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Heavy Metal Concentrations in Free-Living Southern Caracaras (*Caracara plancus*) in the Northeast Region of Brazil

Luana Thamires Rapôso da Silva¹, Emanuel Felipe de Oliveira Filho¹, Taciana de Holanda Kunst², Vitor Pereira Matos Rolim¹, José Sérgio de Alcântara e Silva³, Rodrigo Farias Silva Regueira³, Ana Paula Silveira Paim², Pierre Castro Soares¹ & Andréa Alice da Fonseca Oliveira¹

ABSTRACT

Background: With the continuously increasing release of heavy metals in the environment, mostly from anthropogenic sources, there is a need to find ways of evaluating and managing the issuance of these contaminants and correct its damages. The birds being at the top of some food chains reflect the presence of metals in the environment, keeping this in perspective, raptors have been successfully used for heavy metals biomonitoring studies in the past. The purpose of this study was to identify and quantify cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), and chromium (Cr) in the feathers and livers of free-living southern caracaras, live and dead, from Recife, Pernambuco state, Northeast of Brazil.

Material, Methods & Results: Sixty-two feathers from live and dead southern caracaras and 21 livers from dead southern caracaras were analyzed by atomic absorption spectrometry for Hg and by inductively coupled plasma optical emission spectrometry for Cd, Cr, Pb, and Cu. Concentrations of Cr and Cu elements were detected in all feather and liver samples analyzed from live and dead caracaras. There was no difference in the concentration of metals between feathers of dead and live caracaras: Pb ($P = 0.3576$), Cd ($P = 0.0792$), Cr ($P = 0.5475$), and Cu ($P = 0.3603$), but significant variation was observed for Hg ($P = 0.0459$). The highest concentrations of Pb ($P < 0.0001$) and Cr ($P < 0.0001$) were found in the feathers than in the liver. On the other hand, the highest concentrations of Cu were found in liver samples ($P = 0.0011$). No significant variation in the concentrations of Cd ($P = 0.7770$) and Hg ($P = 0.3669$) was found between feathers and liver samples.

Discussion: Chromium, as well as Hg, and Cu have a high affinity for keratin, which may explain the higher concentrations of Cr in caracaras feathers in this research. Lead was detected in all liver samples analyzed. Elevated levels were also found in the feathers of dead (95.2%) and live (75.6%) caracaras. The presence of Pb may be due to external contamination by pollutants, such as fossil fuels. Higher concentrations of Hg were observed in dead caracaras feathers, this concentrations change during molting but are not affected by external contamination. Metals, such as Hg, and Cd, accumulate in organisms from different trophic levels, which may indicate that this contamination comes mainly from feed. Pearson's coefficient here showed no correlation between metals from dead caracaras feathers and livers, although a few previous studies have shown a correlation between metal concentrations from tissues and feathers. This pattern can be attributed to the different time of exposure of feathers to metals in relation to livers. The concentrations of metals in hepatic tissues reflect the levels of elements in the diet of these birds, characterizing acutely the contamination of the ecosystem. On the other hand, feathers represent the exposure in the time of molting, when the artery supplies metals, thus representing a chronic exposure. In feathers from live birds, the Pearson's correlation analysis showed a high to moderate correlation between metals, which may suggest that they come from a similar source, but there is no way to specify the origin since the metals surveyed could be present in the soil, water, or in the prey eaten by these animals. For the purpose of environmental analyses, it is required to determine the exact source of contamination. Considering that it is possible to confirm the presence of these heavy metals in southern caracaras, these birds may be important environmental bioindicators. The development of systematic research on animals and the environment is essential for monitoring the levels of metal pollutants and evaluating their impact in order to guide measures to protect fauna and human health.

Keywords: environmental bioindicators, feather, poisoning, raptors, airport area.

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INTRODUCTION

Concerns about environmental contamination are increasing worldwide because of the influence that many common contaminants can have on fauna and flora [1,20]. Heavy metals contribute to this contamination as one of the major elements threatening ecosystem sustainability. They are present in every environment as a result of either natural geological cycles or human activities and can cause endocrine problems, nervous disorders, and genotoxicity in animals and humans [1,5].

