

FEATHERS AS INDICATORS OF EXPOSURE TO METALS: STUDY IN *Anas crecca* AND *Anser caerulescens* IN DURANGO, MEXICO

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ABSTRACT

The aim of this study was to evaluate the presence of inorganic elements in feathers of green-winged teals (*Anas crecca* Linnaeus, 1758) and snow geese (*Anser caerulescens* Linnaeus, 1758) that hibernate at Laguna de Santiaguillo in Durango. Additionally, the use of feathers as exposure indicators to metallic pollutants was determined. The hypothesis proposed was that the feathers of both bird species contain detectable concentrations of metals, indicating varying levels of exposure to environmental pollutants based on their habits and migration routes. During the 2021–2022 hunting season, a total of 30 green-winged teals and 27 snow geese were collected. The primary P9 and P10 feathers from the left wing of each bird were gathered for analysis. The feathers were cleaned, dehydrated, and analyzed using voltammetry to quantify the concentrations of Zn, Cd, Pb, Cu, Cr, Sn, Al, As, Ni, and Hg. The results revealed significant differences between species. The teals displayed higher concentrations of As, Cr, and Ni, whereas the geese had higher levels of Ni and Cu. Although essential elements like Zn and Cu were present in high concentrations, non-essential elements such as Cd and Pb were also detected. Particularly, Pb levels in some teal individuals were concerning due to their potential toxicity. Significant correlations were identified between certain metals (As-Cr and Pb-Zn), suggesting common exposures to anthropogenic sources, possibly related to agricultural and industrial activities. This study confirms that feathers serve as effective and non-invasive biomarkers to detect the exposure to metallic pollutants, providing a “chemical memory” of accumulation during growth. Consequently, the working hypothesis is accepted, establishing a foundation for future research and environmental conservation efforts focused on priority wetlands, such as Laguna de Santiaguillo.

Keywords: Heavy metals, metalloids, biomarkers, migratory aquatic birds.

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INTRODUCTION

Heavy metals and other inorganic elements are widely distributed in the environment through the geological cycle (Abbasi *et al.*, 2015; Vizuetete *et al.*, 2018). They are also released by diverse human activities (Abbasi *et al.*, 2015; Abraham-Covarrubias and Peña-Cabriales, 2017), such as industrialization (Kolf-Clauw *et al.*, 2007; Vizuetete *et al.*, 2018), combustion (Haygarth and Jones, 2017), smelting processes (Abdullah *et al.*, 2015), motor vehicle emissions (Estrada-Guerrero and Soler-Tovar, 2014; Abraham-Covarrubias and Peña-Cabriales, 2017), mining (Abraham-Covarrubias and Peña-Cabriales, 2017; He *et al.*, 2019), agricultural runoff (Abdullah *et al.*, 2015; Abraham-Covarrubias and Peña-Cabriales, 2017), and petroleum-related activities (Borah and Deka, 2023).

Some inorganic elements, including zinc (Zn), copper (Cu), and selenium (Se), are essential for living organisms and play a crucial role in their biological functions. Cadmium (Cd), mercury (Hg), and lead (Pb), on the other hand, are not necessary for birds and have been shown to be bad for their health. These elements are naturally present in the environment, resulting in stable exposure levels, although birds have evolved to endure this exposure. The concentrations of essential elements in birds are typically balanced, unless exposure is excessively high, disrupting normal homeostatic processes (Custer, 2011).

Feathers serve as effective biomarkers for metal and metalloid exposure, as these elements bind to proteins in the bloodstream (Dauwe *et al.*, 2003) and accumulate throughout their development and growth process (Lodenius and Solonen, 2013). Within the feather, the metals bind with the metallothioneins and become immobilized. Once the feather stops growing, it keratinizes and stops interacting with the physiology of the bird, which reflects the levels of metals accumulated during its period of growth or molt (Dauwe *et al.*, 2003).

In this context, it was hypothesized that the feathers of green-winged teals and snow geese collected at Laguna de Santiaguillo in Durango, Mexico, contain detectable concentrations of inorganic elements that reflect a differential exposure to environmental contaminants, reflecting their ecological habits and migration routes. Likewise, it was considered that feathers can be used as non-invasive biomarkers to monitor the presence of metallic contaminants in migratory birds. The aim of this study was to evaluate the presence and concentration of inorganic elements in feathers from both species and determine their use as indicators of environmental exposure to generate information for environmental monitoring and the conservation of priority wetlands.

MATERIALS AND METHODS

Site of study

The study area covers an extension of 2542.16 km², encompassing territories within the municipalities of Nuevo Ideal, Canatlán, Santiago Papasquiario, Coneto de Comonfort,

San Juan del Río, and El Oro in the basin of Laguna de Santiaguillo (Figure 1). The predominant climate in this region is temperate semi-dry, characterized by summer rains and scarce precipitation throughout the rest of the year. A subhumid temperate climate with summer rains is also found, along with a semi-cold subhumid climate. The mean monthly temperature in the region is approximately 17 °C, with a mean annual rainfall of 426.5 mm, concentrated between June and September (RAMSAR, 2012).

