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Original

Mercury and lead bioaccumulation in the Dendrocygna autumnalis duck in the subregion of La Mojana, Colombia

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ABSTRACT

Objective. La Mojana is a biodiverse area of wetlands that is in the Caribbean region, being the habitat of numerous species. Mining, upstream, and agricultural activities have generated the accumulation of heavy metals in these ecosystems. Mercury (Hg) and Lead (Pb) concentrations in 47 samples of blood and feathers of *Dendrocygna autumnalis* (Pisingo) were determined in two sites at the Mojana, Colombia. **Materials and methods.** Concentrations of Hg and Pb in blood and feathers were quantified using Cold Vapor-Atomic Absorption Spectrophotometry (CVAAS) and graphite furnace atomic absorption spectrometry (GFAAS), respectively. **Results.** The highest concentrations of Hg and Pb in feathers and blood were 475.4±180.5 ng/g, 873.59±412.06 ng/g and 9.87±10.6 ng/mL, 218.5±216.6 ng/mL, respectively. 100% of the feather samples from the captured individuals exceeded the permissible limit of 40 ng/g for Hg in duck meat established by the European Union. Likewise, 47% of the blood samples and 53% of the feathers from the captured individuals exceeded the European Union permissible limits for lead in duck meat of 100 ng/g. **Conclusions.** Mining and agricultural activities have generated heavy metal accumulation processes in *Dendrocygna autumnalis*, which represents a threat to the conservation of this species and a risk to the health of the inhabitants of the region due to its consumption.

Keywords: Birds; bioindicators; wetlands; heavy metals; mining; Environmental toxicology (*Sources: ICYT Animal Biology Thesaurus, Environmental Thesaurus for Colombia*).

RESUMEN

Objetivo. La Mojana es una zona biodiversa de humedales que se ubica en la región Caribe, siendo el hábitat de numerosas especies. La minería, aguas arriba, y las actividades agrícolas han generado la acumulación de metales pesados en estos ecosistemas. Se determinaron las concentraciones de mercurio (Hg) y plomo (Pb) en 47 muestras de sangre y plumas de *Dendrocygna autumnalis* (Pisingo) en dos sitios de La Mojana, Colombia. **Materiales y métodos.** Las concentraciones de Hg y Pb en sangre y plumas se cuantificaron mediante espectrofotometría de absorción atómica de vapor frío (CVAAS) y espectrometría de absorción atómica en horno de grafito (GFAAS), respectivamente. **Resultados.** Las más altas concentraciones de Hg y Pb en plumas y sangre fueron 475.4±180.5

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ng/g, 873.59±412.06 ng/g y 9.87±10.6 ng/mL, 218.5±216.6 ng/mL, respectivamente. El 100% de las muestras de plumas de los individuos capturados sobrepasó el límite permisible de 40 ng/g para Hg en carne de pato establecido por la Unión Europea. Así mismo, el 47% de las muestras de sangre y el 53% de las de plumas de los individuos capturados excedieron los límites permisibles para plomo en carne de pato de 100 ng/g de la Unión Europea. **Conclusiones.** Las actividades mineras y agrícolas han generado procesos de acumulación de metales pesados en *Dendrocygna autumnalis*, lo cual representa una amenaza para la conservación de esta especie y un riesgo a la salud por consumo para los habitantes de la región.

Palabras clave: Aves; bioindicadores; humedales; metales pesados; minería; toxicología ambiental (*Fuentes: Tesauro ICYT de Biología Animal, Tesauro ambiental para Colombia*).

INTRODUCTION

La Mojana ecoregion is one of the most important wetland systems in the Colombian Caribbean, which originates from the interaction of the basins of three rivers: Cauca, Magdalena, and San Jorge (1). These wetlands occupy 30.781.149 hectares of the country (2), of which 760.340 hectares are recognized as RAMSAR territories (3), ecosystems with a vast biodiversity fundamental in ecological cycles, in addition to providing ecosystem services to human populations associated with them (4).

