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Determination of Metal and Metalloids in Bottlenose Dolphins' (*Tursiops truncatus*) Skin from the Yucatan Peninsula, Mexico

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Abstract

Contamination by metals poses a significant threat to marine ecosystems and the health of marine organisms, including cetaceans. This study aimed to assess the concentrations of some metal and metalloids in the skin of bottlenose dolphins (*Tursiops truncatus*) from the Yucatan Peninsula in Mexico using Flame Atomic Absorption (FAA) spectrometry. A total of 41 skin samples were collected, comprising 22 samples from Yucatán in the Gulf of Mexico and 19 samples from Chetumal Bay in the Caribbean. The results of our analysis revealed the presence of various elements in the skin of bottlenose dolphins from coastal waters in the Yucatan Peninsula. Among the detected metals, zinc (Zn), iron (Fe), and arsenic (As) exhibited notable concentrations, with the order of abundance being Zn > Fe > As > Cu > Cr > Cd > Ni. The elevated concentrations of Zn and Fe suggest potential exposure and accumulation of these metals in the studied dolphin population. Interestingly, measurable concentrations of lead (Pb) and manganese (Mn) were not detected in the skin samples, indicating either low levels or the absence of their accumulation in the studied dolphin populations. The absence of measurable Pb concentrations is particularly encouraging as Pb is known to have detrimental effects on marine organisms and is commonly associated with anthropogenic activities. This study provides valuable insights into the metal contamination status in bottlenose dolphins from the Yucatan Peninsula, Mexico. The notable concentrations of Zn, Fe, and As raise concerns about potential health risks to these charismatic marine mammals. Further research is warranted to investigate the sources and pathways of heavy metal exposure in this population and to understand the potential consequences of such exposure on the health and well-being of bottlenose dolphins in the Yucatan Peninsula.

Key Words: Delphinidae, *Tursiops truncatus*, cetacean, pollution, high concentration, metals

Introduction

The dolphin commonly known as the bottlenose dolphin (*Tursiops truncatus*) is a marine mammal that inhabits oceans and coastal waters around the world (Wells & Scott, 2009). As a top predator in the marine ecosystem, bottlenose dolphins are exposed to various pollutants, including heavy metals, which can have detrimental effects on their health (Roditi-Elasar et al., 2003). Among toxic elements, there are a group of metallic and metalloid elements that usually have a high density and are toxic to living organisms at certain concentrations (Pandey & Madhuri, 2014). They occur naturally in the environment but are also released into water bodies through human activities such as industrial processes, mining, and urban runoff (Sankhla et al., 2016). Some common metals of concern include mercury (Hg), lead (Pb), nickel (Ni), cadmium (Cd), and arsenic (As).

These metals can enter the marine environment through different pathways. For example, industrial discharges and wastewater treatment plants can release heavy metals directly into rivers and coastal waters (Sharifuzzaman et al., 2016). Atmospheric deposition, where pollutants settle from the air onto the water surface, is another important pathway (Gunawardena et al., 2013). Once in the water, these elements can accumulate in sediments, as well as in the tissues of aquatic organisms, including bottlenose dolphins (Das et al., 2003).

Bottlenose dolphins are particularly vulnerable to metal pollution due to their long lifespan, high trophic level, and position in the marine food web (Das et al., 2003). They can bioaccumulate toxic metal and metalloids over time, meaning that these toxic substances can accumulate in their bodies at concentrations higher than what is present in their environment (Shoham-Frider et al., 2009). This occurs because dolphins ingest contaminated prey and absorb the toxic element through their food and skin (Das et al., 2003).

The accumulation of metals in bottlenose dolphins can lead to a range of health effects (Pellisó et al., 2008). Cd and other elements can impair the central nervous system and cause neurological disorders (Gajdosechova et al., 2016). Pb can interfere with the functioning of organs such as the kidneys, liver, and brain. Cd is known to be toxic to the kidneys (Rana et al., 2018), while As can cause various forms of cancer and disrupt cellular processes (Jomova et al., 2011). Copper (Cu), although an essential element, can be harmful at elevated concentrations and may result in oxidative stress (Gaetke & Chow, 2003).

