Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2026, 17(11), 694-714
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Physical Abnormalities and Heavy Metal Accumulation in Embryos of Marine Turtle *Chelonia mydas agassizii*—Colola Beach, Southern Mexico

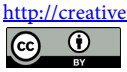
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How to cite this paper: Pérez-Cruz, L., Fuentes-Farías, A.L., Urrutia, A.O., Meléndez-Herrera, E., Montoya-Pérez, R., Gutiérrez-Ospina, G. and Urrutia-Fucugauchi, J. (2026) Physical Abnormalities and Heavy Metal Accumulation in Embryos of Marine Turtle *Chelonia mydas agassizii*—Colola Beach, Southern Mexico. *Advances in Bioscience and Biotechnology*, **17**, 694-714.
https://doi.org/10.4236/abb.2026.1711041

Received: September 28, 2026
Accepted: November 8, 2026
Published: November 11, 2026

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Abstract

Results of a study of heavy metal concentrations in malformed embryos of marine turtle *Chelonia mydas agassizii* from the Colola nesting beach are presented. Finding malformed embryos of marine turtles from artificial nests is increasingly frequent along the Pacific Ocean coast in southern Mexico. The beaches are being affected by coastal developments and increasing activities of extractive mining industries. We analyzed heavy metal concentrations in muscle, carapace and bowel samples dissected from twelve non-hatched embryos recovered from ten artificial nests. The trace element concentrations were determined by inductively coupled plasma-mass spectrometry. Mercury was determined by cold vapor atomic absorption spectrometry. The metals found with highest concentrations among samples were iron, zinc, aluminum and barium, followed by manganese, copper, chromium, vanadium, nickel, lead, cobalt, cadmium, indium, bismuth and mercury. Our results show coincidental occurrences of physical malformations with presence of heavy metals in tissues of *C. mydas agassizii* embryos. The results show individual embryos differed in their levels of heavy metal accumulation and did not accumulate uniformly in the tissues. The study provides critical baseline ecotoxicological

data for sea turtle conservation management along the Pacific coast of Mexico.

Keywords

Colola Beach, Southern Mexico Continental Margin, Phenotypic and Craniofacial Abnormalities, Heavy Metals, Marine Turtles

1. Introduction

Marine turtles are long known for their remarkable navigation abilities and their open sea migrations span hundreds to thousands of kilometers between feeding grounds and nesting beaches [1] [2]. Hatchlings emerge from nests and enter the ocean, where they spend years in pelagic habitats before adult turtles return to coastal feeding grounds. Natal homing relies on a complex integration of sensory abilities that allow turtles to orient and navigate across vast ocean basins [2]-[6].

A number of marine turtle species nest along the coasts of Mexico and several of their populations have been subject of long-term investigations [7]-[9], including the *Chelonia mydas agassizii.* The *C. mydas agassizii* nests along the Michoacan coast in the southern Pacific Ocean margin (**Figure 1**). Crucially, we have observed an increase in the number of malformed embryos of *C. mydas agassizii* recovered from non-hatched eggs in artificial nests in the Colola beach.

Encountering malformed embryos from non-hatched eggs laid by marine turtles is being increasingly documented in a number of species including *Caretta caretta* [10]-[13], *Lepidochelys olivacea* [14] [15] and *Chelonia mydas* [16] [17]. Occurrence of deformed embryos might be related to genetic factors such as interbreeding, mutations and chromosomal aberrations. Non-genetic factors may include environmental effects, nutrition, moisture, hyperthermia, radiation and pollutants. Identifying underlying factors remains difficult, with multiple involved in congenital malformation and embryos exposed to environmental pollutants [18] [19].

Because of their long lifespans, time to reach sexual maturity and their migratory behaviour marine turtles are highly vulnerable to environmental factors. In recent years, heavy metal pollution has received increased attention because of their persistence and bioaccumulation [20]-[22]. Anthropogenic sources of heavy metals include mining, industrial discharges, agricultural runoffs, urban wastewater, oil and gas operations, atmospheric transport and harbor and marine traffic. Marine turtles are particularly vulnerable to heavy metal pollution at sea because they occupy a high position in the trophic chain and higher concentrations of heavy metals in the coastal nesting areas because of extractive industries and coastal area urban development, with large-scale mining operations for iron, lead, zinc, silver and gold [14] [19] [23] [24].

Marine turtles accumulate heavy metals through ingestion of contaminated prey, polluted sediment, seawater uptake and maternal transfer to eggs [25]. Adult