La Mojana's biodiversity and ecosystems have been affected by gold mining, which discharges heavy metals into the San Jorge, Cauca, and Magdalena rivers. These rivers surround the wetlands of La Mojana region and transport heavy metals to it (1).

Many studies have recorded high levels of contamination by heavy metals, such as As, Cr, Cd, Hg, Pb, Ni in various ecosystem components of La Mojana. Heavy metal concentrations have been recorded in sediments and organic matter, such as Cu (48400 ng/g), Zn (79200 ng/g), Ni (58100 ng/g), Pb (3200 ng/g), Cd (560 ng/g), Mn (41.100 ng/g), and Hg (100 ng/g) (5), T-Hg concentrations of 0.097±0.049 µg/g in sediments, 0.191± 0.017 µg/g in macrophytes (1), 17.05 ng/g in rice (6), in fish, such as *Caquetaia kraussi* (150 ± 57 ng/g), *Hoplias malabaricus* (239±67 ng/g), *Plagioscion surinamensis* (578±392 ng/g) and *Pseudoplatystoma fasciatum* (568±224 ng/g) (1).

Such contamination is originated mainly in the mining of the gold and agrochemical inputs used in crops of commercial interest (1). Heavy metals that generate pollution processes in La Mojana are of great importance for its abundance, distribution, and effects on the biota are the mercury (Hg) and lead (Pb) (5). Hg causes reproductive, embryological, ethological, nervous, immunological, and genetic problems in the development of birds (7). On the other hand, contamination with Pb in birds generates neuromotor problems, anemia, lethargy, decrease in body mass, and behavioral and reproductive alterations, in addition to affecting embryonic development (8).

Out of the total bird species that Colombia has, 266 species are birds associated with aquatic ecosystems (9), which are of great importance as bioindicators of the conservation status of wetlands and in the ecological processes that occur in these ecosystems (10). La Mojana permanent and temporarily is home to many bird species distributed among the several niches provided by these ecosystems (11).

Among these birds, the Pisingo (*Dendrocygna autumnalis)* is of utmost importance, since it is used as a source of protein of animal origin and is one of the most valued gastronomic dishes in the region (12). Ecology and life habits of *D. autumnalis* can be used to measure the effect of heavy metals on ecosystems where they inhabit. The pisingo is the most abundant neotropical duck. It inhabits wetlands, floodplains, and rice fields. It adapts very well to agroecosystems and reproduces easily. Because their eating habits are based on the consumption of maize kernels and rice, this species becomes a suitable bioindicator of contamination with heavy metals. The eating habits and abundance of *D. autumnalis* make it a good bioindicator of environmental pollution (13,14).

Therefore, the bioaccumulation of mercury and lead in *D. autumnalis* belonging to the assemblage of birds present in La Mojana region, which is being affected by gold mining and agricultural practices was studied.

The purpose of this study was to determine first, the concentrations of Hg and Pb in blood and feathers of *D. autumnalis* (Pisingo) in La Mojana region, in virtue of the absence of records of studies of heavy metals in waterfowl in the region.