To assess the impact of metal pollution on bottlenose dolphins, researchers have analyzed the concentration of these elements in dolphin tissues such as in the liver, kidney, muscle, and skin samples (Das et al., 2003; Roditi-Elasar et al., 2003; Shoham-Frider et al., 2009). These analyses help in understanding the extent of metal exposure and its potential health risks to these marine mammals (Das et al., 2003). Therefore, this study aimed to assess the concentrations of some metal and metalloid elements in the skin of bottlenose dolphins from two regions in the Yucatan Peninsula.

Understanding the presence and effects of metals in bottlenose dolphins is crucial for monitoring and managing marine pollution (Carvalho et al., 2002; Shoham-Frider et al., 2009). It not only provides insights into the health of dolphin populations but also serves as an indicator of the overall environmental quality of coastal and marine ecosystems (Zhou et al., 2001). Efforts to reduce metal pollution and protect the habitats of bottlenose dolphins are vital for their conservation and the preservation of marine biodiversity (Barratclough et al., 2019).

Methods

Field

Since 2010, bottlenose dolphin skin samples were collected from the stranded events taking place in two main areas: the coasts of Yucatán State (YUC) in the Gulf of Mexico and Chetumal Bay (BCH) of Quintana Roo State in the Caribbean, both in the Yucatan Peninsula (Figure 1). Samples were labeled, stored in plastic bags, and transported on ice to Laboratorio de Ecología y Ordenamiento Territorial in the Universidad Autónoma del Estado de Quintana Roo, where all samples were stored at -20°C until the analysis was performed.

Laboratory

All laboratory material used in the quantification of the trace element was washed with phosphate-free soap (Extran, Merck, Germany) and rinsed with abundant tap water. Following this, the material was placed overnight into a 20% HNO_3 solution (J. T. Baker, Phillipsburg, NJ, USA), rinsed again with ultrapure water, and dried until used.

One gram of the *Tursiops truncatus* skin samples was weighed over plastic dishes at an ultra-analytic scale, Citizon CM-5. Following this, they were dried for 48 h at 70°C in a laboratory oven and then reweighed. The percentage of humidity was determined gravimetrically as the difference between both weights and multiplied by 100. Dried samples were then put into Teflon extraction vessels (CEM Corp., Matthews, NC, USA) and digested with 10 ml of acid solution (4 ml HCl and 6 ml HNO_3 70.0% [both from J. T. Baker]) in a CEM MARs X microwave digestor (CEM Corp.). The digestion method consisted of a temperature pressure ramp to reach 150°C and 13.79 Bar (200 PSI), which were maintained for 20 min. The resulting solution was then carried out to 50 ml, adding ultrapure water into a borosilicate volumetric flask (Kimble/Kontes, USA).

Each of the 12 samples were processed at the same time with one reactive blank and an element target solution of Pb at 1 mg L^{-1}. Element quantification was performed by flame atomic absorption spectrometry on a FAA spectrometer (ICE 3000 series; Thermo Fisher Scientific, Waltham, MA, USA). Air/acetylene mixture and air/nitrous oxide were used to vaporize and ionize the elements present in the digested solution. Calibration curves (absorbance vs element concentration) were constructed for Pb, Ni, As, Cd, chromium (Cr), zinc (Zn), Cu, iron (Fe), and manganese (Mn). A correlation coefficient (R^2) was used to determine accuracy of linearity. Only high linear correlations (≥ 0.95) were used to quantify elements. Blank correction was applied to each sample to prevent false positives caused by external or background contamination. Limits of quantification (LOQ) and detection (LOD) were defined as three and ten times the standard deviation of the signals of blank samples (European Commission, 2007). Results are reported in mg kg^{-1} on a dry weight (dw) basis.

Statistical Comparisons

The Mann-Whitney and Kruskal-Wallis tests were employed to assess the presence of significant