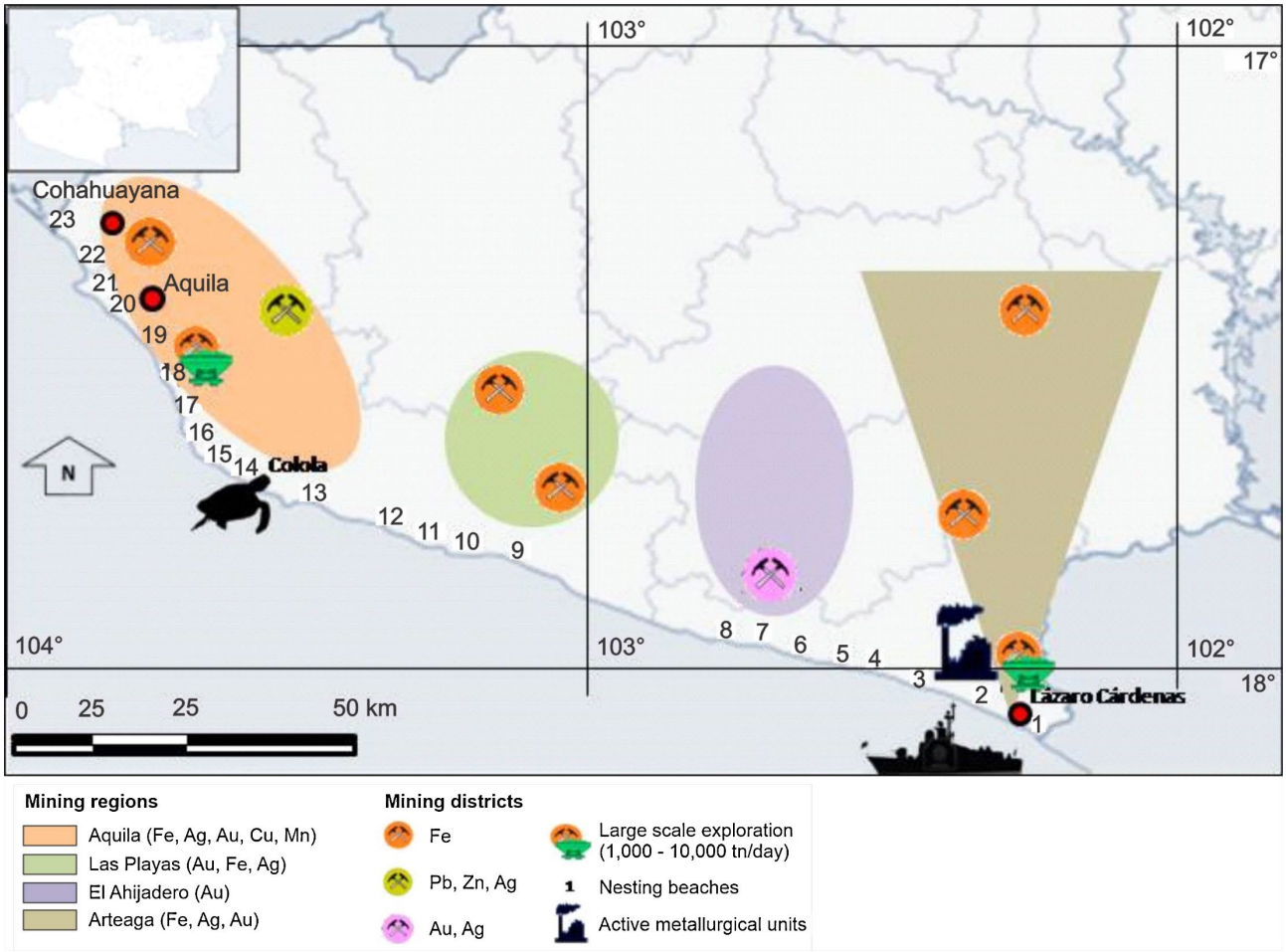


Figure 1. Map depicting the geographical overlap of nesting sites of *Lepidochelys olivacea, Chelonia mydas agassizii* and *Dermochelys coriacea* with the mining districts along the southern Pacific margin. Nesting sites are identified as Arabic numerals. In the Municipality of Lázaro Cárdenas: **1.** Balsas, **2.** Barra de Pichi, **3.** Taracosta, **4.** Barra de Tigre, **5.** Las Peñas, **7.** Boca Seca, **8.** Chuquiapan. In the Municipality of Aquila: **9.** Pichilinguillo, **10.** Cachan, **11.** Paso de Noria, **12.** Maruata, **13.** Colola, **14.** Motín del Oro, **15.** El Faro, **16.** Ixtapilla, **17.** La Ticla, **18.** El Tuano, **19.** La Placita, **20.** San Juna de Alima. In the Municipality of Coahuayana: **21.** El Ticuiz (Source: [22] [71]).

females can transfer stored contaminants to developing eggs during vitellogenesis, exposing embryos to toxic substances during critical stages of development [26]. Heavy metals detected in marine turtles include mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), nickel (Ni), copper (Cu) and zinc (Zn) and have been found in tissues such as blood, liver, kidney, muscle tissue, eggs, and hatchlings, demonstrating the potential for transgenerational exposure [25] [27]. Embryonic development is particularly sensitive to heavy metal toxicity. During organogenesis, contaminants may disrupt cellular processes and interfere with normal tissue differentiation. Potential developmental effects include neural tube defects, craniofacial abnormalities, limb malformations, eye deformities, cardiac abnormalities, shell and skeletal defects and growth retardation [28]. Because of the geographical closeness of Colola beach to the Aquila-Coahuayana mining district, it is possible that malformed embryos in *C. mydas agassizii* found at this

location might present measurable quantities of heavy metals.

Here we assess heavy metal concentrations in twelve malformed embryos of *C. mydas agassizii* recovered from artificial nests at the Colola nesting beach (Figure 1). Interest in this study was prompted from the observations of malformed embryos and the presence of nearby potential sources of heavy metal contaminants.

2. Materials and Methods

2.1. Study Site and Sample Collection

Sampling was conducted at Colola Beach, located in the municipality of Aquila on the Pacific coast of Michoacán, Mexico (approx. 18˚18'N, 103˚26'W; Figure 1). The geographic, physical, and magnetic substrate featured by Colola Beach have been described previously [7] [29].

2.2. Sample Collection

Sampling was performed during the 2010 nesting season. Ten artificial relocation nests located in the central third of the beach were monitored. Following the completion of hatchling emergence, non-hatched eggs (n = 125) were excavated. Embryos were extracted using sterile plastic tools to avoid metallic contamination and thoroughly rinsed with filtered distilled water. Extracted embryos were preserved in 4% phosphate-buffered paraformaldehyde at 4˚C for one week prior to tissue dissection.

The 125 excavated, non-hatched eggs contained embryos exhibiting morphological malformations. No-normally developed embryos were recovered from these non-hatched eggs. Consequently, direct analytical comparisons between malformed and unaffected embryos from the same clutches were not possible. Gross phenotypes were photographed and classified. Field collection protocols were authorized by the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT Permission No. SGPA/DGVS/04011/08).

2.3. Trace Element Analyses

Twelve deformed embryos (n = 12) were randomly selected for tissue analysis, ensuring representation from each of the ten artificial nests. Tissue dissections were performed under clean laboratory conditions. Three tissue matrixes were collected per embryo:

1) Muscle: Pooled tissue from the neck, abdominal wall, and flippers.

2) Carapace: Combined central and costal scutes.

3) Bowel: Small and large intestinal tracts.

Tissue samples were rinsed with reptile Ringer's solution to remove extraneous debris, oven-dried at 80˚C for 12 hours, and weighed (100 μg dry weight). Samples were acid-digested with ultra-pure nitric acid (HNO_3) in sealed polytetrafluoroethylene (PTFE) vessels [30].