MATERIALS AND METHODS

Study area. This research was developed in two sampling stations located in La Mojana, at the intersection of the Magdalena, Cauca, and San Jorge Riverbeds, in the Momposina depression. The first station was located in an area of the San Jorge River near the Ayapel swamp (8°27'34" N - 75°2'37" W), in the district of Cecilia (Ayapel-Córdoba), through which flows the Viloria channel, a sub-basin of the Ayapel swamp, which receives the influx of the San Jorge and Cauca rivers. The second was located in an area of the San Jorge River adjacent to the San Marcos swamp (8°35'19.30" N - 75°4'43.40" W), in the town of El Torno (San Marcos-Sucre), which is a flooded area of the San Jorge River and borders the San Marcos swamp. In the study area, contamination by heavy metals has been documented in various matrices of the ecosystem due to the loads contaminated with heavy metals from the rivers that surround La Mojana. Heavy metal concentrations have been recorded in sediments and organic matter, such as Cu (48400 ng/g), Zn (79200 ng/g), Ni (58100 ng/g), Pb (3200 ng/g), Cd (560 ng/g), Mn (41.100 ng/g), and Hg (100 ng/g) (5), T-Hg concentrations of 0.097±0.049 µg/g in sediments, 0.191±0.017 µg/g in macrophytes (1), 17.05 ng/g in rice (6), in fish, such as *Caquetaia kraussi* (150±57 ng/g), *Hoplias malabaricus* (239±67 ng/g), *Plagioscion surinamensis* (578±392 ng/g), and *Pseudoplatystoma fasciatum* (568±224 ng/g) (1)(Figure 1).

Collection of samples. The samplings were carried out in April and December 2019, which correspond to the months of the rainy and dry season, respectively. 10 nets of mist (9m x 2.5m) were implemented in the capture of the individuals, which were placed in places considered of transit, feeding, and/ or refuge of the birds. The capture hours were between 7:00 and 18:00. The taking of sampling was carried out with the appropriate permits granted by the University of Córdoba and CORPOMOJANA. Captured individuals were identified following the taxonomic keys of Ayerbe (15). With the assistance of a veterinarian and two biologists, between 1 and 3 ml of blood were collected from each bird by puncturing the jugular vein with an insulin needle. The samples were stored in tubes with ethylenediaminetetraacetic (EDTA) anticoagulant acid and kept in expanded polystyrene boxes with refrigerants gels at a temperature of 4-6°C for storage (16). About 10 feathers were collected for each individual. Having the bird properly immobilized, 4-5 feathers were extracted from the chest and 4-5 from another part of the body. The samples were preserved in hermetically sealed plastic bags, labeled, and stored for processing in the laboratory of the Water, Applied and Environmental Chemistry research group of the University of Córdoba (16).

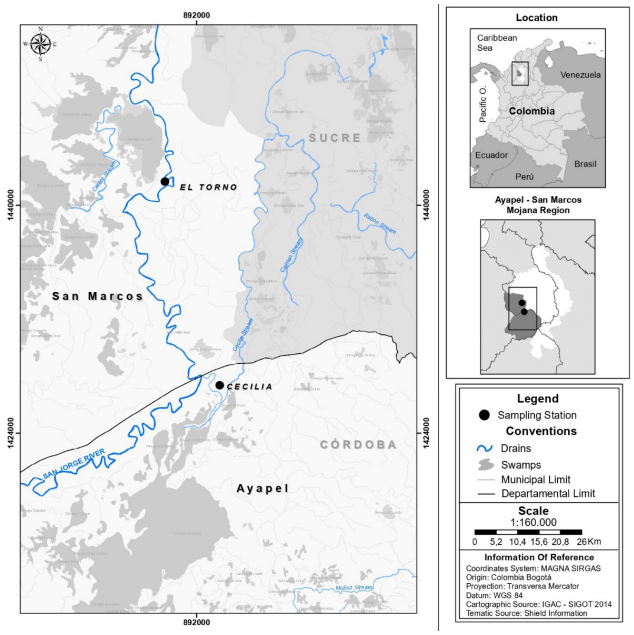

Figure 1. Region of La Mojana (Colombia). Sampling stations: 1. Caño Viloria–Cecilia. 2. San Jorge River – El Torno.