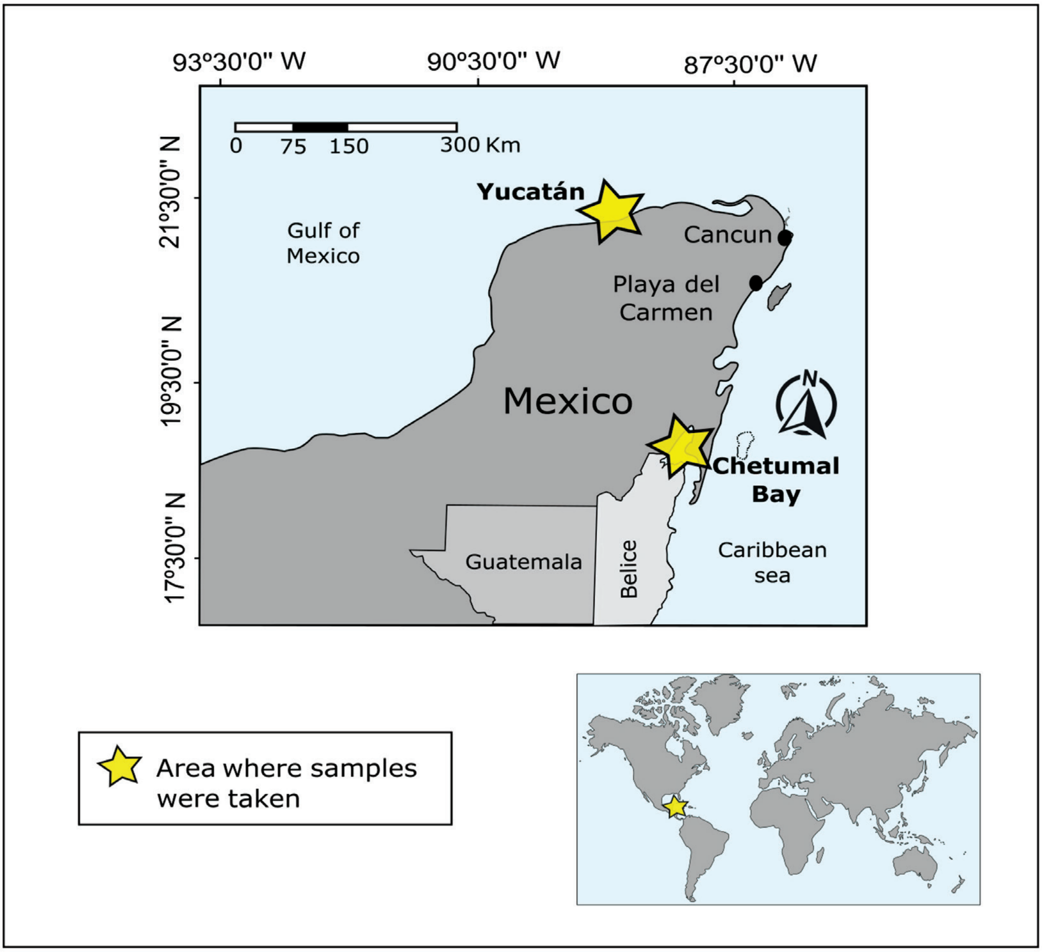

Figure 1. Geographical location of sampling sites in the Yucatan Peninsula where skin samples of *Tursiops truncatus* were collected. Stars show Yucatán State (YUC) and Chetumal Bay (BCH) areas, respectively.

differences in trace element concentrations in bottlenose dolphin skin among both areas (YUC vs BCH), sexes (male vs female), and age categories (adult vs juvenile vs calf). All analyses were conducted using the *PAST* software and *R studio*. Statistical differences among groups were identified at $p \leq 0.05$.

Results

A total of 41 skin samples were collected and analyzed for bottlenose dolphins: 22 for Yucatán in the Gulf of Mexico (11 females, 9 males, two non-identified), and 19 for Chetumal Bay in the Caribbean (6 females and 13 males).

Concentrations of the analyzed elements were summarized for both areas, YUC and BCH, and are reported in Table 1. For bulk samples analyzed regardless of area, sex, or age category, the order of trace element concentration, ranked from highest to lowest, was Zn > Fe > As > Cu > Cr > Cd > Ni > Mn > Pb (Figure 2).

For Yucatán

For all bottlenose dolphins analyzed in YUC, the element concentration presented the Zn > Fe > As > Cu > Cr > Cd > Ni > Mn > Pb pattern with Zn, Fe, and As as the elements with higher concentrations. Zn concentrations in dolphins from YUC were determined to be 1,205.43 ± 724.62 mg kg^{-1}; Fe concentrations were measured at 479.18 ± 271.44 mg kg^{-1}; and As was found to be 110.20 ± 223.84 mg kg^{-1}. Of the other elements, Cu concentrations were found to be 38.77 ± 75.85 mg kg^{-1}; Cr concentrations were measured at 4.95 ± 6.68 mg kg^{-1}; Cd concentrations were

Table 1. Concentrations of the analyzed elements. The element concentration is reported in milligrams per kilogram (mg kg^{-1}) dry weight (dw) and are presented as means ± standard deviations (SD). Parentheses are the minimum and maximum values found (max-min). YUC = Yucatan State; BCH = Chetumal Bay.