The basin of the Laguna de Santiaguillo presents a notable plant diversity, which is attributed to the topographic complexity of the region. Different types of forests are identified, including pine, pine-oak, oak-pine, and oak. There are also areas with riparian vegetation, xerophytic shrubland, grasslands, halophytic vegetation, and aquatic and semi-aquatic vegetation, as well as the typical vegetation of disturbed areas (RAMSAR, 2012).

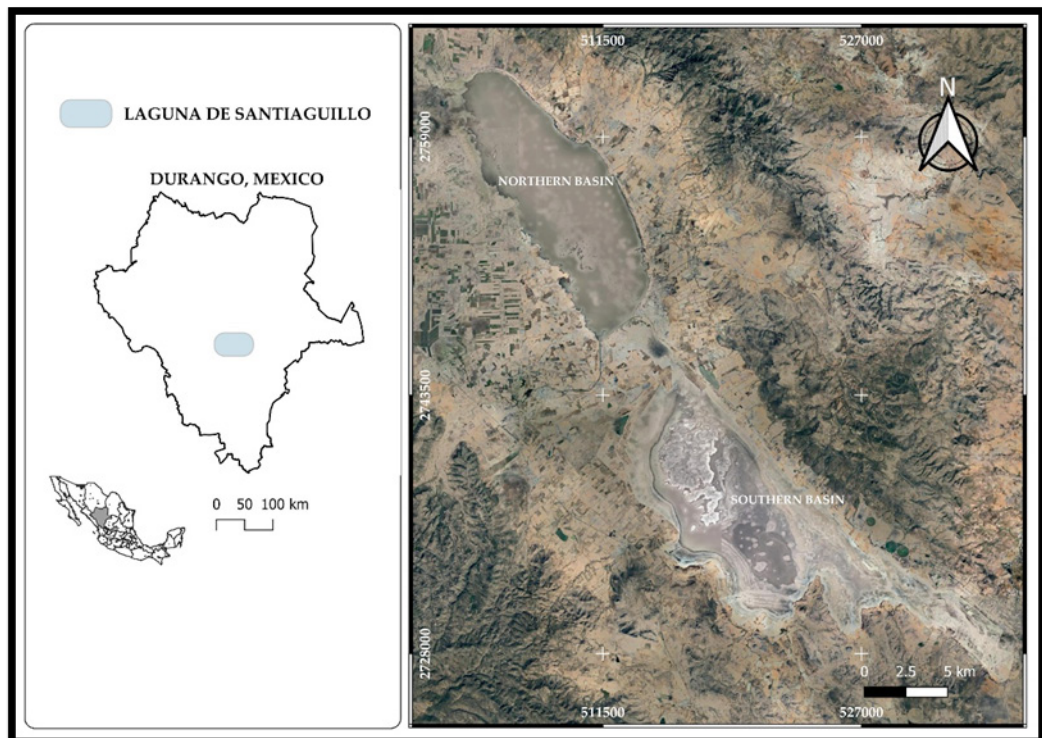


Figure 1. Location of the study site in Laguna de Santiaguillo, Durango, Mexico.

Bird collection

During the 2021–2022 hunting season, 27 snow geese (*Anser caerulescens* Linnaeus, 1758) and 30 green-winged teals (*Anas crecca* Linnaeus, 1758) were captured. The birds were collected with the help of service providers from the Wildlife Conservation

Management Unit, registered under SEMARNAT-UMA-EX-0150-DGO, with authorization document SGPA/DGVS/07540/21. In addition, volunteer hunters helped by donating the birds they hunted to this study.

Analysis of metals

Samples of primary feathers P9 and P10 of the left wings from captured birds were collected and stored in paper envelopes labelled for transportation to the laboratory of the Faculty of Veterinary Medicine and Animal Science (FMVZ) of the Juárez University of the State of Durango. The feathers were cleaned using a 1 % detergent solution (Triton® X-100, CAS 9002-93-1 Biopharm) for 4 h. Then, they were weighed in a fresh state on a precision scale (Sartorius CP224S) and dehydrated in a stove (Thermo Scientific 6964) at 35–45 °C for 48 h.

The dehydrated feathers were packaged and sent to the Institute of Ecology, Fisheries, and Oceanography of the Gulf of Mexico (EPOMEX) of the Autonomous University of Campeche. The samples were cut into small fragments to ensure an even representation of contaminants. From each feather, 0.3 g was transferred to Teflon tubes containing 3 mL of a 9:1 mixture of 65 % nitric acid and 37 % hydrochloric acid. The digestion of the samples was carried out using a MARS 5 microwave digestion system (EPA, 2007). The metals were determined using voltammetry (van den Berg, 1991) with a Metrohm 797 VA Computrace voltamperometer. The analyzed metals and their limits of detection (LOD) were Cd 0.05, Pb 0.03, Cu 0.05, Cr 0.05, Hg 0.02, As 0.02, Zn 0.05, Sn 0.03, Ni 0.4, and Al 0.05 ng g⁻¹.

