

Mercury in liver and feathers of some birds of prey in Rio de Janeiro State, Brazil:

Preliminary results

Mercúrio no fígado e nas penas de algumas aves de rapina no estado do Rio de Janeiro, Brasil:

Resultados preliminares

Mercurio en hígado y plumas de algunas aves rapaces del Estado de Río de Janeiro, Brasil:

Resultados preliminares

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Abstract

Birds of prey at higher trophic levels are particularly vulnerable to mercury pollution and can serve as indicators of trace elements in different ecosystems. Thus, the aim of this study was to evaluate the presence of mercury in feathers and livers in some birds of prey that occur in the State of Rio de Janeiro, Brazil. Eleven livers were collected from birds of prey during routine necropsies performed at the Rio de Janeiro Zoological Garden, and the feathers from animals in rehabilitation/release process. The results showed mercury concentrations ranged from 0.5 mg / kg to 1.3074 mg / kg in livers and from 0.3376 mg / kg to 3.000 mg / kg in feathers. Liver samples can be collected through invasive methods like biopsies, which involve anesthesia and handling of the bird, or during routine necropsies in

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zoological collections, breeding grounds, and from deceased birds found in the field, especially those affected by trampling or firearm injuries. Alternatively, analyzing feathers for mercury provides a practical, cost-effective, and non-invasive method that avoids risking harm to the bird's physical well-being.

Keywords: Birds of prey; Feathers; Liver; Mercury.

Resumo

As aves de rapina de níveis tróficos mais elevados são particularmente vulneráveis à poluição por mercúrio e podem servir como indicadores de elementos-traço em diferentes ecossistemas. Assim, o objetivo deste estudo foi avaliar a presença de mercúrio em penas e fígados de algumas aves de rapina que ocorrem no Estado do Rio de Janeiro, Brasil. Onze fígados foram coletados de aves de rapina durante necropsias de rotina realizadas no Jardim Zoológico do Rio de Janeiro, e as penas de animais em processo de reabilitação/soltura. Os resultados mostraram que as concentrações de mercúrio variaram de 0,5 mg / kg a 1,3074 mg / kg nos fígados e de 0,3376 mg / kg a 3,000 mg / kg nas penas. As amostras de fígado podem ser coletadas por meio de métodos invasivos, como biópsias, que envolvem anestesia e manuseio da ave, ou durante necropsias de rotina em coleções zoológicas, criadouros e de aves mortas encontradas no campo, especialmente aquelas afetadas por atropelamento ou ferimentos por arma de fogo. Como alternativa, a análise de penas para detecção de mercúrio oferece um método prático, econômico e não invasivo que evita o risco de prejudicar o bem-estar físico da ave.

Palavras-chave: Aves de rapina; Penas; Fígado; Mercúrio.

Resumen

Las aves de presa de niveles tróficos superiores son particularmente vulnerables a la contaminación por mercurio y pueden servir como indicadores de elementos traza en diferentes ecosistemas. Así, el objetivo de este estudio fue evaluar la presencia de mercurio en plumas e hígados de algunas aves de presa que ocurren en el Estado de Río de Janeiro, Brasil. Se recogieron 11 hígados de aves de presa durante necropsias rutinarias realizadas en el Jardín Zoológico de Río de Janeiro, y las plumas de animales en proceso de rehabilitación/liberación. Los resultados mostraron que las concentraciones de mercurio oscilaron entre 0,5 mg / kg y 1,3074 mg / kg en los hígados y de 0,3376 mg / kg a 3.000 mg / kg en las plumas. Las muestras de hígado pueden recogerse mediante métodos invasivos como las biopsias, que implican anestesia y manipulación del ave, o durante necropsias rutinarias en colecciones zoológicas, criaderos y de aves fallecidas encontradas en el campo, especialmente las afectadas por pisoteo o heridas de arma de fuego. Como alternativa, el análisis de las plumas en busca de mercurio ofrece un método práctico, rentable y no invasivo que evita el riesgo de dañar el bienestar físico del ave.