	YUC			BCH		
	Females (n = 11)	Males (n = 9)	Total (n = 22)	Females (n = 6)	Males (n = 13)	Total (n = 19)
As	185.55 ± 297.28	29.12 ± 65.70	110.20 ± 223.84	247.00 ± 166.69	135.68 ± 145.73	170.93 ± 157.22
	(< LD-813.90)	(< LD-193.56)	(< LD-813.90)	(< LD-438.02)	(< LD-438.02)	(< LD-438.02)
Pb	< LD ± 0.00	< LD ± 0.00	< LD ± 0.00	< LD ± 0.00	0.36 ± 0.53	0.28 ± 0.47
	(< LD)	(< LD)	(< LD)	(< LD-0.71)	(< LD-1.82)	(< LD-1.82)
Ni	1.07 ± 3.55	(< LD)	0.53 ± 2.51	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	(< LD-11.77)	(< LD)	(< LD-11.77)	(< LD ± 0.00)	(< LD ± 0.00)	(< LD ± 0.00)
Cd	0.42 ± 0.70	2.21 ± 2.06	1.11 ± 1.65	0.36 ± 0.76	0.20 ± 0.74	0.25 ± 0.73
	(< LD-2.08)	(< LD-5.83)	(< LD-5.83)	(< LD-1.90)	(< LD-2.68)	(< LD-2.68)
Cr	7.84 ± 12.30	0.11 ± 0.34	4.39 ± 9.39	4.81 ± 5.57	5.01 ± 7.37	4.95 ± 6.68
	(< LD-37.35)	(< LD-1.04)	(< LD-37.35)	(< LD-12.35)	(< LD-19.55)	(< LD-19.55)
Zn	1,306.38 ± 746.09	1,052.96 ± 664.12	1,205.43 ± 724.62	931.41 ± 689.23	747.12 ± 487.23	805.31 ± 545.40
	(387.92-2,809.34)	(377.49-2,233.94)	(377.49-2,809.34)	(362.53-2,051.31)	(222.00-1,670.88)	(222.00-2,051.31)
Cu	48.27 ± 107.92	29.67 ± 16.56	38.77 ± 75.85	18.69 ± 11.09	5.36 ± 9.84	9.57 ± 11.80
	(< LD-369.93)	(10.92-58.51)	(< LD-369.93)	(< LD-33.49)	(< LD-34.81)	(< LD-34.81)
Fe	461.94 ± 305.16	514.15 ± 254.35	479.18 ± 271.44	944.91 ± 591.34	541.59 ± 284.97	668.95 ± 434.02
	(190.95-1,309.01)	(118.20-920.18)	(118.20-1,309.01)	(1,799.45-5,669.50)	(227.04-1,211.91)	(227.04-1,799.45)
Mn	< LD ± 0.00	< LD ± 0.00	< LD ± 0.00	< LD ± 0.00	< LD ± 0.10	< LD ± 0.00
	(< LD-0.02)	(< LD-0.10)	(< LD-0.29)	(< LD-0.20)	(< LD-0.03)	(< LD-0.03)

found to be 0.25 ± 0.73 mg kg^{-1}; Ni concentrations were measured at 0.53 ± 2.51 mg kg^{-1}; Pb concentrations were 0.28 ± 0.47 mg kg^{-1}; and Mn concentrations were below the limit of detection (< LD) in almost all samples (Table 1).

For Chetumal Bay
Bulk bottlenose dolphin skin samples were analyzed for BCH in the State of Quintana Roo. The concentration of metals were Zn > Fe > As > Cu > Cr > Cd > Ni > Pb > Mn.