Concentrations of fourteen trace elements (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, In, Ba, Pb, and Bi) were measured by Inductively Coupled Plasma-

Mass Spectrometry (ICP-MS) [31]. Total mercury was determined by Cold Vapor Atomic Absorption Spectrometry (CVAAS). Analytical quality control was maintained using procedural blanks, standard addition calibrations, and certified reference materials from the National Institute of Standards and Technology (NIST SRM). Detection limits ranged between 0.001 and 0.05 μg/g dry weight, and matrix spike recoveries averaged between 92% and 106%.

2.4. Statistical Analyses

Element concentrations were converted to parts per million on a dry weight basis (ppm; mg/kg d.w.) and date were log-transformed prior to statistical analysis. Inter-individual and inter-tissue variations were evaluated using a two-way Analysis of Variance (ANOVA) incorporating embryo identity, tissue matrix, metal type, and interaction terms. Nested observational units were used to account for embryos originating from the same nest. Analyses were executed using FAUANL software (Microsoft Office Excel 2007) and plots were constructed in SigmaPlot 10.0.

3. Results

3.1. Anatomical Malformations

Assuming an average clutch size of 70 eggs for *C. mydas agassizii*, an average of 12 ± 5 non-hatched eggs containing malformed embryos was recovered per artificial nest. This yields an estimated embryonic failure rate of <18% per clutch, which aligns with malformation frequencies reported in sea turtle populations elsewhere [14] [32].

The anatomical defects varied considerably among individuals. Observed abnormalities included equal/unequal twinning, bicephaly, abdominal dysraphism, and severe kyphosis (**Figure 2**). Craniofacial dysmorphias included exophthalmia, anophthalmia, monophthalmia, synophthalmia, arrhinia, micrognathia, and severe jaw malformations (**Figure 3**). Multiple defects frequently co-occurred within single embryos.

The type of abnormalities in the non-hatched eggs varied considerably. **Figure 2** illustrates the abnormal phenotypes encountered in our sample of *C. mydas agassizii* malformed embryos. Various craniofacial dysmorphias, abdominal dysraphism and kyphosis were found alone but mostly combined (**Figure 3**). We note that the embryo malformations are comparable to those previously recorded for other marine turtle species in southern Mexico coastal zones [14].

3.2. Heavy Metals

Trace element concentrations in muscle, carapace, and bowel tissues are presented in **Table 1** and **Figure 4**. The metals exhibiting the highest overall concentrations across all tissue matrixes were Fe, Zn, Al, and Ba. These were followed by Mn, Cu, Cr, V, Ni, and Pb. The lowest concentrations were consistently recorded for Co, Cd, In, Bi, and Hg. More specifically, Fe (195 ppm),

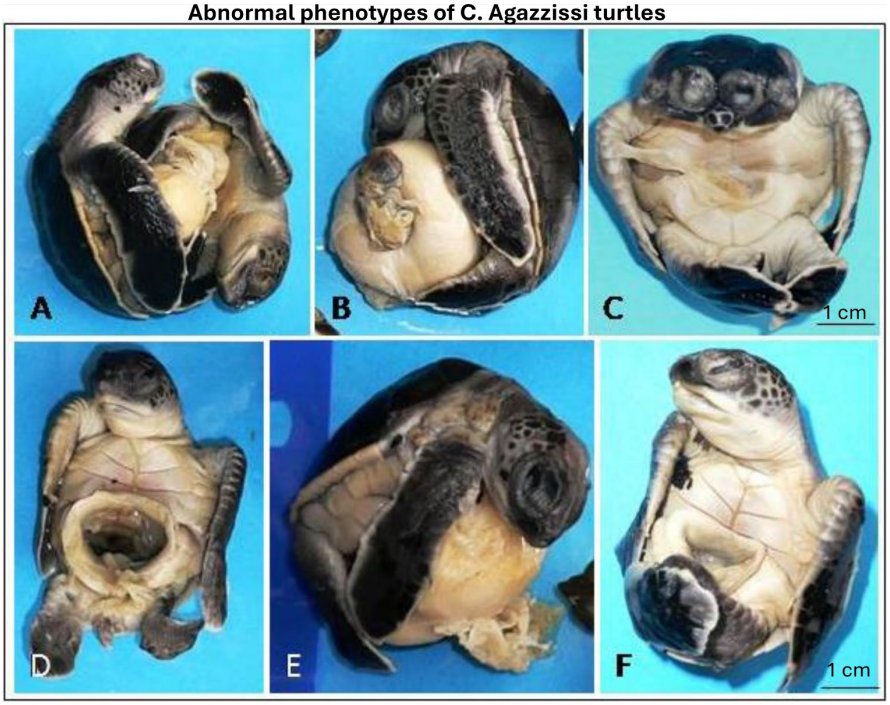


Figure 2. Photographs of phenotypic abnormalities observed in *C. mydas agassizii* embryos recovered in Colola Beach. (A) and (B) Equal and inequal twins, (C) Bicephalia, (D) abdomionoshisis, (E) kyphosis, (F) abdomionoshisis. Scale bar A-F = 1 cm.

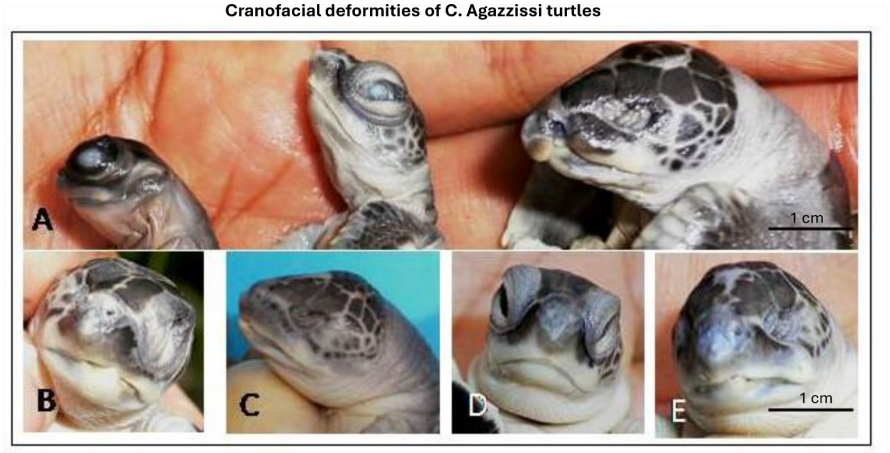


Figure 3. Craniofacial deformities of *C. mydas agassizii* turtles. (A) Exophthalmia and anophtalmia, (B) Monophtalmia and mandibular deformities, (C) Anophthalmia and micrognathia, (D) Synophtalmia, (E) Anophthalmia, arrhinia and mandibular deformities. Scale bar A-E = 1 cm.