Exposure to heavy metals and other pollutants is generally higher in animals that are at the top of a food chain, such as raptors [1,9,19] and many are acquired by animals through food; once in the body, they undergo biochemical and physiological processes and are distributed or accumulated. In birds, a large proportion of metal is found in the feathers [19].

The southern caracara (*Caracara plancus*) is a raptor from the order Falconiformes and family Falconidae. In Brazil, it has large populations in the northeastern and southeastern regions [24]. Studies on the feeding habits of these birds have shown that they are opportunistic in nature [25] and are adapted to urban environments, as well as natural areas, and thus, are exposed to a diversity of chemical compounds [24].

The purpose of this study was to identify and quantify cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), and chromium (Cr) in the feathers and livers of free-living southern caracaras, live and dead, from Recife, Pernambuco state, Northeast of Brazil.

MATERIALS AND METHODS

Collection site and sampling

The study was carried out with southern caracaras (*Caracara plancus*) captured at Recife/Guararapes Gilberto Freyre International Airport (08°07'53"S and 34°55'05"W), which is an urban area located approximately 10 km from the central region of the Recife area, Pernambuco State, Northeast of Brazil, in partnership with the team of Fauna Project at the Brazilian Airports.

The site is characterized by disordered urban growth, presence of domestic effluents, and degraded environmental area near the airport. Furthermore, several commercial ventures that store fuels, such as gas stations and gas tanks are present nearby.

Sixty-two southern caracaras (*Caracara plancus*) collected by the team of Fauna Project at the Brazilian Airports were used in the study. The birds were captured using Tomahawk type traps that were arranged at the collection site. From the total number of birds collected, feather samples were obtained from 41 live birds and both feather and liver samples of 21 dead birds were obtained; the dead birds were victims of traumas resulting from collisions with aircrafts. The captured live birds were sent to the Technological Development Support Center located at the airport for collection of biological samples.

This research was carried out according to the license issued by Brazilian Institute of Environment and Renewable Natural Resources and the Ethics Committee on the Use of Animals from Federal Rural University of Pernambuco.

Biological material collection

Birds captured live were initially submitted to clinical examination meant to observe the general state of health. Later, two primary feathers were collected from each bird, conditioned in collectors (metal-free plastic)¹, correctly identified, and stored in a freezer at -20°C until the moment of digestion. From the dead birds, two primary feathers were collected from each wing and liver samples per bird, which were likewise stored in a freezer at -20°C until the moment of digestion. Data were recorded in individual sheets and after analysis were arranged in a spreadsheet.

Heavy metal analysis in feathers and livers

Feathers were initially washed with acetone thrice while alternating with deionized water to remove any external contamination. Afterward, the feathers were oven dried at 60°C for 48 h, ground, and weighed. The dry weights were adjusted from 0.01 to 0.09 g followed by the feathers being put in tubes containing 4 mL of HNO₃ (65%) and 4 mL of H₂O₂ (30%). They were then placed in Mars® Microwave Digester². The digestion was performed according to protocol previously established [7]. Subsequently, the samples were placed in falcon tubes (15 mL) and stored at room temperature until analysis.

For analysis of heavy metals in the liver samples, they were fragmented and cooked at 60°C for 24 h. The dried livers were ground, placed in collectors, and

the dry matter obtained was weighed. The weights were adjusted between 0.1 and 0.2 g. The samples were then placed in tubes containing 4 mL of HNO_3 (65%) and 4 mL of H_2O_2 (30%) followed by being placed in a Mars® Microwave Digester² [7].

For quantification of Pb, Cd, Cr, and Cu ($\mu\text{g/g}$) the digested samples were diluted using deionized water (1:10) and analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 7000 DV[®])³ with axial configuration. For quantification of Hg, the samples were analyzed using an atomic absorption spectrophotometer (Varian AA240FS[®])⁴, with Hydride Generator Systems (model VGA 77)⁵. All samples were analyzed in batches with blanks and appropriate standards. The detection limits for each metal used in this study were established according to the equipment manufacturer.