Sample analysis. The determination of Hg and Pb in *D. autumnalis* blood was carried out by means of cold vapor atomic absorption spectrometry (CVAAS) and graphite furnace atomic absorption spectrometry (GFAAS) respectively. 2 mL of each blood sample were transferred to a 100 mL capacity Teflon glass, to which 8 mL of concentrated nitric acid and 2 mL of hydrogen peroxide (30%) were added later on. The vessels closed and their digestion was carried out in a microwave oven at a temperature of 280°C, with a pressure of 80 bars, and a power of 1,400 W for 30 minutes. For the determination of Hg, once cooled, the solutions were transferred to a marked reactor and the final volume was adjusted to 70 mL by adding deionized water for

subsequent analysis (17). For the determination of Pb, once cooled, the solution was taken to a clean and dry container. A 500 µL aliquot of the digested solution was placed in a sample container and its volume adjusted to 1 mL with deionized water (17).

The determination of Hg and Pb in feathers of *D. autumnalis* was carried out by CVASS and GFAAS respectively. The feather samples were washed with deionized water and acetone to eliminate free metals; later on, they were dried at 60°C for 48 h. Feather samples were digested with a mixture of H_2SO_4-HNO_3 (2:1, v/v) at 100-110°C for 3 h, and $KMnO_4$ (5%, p/v) at 100°C for 30 min (18).

Statistical analysis. For the statistical treatment, the means ± standard deviation were determined to express the concentrations of mercury and lead found in the blood and feathers of the 47 captured individuals. In this way, the Shapiro-Wilk test was carried out from which the normality ($p<0.05$) of the distributions of the concentrations of Hg and Pb in the tissues studied was corroborated. Subsequently, the ranges of the concentrations of both metals in blood and feathers were calculated for Ayapel and San Marcos with a significance interval of 95%. The analyzes were carried out with the statistical package R version 3.5.3.

RESULTS

The concentrations of Hg and Pb in the tissues of the birds collected are presented in Table 1, Figure 2 and Figure 3. 47 *D. autumnalis* bird species samples in total were obtained: 22 in the first sampling station (Cecilia) and 25 in the second (El Torno). The mean concentrations of Hg in blood were similar in birds sampled in Cecilia and El Torno, 9.87±10.6 ng/mL, and 5.47±3.45 ng / mL, being slightly higher in Cecilia. With respect to the concentration of Hg in feathers, the concentrations of the first station (475.4±180.5 ng/g) were higher than those of the second (438.46±131.25 ng/g). For Pb in blood, the higher concentration occurred in the first sampling station (218.5±216.6 ng/mL) while lead in feathers was considerably higher in the second station (873.59±412.06). The higher concentration of Hg intervals and Pb were found in the feathers of individuals captured in the first one (179.2 ng/g - 756.9 ng/g) and the second sampling station (243.99 ng/g - 1643.70 ng/g) respectively. The concentrations of Hg and Pb in feathers were higher than those found in blood.

Table 1. Concentration (ng/mL) of heavy metals (Hg and Pb) in blood and feathers of *D. autumnalis* in La Mojana region.

Station	Mean concentration ± SD (Intervals)				n
	Blood (ng/mL)		Feathers (ng/g)		
	Hg	Pb	Hg	Pb	
E1	9.87 ±10.6 1.23 - 4.01	218.5 ±21.6 8.73 - 929.5	475.4 ±180.5 179.2 - 756.9	5.5 ±1.2 0.3 - 37	22
E2	5.47 ±3.45 1.87 - 14.58	94.11 ±94.3 5.58 - 280.55	438.46 ±131.25 186.37 - 676.32	873.59 ±412.06 243.99 - 1643.70	25
Total					47

E1: Sampling station 1. E2: Sampling station 2

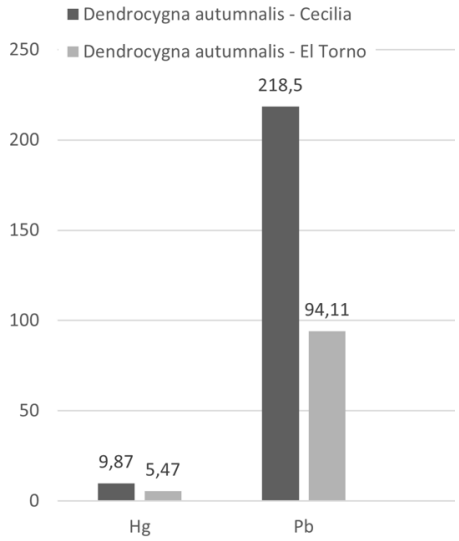

Figure 2. Mean concentrations of Hg and Pb in blood (ng/mL) of *Dendrocygna autumnalis* in Cecilia and El Torno.

