Contamination of heavy metals in birds from Embalse La Florida (San Luis, Argentina)

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Embalse La Florida is an artificial lake located in midwestern Argentina's San Luis province. It provides drinking water to ∼70% of the province's human population and ∼20% of the province is irrigated with water from the reservoir. The presence of heavy metals in Embalse La Florida's water has previously been reported. Nevertheless, no information about the levels of these contaminants in birds is available for this region. The aim of this study, therefore, is to (1) establish baseline data on lead (Pb) and cadmium (Cd) levels in birds from Embalse La Florida, (2) assess metal accumulation patterns between organs and bird species, and (3) evaluate the potential risk that these heavy metals pose for the local avifauna. We measured Pb and Cd in bone, pectoralis muscle, liver, gonad, and brain of three bird species representative of the Embalse La Florida ecosystem: *Podiceps major* (Great Grebe), *Phalacrocorax brasilianus* (Neotropic Cormorant), both of which are piscivorous, and *Pitangus sulphuratus* (Great Kiskadee), which is omnivorous. We also measured both heavy metals in Great Grebe eggs. Pb and Cd were detected in all of the tissues we assayed, and Pb concentrations were significantly higher than those for Cd in all tissues. The patterns of Pb and Cd accumulation differed between tissues, however. In general, gonads had the highest concentrations of Pb while Cd tended to accumulate in the liver. An interspecific analysis revealed that the omnivorous species had higher levels of both metals in bone, liver, and brain compared to both piscivorous species. There were no differences in Pb and Cd concentrations between males and females. The highest liver level of Pb (4.69 ppm wet weight) detected in Great Kiskadee, was comparable to those associated with toxic effects in birds, and Pb concentrations found in the liver of two females and two males (2.07 to 2.32 ppm wet weight) were also similar to those that could be physiologically detrimental in other species. In all birds assayed, Cd levels in liver tissue were lower than those typically shown to be harmful. Our results indicate that Great Kiskadees are highly polluted by Pb and their exposure to this contaminant exceeds the level reported to trigger adverse effects. This is the first study to assay heavy metals in birds from midwest Argentina and provides a starting point for studies examining the impact that these metals have on both wildlife and humans in the region.

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Introduction

Heavy metals occur naturally in all ecosystems, but industrial activities and urbanization have greatly increased their concentration in the environment. Recently, concern has grown over the impact that heavy metal contamination may have on both human and wildlife food resources.[1,2] For this reason, monitoring heavy metals in ecosystems is one important step in assessing and preventing health risks to both wildlife and humans.

Environmental impact

Heavy metals are associated with deleterious effects on fauna and humans. Thus, monitoring heavy metals in the environment is mandatory to establish the magnitude of the contamination, anticipate risks of planned actions, take decisions about public health or environmental protection, establish research priorities, and provide scientific basis for regulatory actions. In this paper, we report for the first time levels of lead and cadmium in birds of the Midwest of Argentina and discuss the potential risk of the observed contamination for the avifauna of this region. This heavy metal monitoring was intended as a starting point for future studies and as a base for researchers and land managers to initiate policies to remediate or reduce the effects of the contamination.

Lead (Pb) and cadmium (Cd), both nutritionally nonessential metals, are relatively persistent pollutants in nature. They accumulate in organisms and are biomagnified in the food chain.[3–6] The teratogenic, carcinogenic, and likely mutagenic properties of both metals are responsible for a variety of acute and chronic toxic effects.[3,4,7,8]

Recently, heavy metal contamination in both the water and sediments of our study lake, Embalse La Florida (Fig. 1), have been measured. Embalse La Florida is located in the San Luis province of midwestern Argentina (66° W, 33°07′ S). In addition to providing drinking water to roughly 70% of this province's human population, approximately 20% of the agricultural land in this province is irrigated with water from Embalse La Florida.[9] The quality of the water in Embalse La Florida, however, seems poor. That is, several sectors of the reservoir have Pb and Cd concentrations above those permitted for drinking water (Pb range: 24.41–103.56 ppb; Cd range: 0.29–1.05 ppb) which have probably resulted from human activities.[10] The health of the Embalse La Florida reservoir and ecosystem are of interest because the area is home to a rich diversity of wildlife and is, as previously mentioned, an important source of water for humans, animals and irrigation in the region. Furthermore, having been constructed on the Río Quinto river, the water in Embalse La Florida also impacts downstream areas, including those in the provinces of Buenos Aires, Santa Fé, Cordoba, and La Pampa. Despite the high levels of Pb and Cd contamination and the importance of this aquatic ecosystem, no studies have measured heavy metals in animals or humans that utilize resources from Embalse La Florida. Therefore, our work is intended to (1) establish baseline concentration data of these heavy metals in organisms associated with the reservoir, (2) examine the distribution of Pb and Cd between different tissues, (3) compare the Cd and Pb burden in species belonging to different positions in the food chain, and (4) consider the potential for adverse physiological effects based on the Pb and Cd concentrations we measured.