Data analysis

The data were expressed considering measures of central tendency (mean, standard error, median, and percentiles of 25 and 75). The concentrations of metals, Pb, Cd, and Hg, were treated with a root transformation of $x + 1$, whereas those of Cr and Cu were treated with \log_{10} base transformation. After transformation, the data was treated with analysis of variance (Test F) to evaluate the effect of different variability factors (feathers of live and dead caracaras). Wherever there was significance in the F test, the means of the treatments were compared by the Student-Newman-Keuls test. Pearson's correlation coefficient (r) was determined for the categories: concentration of metals in feathers and liver of birds as well as feathers of live birds and the significance obtained in the correlation was analyzed [18]. The data was analyzed through the Statistical Analysis System (version for windows 9.2)⁶, using the General Linear Model (GLM) procedure of SAS. Samples yielding concentration values below the detection limits were removed from analysis and for all analysis, the significance level was set at $P < 0.05$.

RESULTS

All samples of feathers and livers of the analyzed birds contained Cr and Cu. Among the live birds, feathers of 75.6% (31/41) birds had Pb, 26.8% (11/41) had Cd, and 24.4% (10/41) had Hg. Among dead birds, feathers of 95.2% (20/21) birds had Pb,

52.4% (11/21) had Cd, and 47.6% (10/21) had Hg. Lead was found in all liver samples along with Cd that was found in 80.9% (17/21) of birds and Hg in 23.8% (5/21).

Mean values, standard error, median, and lower and upper limits of concentration of heavy metals in live and dead caracaras feathers are shown in Table 1. There was no significant difference in the concentrations of metals present in feathers of live and dead birds: Pb ($P = 0.3576$), Cd ($P = 0.0792$), Cr ($P = 0.5475$), Cu ($P = 0.3603$), but there was a significant variation in relation to Hg ($P = 0.0459$) as shown in Figure 1.

Mean values, standard error, median, and lower and upper concentration limits of heavy metals in feathers and livers of dead caracaras are shown in Table 2.

Higher concentrations of Pb ($P < 0.0001$) and Cr ($P < 0.0001$) were found in feathers as compared to hepatic tissues in dead caracaras. The highest concentrations of Cu were found in liver samples ($P = 0.0011$) from dead birds as well. As for the concentrations of Cd ($P = 0.7770$) and Hg ($P = 0.3669$), no significant difference was found between feathers and liver samples from dead caracaras.

In feathers of live caracaras, high positive correlation was observed between Cd and Cr ($r = 0.72$, $P < 0.0001$) as well as between Cd and Cu ($r = 0.63$, $P < 0.0001$). Moderate positive correlation was observed between Pb and Cd ($r = 0.46$, $P = 0.0002$), Pb and Cr ($r = 0.48$, $P = 0.0017$), Pb and Cu ($r = 0.41$, $P = 0.0073$), Cd and Hg ($r = 0.31$, $P = 0.0493$), Cr and Hg ($r = 0.36$, $P = 0.0228$) as well as Cu and Hg ($r = 0.32$, $P = 0.0407$).

In the samples of dead caracaras feathers, there was a high positive correlation between Pb and Cd ($r = 0.74$, $P < 0.0001$) and between Cr and Cu ($r = 0.80$, $P < 0.0001$). A moderate positive relationship was observed between Pb and Cr ($r = 0.49$, $P = 0.0258$) and between Pb and Cu ($r = 0.57$, $P = 0.0069$). In the samples of dead caracaras livers, a high positive correlation was observed between Cu and Hg ($r = 0.79$, $P < 0.0001$). Moderate positive relation was observed between Cd and Cu ($r = 0.43$, $P = 0.0532$) and between Cd and Hg ($r = 0.51$, $P = 0.0172$) [Table 3].

No relationship was found between the concentrations of heavy metals in feathers and livers of dead caracaras according to Table 4.

Table 1. General media of metals from live and dead caracaras feathers in Pernambuco state, Brazil.