Palabras clave: Aves de presa; Plumaz; Hígado; Mercurio.

1. Introduction

Metals endure in the environment for a long time due to their slow natural cycles, building up in food chains, particularly in water environments. Around 30 of them have significant toxicological concern mainly: cadmium, aluminum, lead, selenium, and mercury (Vajargah, 2021). This last, mercury (Hg), is found in the environment associated with at least 25 other elements, the most common being sulfur (S) forming the mercury sulphide II (HgS) also known as cinnabar, compound of red color, from which the metallic mercury is obtained by heating followed by condensation (Peterson et al., 2023). Volcanic eruptions, natural evaporation and mercury mines are other natural sources of mercury. The amalgamation of mercury in gold extraction, industries that burn fossil fuels, garbage incinerators, paper pulp, paints, fungicides, mercury vapor lamps, batteries and the production of fertilizer from sewage sludge with industrial waste are some sources of anthropogenic mercury in the environment (Panzenhagen et al. et al., 2024).

Mercury and its compounds are commonly present in both biotic and abiotic environments. However mercury is not necessary for the vital processes of plants and animals and is considered an environmental contaminant, especially when in its organic forms (Amundsen et al, 2023), being well known the toxicity of methyl mercury (MeHg). Mercury entering the oceans and inland waters can be converted methyl-mercury by bacteria and accumulated in the aquatic food chain. Several factors, including pH, oxygen availability, temperature, and nutrient concentration, are important in the processes of methylation of Hg in the environment (Gojkovic et al., 2023). In mercury biotransformation, methylation and demethylation are essential steps that affect both the passage and effects of mercury in the ecosystem as well as kinetics and toxicity to animals (Kumar et al., 2023).

Although raptors are not very easy to identify, they have well established systematics and there is little risk of misunderstanding due to uncertainties about the identity or relationships between the species studied. This increases the usefulness of these birds as bioindicators, reducing the risk of misinterpretation (Monserate-Maggi et al., 2023). Similarly, birds of prey occupy mainly the upper trophic levels, which helps to show chemical contamination in various compartments of the ecosystems by biomagnification of persistent elements such as mercury (Bahamonde et al., 2023). A study of the wild fauna of the Arctic showed that exposure to mercury began in the latter half of the 18th century, and currently the source of anthropogenic contamination is approximately 92% (Grunst et al., 2023). The concentration of trace elements in birds can be evaluated using liver, kidneys and tissues such as bones, muscles, fat, eggs, feathers and excreta (Masad et al., 2023; Ashraf et al., 2023).

The concentration of mercury in feathers may reflect both the blood concentration (Carravieri et al., 2022; Viana et al., 2024) and the concentration in the tissues (Skibniewska & Skibniewski, 2023) during the period in which the feather was developed. Birds deposit metals and other elements in the feathers, which can be a significant detoxification route for the metal. The feathers in some cases contain from 49% to 93% of the body's mercury load, mainly as methyl mercury (Adeogun et al., 2022; Zubair & Ullah, 2022). Another preponderant factor for the use of feathers is that sampling is relatively easy, even with the use of non-destructive techniques, which eliminate the need for euthanasia of birds. Feather samples are easy to collect and reduce sampling costs.

The birds liver examination has special importance, as it affects about 90% of the tumor lesions cases and infectious problems besides the metabolic alterations. Likewise, its size in relation to the other visceral organs offers during necropsy the best conditions to highlight the pathological modifications (Zhao et al., 2022). Due to its role in excretion, the liver is exposed to various concentrations of toxic substances and their metabolites, subjecting liver cells to these non-metabolized substances; thus, the liver can provide information on the conditions of the environment in which the bird lived (Kraikivska et al., 2023; Wang et al., 2023; Zhou et al., 2023).