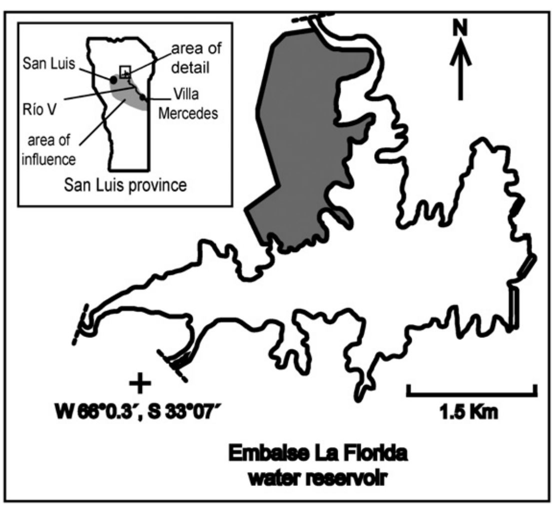

Fig. 1 Geographic location of the Embalse La Florida water reservoir. This artificial lake provides drinkable water to around 70% of the human population of San Luis province and irrigation water to approximately one fifth of this province's territory (gray-shaded area in the San Luis province map). The gray area in the map represents the natural reserve "Reserva Natural La Florida" created in 1992, along the northern shoreline of the Embalse La Florida water reservoir, to protect the natural flora and fauna.

We chose birds as bioindicators for this study because they are easy to identify, sample, and track and the contaminant levels in their tissues integrate temporal and spatial exposure.[11] Additionally, birds at the top of food chains often accumulate high levels of pollutants in their tissues and are particularly susceptible to physiological disruption.[12,13] Studies in birds report that Cd is associated with behavior and metabolism alterations, gonad and kidney damage, egg laying inhibition, anemia, and growth retardation.[14,15] With respect to Pb, it has been shown to negatively effect most body systems.[4,16–18] For instance, in birds, Pb impairs survival, growth, locomotor and thermoregulatory capacity, development, learning, and metabolism.[14,19–22]

Pollutant accumulation occurs when organisms are unable to degrade and/or eliminate compounds at the same rate at which they are incorporated. Following absorption in the intestinal tract, metals may accumulate in body tissues or be excreted.[1,11,23] Additionally, reproductive females may also transfer metals into their eggs.[24–27]

The three bird species we used in this study exhibit different degrees of piscivory. Neotropic Cormorant (*Phalacrocorax brasilianus*) and Great Grebe (*Podiceps major*) are specialized piscivores; Great Kiskadees (*Pitangus sulphuratus*) are omnivorous, but also predate on fish.[9] All three species are at the top or near to the top of the food chain in the Embalse La Florida ecosystem.

Materials and methods

Collection of material

During the austral spring of 2002, we captured 15 adult birds (>one year): six Great Kiskadees (three of each sex), seven Neotropic Cormorants (four males, three females), and two Great Grebe males at Embalse La Florida (Fig. 1). We also collected three Great Grebe eggs. All captures were performed under authorization of the Government of the Province of San Luis, Ministry of Human and Social Development, Program of Planning and Environmental Management (Resolution No. 027, 01/11/2005).

Each animal was examined carefully for external abnormalities and measured (body mass, body length, wingspan, tarsus length, and wing length). Table 1 shows the sample list and biometry data. The birds were then stored individually in plastic bags, refrigerated, and sent immediately to the laboratory. Upon arriving at the laboratory, we weighed the animals (Table 1), opened their abdominal cavities, and inspected the external appearance of internal organs. During these dissections we sexed the birds by examining gonads.

Samples of the liver, muscle, bone, gonad, and brain were excised, and an aliquot of each tissue was used for quantifying Pb and Cd burden. In Great Grebe eggs, Pb and Cd were determined in both eggshells and content.