Zn (143 ppm), Ba (37 ppm), and Al (33 ppm) were the elements with the maximum concentration levels in the muscle. Similarly, Fe (246 ppm), Zn (187 ppm), Ba (36 ppm), and Al (25 ppm) with the highest concentrations in the carapace. Lastly, Fe (397 ppm), Zn (127 ppm), Al (21 ppm), and Ba (13 ppm) were those elements with the greater concentration in the bowel.

The two-way ANOVA revealed statistically significant differences in total metal

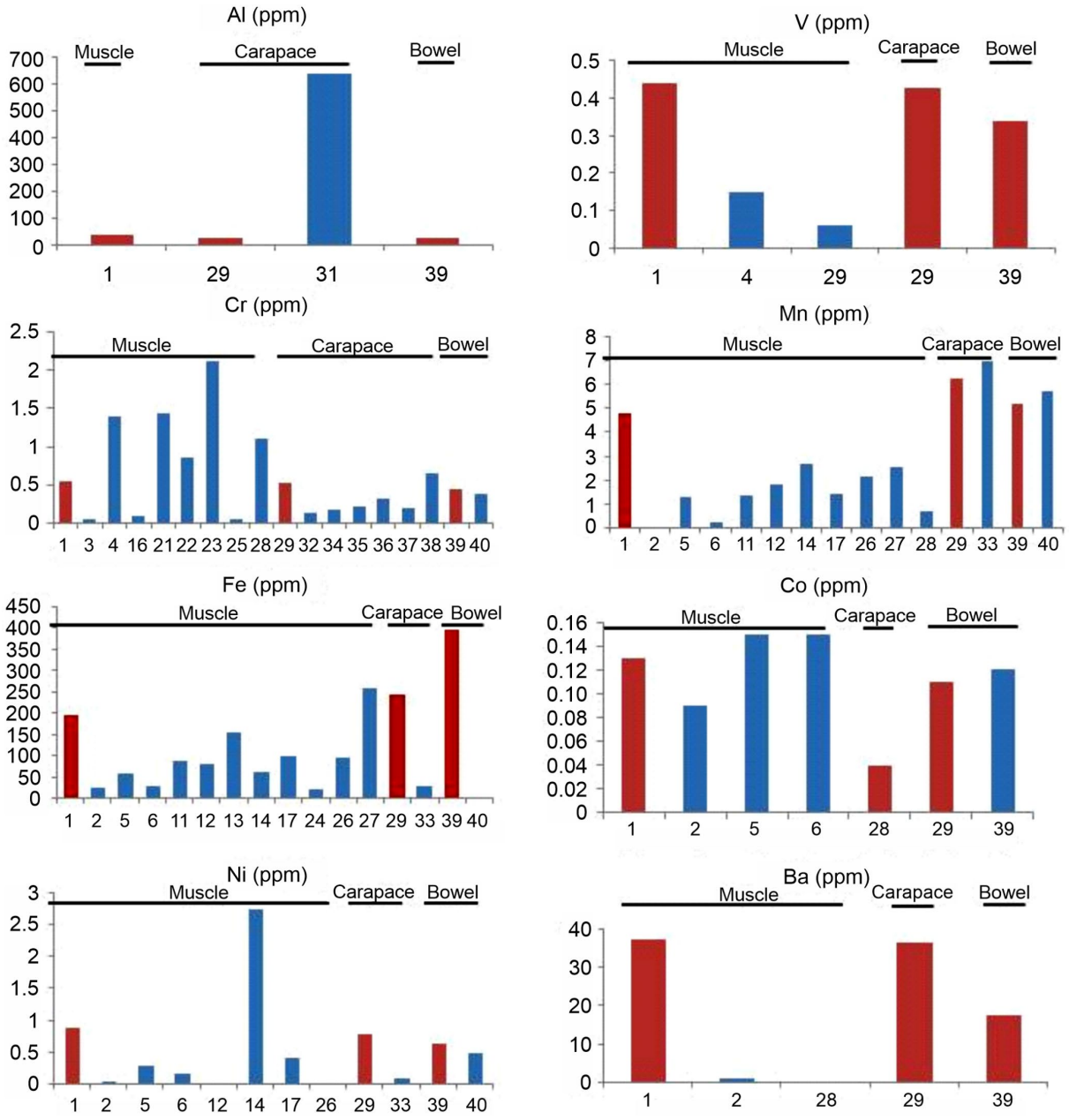
Table 1. Trace element concentrations in tissues of marine turtles.