Statistical analysis

The Minitab 19 (Minitab, LLC 2019) computer program was utilized for analysis. Since the data did not meet the assumptions of normality and homogeneity of variance necessary for parametric tests, the Kruskal-Wallis test was used to compare metal concentrations among species ($p < 0.05$). To identify the most prevalent metals in the feathers of the studied birds, prevalence was calculated as the percentage of samples with detectable concentrations (above LOD). Furthermore, a Spearman correlation analysis was conducted to assess the relationships between metal concentrations detected in both species, which facilitated the identification of shared or divergent bioaccumulation patterns.

RESULTS AND DISCUSSION

The concentrations of inorganic elements (Zn, Cd, Pb, Cu, Cr, Sn, Al, As, Ni, and Hg) found in feathers (Table 1 and Figure 2) varied according to the following pattern: for *A. crecca*, As > Cr > Cu > Ni > Hg > Zn > Sn > Pb > Al > Cd, whereas for *A. caerulescens*, Ni > Cr > Cu > As > Hg > Al > Sn > Zn > Pb > Cd. Coincidentally, in both species, Cr and Cu were found with the highest concentrations (second and third places, respectively), whereas Cd displayed the lowest concentrations. The rest of the elements displayed different distributions among species.

Table 1. Concentration (mg kg⁻¹ dry base) of inorganic elements in feathers of green-winged teals (*Anas crecca*) and snow geese (*Anser caerulescens*) collected at Laguna de Santiaguillo, Durango, Mexico.

[†] Element	<i>Anas crecca</i> (n = 30)	<i>Anser caerulescens</i> (n = 27)	[‡] p-value
Zn	3.46 (8.53), 0.92–48.31	0.34 (0.13), 0.12–0.65	0.00
Cd	0.13 (0.48), 0.00–2.18	0.00 (0.02), 0.00–0.12	0.33
Pb	1.29 (3.35), 0.00–18.73	0.01 (0.04), 0.00–0.16	0.00
Cu	19.13 (25.57), 0.00–146.44	2.41 (1.12), 0.00–4.91	0.00
Cr	22.65 (20.54), 1.71–87.62	3.49 (3.40), 0.00–12.89	0.00
Sn	2.29 (7.20), 0.00–38.12	0.47 (1.77), 0.00–9.30	0.00
Al	0.56 (1.45), 0.00–7.39	1.49 (1.90), 0.00–6.88	0.05
As	24.64 (30.47), 0.00–122.28	2.01 (1.56), 0.17–5.47	0.00
Ni	18.57 (17.44), 0.71–71.98	4.61 (4.97), 0.00–15.57	0.00
Hg	4.09 (16.07), 0.00–76.52	1.52 (3.80), 0.00–15.08	0.22

[†]Values are reported as averages, (standard deviation), minimum–maximum.

[‡]Comparison between species (Kruskal-Wallis, $\alpha = 0.05$).

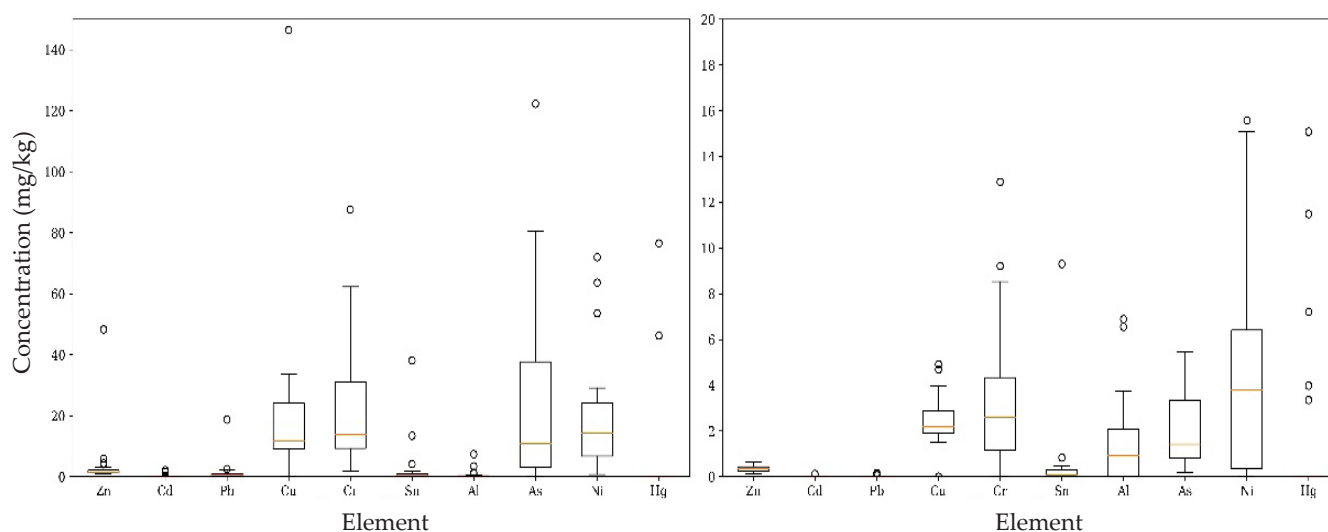


Figure 2. Median, quartiles, and extreme values of inorganic element concentrations (mg kg⁻¹ dry base) in feathers of green-winged teals (*Anas crecca*, left) and snow geese (*Anser caerulescens*, right) captured at Laguna de Santiaguillo, Durango, Mexico.