Solvents and chemicals

Nitric acid 60% (Merck, Ultrapur), hydrogen peroxide 31% (Merck, Ultrapur), perchloric acid 70% (Merck, Suprapur), magnesium matrix modifier 50 ml (Mg) = 10.0 ± 0.2 g/l in

Table 1 Biometry of bird (length and weight values are mean ± SD) captured from Embalse La Florida

Species	Great Kiskadee[a] (*Pitangus sulphuratus*)		Neotropic Cormorant[a] (*Phalacrocorax brasilianus*)		Great Grebe (*Podiceps major*)
Sex	male	female	male	female	male
Number	3	3	4	3	2
body mass/g	67.0 ± 3.4	58.9 ± 6.0	1393.8 ± 104.9	1355.8 ± 36.5	1325.5 ± 46.2
tarsus length/mm	25.2 ± 0.8	23.0 ± 2.9	46.5 ± 1.4	46.5 ± 3.7	60.9 ± 0.9
wing length/cm	18.3 ± 0.4	17.8 ± 0.7	53.4 ± 1.4	49.3 ± 0.9	33.3 ± 5.8
wingspan/cm	40.0 ± 0.3	38.3 ± 0.8	112.6 ± 2.0	107.7 ± 1.3	86.1 ± 0.1
body length/cm	24.3 ± 0.2	24.9 ± 0.3	75.9 ± 1.0	71.7 ± 1.5	71.8 ± 4.8

[a] For Great Kiskadee and Neotropic Cormorant, no significant statistical difference was found in biometry measures between males and females (Mann-Whitney U Test, $P \leq 0.05$).

nitric acid 15% (Merck), Palladium matrix modifier 50 ml (Pd) = 10.0 ± 0.2 g/l in nitric acid 15% (Merck). Stock standard solution of Cd Merck of 1003 ± 2 mg/l, lot OC 105824, traceable to SRM from NIST Cd $(NO_3)_2$ in HNO_3 0.5 mol/l. Stock standard solution of Pb Merck of 1000 ± 2 mg/l, lot OC186400, traceable to SRM from NIST Pb $(NO_3)_2$ in HNO_3 0.5 mol/l. All water used in this study was deionized (Milli-Q Element). All plastic-ware and glassware were acid-washed in 1:10 nitric acid (analytical grade) followed by a thorough rinsing with water.

Analytical procedure

Wet samples were weighed and then dried at 90–100 °C for about 24 h until constant mass. Dry samples were reweighed and subsequently digested in a 2:1 (v/v) mixture of nitric and perchloric acid. The sample (about 0.1 g), was placed in a digestion tube and treated with 2 ml of concentrated nitric acid. Next, it was heated progressively at 150 °C for 1 h, after which 1 ml of perchloric acid was added and it was heated at 200 °C. Lastly, we added 1 ml of oxygenated water. The digestion was finalized when no fumes were observed and the mixture was pale and without sediments. After decomposition, the solution was transferred quantitatively to a 5 ml volumetric flask. Method 200.9 revision 1.2 4/91 (Determinations of trace elements by stabilized temperature graphite furnace atomic absorption spectrometry) was used to determine Cd and Pb in samples in a Perkin-Elmer graphite furnace atomic absorption spectrometry model AAnalyst 200 (GFAAS - GF 900).

Metal concentrations are expressed as ppm wet weight (ppm ww). To facilitate comparisons, dry weight concentrations (ppm dw) are also reported in Table 2 and Table 3.

Quality assurance

Validation was carried out on a synthetic sample (cow liver homogenate) with the addition of Cd and Pb standard solutions traceable to SRM from NIST, following method 200.9 revision 1.2 4/91 protocol. Metal concentrations of reference material and recoveries are shown in Table 4. Blanks, reference material and spiked samples were always run with samples. Spike recoveries accepted ranged from 95% to 105%.

Additionally, an inter-laboratory comparison with the ECO-CHEM S.A. Laboratory (San Luis, Argentina), accredited for ISO 9001, ISO 14000, and in process of accreditation for IRAM 301 and ISO 17025 was carried out. The results for this inter-comparison exercise were acceptable and are shown in Table 4.

Statistical analyses

All statistical analyses were made using the concentrations expressed as ppm ww.