According to Mallet-Rodrigues and Pacheco (2015), there are at least 50 species of birds of prey in Rio de Janeiro State. Some of those are threatened due to several factors, especially the anthropogenic ones. Three species are considered extinct in the state: crested eagle (*Morphnus guianensis*) and the orange-breasted falcon (*Falco deiroleucus*), although these birds do not appear in the last list of the Ministry of the Environment (Silveira & Straube, 2008). Nevertheless, birds of prey are mainly carnivorous, varying feeding habits among different species as piscivorous, which some are specialized in removing mollusks from their shells (Table 1). Others capture reptiles, mammals and birds, or hunt insects or take advantage of carcasses of dead animals and also the fruits of palm trees (Duque-Correa et al., 2022; Lindberg & Odsjoe, 1983; Lemos & Freitas, 2009; Bressan & Lemos, 2013).

Table 1 - Birds of prey diet cited in this research.

<i>SPECIES</i>	<i>DIET</i>
<i>Rupornis magnirostris</i>	Birds, lizards, insects. Captures bats in its dorns. Prey on birds nests
<i>Geranoaetus albicaudatus</i>	Insects, mammals, repteis, amphibians, birds. Eats animal carcasses run over on highways.
<i>Buteo brachyurus</i>	Although specialized in catching birds also preys lizards.
<i>Urubitinga coronata</i>	It catches medium mammals like possums, rabbits, armadillos, wild rats, besides birds and reptiles. Eventually it consumes dead mammals and birds.
<i>Harpia harpyja</i>	Large mammals such as monkeys, sloths, armadillos, cotias, deer pups, wild dogs and large birds such as macaws and seriemas.
<i>Falco sparverius</i>	Small rodents, lizards and large insects. It also captures birds.
<i>Falco peregrinus</i>	It catches birds such as pigeons, southern lapwing, sandpipers, ducks, but also feed on bats and small mammals.
<i>Megascops choliba</i>	It catches large insects like grasshoppers and moths. Also hunting small vertebrates such as mice and frogs.
<i>Asio clamator</i>	Hunting mice, bats, birds, amphibians, reptiles and large insects.

Source: Brown e Amadon (1989), Sick (2001), Lindberg e Odsjoe (1983).

While the total amount of mercury on the planet has remained constant, the amount released into the biosphere has dramatically increased over the past century. Emissions from fossil fuel combustion (such as coal and oil-fired power plants) are responsible for generating the greatest amounts of mercury into the atmosphere, where it is then precipitated into the food chain through soil contamination and bioaccumulation in fish and in others animals tissues, like muscle and liver. Other contamination sources include medical and hazardous waste incineration, industrial (paint, pesticides, batteries, paper) waste, and even broken thermometers and manometers. The hazards of mercury contamination make accurate detection extremely important. Therefore, the aim of this study was to evaluate the presence of mercury in feathers and livers in some birds of prey that occur in the State of Rio de Janeiro, Brazil.

2. Material and Methodos

2.1 Sample collection

This research was an ecological descriptive field study (Toassi & Petry, 2021), in which 11 livers were collected from two species of *Accipitridae* and two species of *Strigidae* (Table 2), during routine necropsies performed at the RIOZOO Foundation. Before the necropsy, the birds were weighed using an Avinet scale with a gram precision for the smaller birds and a mechanical scale with a precision of five grams for the larger birds. After opening the carcass and access to the celoma cavity, the liver was removed, weighed and each lobe was measured in its length and width, using a caliper. The livers were then photographed together with an identification number and preserved in 10% formaldehyde buffer solution. For the mercury analysis, the formalin preserved livers fragments were removed and allowed to dry at room temperature for 24 hours.