These findings are similar to metal concentrations found in the feathers of two aquatic birds, *Ardea alba* and *Nycticorax nycticorax*, with Cr having the highest concentration. Furthermore, the species were found to accumulate metals in a different order (González *et al.*, 2018).

Zinc

Zinc was detected in all samples of each species. The amount in the teal feathers was greater (Kruskal-Wallis, $h = 41.9$, $p = 0.00$) compared to those from geese. Zinc is an essential element that plays important parts in diverse metabolic reactions. To date, the effects of chronic exposure to Zn on organisms are not entirely known (Abdullah *et al.*, 2015). This element has natural and anthropogenic origins (Ullah *et al.*, 2014). It is used as a protective coating and in galvanization processes to prevent corrosion. It is also used in alloys and as a catalyst in the synthesis of certain polymers. In addition, it is less toxic than the other metals analyzed in this study (Newman and Unger, 2003).

Cadmium

The content of Cd in the feathers of teals and geese was below the toxicity threshold ($>2 \text{ mg kg}^{-1}$ dry base (DB)) (Burger and Gochfeld, 2000). This element was the least prevalent, as it was detected in 10 % of the teals and 3.7 % of the geese. The amount of Cd found in the teal and goose feathers was similar ($h = 0.95$, $p = 0.33$). Cd is not essential to organisms, as well as being toxic and carcinogenic (Laws, 1993). The harmful effects on birds include alterations in the formation of eggs, damage to testicles, malfunctioning of the oviduct, and kidney damage (Malik and Zeb, 2009). This element is used to manufacture alloys, as a plastic stabilizer, in electroplating processes, galvanization, and the production of pigments, batteries, and other products. Cadmium is found in low concentrations in the rocks and soils, but it is chemically similar to Zn. In addition, it is obtained as a byproduct during Zn processing (Laws, 1993). Sewage sludge is often contaminated with Cd, and its application on agricultural soils can significantly contribute to the accumulation of this element (Jackson and Alloway, 1992).

Lead

The prevalence of Pb in teal feathers was 73.33 %, whereas for geese it was 14.81 %. The amount of Pb in teal feathers was higher than in geese ($h = 24.03$, $p = 0.00$). Although the average concentration in both species does not seem to indicate a significant exposure to this element, some teal individuals were observed to have concentrations of up to 18.7 mg kg^{-1} , suggesting a certain level of exposure.

Reports that suggest that concentrations of over 4 mg kg^{-1} of Pb in feathers are associated with alterations in behavior, thermoregulation, and locomotion, and consequently, a lower survival of chicks, as well as other physiological alterations such as hemolytic anemia and damage to the digestive tract and nervous system, which can even lead to death (Tsipoura *et al.*, 2011; Estrada-Guerrero and Soler-Tovar, 2014). This toxic metal is ubiquitous due to its prolonged and widespread use in gasoline, batteries, welding, pigments, pipes, ammunition, paints, ceramics, and other uses. Bird poisoning can take place due to the ingestion of Pb pellets (Newman and Unger, 2003).

Copper

The prevalence of Cu in teal and geese feathers was 96.67 and 92.59 %, respectively. These values were the highest, considering all the inorganic compounds analyzed in this study. Teals presented a notably higher concentration compared to geese ($h = 31.29$, $p = 0.00$). These values are found within the range reported by Pandiyan *et al.* (2020) in feathers from 15 shorebirds in two hibernation sites in their migratory route in central Asia. In that study, the concentration of Cu in feathers had a range of 0.2–82.3 and 0.2–87.2 mg kg⁻¹ DB in each location.

Copper is an essential element, required at low concentrations for several physiological processes such as red blood cell formation, blood vessel maintenance, the immune system, bones, glucose and cholesterol metabolism, the contractility of the myocardium, and hormonal development (Calvo-Bruzos *et al.*, 2016). However, high doses or chronic exposures can lead to seriously harmful effects, including reproductive, respiratory, gastrointestinal, hematological, hepatic, endocrine, and ocular damage (Chen *et al.*, 1993). Copper is widely used in the manufacture of cables, electronic devices, and pipes. It is also used to control the growth of algae, bacteria, and fungi. Although it is toxic in high concentrations, it can easily form complexes with organic matter dissolved in a solution, reducing the biologically available fraction (Newman and Unger, 2003).