The parametric tests were performed on log-transformed data to satisfy the normality and homogeneity of the variance. The normality of the data was tested using the Shapiro-Wilk W test[28]

Table 2 Mean ± standard error of the mean and range of Pb concentrations (ppm, ww: wet weight and dw: dry weight) in the tissues of N birds from Embalse La Florida

	Great Kiskadee (*Pitangus sulphuratus*) (N = 6)		Neotropic Cormorant (*Phalacrocorax brasilianus*) (N = 7)		Great Grebe (*Podiceps major*) (N = 2)	
Tissue	(ppm ww)	(ppm dw)	(ppm ww)	(ppm dw)	(ppm ww)	(ppm dw)
Muscle	1.76 ± 0.50	3.81 ± 0.81	0.77 ± 0.12	2.17 ± 0.38	1.74 ± 1.41	3.88 ± 2.95
	(0.45_3.26)	(1.39_6.19)	(0.40_1.15)	(1.08_3.30)	(0.32_3.15)	(0.93_6.83)
Bone	3.31 ± 0.60	3.60 ± 0.63	1.19 ± 0.30	1.33 ± 0.34	0.75 ± 0.54	0.85 ± 0.60
	(1.89_5.39)	(2.07_5.86)	(0.39_2.08)	(0.46_2.34)	(0.22_1.29)	(0.26_1.45)
Liver	2.48 ± 0.46	4.78 ± 0.92	0.71 ± 0.09	1.88 ± 0.21	0.77 ± 0.30	2.13 ± 0.59
	(1.40_4.69)	(2.94_9.21)	(0.35_1.05)	(1.14_2.76)	(0.47_1.07)	(1.54_2.72)
Gonad	5.02 ± 0.80	8.05 ± 0.72	3.57 ± 0.68	9.68 ± 1.48	1.56 ± 0.85	5.81 ± 2.23
	(2.30_7.87)	(4.85_9.86)	(1.37_6.19)	(4.16_14.39)	(0.71_2.40)	(3.59_8.04)
Brain	5.16 ± 2.44	11.15 ± 5.04	0.44 ± 0.05	1.76 ± 0.17	1.27 ± 0.43	5.34 ± 1.84
	(0.93_16.23)	(2.44_33.36)	(0.23_0.57)	(0.95_2.29)	(0.85_1.70)	(3.50_7.18)

Table 3 Mean ± standard error of the mean and range of Cd concentrations (ppm, ww: wet weight and dw: dry weight) in tissues of N birds from Embalse La Florida

	Great Kiskadee (*Pitangus sulphuratus*) (N = 6)		Neotropic Cormorant (*Phalacrocorax brasilianus*) (N = 7)		Great Grebe (*Podiceps major*) (N = 2)	
Tissue	(ppm ww)	(ppm dw)	(ppm ww)	(ppm dw)	(ppm ww)	(ppm dw)
Muscle	0.09 ± 0.03 (0.03_0.21)	0.21 ± 0.05 (0.09_0.38)	0.07 ± 0.02 (0.04_0.18)	0.20 ± 0.07 (0.09_0.54)	0.09 ± 0.05 (0.04_0.14)	0.20 ± 0.09 (0.11_0.30)
Bone	0.27 ± 0.02 (0.19_0.37)	0.30 ± 0.03 (0.21_0.41)	0.05 ± 0.01 (0.02_0.13)	0.06 ± 0.02 (0.02_0.15)	0.03 ± 0.01 (0.02_0.04)	0.04 ± 0.01 (0.02_0.05)
Liver	0.26 ± 0.02 (0.20_0.34)	0.50 ± 0.03 (0.41_0.61)	0.09 ± 0.02 (0.04_0.18)	0.26 ± 0.06 (0.12_0.51)	0.05 ± 0.02 (0.03_0.06)	0.13 ± 0.03 (0.10_0.17)
Gonad	0.65 ± 0.21 (0.10_1.38)	1.11 ± 0.40 (0.21_2.87)	0.25 ± 0.08 (0.01_0.50)	0.71 ± 0.21 (0.01_1.56)	0.08 ± 0.06 (0.03_0.14)	0.30 ± 0.18 (0.13_0.48)
Brain	0.21 ± 0.08 (0.07_0.58)	0.49 ± 0.18 (0.17_1.33)	0.03 ± 0.00 (0.02_0.05)	0.13 ± 0.02 (0.07_0.22)	0.10 ± 0.04 (0.06_0.14)	0.41 ± 0.17 (0.24_0.57)