Muscle Sp/Study	Location	Ages/Weight	Graph ID.	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	In	Ba	Pb	Bi	Hg
***Chelonia agassizii* (this study)**	**Colola, México**	**Fetuses DW**	**1**	**32.78**	**0.44**	**0.54**	**4.82**	**195.19**	**0.13**	**0.88**	**6.85**	**142.56**	**0.10**	**0.05**	**37.42**	**0.75**	**0.08**	**0.11**
Chelonia mydas (Gardner et al. 2006)	Baja Ca. México	Adults Stranded DW	2				0.00	20.99		0.03	0.03	38.26	0.01			0.01		
Chelonia mydas (Wang, 2003)	Texas, EU	Adults Stranded DW	3			0.05					1.32	23.10	0.11			0.30		0.06
Chelonia mydas (Anan et al. 2001)	Japan	Adults in Wild DW	4		0.15	1.4	0.46		0.01		0.87	47.7	0.24		0.65	0.09		0.04
Chelonia mydas (Sakai et al. 2000a)	Japan	Adults Stranded DW*	5				1.3	56.9	<0.15	0.29	1.3	48	0.1			<0.15		0.02
Chelonia mydas (Sakai et al. 2000b)	Japan	Adults Stranded DW*	6				0.2	26.4	<0.15	<0.15	1.8	44	0.3			<0.15		0.1
Chelonia mydas (Godley et al. 1998)	Cyprus	Adults Stranded DW	7										0.37			1.81		0.09
Chelonia mydas (Godley et al. 1998)	Cyprus	Embryo DW	8										0.33			0.66		ND
Chelonia mydas (Godley et al. 1998)	Cyprus	Hatchling DW	9										0.23			BDL		ND
Caretta caretta (García et al. 2009)	Spain	Adults Stranded DW	10								5.04	65.39	0.20			0.26		
Caretta caretta (Andreani et al. 2008)	Adriatic Coast	Adults Stranded DW	11				1.35	85.4			2.4	105	0.81			ND		
Caretta caretta (Gardner et al. 2006)	Baja Ca. México	Adults Stranded DW	12				1.82	77.44		0.01	0.41	31.11	0.1			0.01		
Caretta caretta (Storelli et al. 1998a)	Adriatic Sea	Adults Stranded DW*	13					155.4			0.29	139.5	0.35			0.2		0.9
Caretta caretta (Frazelleti et al. 2005)	Adriatic Sea	Adults Stranded DW	14				2.7	60.9		2.76	1.5	30.9	0.36					
Caretta *caretta* (Maffucci et al. 2005)	Italy	Adults Stranded DW	15								2.7	107.0	0.20					0.40
Caretta caretta (Wang, 2003)	Texas, EU.	Adults Stranded DW	16			0.09					2.98	30.70	0.54			0.75		0.09
Caretta caretta (Sakai et al. 2000a)	Japan	Adults Stranded DW*	17				1.4	99		0.415	4.05	125	0.3			0.1		472
Chelonia mydas (Godley et al. 1998)	Cyprus	Adults Stranded DW	18										0.57					0.48
Chelonia mydas (Godley et al. 1998)	Cyprus	Embryo DW	19										0.21			BDL		0.01
Chelonia mydas (Godley et al. 1998)	Cyprus	Hatchling DW	20										0.34			0.13		0.02
Caretta caretta (Storelli et al. 1998a)	Adriatic Sea	Adults Stranded DW	21			1.43							0.55			0.54		0.69
Caretta caretta (Storelli et al. 1998b)	Adriatic Sea	Adults DW	22			0.85							0.21			0.47		0.89
Caretta caretta (Storelli et al. 1998b)	Adriatic Sea	Youngs DW	23			2.13							0.96			0.66		0.43
Caretta caretta (Sakai et al. 1995)	Japan	Adults DW-M	24					20.1			0.83	24.2	0.29			ND		0.51
Lepidochelys kempii (Wang, 2005)	Texas, EU.	Adults Stranded DW	25			0.05					2.35	31.4	0.05			0.14		0.43
Lepidochelys olivacea (Gardner et al. 2006)	Baja Ca. México	Adults Stranded DW	26				2.1	93.09		0.01	1.28	85.78	0.48			ND		
Eretmochelys imbricata(Gardner et al. 2006)	Baja Ca. México	Adults Stranded DW	27				2.53	258		ND	3.68	102	1.02			0.38		
Eretmochelys imbricata (Anan et al. 2001)	Japan	Adulst in Wild DW	28		0.06	1.1	0.69		0.04		0.96	48.6	0.07		0.086	0.04		0.04

Carapace Sp/Study	Location	Ages	Graph ID	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	In	Ba	Pb	Bi	Hg
***Chelonia agassizii* (this study)**	Colola, México	Fetuses DW	29	24.59	0.43	0.52	6.25	245.55	0.11	0.77	7.34	186.76	0.23	0.06	36.49	0.83	0.11	0.10
Chelonia agassizii (Presti et al 1999)	Golfo Ca.México	Adults DW	30															0.05
Chelonia mydas (Komoroske et al 2011)	Sn. Diego Ca. EU	Adults in Wild DW	31	641							7.09		0.44			7.23		0.05
Chelonia mydas (Wang, 2003)	Texas, EU	Adults Stranded DW	32			0.13					0.953	380	0.21			0.53		0.24
Chelonia mydas (Sakai et al 2000a)	Japan	Adults Stranded WW	33				7.01	26.2		0.09	0.25	198	0.13			2.42		43.2
Lepidochelys Kempii (Wang, 2005)	Texas, EU.	Adults Stranded DW	34			0.16					1.56	270	0.15			1.38		1.18
Lepidochelys Kempii (Wang, 2005)	Texas, EU.	Adults in Wild DW	35			0.20					3.14	311	0.14			0.98		1.61
Lepidochelys Kempii (Wang, 2005)	Texas, EU.	Adults DW	36			0.33					3.1	329	0.13			1.22		1.12
Lepidochelys Kempii (Wang, 2005)	Atlantic SE	Adults DW	37			0.18					1.07	306	0.05			0.73		0.72
Lepidochelys Kempii (Wang, 2005)	Tams. México	Adults DW	38			0.64					0.73	260	0.04			1.18		2.42
Bowel Sp/Study	**Location**	**Ages**	**Ref.**	**Al**	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Cd**	**In**	**Ba**	**Pb**	**Bi**	**Hg**
***Chelonia agassizii* (this study)**	**Colola, México**	**Fetuses DW**	**39**	**20.92**	**0.34**	**0.44**	**5.21**	**397.40**	**0.12**	**0.62**	**8.9**	**127.06**	**0.13**	**0.06**	**17.75**	**1.11**	**0.18**	**0.15**
Chelonia mydas (Sakai et al. 2000a)	Japan	Adults Stranded WW	40			0.37	5.72	<0.03	0.043	0.49	28.3	4.38			<0.03			7.46

concentrations among individual embryos ($F = 22.6367$, df $= 14$, $p = 0.0001$), confirming substantial inter-individual variability. Metals accumulated non-uniformly across tissues, demonstrating matrix-specific affinity; iron was preferentially concentrated in bowel tissue, whereas zinc and barium showed higher affinities for carapace scutes (Figure 4).