Chromium

This element was found in 100 % of teals and in 88.89 % of geese. A concentration comparison between species displayed a higher value in teals ($h = 30.76$, $p = 0.00$). The presence of Cr in feathers at levels greater than 4 ppm is considered alarming (Pandiyan *et al.*, 2020). In this study, teals displayed a high concentration (22.65 mg kg⁻¹ BS), which could be harmful for them (Burger *et al.*, 2015). The absorption of Cd, Cr, and Pb in bird feathers can be due to secretions of the uropygial gland and the nasal gland and may be enhanced by the binding affinity of keratin for heavy metals (Morais *et al.*, 2012).

Intoxication by Cr is relevant, and some researchers are carrying out complementary studies to understand its impact on bird physiology, particularly on reproductive toxicology, which could provide clues towards wildlife management (Pandiyan *et al.*, 2020). This element is used to manufacture alloys, catalysts, pigments, and wood preservatives. It is also used in the tanning of leather products. It can be found in hexavalent [Cr(VI)] and trivalent forms [Cr(III)]. Hexavalent Cr is carcinogenic, and it is the more toxic of the two (Newman and Unger, 2003).

Tin

In teals, the prevalence of this element was 73.33 %, and for geese, it was 59.26 %. Teals presented a higher Sn concentration in relation to the geese ($h = 7.05$, $p = 0.00$). Sn has no known function in humans, and the documentation of adverse effects is scarce (Thomas and McGill, 2008). As with some essential elements such as Cu and

Zn, there can be situations in which extreme Sn levels may lead to adverse effects (He *et al.*, 2019).

Tin can be released into the environment through diverse anthropogenic and natural sources, including the flow of continental dust, volcanic emissions, and forest fires. The burning of fossil fuels, such as coal and oil, waste burning, and the production of Sn, organotin, iron, steel, and non-ferrous metals are significant sources of contamination. The release of Sn through these processes is approximately 10 times higher than the release from natural sources. These industrial and burning activities contribute to the presence of Sn in the atmosphere and ecosystems, which leads to potentially harmful environmental impacts (Cima, 2011).

Aluminum

This element had a prevalence of 63.33 % in teals and 74.07 % in geese. Average concentrations between species were similar ($h = 3.68$, $p = 0.05$) and lower than those reported by Solonen *et al.* (1998) in species in different habitats and trophic chains such as *Columbia livia* (5–250 mg kg⁻¹ DB, $n = 26$), *Strix aluco* (5–200 mg kg⁻¹ DB, $n = 33$), *Buteo buteo* (5–90 mg kg⁻¹ DB, $n = 21$), *Accipiter nisus* (5–94 mg kg⁻¹ BS, $n = 20$), *Accipiter gentilis* (5–140 mg kg⁻¹ DB, $n = 21$), and *Pandion haliaetus* (33–110 mg kg⁻¹ BS, $n = 17$).

Aluminum is the most abundant metallic element in the Earth's crust, accounting for 8 % of its composition. In normal pH conditions, it is found mainly as a component of minerals like gibbsite (Al₂O₃·3H₂O) and kaolinite (Al₂Si₂O₅(OH)₄) (Stumm and Morgan, 1981). However, under low pH conditions, such as those caused by acid rain or drainage from mines, the presence of free Al (Al³⁺) can increase to unusually high dissolved concentrations, which can be lethal to aquatic species (Newman and Unger, 2003).

Arsenic

The prevalence of As in teals and geese was 96.67 and 100 %, respectively. The concentrations observed in teals were higher than those in geese ($h = 14.84$, $p = 0.00$). In teals, As was the element with the highest concentrations in relation to the nine inorganic elements found in feathers (up to 122.28 mg kg⁻¹ DB). The average values in the teals (24.6 mg kg⁻¹) in the study site are similar to those reported in two areas contaminated with As in Pakistan, where concentrations of 17–24.5 mg kg⁻¹ were reported for herons (*Bubulcus ibis*) (Abdullah *et al.*, 2015).

This element is a metalloid used in the manufacturing of diverse products, such as pesticides, wood preservatives, plant desiccants, and herbicides. It is also linked to coal fly ash and is released when gold and lead are mined. Some fungi can methylate As, producing methylated, demethylated, and trimethylated arsines, which are volatile and highly toxic compounds (Atlas and Barta, 1981). Exposure to As occurs when birds spend their breeding season in areas affected by mining or intensive agriculture, where they eat grains contaminated with pesticides and herbicides.

Arsenic is one of the most toxic inorganic elements, as it can cause serious reproductive, genetic, immunological, cellular, and biochemical disorders (Abdullah *et al.*, 2015). It

is also a carcinogenic compound (Newman and Unger, 2003). There are few reports on bird exposure to this element; however, it is important to know the shared risk that can exist with human populations that live near bodies of water where these birds are found.