Metal (µg/g)	Caracaras feathers	Statistical measures					Level of <i>P</i>	General Media
		Mean	SE*	Median	Lower	Upper		
Pb	Live	5.27a	1.22	3.86	1.77	6.11	0.3576	5.36
	Dead	5.44a	0.78	5.30	3.15	7.31		
Cd	Live	0.43a	0.13	0.00	0.00	0.89	0.0792	0.59
	Dead	0.74a	0.17	1.01	0.00	1.25		
Cr	Live	3.84a	0.48	2.71	2.27	4.18	0.5475	3.92
	Dead	3.99a	0.52	3.20	2.50	4.21		
Cu	Live	10.75a	1.48	7.50	6.29	10.67	0.3603	10.94
	Dead	11.12a	1.32	9.52	7.50	11.77		
Hg	Live	0.12b	0.04	0.00	0.00	0.00	0.0459	0.52
	Dead	0.91a	0.54	0.00	0.00	0.26		

*Standard Error. Different lowercase letters in the same column represent a significant variation in concentration of metals on feathers and livers of dead caracaras and captured at the airport, for each metal, at a level of 5% probability.

Table 2. General media of metals from feathers and liver of dead caracaras in Pernambuco state, Brazil.

Metal (µg/g)	Sample	Statistical measures					Level of <i>P</i>
		Mean	SE*	Median	Lower	Upper	
Pb	Feathers	5.44a	0.78	5.30	3.15	7.31	<0.0001
	Liver	1.36b	0.11	1.29	1.19	1.54	
Cd	Feathers	0.74a	0.17	1.01	0.00	1.25	0.7770
	Liver	0.45a	0.09	0.37	0.19	0.68	
Cr	Feathers	3.99a	0.52	3.20	2.50	4.21	<0.0001
	Liver	1.35b	0.09	1.28	1.14	1.46	
Cu	Feathers	11.12b	1.32	9.52	7.50	11.77	0.0011
	Liver	17.03a	2.07	15.17	13.02	18.46	
Hg	Feathers	0.91a	0.54	0.00	0.00	0.26	0.3691
	Liver	0.44a	0.30	0.00	0.00	0.00	

*Standard Error. Different lowercase letters in the same column represent a significant variation in concentration of metals on feathers and livers of dead caracaras and captured at the airport, for each metal, at a level of 5% probability.

Table 3. Pearson correlation matrix for concentration of metals (µg/g) in liver from dead caracaras in Pernambuco state, Brazil.

Metal	Pb	Cd	Cr	Cu	Hg
Pb	1	-0.05 0.8264	-0.13 0.5681	0.06 0.8005	-0.09 0.6963
Cd		1	0.14 0.5463	0.43 0.0532	0.51 0.0172
Cr			1	-0.02 0.9353	-0.18 0.4461
Cu				1	0.79 <0.0001
Hg					1

Table 4. Pearson correlation matrix for concentration of metals ($\mu\text{g/g}$) in feathers and liver of dead caracaras in Pernambuco state, Brazil.

Metal	Feather Pb	Feather Cd	Feather Cr	Feather Cu	Feather Hg
Liver Pb	-0.16576 0.4727				
Liver Cd		0.18967 0.4102			
Liver Cr			-0.13383 0.5630		
Liver Cu				-0.06944 0.7649	
Liver Hg					0.26095 0.2532

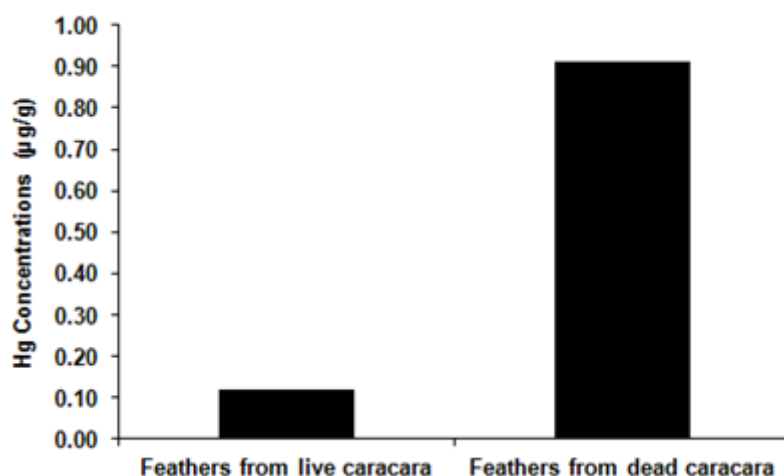


Figure 1. Concentrations of Hg ($\mu\text{g/g}$) in live and dead caracaras feathers, Pernambuco, Brazil.