Heavy metals did not accumulate uniformly in the different tissues. Differences between the muscle, carapace, and bowel tissues suggest that tissue types influence the distribution and retention of heavy metals within the body. Tissues show different capacities for absorbing, storing, transporting, and eliminating contaminants. Concentrations for metals across tissue samples were similar, although they accumulated differently in the muscle, carapace and bowel (Figure 4).



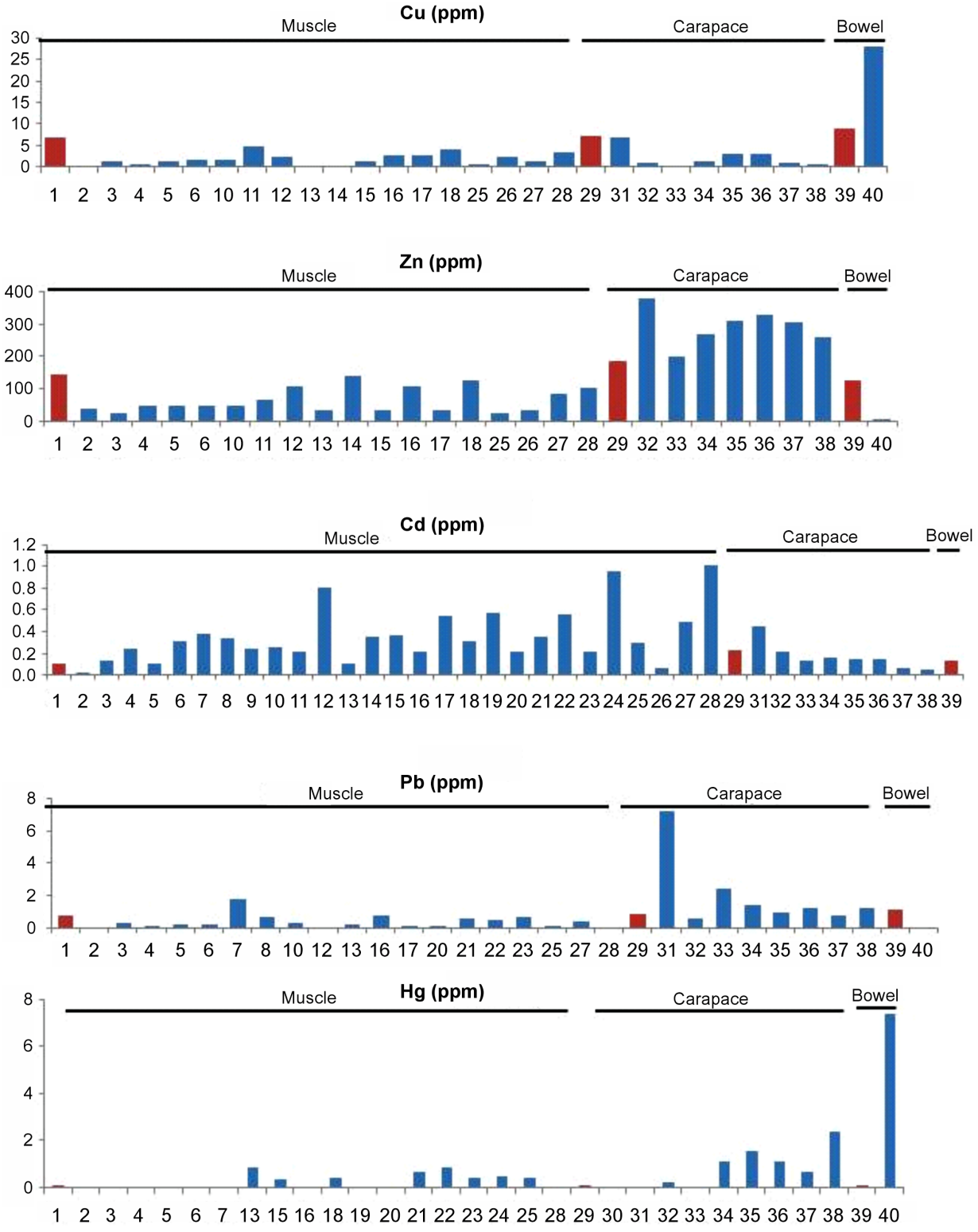


Figure 4. Heavy metal concentrations in malformed embryos. Bar graphs with heavy metal concentrations in muscle, carapace and bowel tissue samples. Data bars for *C. mydas agassizii* from Colola Beach are in red. Data bars for other turtle species and localities are in blue (Graph identification numbers are listed in **Table 1**). Data for *C. mydas agassizii, Chelonia mydas, Caretta caretta, Lepidochelys olivacea Eretmochelis imbricata and Lepidochelys Kempii*. (A) Aluminum, (B) Vanadium, (C) Chromium, (D) Manganese, (E) Iron, (F) Cobalt, (G) Nickel and (H) Barium, (I) Copper, (J) Zinc, (K) Cadmium, (L) Lead, (M) Mercury.

4. Discussion

Studies on dysmorphic turtle embryos in natural and artificial nests have investigated the occurrence of malformations, natal beach characteristics, and underlying etiologies; however, factors that trigger developmental deformities remain difficult to unravel. Embryonic development is orchestrated by precise genetic and epigenetic networks controlling cell division, tissue differentiation, organogenesis, and body patterning. While inherited harmful genetic mutations increase the probability of structural abnormalities in offspring, exogenous environmental contaminant, such as heavy metals, petroleum-derived hydrocarbons, pesticides, and persistent organic pollutants, act as potent endocrine disruptors that interfere with developmental hormonal pathways, alter gene expression through epigenetic mechanisms, and cause severe cellular damage [11] [18] [19] [33]-[35].

4.1. Tissue-Specific Accumulation and Inter-Individual Variability

In our survey of *C. mydas agassizii* embryos exhibiting major anatomical abnormalities, heavy metals accumulated non-uniformly across the analyzed tissue matrixes (**Figure 4**). Concentrations of Fe, Zn, Al, and Ba were highest across muscle, carapace, and bowel tissues, followed by Mn, Cu, Cr, V, Ni, and Pb, whereas Co, Cd, In, Bi, and Hg were present at lower concentrations.