Nickel

This element was found in 100 % of teal feather samples and 74.07 % from geese. Teals exhibited significantly higher concentrations ($h = 18.22$, $p = 0.00$), with some individuals reaching elevated levels ($71.98 \text{ mg kg}^{-1} \text{ DB}$). The average concentrations in geese ($4.6 \text{ mg kg}^{-1} \text{ DB}$) were lower than the values reported by Malik and Zeb (2009) in western cattle egret (*Bubulcus ibis*, $7.8\text{--}9 \text{ mg kg}^{-1} \text{ DB}$) in agricultural areas. High Ni concentrations were recorded in *B. ibis* feathers in places near areas with industrial activity related to the manufacturing of electric devices and Ni-Cd batteries, with ranges of $30\text{--}47.5$ and $77\text{--}89 \text{ mg kg}^{-1} \text{ DB}$ (Abdullah *et al.*, 2015). Excess amounts of Ni may have an impact on feather molt (Malik and Zeb, 2009). It is used in alloys such as stainless steel and nickel-plating processes and has countless applications, including the production of batteries. At high enough concentrations, it is toxic and carcinogenic (Newman and Unger, 2003).

Mercury

The prevalence of mercury was 6.67 % in the teals and 18.52 % in the geese. The teals presented extreme values of up to $76.52 \text{ mg kg}^{-1} \text{ DB}$. A comparison of average values in both species resulted in similar values ($h = 1.45$, $p = 0.22$). The average values detected in teal feathers (4 mg kg^{-1}) seem to indicate some level of exposure to this element, as experimental references (Jackson *et al.*, 2011) suggest that values of more than 3 ppm (mg kg^{-1}) may generate some signs of intoxication. González *et al.* (2018) conducted measurements in feathers from *Ardea alba*, *Egretta thula*, and *Nycticorax nycticorax* from Chapala Lake and found maximum values of 1.1 mg kg^{-1} . The source of Hg in aquatic systems may be due to the discharge of industrial wastewater.

Mercury is used in the electronics industry, dental amalgam, gold mining, and paint manufacturing. Phenylmercury compounds and Hg salts are used as fungicides for seed treatments and to inhibit the growth of algae and slime on metallic structures in diverse industries (Atlas and Barta, 1981). This element can be released into the environment as a residue from chemical laboratory products, batteries, fungicides, pharmaceutical products, and as a component in wastewater effluents (Manahan, 2000). When Hg enters water systems, it can remain in the water or sediments (García-Herruzo *et al.*, 2010). It is also present in areas where agrochemicals are used (Doadrio-Villarejo, 2004).

Correlation analysis

Results show significant correlations between diverse metals found in the feathers of both species (Figures 3 and 4), which suggests possible sources of contamination or shared bioaccumulation mechanisms.

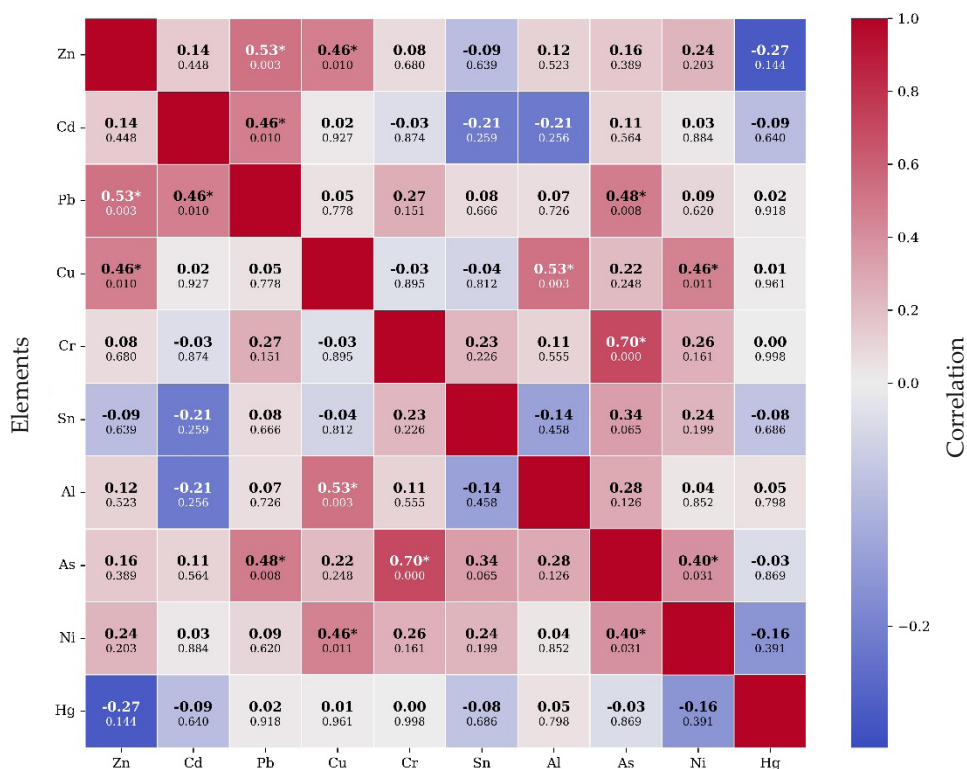


Figure 3. Correlation between the concentrations of metals and metalloids in feathers of green-winged teals (*Anas crecca*) collected at Laguna de Santiaguillo, Durango, Mexico. Each cell shows the Spearman coefficient correlation and *p*-value. Significant correlations ($p < 0.05$) are marked with an asterisk (*).