The feathers used in this study were obtained from specimens received for rehabilitation, supported by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, Cooperation Agreement IBAMA/RJ number 02022.003488/98-36), except for Harpy eagle (*Harpia harpyja*) which was under the care of the Wildlife Rehabilitation Center of Estácio de Sá University, Rio de Janeiro, Brazil. When it was possible, the oldest secondary remex were selected because they emerged before the other contour feathers, and an older pen is more likely to accumulate the contaminant than a newer pen. Also the removal of a secondary remige compromises the flight less than the removal of a primary remige. In some cases the feathers dropped during molting were used. The feather fragments were removed at three different sites of the vexillum. The feather fragments were mixed and three replicates were made for each bird. Four species of *Accipitridae* and two species of *Falconidae* were evaluated (Table 2).

Table 2 - Species of birds of prey in which mercury concentrations were measured in livers and feathers.

FAMILIES	SPECIES	N	ORIGIN	SAMPLES
STRIGIDAE	<i>Asio clamator</i>	2	RIOZOO *	Liver
	<i>Megascops choliba</i>	1	RIOZOO	Liver
	<i>Buteo brachyurus</i>	1	Niterói **	Feather
	<i>Geranoaetus albicaudatus</i>	2	RIOZOO/Niterói	Liver and Feather
ACCIPITRIDAE	<i>Harpia harpyja</i>	1	CRAS/UNESA ***	Feather
	<i>Rupornis magnirostris</i>	7	RIOZOO	Liver
	<i>Urubitinga coronata</i>	1	Teresopolis *	Feather
FALCONIDAE	<i>Falco peregrinus</i>	1	Niterói	Feather
	<i>Falco sparverius</i>	1	Niterói city	Feather

* RIOZOO = Rio de Janeiro Zoological Garden at the Rio de Janeiro city.** Niterói and Teresópolis are cities near the city of Rio de Janeiro.

***CRAS/UNESA = Wildlife Rehabilitation Center, Estácio de Sá University, located in Rio de Janeiro city. Source: Authors.

2.2 Mercury analysis

The mercury analysis was performed with a mercury direct analyzer (DMA-80 Milestone, Sorisole, Italy), following by HG / EM-01 Environmental-Feathers protocol using a maximum of 10.0 mg of feathers. As there was no specific protocol for birds liver, the HG / FO-21 Food-Bovine muscle and liver protocol was used with a maximum of 250 mg of sample (Milestone Application Book, 2011).

2.3 Statistical analysis

The study was performed with 17 bird specimens, which the results was presented as the mean \pm standard deviation. Statistical analysis was conducted using analysis of variance (One-way ANOVA), followed by Tukey's posthoc test with a 95% confidence interval ($p < 0.05$) to determine significant differences between sample means. Additionally, correlation analysis using Pearson's linear coefficient was employed to assess the relationship between antioxidant compounds. The tabulation and data analysis were carried out using the XLSTAT® software (Addinsoft, Paris, France).

3. Results and Discussion

Table 3 shows mercury concentration in some birds of prey livers and feathers, as well; in Table 4 are presented in the three families surveyed in this study. This metal concentration ranged between 0.5174 to 1.1661 mg/Kg (Table 3 and Table 4). Table 5 introduces weights of the birds and their livers used in this research. As expected, a positive relationship was observed between bird weight and their respective livers weight (Figure 1), and an inverse relationship between liver weight and mercury concentration (Figure 2). The highest mercury concentration in livers was found in *Rupornis magnirostris* and the lowest in *Geranoaetus albicaudatus* (Table 4), while the mercury concentration in the feathers was higher in *Urubitinga coronata* and smaller in *Harpia harpyja* (Table 4).

Table 3 - Mercury concentrations in livers and feathers of some birds of prey at Rio de Janeiro state, Brazil.