Four individuals of *D. autumnalis*, corresponding to 8.5% of the individuals sampled exceeded the value of 15 ng/mL indicator of intoxication by Hg in the blood and the possibility of death described by Scheuhammer et al (19). All the birds presented Hg concentrations in feathers higher than the 5.0 ng/g toxicity indicator described by Eisler (20).

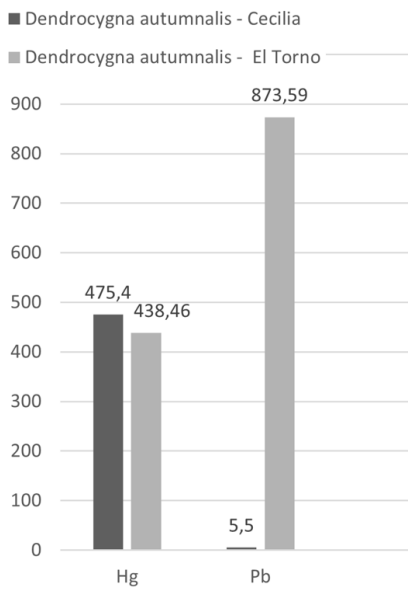

Figure 3. Mean concentrations of Hg and Pb in feathers (ng/g) of *Dendrocygna autumnalis* in Cecilia and El Torno.

Regarding Pb in blood, eleven copies of *D. autumnalis*, corresponding to 23.4% of the individuals sampled presented subclinical intoxication (between 200 and 500 ng/mL), while two individuals (9%) manifested clinical intoxication (between 500 and 1000 ng/mL) as described by Pain (8). Subclinical intoxication manifests with subcellular toxicity in the liver, decreased egg size, impacts on the immune response, reduced egg hatching rate, decreased sperm motility and oxidative stress, with the risk of damaging the components of the cell (8). Clinical manifestations include manifestations such as anemia, lethargy, muscle wasting, and loss of fat reserves, green diarrhea, wing drop, loss of balance and coordination, and other neurological signs, such as paralysis or convulsions (8). On the other hand, none of the *D. autumnalis* individuals exceeded the Pb concentration limit in feathers used as an indicator of toxicity in birds equivalent to 4000 ng/g described by Tsipoura et al (21).

All the feathers sampled exceeded the allowable limit for Hg established by the EU Regulation 2018/73 (22) of the Union European for duck meat (farmed and wild) equal to 40 ng/g. The 22 individuals from the first sampling station recorded a mean concentration of lead in blood (218.5 ± 216.6 ng/mL) higher than the allowable limit for Lead in duck meat recommended by the European Union for maximum levels for certain contaminants in food products (23) of 100 ng/g. Similarly, the 25 individuals from the second sampling station recorded an average value of concentration of lead in feathers (873.59±412.06 ng/g) considerably higher than the above limit.