Correlation of metals in green-winged teals

A significant correlation was identified between Pb and Zn ($r = 0.53$, $p = 0.003$), suggesting that both metals could share a common source of exposure. This association can be attributed to anthropogenic activities, such as industrial emissions or urban waste (Newman and Unger, 2003; Ullah *et al.*, 2014). On the other hand, As and Cr displayed the strongest correlation among the metals analyzed ($r = 0.7$, $p < 0.001$), which suggests that these elements come from similar sources, such as pesticides (Atlas and Barta, 1981; Izydorczyk *et al.*, 2021) or contaminants found in bodies of water near feeding sites. Likewise, Cu and Al displayed a significant correlation ($r = 0.53$, $p = 0.003$), probably related to mining activities or industrial discharges (Izydorczyk *et al.*, 2021) that impact the reproduction and resting areas of birds in their migration path. As and Pb showed a moderate yet significant correlation ($r = 0.48$, $p = 0.008$), indicating a possible relationship between both metals in the exposure environment. On the other hand, the relation between Ni and Cu ($r = 0.46$, $p = 0.011$) suggests possible interactions in bioaccumulation processes or coincidental sources of environmental

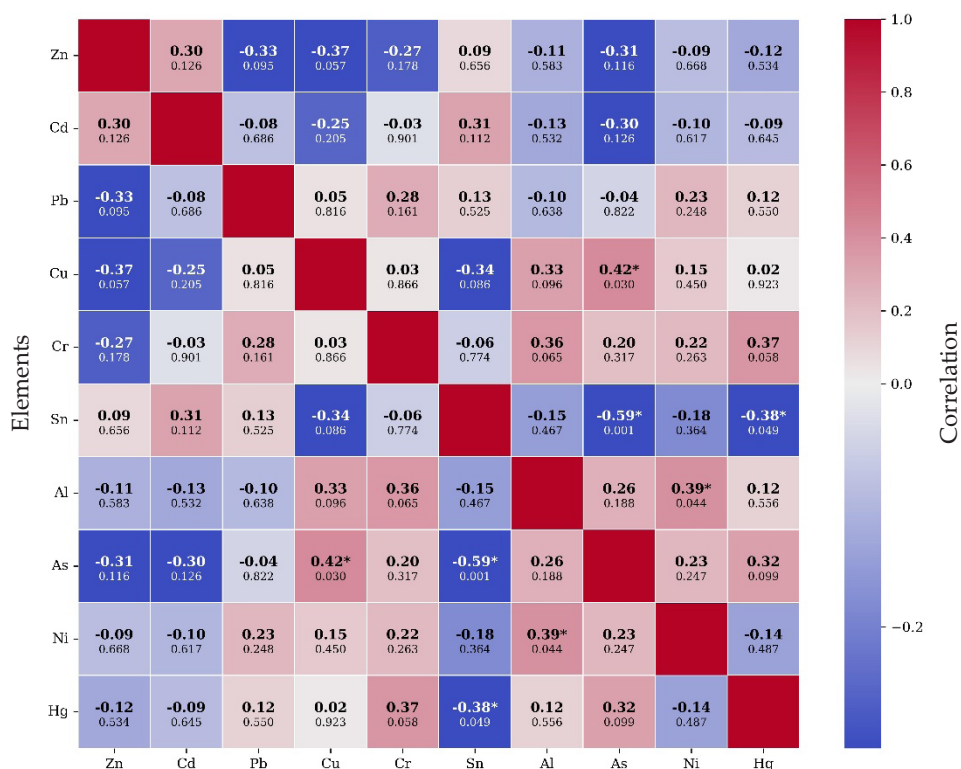


Figure 4. Correlation between the concentrations of metals and metalloids in feathers of snow geese (*Anser caerulescens*) collected at Laguna de Santiaguillo, Durango, Mexico. Each cell shows the Spearman coefficient correlation and p -value. Significant correlations ($p < 0.05$) are marked with an asterisk (*).

contamination. Mercury exhibited weak or negative correlations with the majority of the analyzed elements, suggesting distinct sources or bioaccumulation mechanisms, likely associated with atmospheric emissions or accumulation via aquatic food webs (Charvát *et al.*, 2020). Tin displayed low and non-significant correlations with the metals analyzed, such as with Pb ($r = 0.08$, $p = 0.666$); therefore, it could be associated with independent contamination sources, such as electronic waste or specific manufacturing activities (Cima, 2011).

Correlation of metals in snow geese

Cu and As ($r = 0.42$, $p = 0.03$) presented a significant association that can be attributed to common sources of contamination, such as pesticides or fertilizers used in agricultural fields. Arsenic is a known component in agrochemical products (Atlas and Barta, 1981), while Cu is used as a fungicide (Newman and Unger, 2003) and may be found in the same environments. Geese may be simultaneously exposed to both elements through their diets or the water in wetlands where they feed. Likewise, a significant

correlation was found between Ni and Al ($r = 0.39$, $p = 0.044$), which indicates that these elements could share an environmental source, such as industrial emissions or sediments contaminated in wetlands (Newman and Unger, 2003).