The elements Zn and Fe had the higher concentrations. Zn concentration was 805.31 ± 545.40 mg kg^{-1}; and Fe was measured at 479.18 ± 271.44 mg kg^{-1}. For the other metals, As was found to be 668.95 ± 434.02 mg kg^{-1}; Cu concentrations were found to be 9.57 ± 11.80 mg kg^{-1}; Cr concentrations were measured at 4.39 ± 9.39 mg kg^{-1}; Cd concentrations were found to be 1.11 ± 1.65 mg kg^{-1}; Ni concentrations were measured at 0.53 ± 2.51 mg kg^{-1}; Pb concentrations were found to be 0.28 ± 0.47 mg kg^{-1}; and Ni and Mn concentrations were < LD in all samples (Table 1).

Comparison Between Both Locations
Significant differences (Mann-Whitney, $p \leq 0.05$) between locations were found for As, Cu, and Zn (Figure 3).

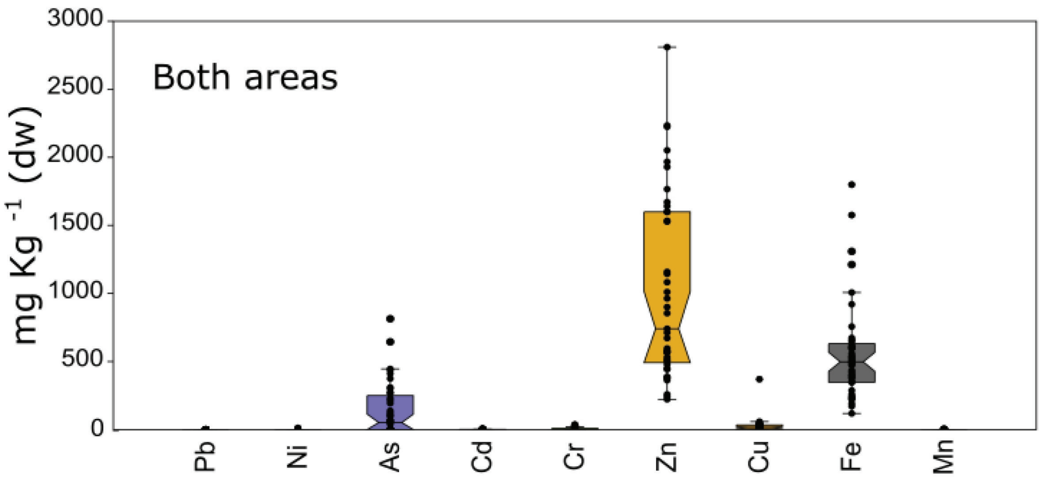

Figure 2. Notched-Boxplots (mean ± Q1, Q3; whisker: 95% confidence interval) for metal and metalloid elements found in bottlenose dolphin (*Tursiops truncatus*) skin samples for YUC and BCH in Mexico. Concentration expressed in mg kg^{-1} dry weight (dw).