DISCUSSION

Concentrations of Cr and Cu elements were detected in all feather and liver samples analyzed from live and dead caracaras. Chromium, as well as Hg, Cu, As, and Se have a high affinity for keratin [19], which may explain the higher concentrations of Cr in caracaras feathers in this research. High concentrations of Cr in tissues are only observed when body's homeostasis malfunctions and Cr is assimilated beyond what is necessary for nutrition, causing intoxication [27]. However, this effect was not observed in this study because the levels of Cr in the liver were lower than those in the feathers studied from dead caracaras.

Pigmentation also influences the endogenous deposition of metals, such as copper, zinc, manganese, and iron due to eumelanin, which is common in black and dark brown feathers like those of caracaras, and

has significant binding capacity with these metallic ions [9,22]. High concentrations of Cu were observed in feathers, which may be attributed to the participation of this element in the keratinization process. However, concentrations were even higher in the liver, which is corroborated by the results obtained in study carried out in Sweden [10], who demonstrated that Cu is heavily accumulated in the liver and kidneys.

Lead was detected in all liver samples analyzed. Elevated levels were also found in the feathers of dead (95.2%) and live (75.6%) caracaras. The presence of Pb may be due to external contamination by pollutants, such as fossil fuels [8,9,15]. The deposition of compounds in external parts of the feathers can occur through chemical products, the air, or the uropygial gland of birds [15]. According to research, 50% to 98% of the concentrations of Pb detected in organisms

can originate from external contamination, although it may also occur due to contaminated feed [10]. It should be considered that the origin of the birds in this study contributes significantly to the results obtained for some metals since the airport region, as well as the surrounding area, is rich in fossil fuels.

Higher concentrations of Hg were observed in dead caracaras feathers ($P = 0.0459$) as compared to live ones. Dauwe *et al.* [9] demonstrated that Hg concentrations change during molting but are not affected by external contamination. Metals, such as Hg and Cd, accumulate in organisms from different trophic levels, which may indicate that this contamination comes mainly from feed [19].

In study carried out in Pakistan with laggar falcon (*Falco jugger*) [20] were found concentrations up to 0.10 µg/g for Cd, 1.56 µg/g for Pb, 3.09 µg/g for Hg, and 1.98 µg/g for Cr in the feathers. The concentrations found by us, when compared to this study, were higher only for Hg, which suggests lower environmental contamination or less access to contaminated prey in the region under consideration here. In a similar study in Italy [27] using livers of 52 little owls (*Athene noctua*), concentrations of 0.17 µg/g for Cd, 0.297 µg/g for Cr and 0.312 µg/g for Pb were observed. Higher concentrations of metals in both feathers and livers from live and dead birds in our study suggests that Italian birds were less exposed to environmental contamination than the caracaras studied here.

Pb was investigated in seven birds of the order Falconiformes in Korea [21]. They observed concentrations from 2.9 to 8.1 µg/g, on average, for Pb in the liver of these birds. The concentrations found in our work are lower for both liver and feathers, possibly due to the greater urbanization in Korea, which increases the number of pollutants in the atmosphere and consequently exposes more birds. In studied four birds from the order Falconiformes in Pakistan [1] the authors to report concentrations of 2.70 µg/g for Pb, 1.00 µg/g for Cd, 1.55 µg/g for Cr, and 5.06 µg/g for Cu. When compared, the concentrations of metals in caracaras were higher for Pb, Cr, and Cu indicating that they were more exposed to environmental contamination either through atmospheric and feather deposition or through contaminated prey.