The differential accumulation across tissue matrixes reflects organ-specific differences in transport mechanisms, buffering capacity, metabolic clearance, and storage dynamics [34]. Muscle tissue reflects active metabolic accumulation; carapace scutes serve as long-term deposition sinks for divalent cations; and the bowel reflects mucosal absorption and embryonic excretion pathways.

On the other hand, individual embryos exhibited marked variations in overall heavy metal burdens. Inter-individual variability may stem from differences in maternal exposure history, foraging ecology, diet composition, trophic position, or maternal physiological condition [25] [27] [34] [36] [37]. Also, because heavy metals enter marine food webs and accumulate over time, foraging adult females encounter heterogeneous contamination levels depending on their specific migratory corridors and feeding grounds [25] [27] [37].

4.2. Disruption of Developmental Signaling Cascades May Explain Fetal Abnormalities

While trace elements like Fe, Zn, Cu, and Mn perform essential metabolic and structural functions during embryogenesis, excess concentrations and exposure to non-essential metals (Pb, Cd, As, Hg) disrupt critical developmental signaling cascades. Accordingly, the craniofacial dysmorphias, ocular defects, axial skeletal deformities, and abdominal wall dysraphism observed in Colola Beach embryos coincide with critical organogenesis windows where specific heavy metals disrupt embryonic patterning through well-defined molecular mechanisms [38]-[42]. For instance, embryonic exposure to Cd inhibits metallothionein gene

expression, causing severe head and craniofacial malformations [41]. Pb linked to microcephaly, jaw hypoplasia, and facial dysmorphias inhibiting the canonical Wnt/β-catenin pathway, which downregulates facial identity factors (Pax3, Sox9, Sox10) in cranial neural crest cells (CNCs). Pb also disrupts retinoic acid (RA) receptor signaling across the pharyngeal arches and mimics Ca^{2+} in the Ca^{2+}/Calmodulin (CaMKII) pathway, causing premature neural progenitor cell loss in the rostral neural tube [43]. As, Co, Cu, Fe, and Mn promote hydroxyl radical production through Fenton reactions, inhibiting PAX2 (optic stalk specification) and MITF (retinal pigment epithelium development), which restricts final ocular volume (microphthalmia) [44] [45]. Hg induces anophthalmia and microphthalmia by downregulating the master ocular gene PAX6 and suppressing the Sonic Hedgehog signaling cascade emanating from the precordal plate. Furthermore, Hg binds to sulfhydryl (–SH) groups on tubulin, causing microtubule depolymerization that impairs neuroectodermal optic vesicle evagination and triggers p53-mediated apoptosis in neural progenitor cells [46].

Regarding vertebral, axial, and limb deformities As, Fe and Mn disrupt the "somitogenesis clock" and morphogen gradients of FGF8 and Wnt3a in the presomitic mesoderm [39] [42] [43]. This asymmetrical segmentation of cervical somites causes hemivertebral fusions, resulting in severe torticollis and axial skeletal twisting (twisted neck) As, Co and Cu induce micromelia (shortened limbs) [44] [45] by disrupting reciprocal SHH/FGF signaling between the apical ectodermal ridge (AER, source of FGF4/8) and the zone of polarizing activity (ZPA, source of SHH). These metals inhibit focal adhesion kinase (FAK) and suppress the BMP/Runx2 cascade, arresting precartilaginous mesenchymal condensation [44] [45]. Pb, Cd and Al interfere with Notch-dependent signaling and sclerotome proliferation during axial patterning leading to extensive vertebral arch malformations, kyphosis, and scoliosis [39] [42] [43].

Although the precise molecular signaling cascades mediating ventral wall and visceral defects remain obscure, it has been shown that excess Zn and the combined metal exposure impair gut rotation and intestinal looping mechanics, resulting in incomplete gut coiling, as well as the disruption of ventral body wall closure during early lateral fold fusion and extracellular matrix remodeling, producing severe everted viscera, gastroschisis, and schistosomus reflexus syndromes [40] [45].

4.3. Mechanisms of Metal Teratogenesis and Cellular Damage

Exposure to heavy metals stimulates the overproduction of reactive oxygen species, inducing oxidative stress and damaging proteins, membrane lipids, and DNA [33]. Embryonic tissues are exceptionally vulnerable to oxidative injury because their endogenous antioxidant defense systems are not yet fully mature. Metals such as Hg, Cd, and Pb damage DNA, disrupt chromatin architecture, and alter developmental gene expression [41] [46] [47]. Furthermore, heavy metals inter-

fere with calcium metabolism during skeletal formation, disrupting cartilage mineralization and resulting in severe bone and shell dysmorphias (see below).

Heavy metals are recognized as teratogenic agents across diverse metazoan taxa, including crustaceans [48], echinoderms [49], anurans [21] [50]-[52], fish [53]-[55], birds [44] [56], and mammals [57] [58]. Even essential trace elements with vital physiological roles can become toxic and teratogenic when systemic concentrations exceed physiological thresholds. The magnitude of fetal deformities is greatest, and overall embryonic viability is lowest, when metal overexposure occurs during critical early windows of fertilization and initial organogenesis [55] [59]-[61].

4.4. Environmental Pathways, Epigenetics, and Idiosyncratic Responses

The transport of heavy metals to developing *C. mydas agassizii* embryos likely involves a two-stage environmental mechanism [60] [61]. The first stage would be defined by regional transport and substrate absorption. Heavy metals discharged from inland mining operations (e.g., the Aquila iron district) [22] may be transported via river networks and ocean currents to nesting beaches. Sand substrates inundated with contaminated seawater or dust fall allow trace elements to penetrate eggshells via passive diffusion and absorptive processes during incubation [60]-[62]. Transgenerational maternal transfer would then take place. Foraging females exposed to heavy metals in polluted feeding grounds transfer circulating toxicants across the uterine epithelium into the egg yolk during vitellogenesis, directly exposing embryos to toxic loads from early cleavage [25] [27] [60] [62]. Finally, *in situ* nest substrate absorption would add an additional source of pollutants. Colola beach receives hydrological run-off carrying eroded tailings from the nearby Aquila iron mining district via regional rivers [22]. Trace elements deposited in beach sand can cross the porous eggshell during incubation, altering eggshell gas/moisture permeability and directly exposing embryonic membranes [60]-[62].