Table 4 Validation was carried out on a synthetic sample (cow liver homogenate) with the addition of Cd and Pb standard solutions traceable to SRM from NIST, following method 200.9 revision 1.2 4/91 protocol. Additionally an inter-laboratory comparison exercise with ECOCHEM S.A. Laboratory (San Luis, Argentina) was conducted using an inductively coupled plasma-optical emission spectrometer (ICP-OES), Perkin Elmer Optima-5300 DV ICP-OES equipped with a SeaSpray concentric nebulizer and cyclonic spray chamber. Certified, mean observed and external laboratory (±1 S.D.) values in ppm per dry weight and percent recoveries are shown

			Our laboratory		
Reference material (RM)	Heavy metal	Certified value	Observed value	Recovery/%	Ecochem S.A. Laboratory Inter-laboratory exercise
RM1	Pb	15.00	14.92	99.48	15.03 ± 0.12
	Cd	2.00	1.97	98.31	2.02 ± 0.001
RM2	Pb	7.00	6.86	98.00	7.02 ± 0.15
	Cd	0.10	0.10	98.88	0.10 ± 0.002

and the homogeneity of the variance using the Levene test.[29] All nonparametric tests were made on nontransformed data. Pearson's product moment correlation (r) was used to analyze the relationship between Pb and Cd for each tissue and to evaluate the correlation of each metal between different tissues. This test was not applied for Great Grebe tissue or egg values, on account of the small size of the sample (Table 1). We used repeated measures analysis of variance (RM-ANOVA) with Fisher's Least Significant Difference (LSD) post hoc tests to check for differences between tissue concentration of Pb and Cd. A paired Student's t-test was also used to compare the Cd and Pb concentration in each tissue. Student's t-test[30] for independent samples was applied to check the difference in the metal concentration between piscivores and the omnivore. To compare the metal concentration between sexes, we utilized the U-Man Whitney test.[31] For all statistical tests, we used $\alpha = 0.05$ as the level of significance.

Results

Levels of Pb and Cd were detected in all the tissues we assayed (Table 2 and Table 3). Pb and Cd were not correlated (Pearson correlation, $P > 0.05$), and Pb concentrations were significantly higher (7.5–24.4 times higher; Table 2) than Cd concentrations in all of the tissues we examined (Paired Student's t-test, $P < 0.05$).

There was no correlation between Pb and Cd concentrations in the internal tissues of Great Kiskadees and Neotropic Cormorants (Pearson correlation, $P > 0.05$, Great Kiskadee: N = 6, Neotropic Cormorant: N = 7). Pb concentrations differed among tissues in Neotropic Cormorants (RM-ANOVA $F_{4,20} = 13.58$, $P < 0.001$), but not in Great Kiskadees (RM-ANOVA $F_{4,20} = 2.30$, $P = 0.093$). Gonads of Neotropic Cormorants exhibited significantly higher Pb concentrations than those detected in muscle ($P < 0.001$), bone ($P < 0.001$), liver ($P < 0.001$), and brain tissues ($P < 0.001$); Pb levels were higher in bone compared to brain tissue ($P < 0.05$).

Cd concentrations were positively correlated between the liver and muscle of Great Kiskadees (Pearson correlation, N = 6, $r = 0.93$, $P < 0.05$) and between muscle, bone, and liver tissues of Neotropic Cormorants (Pearson correlation, muscle *vs.* bone: N = 7, $r = 0.78$, $P = 0.04$; muscle *vs.* liver: N = 7, $r = 0.80$, $P = 0.03$; bone *vs.* liver: N = 7, $r = 0.83$, $P = 0.02$). Cd concentrations varied between tissues of Great Kiskadees (RM-ANOVA $F_{4,20} = 6.17$, $P = 0.002$) and Neotropic Cormorants (RM-ANOVA $F_{4,20} = 3.31$, $P = 0.027$). Cd concentrations of muscle were significantly lower compared to the concentrations of bone, liver, and gonad tissues in Great Kiskadees (Fisher's LSD: muscle *vs.* bone: $P = 0.004$; muscle *vs.* liver: $P = 0.005$; muscle *vs.* gonad: $P < 0.001$). Also, the brain had a lower Cd concentration than gonads in Great Kiskadees (Fisher's LSD: $P = 0.013$). In the Neotropic Cormorant, Cd levels of liver tissue were significantly higher than the concentrations detected in brain tissue (Fisher's LSD: $P = 0.031$). Gonad and liver tissue had similar concentrations of Cd, and these concentrations were higher than those detected in both brain and bone (Fisher's LSD: gonad *vs.* brain: $P = 0.003$; gonad *vs.* bone: $P = 0.019$).