SPECIES	LIVER(mg/Kg)		FEATHER (mg/Kg)	
	n	means/sd	n	means/sd
<i>Asio clamator</i>	2	0.8031 ± 0.12	-	---
<i>Megascops choliba</i>	1	1.1661 ± 0.00	-	---
<i>Buteo brachyurus</i>	-	---	1	0.8685 ± 0.04
<i>Geranoaetus albicaudatus</i>	1	0.5174 ± 0.19	1	1.0004 ± 0.09
<i>Harpia harpyja</i>	-	---	1	0.3556 ± 0.03
<i>Rupornis magnirostris</i>	7	0.9029 ± 0.21	-	---
<i>Urubitinga coronata</i>	-	---	1	2.9852 ± 0.02
<i>Falco peregrinus</i>	-	---	1	0.6575 ± 0.03
<i>Falco sparverius</i>	-	---	1	0.9490 ± 0.03

Source: Authors.

Table 4 - Mercury concentrations in livers and feathers in the three families surveyed in this study.

FAMILIES	N	LIVER	N	FEATHER
		MEANS/SD (mg/Kg)		MEANS/SD (mg/Kg)
STRIGIDAE	3	0.9846 ± 0.06 ^a	----	-----
ACCIPITRIDAE	8	0.7102 ± 0.20 ^{a, A}	4	1.3024 ± 0.05 ^{a, A}
FALCONIDAE	----	-----	2	0.8033 ± 0.03 ^a

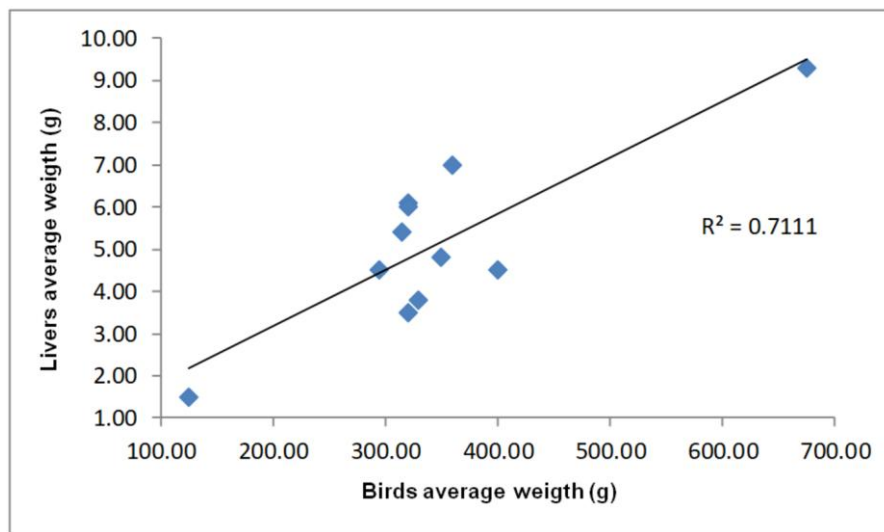
* Equal lowercase letters in the same column indicate that there are no differences between means (p> 0.05). Equal capital letters on the same row indicate that there are no differences between means (p>0.05). Source: Authors.

Table 5 - Weights of the birds and their livers.

SPECIES	GENDER	BIRD WEIGHT (g)	LIVER WEIGHT (g)
<i>Megascops choliba</i>	Male	125,0	1,50
<i>Asio clamator</i>	Female	320,0	6,10
<i>Asio clamator</i>	Male	295,0	4,50
<i>Rupornis magnirostris</i>	Female	315,0	5,40
<i>Rupornis magnirostris</i>	Female	320,0	3,50
<i>Rupornis magnirostris</i>	Male	360,0	7,00
<i>Rupornis magnirostris</i>	Female	320,0	6,00
<i>Rupornis magnirostris</i>	Male	330,0	3,80
<i>Rupornis magnirostris</i>	Female	400,0	4,50
<i>Rupornis magnirostris</i>	Female	350,0	4,80
<i>Geranoaetus albicaudatus</i>	Male	675,0	9,30

