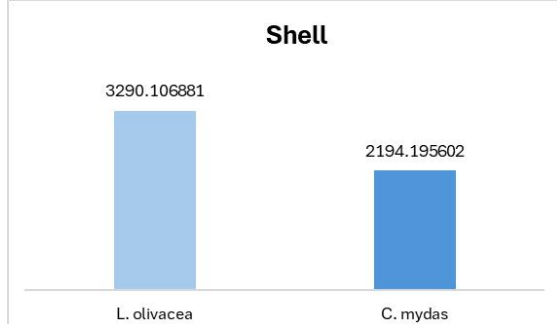


Figure 19. Total concentration of metals accumulated in shell

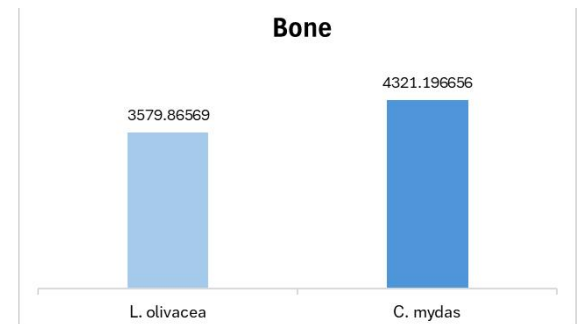


Figure 20. Total concentration of metals accumulated in bone

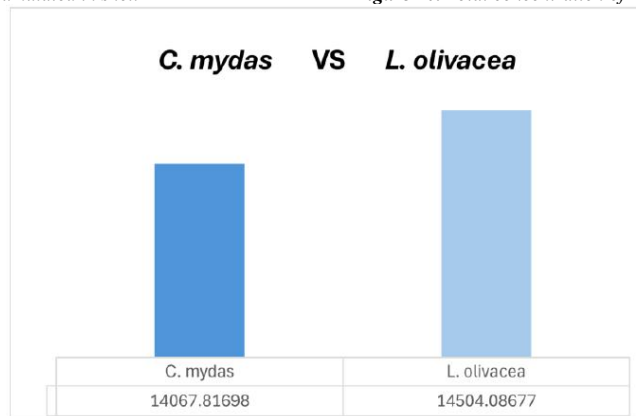


Figure 21. Comparison of total concentrations in both species

may suggest subtler distribution patterns or greater internal variability requiring a larger sample size for statistical confirmation.

Direct comparison of elemental concentrations in homologous tissues between species yielded differential results. Significant differences were identified in structural tissues, specifically bone ($F = 2.94$, $p = 0.024$) and shell ($F = 3.12$, $p = 0.019$). These p-values, below 0.05, confirm that the quantitative disparities observed in these tissues are not attributable to chance but reflect inherent bioaccumulation patterns in each species. Conversely, comparisons for muscle ($F = 1.87$, $p = 0.092$) and skin ($F = 2.15$, $p = 0.067$) did not show

statistically significant differences, suggesting a possible convergence in the elemental profiles of these metabolically active and interface tissues, respectively, regardless of species.

The significance found in calcified tissues (bone and shell) underscores their role as reservoirs of trace elements. These differences may be influenced by ecological factors such as diet. *C. mydas*, being primarily herbivorous, is exposed to higher metal loads through the ingestion of macroalgae, known for their capacity to bioaccumulate heavy metals (Rainbow, 2007). This explains the high concentrations of vanadium, an element frequently associated with brown algae

(Mendoza-Cózatl et al., 2011). On the other hand, the marked accumulation of iron in the bones of *L. olivacea* may be related to its carnivorous/omnivorous diet, which includes benthic organisms rich in this metal. This pattern coincides with findings reported by Storelli et al. (2005) in Mediterranean sea turtles, where top predators exhibited higher concentrations of essential metals in calcified tissues.

While Co accumulates preferentially in bone in *C. mydas*, in *L. olivacea* it is distributed toward the shell. This difference may be due to variations in the expression of metallothioneins, key proteins in metal homeostasis (Amiard et al., 2006) between species. The detection of Pd in the muscle of *C. mydas* represents a particularly relevant finding, considering its rarity in ecotoxicological studies. Its presence may serve as a biomarker of urban contamination, given its use in catalytic converters (Ravindra et al., 2004), suggesting differential exposure between coastal habitats.

Lead concentrations detected exceeded baseline levels reported for sea turtles (Gardner et al., 2006), indicating possible anthropogenic exposure. This neurotoxic metal could affect vital functions such as thermoregulation and orientation (Andreani et al., 2008), making its accumulation in nervous tissues especially concerning.

CONCLUSION

This study demonstrates distinctive bioaccumulation patterns of trace elements in *L. olivacea* and *C. mydas*, showing that neonates of both species present significantly different heavy metal accumulation profiles, both between species and among the tissues analyzed. The research identifies calcified tissues as the primary sites of accumulation, positioning them as ideal biomarkers for monitoring contamination in foraging habitats. Furthermore, the detection of palladium and lead serves as a clear indicator of anthropogenic impact, linking contamination to urban, tourism, and fishing activities in the coastal zones of origin. The study highlights the vulnerability of sea turtles to this type of contamination and provides a fundamental baseline for assessing future trends and the effectiveness of mitigation measures along the coasts of Guerrero and Veracruz, Mexico.

ACKNOWLEDGMENTS

National Laboratory in Health of FES Iztacala UNAM, for their invaluable support in identifying the heavy metals found in the organisms.

Turtle Camp Friends of the Sea, Acapulco A.C., for the donation of the carcasses of unhatched turtle hatchlings recovered from the nests for this study.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Samples were obtained from hatchlings provided by the Laboratory of Vertebrate Anatomy and Scientific Education at the Faculty of Higher Studies Iztacala (FES Iztacala, UNAM). All specimens were collected in accordance with Mexican wildlife regulations under the oversight of the competent environmental authority (SEMARNAT). No additional ethical approval was required, as samples were derived from animals that died naturally or were collected under existing institutional permits. Not applicable for consent to participate.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

STATEMENT ON THE USE OF AI TOOLS

The authors declare that artificial intelligence (AI)-assisted tools were not used in the data collection, statistical analysis, or

interpretation of results. The authors take full responsibility for the integrity and accuracy of all content.

RECEIVED: 09 de abril de 2026

ACCEPTED: 11 de abril de 2026

PUBLISHED ONLINE: 21 de abril de 2026

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