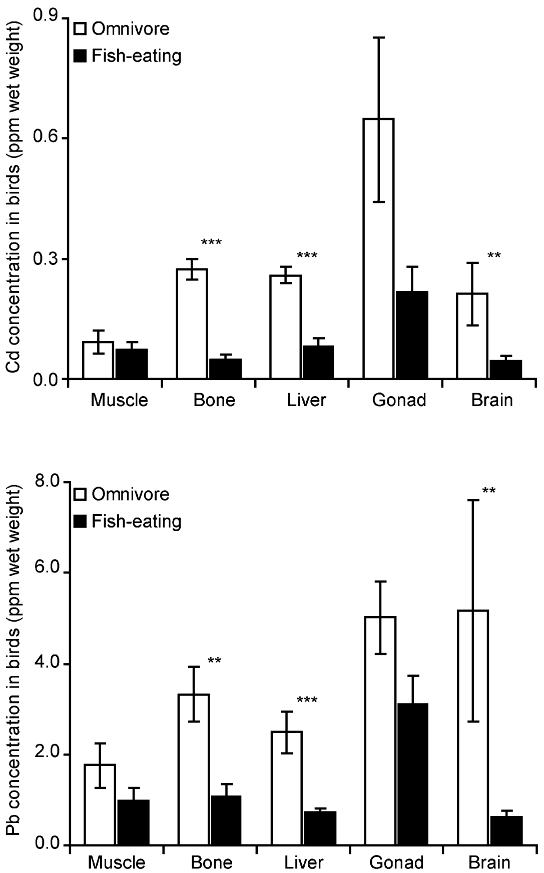

Fig. 2 Comparison of metal burdens (mean ± 1 SEM, ppm ww) between two piscivorous and one omnivorous bird of Embalse La Florida. Asterisks denote a significant difference between dietary categories for the same metal (Student's t-test, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$).

The Pb and Cd concentrations found in bone, liver, and brain tissue were significantly higher in Great Kiskadees (an omnivore) compared to Neotropic Cormorants and Great Grebes (both piscivorous; Student's t-test; Cd-bone: $t = 6.96$, degrees of freedom (df) $= 13$, $P < 0.05$; Cd-liver: $t = 5.07$, df $= 13$, $P < 0.05$; Cd-brain: $t = 4.06$, df $= 13$, $P < 0.05$; Pb-bone: $t = 3.54$, df $= 13$, $P < 0.05$; Pb-liver: $t = 5.92$, df $= 13$, $P < 0.05$; Pb-brain: $t = 4.01$, df $= 13$, $P < 0.05$; Fig. 2).

Pb and Cd were detected in all Great Grebe eggshell and egg content samples we examined. Nonstatistical comparisons due to our small sample size showed similar or greater Cd concentrations in eggshell (0.253 ppm ww, 0.266 ppm dw) and in egg content (0.100 ppm ww, 0.389 ppm dw) than the concentrations found in tissues of adult Great Grebe (Table 3). Pb concentrations in eggshell (0.650 ppm ww, 0.684 ppm dw) were greater than that in egg content (0.056 ppm ww, 0.217 ppm dw) and similar to that found in bone of Great Grebe adults (Table 2).

Concentrations of Pb and Cd in bone, liver, muscle, and brain did not differ between sexes of Neotropic Cormorants and Great Kiskadees (Mann-Whitney U Test: $P > 0.05$).