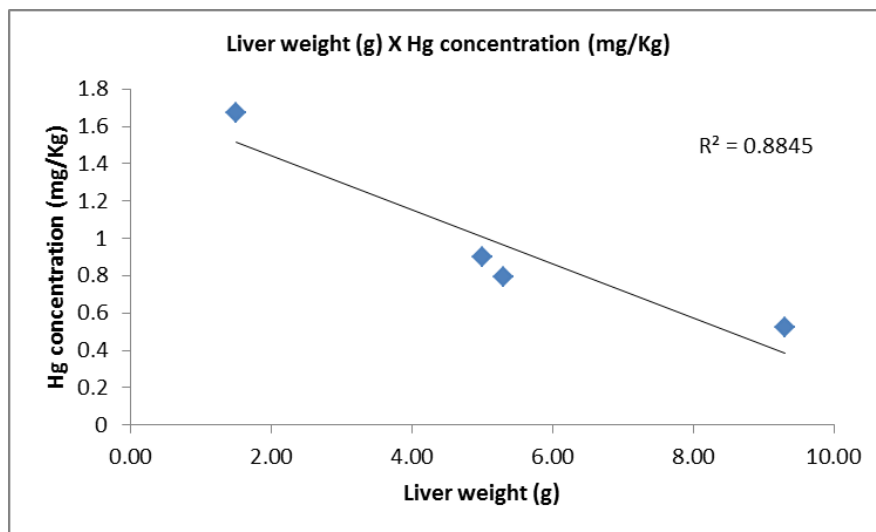
Source: Authors.

Figure 1 - Relationship between birds weight (g) and livers weight (g) in some birds of prey necropsied at Rio de Janeiro Zoological Garden.



Source: Authors.

Figure 2 - Relationship between liver weight (g) and mercury concentration (mg/Kg) in some birds of prey necropsied at Rio de Janeiro Zoological Garden.



Source: Authors.

The concentration of mercury in the smaller livers was higher than that observed in the larger livers, and there was a positive and significant correlation ($p < 0.05$) between the liver weight and the observed mercury concentration (Fig 2). No significant differences ($p > 0.05$) were found between the concentrations of mercury in the feathers and livers of the raptors analyzed in this study (Table 4).

Birds of prey in zoos are fed with small animals (guinea pig, mice, rats, quail and chicks) reared in biotery or purchased from suppliers. In some cases commercial beef is also used. Both animals raised in their own facilities and those purchased from outside suppliers are fed with commercial feed, which uses agricultural products (corn, soybeans, sorghum) as feedstock. These agricultural products are subject to cultural treatments involving chemical fertilization and spraying with herbicides and insecticides for pest control. Accidents caused by the use of methyl mercury as a fungicide for grain seed

treatment confirm the problem of mercury contamination mainly in the form of organomercury compounds (Kumari & Kumar, 2024; Pathak et al., 2024). Mercury poisoning may occur as a result of the accidental delivery of pellets, pelleted feed or concentrates treated with mercury-based antifungal agents, the powders having 5.25% methoxyethylmercury silicate or dicyandiamide methylmercury are the most frequently used products (Martoredjo et al, 2024). Birds of prey fed with rats, quails, chicks, mice fed with rations or grains contaminated with mercury can potentially accumulate mercury in the liver and later on feathers. Birds of prey that are fed with rats, quails, chicks and mice which were fed with rations or grains contaminated with mercury can potentially accumulate mercury in the liver and later on the feathers.

All birds were specimens that were in rehabilitation, and the molting was beginning, therefore the collected feathers represented the situation in the natural environment, despite the change in food items during rehabilitation. The concentration of mercury observed in *Urubitinga coronata* feathers (2,9852 mg / kg) was much higher than in the feathers of the other species analyzed, suggesting a relationship with the place of bird origin, since the municipality of Teresópolis is an area of production of fruits and horticultural crops, where agricultural pesticides are used in large scale. In addition, it was already observed trace elements in *Apis mellifera* honeys from the same region (Ribeiro et al., 2014).