Sn and As ($r = -0.59$, $p = 0.001$) displayed a significant reverse correlation, which suggests different paths of exposure or bioaccumulation in the environment of the snow geese. While As could be related to agricultural sources (Atlas and Barta, 1998), Sn is usually associated with industrial activities or materials used in construction, such as paints or coatings (Cima, 2011). The low co-occurrence of both metals may be due to their environmental availability or the metabolic processes that regulate their deposition on the feathers.

Hg and Sn ($r = -0.38$, $p = 0.049$) also presented a negative relation, which may be due to differences in the environmental distribution of both metals. Mercury is mainly related to contamination from the combustion of carbon and industrial processes (Charvát *et al.*, 2020), whereas Sn could come from local specific activities. The interaction between both metals could be competitive or reflect a spatial segregation of the sources of contamination.

Finally, Cr and Al ($r = 0.36$, $p = 0.065$) displayed a moderate correlation, though not significant. This suggests a possible coexistence of both metals in the wetlands inhabited by geese. Both Cr and Al are transported through water currents and suspended particles (Newman and Unger, 2003), indicating a simultaneous environmental exposure, although not necessarily a joint accumulation in tissues. From a toxicological perspective, simultaneous exposure to multiple toxic metals can negatively affect the health of birds, including reduced fertility, alterations in migratory behavior, and chronic toxicity.

Use of feathers as biomarkers

Evaluating the presence of contaminants in geese and teals requires careful and specific focus due to the migratory nature of these species and the unique characteristics of their feathers, which reflect the accumulation of contaminants during their growth and provide a temporary “memory” of exposure. Focusing on primary feathers has the advantage of being easy to gather in a non-invasive way and covering an adequate exposure period. Collecting the same feathers (P9 and P10) from different birds helps minimize variability and provides a practical and convenient base for biomonitoring (Jaspers *et al.*, 2006). In addition, it is always important to wash the feathers to remove external contaminants.

For the birds studied, the exposure occurs in breeding areas, during hibernation, and throughout the migratory route. Foods and metal concentrations can vary considerably between different areas. The interpretation of metal concentrations in feathers must take into account the metal load found not only in reproductive areas but also the molting patterns of the species involved (Lodenus and Solonen, 2013). The primary feather growth pattern of snow geese and green-winged teals is a well-structured biological and migratory cyclic process. Molting normally takes place after

the breeding season and before migration, ensuring that these birds have new and functional feathers for their migratory flight (Pyle, 2005; Marmillot *et al.*, 2016).

In the case of snow geese, molting takes place between June and November (Mlodinow *et al.*, 2024). During this period, the synchronized replacement of primary and secondary feathers renders the birds incapable of flying for approximately four weeks. The place in which molting takes place varies depending on individual conditions. In general terms, successful breeders molt near their places of birth. However, in many cases, particularly in birds that did not reproduce successfully, it occurs in places far from their places of birth. Other aquatic birds also exhibit this pattern (Bellrose, 2010). For geese, these specific locations are not yet fully known, although it has been suggested that they could include the Great Arctic Lakes (Reed *et al.*, 2003).

The molt of the plumage (or prebasic molt) of the green-winged teal occurs mainly in breeding areas (Johnson *et al.*, 2020). During this stage, adults experience a complete molting of their flight feathers after the reproductive season, which produces a flightless period (Sjöberg, 1988). This process is common in breeding regions located in the northern areas of the continent, such as Canada and Alaska, where the green-winged teal breeds. However, some individuals may move to other nearby areas, before molting, in search of safer places with abundant resources. This phenomenon is known as molt migration. In hibernation areas (such as the southern United States, Mexico, and Central America), molting can be less relevant or incomplete, since the alternative plumage does not imply changes as drastic as in the prebasic molt (Johnson *et al.*, 2020).

The knowledge of molting patterns helps differentiate exposure to metals in breeding areas and hibernating sites independently (Johnels *et al.*, 1979; Burger *et al.*, 1992). The analysis of metal and metalloid concentrations in the collected feathers of teals and geese during winter can reflect the exposure levels to these elements in breeding habitats (Ramos *et al.*, 2009). Examining these feathers is an important way to check the health of both aquatic and terrestrial habitats, find where pollution is coming from, and learn how it might affect ecosystems and biodiversity.

CONCLUSIONS

This study offers a detailed characterization of the presence of inorganic elements on the feathers of green-feathered teals and snow geese that hibernate at Laguna de Santiaguillo, Durango. Both species displayed significant concentrations of metals and metalloids, although the patterns of accumulation differed between them. Teals displayed higher concentrations of As, Cr, and Ni, while geese had higher levels of Ni and Cu, suggesting differences in their exposure and bioaccumulation pathways. Essential metals such as Zn and Cu were detected in high concentrations, as well as non-essential metals such as Cd and Pb, the latter in concerning levels in some teal individuals. Correlations between elements suggest a common exposure to sources of anthropogenic origin and interspecific differences in bioaccumulation.

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