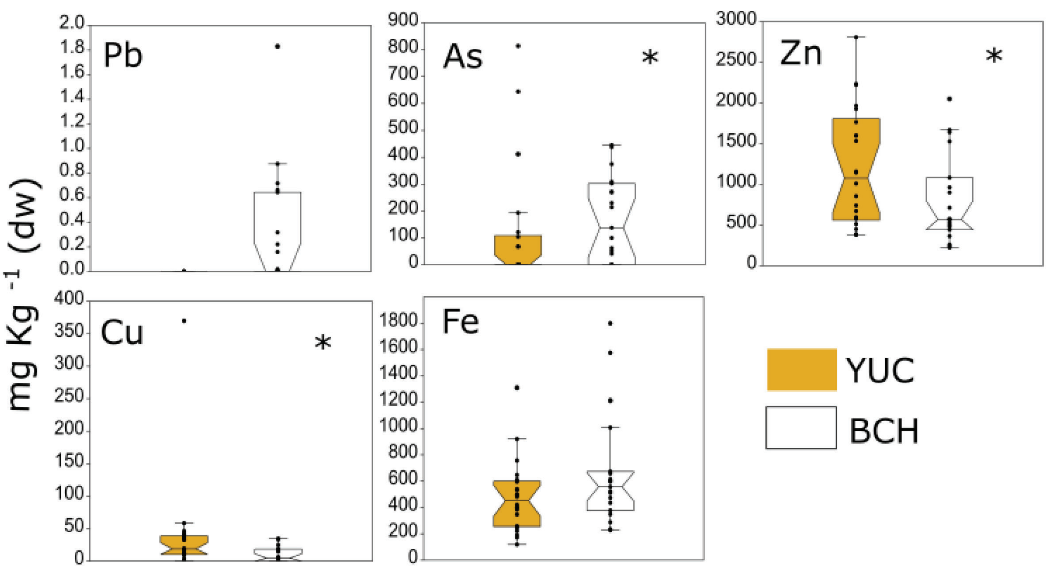

Figure 3. Metal and metalloid elements comparison for bottlenose dolphin skin samples for YUC and BCH in Mexico using Notched-Boxplots (mean ± Q1, Q3; whisker: 95% confidence interval). Concentration expressed in mg kg^{-1} (dw). *Indicates statistical differences in Mann-Whitney, $p > 0.05$.

Comparison According to Sex and Age Categories

When we compare each element's concentration according to sex (male and female) and age (i.e., adult, juvenile, calf) categories for bottlenose dolphins at each location, we do not find statistical differences (Kruskal-Wallis test, $p > 0.05$).

Discussion

Zinc (Zn)

The general pattern of skin samples with Zn as the element with the highest concentration, as found in our analysis, is somewhat common in dolphins (Borrell et al., 2015). Zn plays an essential role in organisms as it is a component of enzymes involved in metabolic and biochemical processes (Borrell et al., 2015; Mouton et al., 2015). It is important to note that this element can become toxic when present in excessive concentrations. Zn plays a crucial role metabolically in the skin, including its enzyme function as a cofactor in various metabolic processes (Decataldo et al., 2004). These enzymes are responsible for catalyzing important biochemical reactions in the skin such as DNA and protein synthesis, wound healing, and antioxidant defense mechanisms. Zn is involved in the synthesis and maintenance of structural proteins, such as collagen and elastin, which are essential for the strength, elasticity, and integrity of the skin. It contributes to the formation and stabilization of the skin's extracellular matrix, ensuring proper skin structure and function (Moulton et al., 2015). Also, Zn is known to play a crucial role in immune function, including the skin's immune response (Monteiro et al., 2020). It is involved in the activation and regulation of immune cells, such as macrophages and lymphocytes, which help protect the skin against pathogens and maintain its overall health (Stavros et al., 2007). As a cofactor for antioxidant enzymes, such as superoxide dismutase (SOD), Zn helps neutralize harmful free radicals and protect the skin from oxidative stress (Chen et al., 2020). This antioxidant defense mechanism is important for maintaining the health and vitality of the skin (Jan et al., 2015).

For bottlenose dolphins, elevated concentrations of Zn have been reported in samples from Israel (1,528 ± 2,185 mg kg^{-1}), the Mediterranean Sea (280 to 3,653 mg kg^{-1}), and Florida (272 to 1,586 mg kg^{-1}) (Roditi-Elasar et al., 2003; Stavros et al., 2007); and also in other delphinids such as Risso's dolphins from Israel (1,087 mg kg^{-1} ww; Shoham-Frider et al., 2002) and Commerson's dolphins from Antarctic waters (Cáceres-Saez et al., 2017). This is evidence of the strong dolphin skin affinity for this element, a general phenomenon among cetaceans (Roditi-Elasar et al., 2003; Borrell et al., 2015; Cáceres-Saez et al., 2017).

Iron (Fe)

Ranking as the second highest element, the concentrations of Fe found here are similar to the results of analyses of other cetaceans' skin samples (Carvalho et al., 2002; Roditi-Elasar et al., 2003; Stavros et al., 2007). However, Fe concentrations in skin samples are similar to those reported for Stavros et al. (2007; ranging from 14 to 1,438 mg kg^{-1} [dw]) for bottlenose dolphins found in Southeast Florida, which are notably high compared with other studies focusing on bottlenose dolphin skin. Unfortunately, the available information about Fe concentrations in the skin tissue of marine mammals is limited (Stavros et al., 2007).