DISCUSSION

In Colombia, the evidence of Hg contamination in birds is scarce. In our study, the largest concentrations of Hg (475.4±180.5 ng/g) were lower than the concentrations recorded in other studies of heavy metal contamination on Colombian birds. Sierra-Márquez et al (24) reported the presence of this metal in feathers (840±50 ng/g) of insectivorous, nectivorous, and frugivorous birds in Las Orquídeas natural park, Antioquia. Burgos-Nuñez et al (25) determined Hg quantities in feathers of *Fregata magnificens* of 10190 ng/g ng/g in the Cispatá bay - Córdoba, which are higher than all the Hg concentrations found in this study. On the other hand, greater amounts of mercury quantified in blood of *Dendrocygna autumnalis* (9.87±10.6 ng/mL) were lower than those obtained by Albuja et al (26) in 54 wild bird species associated to three mining regions in Ecuador with a maximum value of 162.16 ng/mL. Higher concentrations mean of Pb in blood of *Dendrocygna autumnalis* (218.5±216.6 ng/mL) were higher than those found in *Anas platyrhynchos* (112±66 ng/mL) and lower than those found in *Aythya ferina* (926±463 ng/mL) during a research in aquatic birds (27) in Spain. Greater measurements of Pb in feathers of *D. autumnalis* (873.59±412.06 ng/g) were lower than those obtained by Garitano et al (28) in a study done in species of the Tinamidae family associated with mining areas in Bolivia, where mean concentrations of this metal corresponding to 7764.48 ng/g were measured in this tissue. In a study by Ferreyra et al (29), whose purpose was to determine the incidence of ingestion of lead shot used in hunting and consumed by various species of the Dendrocygna gender. Maximum concentrations of this metal of 17.100±5.330 ng/g were determined, higher than those recorded in our research. Previous studies show how human activities are responsible for the release of heavy metals into the environment, which end in the tissues of birds. These metals when they come into contact with water modify their physicochemical parameters (30).

Factors, such as the physicochemical conditions of water and sediment also play a crucial role in the pollutant dynamics of these metals,

since they favor the bioaccumulative process and increase the concentrations thereof in aquatic ecosystems and organisms, through biomagnification and incorporation into the trophic web (5). These processes depend on water flows and the displacement of particulate material, to a great extent, as well as on its circulation in the organisms that make up the food web, through biomagnification from macrophytes to the higher trophic links of the biota associated to the aquatic ecosystems of La Mojana region (1). All this directly influences the biological dynamics of the organisms that make up the food web to which *D. autumnalis* belongs.

The amounts of heavy metals found in *D. autumnalis* can be explained due to specific aspects of the ecology of the species. In this sense, eating habits of individuals play a crucial role in the bioaccumulation and biomagnification processes of pollutants, such as heavy metals (31). *D. autumnalis* is a species of duck with omnivorous eating habits, whose diet is mainly based on plant grains and small invertebrates (13). Wetlands are the main niche for forage birds, such as D. *autumnalis*, because they are highly productive ecosystems offering a large quantity of seeds and aquatic invertebrates to these species. However, wetlands can be very conducive to the production and bioaccumulation of MeHg, since the growth of crops in water limits solar radiation on the wetland generating adequate biophysical conditions to allow little degradation and greater transport of mercury in crops, specifically rice (32). The degrees of contamination by Hg recorded in this research are due to which *D. autumnalis* feeds on rice crops contaminated with this metal. In research works developed in the study area (6,33), contamination by this metal was determined in rice crops from La Mojana, with concentrations of 17.05 ng/g and concentrations of 0.021 µg/g, respectively. Sediments, water, and the consumption of macroinvertebrates also are sources of contamination for this species. Marrugo et al (1) recorded 0.097±0.049 µg/g of T-Hg in sediments and 0.191 ± 0.017 µg/g in macrophytes from La Mojana.

The mercury tends to accumulate on the blanket sediments of wetlands and to be transported to through the column of water. The species *D. autumnalis*, since it is continuously in these environmental matrices receives doses of mercury by direct contact with the skin and through the ingest of the water. In addition, macroinvertebrates from which it feeds have macrophytes plants and sediments as food source. The process of biomagnification of Hg initiates in sediments, passing to macrophytes, and from these to the aquatic macroinvertebrate from which *D. autumnalis* (34) feeds. The concentrations of Pb registered in blood and feathers of *D. autumnalis* can be explained by the accumulation of Pb in environmental matrices that serve as food substrate for this species, such as rice crops, which require high concentrations of agrochemicals, which are rich in heavy metals (35). These pollutants are fixed in rice and are distributed in other producing strata of the food web (5). Another possible reason contamination with Pb is in the fact that this metal is used as ammunition for hunting of *D. autumnalis*, prompting that individuals from these populations ingest ammunition dispersed in the environment to be used as gastroliths, that is, stones that facilitate digestion (29).