Although the municipality of Niterói is cited as having the highest concentration of atmospheric mercury among the four coastal regions analyzed in the State of Rio de Janeiro (Lacerda et al, 2002), the four birds from this municipality presented lower concentrations of the metal, although many industries in Rio de Janeiro metropolitan area have mercury as a component of their atmospheric effluents, especially petroleum refineries, and iron and steel factories.

Kenntner et al. (2003), in a study carried out in Germany, stated that mercury levels did not indicate high exposure to the contaminant in 61 livers of some specimens of northern goshawk (*Accipiter gentilis*), varying from 0.006 to 1.444 mg / kg (mean 0.138 mg / Kg). In this study the results for the Accipitridae family, the same family as *Accipiter gentilis*, presented an average of 0.7061 mg / kg, which is 5 times greater than observed in the study of those authors, although the maximum value found by them (1.444 mg / kg) was similar to that found in the present one (1.3074 mg / kg). The birds used in the study by Kenntner et al. (2003) were found dead or agonizing in several German cities, a situation similar to that of the birds in this study, which were taken to the RIOZOO Foundation by environmental authorities after being rescued from accidents.

Hughes et al. (1997) studied mercury levels in feathers of Osprey (*Pandion haliaetus*), an accipitrid that feed fishes, and found averages ranging from 6.70 to 28.80 ug / g (dry weight) at three different sites. The values obtained by these researchers are significantly lower than the values found for feathers of the family *Accipitridae* (1.30 ± 0.04 mg / kg) in the present study. This event may be explained by the fact that ospreys were breeding pups happens after change of feathers during the breeding season. As well, the feathers collected were new feathers that had not yet accumulated a considerable amount of mercury, while the feathers collected in the present study were old feathers that fell during the molting. Feather molting is the way that birds eliminate the mercury accumulated between one molt and another (Gatt et al., 2021; Tian et al., 2024).

Although this study did not evaluate the differences in mercury levels in different feather regions, nor between different feather types, the results obtained for *Accipitridae* (1.30 ± 0.04 mg / kg) did not differ much from those obtained by Solonen et al. Lodenius (1990), who considered such variations (1.93 ± 0.71 mg / kg).

In the southwest of Iran, Zolfaghari et al (2007) found mercury concentrations of 1.25 ± 0.68 mg / kg and 1.87 ± 0.70 mg / kg with secondary remex of seven species of *Accipitridae* and two species of *Falconidae*, respectively. In this study the mercury concentration in the primary remex of two species of *Falconidae* was 0.80 ± 0.03 mg / kg (range 0.64 to 0.99 mg / kg) and 1.30 ± 0.04 mg/kg (range 0.34 to 3.00 mg / Kg) in four species of *Accipitridae*, which could be influenced by mercury contamination in the area.

4. Conclusion and Suggestions

Birds of prey occupying higher trophic levels are more susceptible to mercury contamination and can be used as indicators of trace elements in various environments. Liver samples can be obtained through biopsies, an invasive method that requires anesthesia and manipulation of the bird, or during routine necropsies in zoological collections, breeding sites, and eventually dead bodies found in the field, especially victims of trampling and firearm projectiles. Otherwise, the use of feathers for mercury analysis is a practical, low-cost and non-invasive method that allows working without endangering the physical integrity of the bird.

Future studies should assess the broader ecological impact of mercury pollution in habitats of birds of prey by analyzing contamination sources in water, soil, and prey species. This could elucidate bioaccumulation and biomagnification in the food web, highlighting risks to other wildlife and humans, which findings could inform local environmental policies and wildlife conservation strategies, leading to regulations to reduce mercury emissions. Thus, public awareness campaigns could emphasize ecosystem preservation and species health, fostering community support for conservation efforts. Together, these actions could protect the environment and its diverse species.

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