Fe plays a crucial role in health as it is an essential mineral required for various physiological processes. Fe serves as a vital component of hemoglobin, which is responsible for transporting oxygen throughout the body (Cáceres-Saez et al., 2017). Adequate Fe levels support immune function, enabling dolphins to combat diseases and infections effectively. Additionally, Fe is involved in enzymatic reactions and the production of neurotransmitters, influencing cognitive abilities and behavior in dolphins (Carocci et al., 2018). However, imbalances in Fe levels can have detrimental effects on their health (Basak & Kanwar, 2022). Excessive Fe accumulation, known as iron overload, can lead to oxidative stress and tissue damage (Puntarulo, 2005; Sousa et al., 2020). Conversely, Fe deficiency can result in anemia and reduced physiological functioning (Romero et al., 2017). Therefore, maintaining optimal Fe levels in dolphin skin is crucial for their well-being and underscores the importance of understanding the implications of Fe to their health.

Arsenic (As)

The third most abundant metal found in bottlenose dolphin skin samples in our analysis was As, which could represent a significant concern for marine mammals and other marine organisms (Francesconi & Edmonds, 1996; Savery et al., 2014). As is a naturally occurring element, and its presence in marine environments can result from both natural sources and human activities such as industrial processes and mining (Garelick et al., 2008). Cetaceans can be exposed to As through the consumption of contaminated prey or through direct contact with As-contaminated water (Savery et al., 2014). As can accumulate in animal tissues over time and disrupt various physiological processes (Page-Karjian et al., 2020). The toxicity of As varies significantly depending on its oxidation states (Akter et al., 2005; Ventura Lima et al., 2011). The predominant form of As typically found in marine biota, including marine mammals, is primarily in an organic form, specifically arsenobetaine. This organic form of As, such as arsenobetaine, is generally considered non-toxic and poses no significant concerns in terms of toxicity (Becker, 2000). Chronic exposure to As can lead to organ damage, including liver and kidney dysfunction (Stavros et al., 2007). In humans, As has been known to impair the immune system, affect reproductive health, and cause neurological and developmental abnormalities (Abdul et al., 2015). Therefore, the impact of As on bottlenose dolphins will depend on multiple factors but underscores the importance of monitoring and reducing pollution in marine ecosystems to safeguard the well-being of these magnificent creatures.

In the region of the Gulf of Mexico and the Caribbean Sea (Yucatan Peninsula), there have been documented cases of elevated As concentrations. For example, studies have revealed that sargassum (*Sargassum* sp.), a type of seaweed, has been found to contain remarkably high levels of As, reaching up to 172 mg kg^{-1} (dw) (Rodríguez-Martínez et al., 2020; Alleyne et al., 2023). Similarly, shrimp species in the area have shown concentrations ranging from 10 to 35 mg kg^{-1} (dw). Bivalves, such as mollusks and clams, have been measured with As levels of 34.4 ± 3.8 and 76.9 ± 22.3 mg kg^{-1} (dw) (Modestin et al., 2022), respectively, while various fish species have exhibited levels ranging from 2 to 26.5 mg kg^{-1} (dw) (Fattorini et al., 2006). These findings highlight the presence of elevated concentrations of As in different components of the ecosystem regionally (Yucatan Peninsula), raising concerns about the potential impacts on marine organisms, including dolphins.

We hypothesize that the elevated concentration of As observed in this study within the Yucatan Peninsula can be attributed to the processes of biomagnification and bioaccumulation triggered by the massive influx of sargassum reaching the Mexican Caribbean and the southern Gulf of Mexico (Rodríguez-Martínez et al., 2020; Alleyne et al., 2023). The Caribbean and YUC currents play a crucial role in facilitating the transportation of millions of tons of sargassum from tropical Atlantic blooms, thereby introducing As into the local food webs (Modestin et al., 2022; Alleyne et al., 2023). Furthermore, significant amounts of As are discharged into the Atlantic Ocean through the Amazonas River system in South America (Scarpelli, 2005; Texeira et al., 2020; Chen & Costa, 2021), which subsequently becomes incorporated into the tissues of seaweed, specifically *Sargassum* sp. The intricate marine current systems then carry this As-laden sargassum into the Caribbean and Gulf of Mexico (Franks et al., 2016; Louime et al., 2016).

Conclusions

Our findings indicate the presence of various elements in bottlenose dolphin skin from coastal waters in the Yucatan Peninsula, with notable concentrations of zinc, iron, and arsenic (Zn > Fe > As > Cu > Cr > Cd > Ni). The absence of measurable lead and manganese concentrations suggests either low levels or absence of their accumulation in the studied bottlenose dolphin population.

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