The species *D. autumnalis* presented low levels of toxicity for Hg in blood; however, the total of individuals sampled of this species exceeded the toxicity limit of 5 ng/g for Hg in feathers described by Eisler (20). A proportion of individuals of *D. autumnalis* presented Pb concentrations in blood indicating some degree of toxicity according to what is described by Pain et al (8). The previous levels of toxicity are a clear indication that this species may be being affected by the adverse effects generated by Hg and Pb in the hematopoietic, vascular, nervous, renal, immune, and reproductive systems of birds (7,8), deriving in the alteration of behavioral traits that are vital for the survival of the species (7,8).

The high biodiversity of La Mojana enables the use of wildlife in feeding human populations seated on it. Hunting and the exploit of reptile species, such as *Trachemys callirostris*, *Iguana iguana*, *Caiman crocodilus fuscus*, *Podocnemis lewyana*, and birds, such as *Anas discors*, *Dendrocygna viduata*, *Dendrocygna autumnalis* (12) are common. Pisingo hunt is done with the purpose of using it for consumption as the basis of animal protein and is part of the gastronomy of the region (12). The ingestion of Pisingo meat with the presence of heavy metals generates behavioral, physiological, and reproductive alterations, oxidative damage to DNA, reduction of growth rates and the number of eggs (21) in animals. In humans, mercury affects the nervous system and lead causes a deficit in IQ, behavioral disorders, as well as a decrease in brain volume in adults (36). Various findings

record contamination with Hg in humans from the consumption of other species associated to the wetlands of La Mojana (17). This way, the consumption of pisingo meat contaminated by Hg is added to the consumption of fish, rice, and turtles also contaminated, putting the health of the inhabitants of the area at risk (1,6,33). In a study carried out in La Mojana, people with the presence of mercury in their body stated that they have symptoms, such as headache, lack of energy, exhaustion, nervousness, or irritability, and excessive worry (37). Mercury and its methylated forms generate toxicity from very low concentrations affecting the central nervous, renal, and hematic system (19). The exposure to Pb affects human health, especially in young children and pregnant women (38). The exposition of fetuses and children to Pb is related to neurological disorders, such as the attention deficit hyperactivity disorder and cognitive deficits (39). Lead can cause disorders during pregnancy, delivery, and in neonates. The presence of heavy metals in food is a current issue due to the contamination of the trophic chain involved and the damage they cause to public health (40). Therefore, consumers may incur in health risks due to prolonged consumption of Pisingo meat contaminated with Hg and Pb.

In conclusion, the species *D. autumnalis* has preoccupant contamination levels of Hg and Pb that reveal a great impact of anthropic activity, such as mining and agricultural activities, in the ecoregion of La Mojana. In order to know more precisely the polluting processes generated by heavy metals in the region's avifauna, studies are necessary in a greater diversity of species, in a greater number of sampling sites, and in longer periods of time. We recommend that studies measuring heavy metals in duck meat already captured for consumption be carried out. Based on the data provided by this research, competent environmental authorities design and implement strategies for the evaluation and conservation of aquatic ecosystems and associated birdlife to these in La Mojana.

The ingestion of contaminated pisingo meat is a public health problem since heavy metals bioaccumulate in these organisms after passing through various levels of the trophic web. For this reason, we need to inform and educate the populations of La Mojana about the consumption of pisingo meat to protect individuals of gestational age, children, and the general population from the effects of mercury and lead on their organisms.

Conflict of interest

There are no conflicts of interest.

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