Pearson's coefficient here showed no correlation between metals from dead caracaras feathers and livers, although a few previous studies have shown a

correlation between metal concentrations from tissues and feathers [2,16,17]. No positive correlation also was found between the metals studied in feathers and livers of three different species of predators in study realized in Galicia, Spain [6]. This pattern can be attributed to the different time of exposure of feathers to metals in relation to livers. The concentrations of metals in hepatic tissues reflect the levels of elements in the diet of these birds, characterizing acutely the contamination of the ecosystem. On the other hand, feathers represent the exposure in the time of molting, when the artery supplies metals, thus representing a chronic exposure.

In feathers from live birds, the Pearson's correlation analysis showed a high to moderate correlation between metals, which may suggest that they come from a similar source, but there is no way to specify the origin since the metals surveyed could be present in the soil, water, or in the prey eaten by these animals. For the purpose of environmental analyses, it is required to determine the exact source of contamination.

A high positive correlation was found between dead caracaras feathers for Pb and Cd ($r = 0.74$, $P < 0.0001$) and Cr and Cu ($r = 0.80$, $P < 0.0001$). This means that higher the concentration of Pb and Cr in feathers, higher was the concentration of Cd and Cu, respectively. These data suggests that metals come from the same source, possibly from the environment, because these concentrations can be influenced mainly by the levels of these metals in water, sediments, and air. The levels of Pb and Cd are directly correlated with prey and slightly with external contamination. A high Pb level is indicative of environmental pollution [14]. Concentration of Cu, on the other hand, can vary with diet and environmental contamination of water, sediments, and effects of industrialization [17]. Similarly, Cr is present in parts of industrial processes, which lead to contaminating the environment [26]. In their study, a high correlation between Cu and Cr ($r = 0.80$; $P < 0.0001$) was found, which may be due to the high affinity of keratin for these elements [19].

In this study, a high positive correlation was found between Cu and Hg ($r = 0.79$, $P < 0.0001$) and a moderate correlation was found between Cd and Cu ($r = 0.43$, $P = 0.0532$) as well as Cu and Hg ($r = 0.51$, $P = 0.0172$), which can be explained by the fact that the liver is a deposition organ for metals. In the case of Hg, accumulation and excretion in the liver is very important, although the deposition of this metal

is greater in feathers [5]. Copper is stored in hepatocytes for the synthesis of copper-dependent enzymes, so higher concentrations in this organ are expected [11,12]. Although Cd concentrations are generally higher in the kidneys, liver also significantly accumulates this metal. In cases of chronic exposure to Cd, liver represents a good biomarker for total exposure to this metal [23]. The concentration of metals can vary considerably, when the feeding habits of birds are being considered. Metals like Cr and Cu exhibit affinity for birds of carnivorous habit, while Pb exhibits affinity for saprophagous birds as well as the caracaras, which are generalist species [1].

As far the toxicity of these metals is concerned, is reported that only concentrations of Cd up to 3 and 10 µg/g are toxic in birds [27]. Concentrations of Pb below 6 µg/g function only as background concentrations and are lethal only when above 25 µg/g [21]. Concentrations of Hg were low in the subjects studied in this research, probably due to the source of Hg being the primary food, i.e., poisoning occurs primarily in piscivorous birds, which is not the case with caracaras [13]. Both Cu and Cr are metals required by the body. It is known that 5-10 mg/kg of Cu are required in the diet of poultry with the maximum concentration that can be tolerated being 250 mg/kg; however, physiology is not the same in different species of birds, and thus, specific studies need to be performed to determine the threshold for intoxication [3]. In case of Cr, it is difficult to evaluate the concentration required to determine intoxication, because the homeostasis mechanism acts as a defense, preventing high concentrations from being absorbed [27].

The development of systematic research on animals and the environment is essential for the monitoring of levels of heavy metals and evaluation of the impact caused by them in order to guide measures to protect flora, fauna, and human health.

CONCLUSION

It was possible to confirm the presence of heavy metals in feathers and livers from free-living southern caracaras in Northeastern region of Brazil, signaling that these birds can be important environmental bioindicators. The detection of heavy metals in birds provides a diagnosis of the environmental situation and raises concerns regarding the exposure of other species as well as humans to these potentially toxic compounds.

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Declaration of interest. The authors declare that they have no conflict of interest that is directly or indirectly related to this research.

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