Multiple interacting factors include heavy metal contamination, organic pollutants, environmental factors, microbial infections, genetic abnormalities, nutritional deficiencies and habitat degradation [63]-[67]. Controlled experimental studies remain scarce because marine turtles are protected species, making field observations and metal contamination analyses important sources of evidence.

How heavy metals reach the developing *C. mydas agassizii* embryos might be related to a two-stage mechanism, with regional long-range transport from their sources by rivers and ocean currents and at nesting beaches bathed by contaminated water, heavy metals may pass through the egg shells by adsorptive/absorptive processes [60] [61] [68]. A possibility is that fertile females and/or males exposed to these pollutants while navigating through contaminated waters could transfer blood borne heavy metal to their gametes and/or fertilized eggs [60] [68]. This mechanism focuses on developmental trajectories though genetic/epigenetic factors [69]. Both alternatives are not mutually exclusive and could be experimen-

tally evaluated.

A paradox in our observations is that embryos from the same clutch displayed different types and severities of malformations, while most eggs in these clutches hatched successfully without gross physical defects. If all eggs within a clutch are exposed to similar maternal or environmental contaminant loads, why are only certain embryos affected, and why do phenotypes vary so widely? This idiosyncratic embryonic response suggests that heavy metal teratogenesis in sea turtles does not depend solely on direct gene mutations, but rather on complex gene-environment interactions and epigenetic chromatin modifications [69] [70]. Differences in micro-location within the nest cavity, variations in microvascular eggshell contact, differential yolk metal partitioning, and individual sensitivity to epigenetic remodeling (such as altered DNA methylation or histone acetylation) could influence whether an embryo suffers developmental arrest, specific malformations, or normal growth [67] [69] [70]. Embryonic developmental heterochrony may be another source of variation, since intra-clutch development across individuals is hardly synchronous.

4.5. Study Limitations and Methodological Constraints

To properly contextualize these findings, several study limitations must be acknowledged explicitly.

The first one relates to the absence of a normal control baseline. The 125 non-hatched eggs recovered from the sampled artificial nests contained malformed embryos. No-normally developed embryos were available to serve as analytical controls. As a result, this study cannot determine whether trace metal burdens in malformed embryos differ significantly from those in healthy hatchlings from the same cohort. In addition, there is no single, standardized regulatory threshold (such as an EPA or WHO limit) that defines a specific metal concentration as universally teratogenic for sea turtle eggs. Because sea turtles are protected species, controlled toxicity experiments (which determine exact dose-response curves like LD_{50} or EC_{50}) are ethically restricted, making established threshold values scarce. Consequently, field observations and tissue bioaccumulation analyses remain indispensable for ecotoxicological assessment [36] [37].

Samples were obtained from artificial relocation hatcheries. Relocation introduces altered incubation microclimates, including thermal spikes, gas exchange alterations, and hydric fluctuations, that independently may induce physical deformities [66]. Isolating the relative contributions of microclimatic stress, maternal transfer, and heavy metal exposure is a labor that must be considered for future field work to reduce confounding factors associated with artificial nesting stressors.

The embryos were stored in 4% paraformaldehyde prior to tissue dissection. Formalin fixation can leach highly soluble, unbound ions (K^+, Ca^{2+}, Mg^{2+}) by over 50% - 80% and induce protein cross-linking that alters sample homogeneity and redistributes metals like Fe, Zn, and Cu. Absolute tissue concentrations should therefore be interpreted with appropriate caution.

Relationships between genetics and environment in marine turtles remain complex to unravel, with genetic factors influencing embryo's vulnerability and environmental pollutants affecting development. Further studies are needed to address these questions on the biological bases of marine turtle fetal malformations and to constrain impacts of genetic mutations and environmental pollutants on marine turtle's conservation.

5. Conclusions

This study provides characterization of heavy metal accumulation in malformed *Chelonia mydas agassizii* embryos recovered from artificial nests at Colola Beach, Michoacán. The highest metal concentrations across muscle, carapace, and bowel tissues were recorded for iron, zinc, aluminum, and barium, followed by manganese, copper, chrome, vanadium, nickel, lead, cobalt, cadmium, indium, bismuth and mercury. Trace elements accumulated non-uniformly across organs, and inter-individual variations were statistically significant ($F = 22.64$, $p = 0.0001$).

The study establishes a coincidental occurrence between heavy metal burdens and congenital malformations in *C. mydas agassizii*. Protocol constraints regulating experimental dosing in endangered species, the lack of control embryos and potential preservation artifacts prevent assigning direct causality. Following conservation research will focus on non-destructive sampling of healthy hatchlings, ecotoxicological analysis of beach substrates, and monitoring of maternal metal transfer to establish baseline safety thresholds for sea turtle embryos.

Acknowledgments

This note is dedicated to the memory of Dr. Felipe Vazquez, a pioneer of research on marine pollution and heavy metals and an esteemed collaborator in this study. We acknowledge ABB Managing Editor Evelyn Gao and the Reviewers for the corrections and comments on the manuscript, which permitted to improve the article. We thank Patricia Ramos, Rafael Venegas, Marysol Valdez, Jesús Ramírez Santos, S. Ivonne Mora, Raymundo Reyes and Edel Pineda López for technical assistance. Thanks to Secretaría de Ciencia Humanidades Tecnologia e Innovacion, SECIHTI—formerly Consejo Nacional de Ciencia y Tecnología, CONACyT—(82879, 94312 and CBF-2025-G-219), Secretaría de Educación Pública (PROMEP 103.5/12/4230), Universidad Michoacana de San Nicolás de Hidalgo and Chicxulub Science Foundation. The protocols of embryo collection and handling were approved by the federal regulatory agency Secretaría del Medio Ambiente y Recursos Naturales (Permission No. SGPA/DGVS/04011/08). Publication IICEAC2026-014.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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