Discussion

Pb and Cd distribution in internal tissues

For all examined tissues of the three species studied, the Pb concentration was higher than Cd (Table 2 and Table 3). This difference may reflect the environmental abundance of each metal as Pb levels in the water of Embalse La Florida are 100 times greater than Cd levels (Cd range: 0.29 to 1.05 ppb and Pb range: 24.41 to 103.56 ppb).[32]

The high concentration of Pb in the water of Embalse La Florida may be the result of human activities. For instance, boats navigating the reservoir have combusted leaded fuel for ∼45 years and leaded plumbs (fishing weights) have been used by anglers for roughly 55 years.[33]

Pb distribution patterns were different between species. In the Neotropic Cormorant, Pb was more concentrated in the gonad compared to liver, bone, muscle, and brain tissue. The pattern of Pb distribution in tissues, however, is not consistent among species or between sexes. For example, Dauwe *et al.*[34] found that Pb levels were higher in bone and liver compared to ovary, muscle, and brain tissue of female Great Tits (*Parus major*). In House Sparrows (*Passer domesticus*), Dauwe *et al.*[34] reported that Pb concentrations were high in liver, intermediate in bone, and low in both muscle and brain tissue. Van Wyk *et al.*[2] detected in five African Whitebacked Vultures (*Pseudogyps africanus*) high levels of Pb in bone, intermediate levels in liver and muscle, and low concentrations in brain tissue. These same authors also reported high Pb concentrations in muscle tissue compared to brain tissue of Lappetfaced Vultures (*Torgos tracheliotos*) and Cape Griffons (*Gyps coprotheres*),[2] which is similar to the pattern we found in Neotropic Cormorants.

In the Neotropic Cormorant, we detected the highest Cd burden in gonad and liver tissue, indicating that these tissues were the dominant targets for Cd accumulation. This tendency for Cd to accumulate in gonad and liver has previously been reported for other species. Dauwe *et al.*[34] found that Cd concentrations in Great Tit (*Parus major*) females were high in liver and ovary and low in muscle, bone, and brain tissue. Saeki *et al.*[35] found levels of Cd that were higher in liver compared to those in the muscle, bone, and brain tissue of Common Cormorants (*Phalacrocorax carbo*). Other studies have also reported high concentrations of Cd in liver and low concentrations in the muscle, bone, and brain of birds.[36,37] This is likely because the principal avian Cd detoxification system is in the liver.[37] The smaller mean concentration of Cd we detected in the brain of Neotropic Cormorants is coincident with others studies.[34,38] It is also known that a blood-brain barrier prevents the transference of Cd to brain tissue.[35]

Comparison between dietary habits

A difference was found for both metals between omnivores and fish-eating birds. Bioaccumulation up the food chain has been reported for a wide variety of pollutants in many different environments.[13,39] Our findings of Pb and Cd in omnivores and fish-eating birds did not reflect the trophic position in the food chain. These differences in contamination between the omnivorous and the fish-eating birds may be explained by the different metabolism of these compounds, a different diet, and/or forage in different regions (migrations). Unfortunately, even though differences in metabolism may be a factor that may explain dissimilar contamination patterns, it is impossible to infer anything due to the scarcity of studies carried out on wild birds[40] and the lack of them analyzing the specific presence of Cd and Pb in the species we studied. Differential use of resources may explain why the omnivorous species differed from the piscivorous birds in the accumulation patterns of both Pb and Cd. Generally, birds that are at the top of the food chain and that predate on large fishes accumulate higher amounts of pollutants than those that are at a lower position and/or eat smaller fishes.[41] However, our findings do not offer support for this phenomenon. A possible explanation is that, in the Embalse La Florida ecosystem, Neotropic Cormorants predate on Argentine Silversides (*Odontesthes bonariensis*). These fish are approximately 10 cm in length (0–1 year old) and have a relatively short exposure time to Cd and Pb, which suggests that they may have low concentrations of the heavy metals. Alternatively, it is possible that Great Kiskadees, the omnivorous species in our study, have a higher level of heavy metals than piscivorous birds because the Great Kiskadees predate on a wide range of aquatic and terrestrial items (*e.g.*, fruits, seeds, invertebrates, amphibians, fish) and the heavy metal concentrations for these prey items is not known. Similarly, Burger[41] found high levels of Pb in eggs of herring gulls; this omnivore species ate invertebrates, fish, and garbage. Blus *et al.*[42] found high Pb levels in tissues of nestling American Robins (*Turdus migratorius*) from northern Idaho, indicating that terrestrial invertebrates probably had high Pb concentrations. Also, species that forage on the ground may have a higher risk, ingesting Pb from the soil.[43] Pb concentrations have been shown to be greater in soil relative to water in some ecosystems when Cd concentrations were approximately equal in both the terrestrial and aquatic areas.[44] In Embalse La Florida, high Pb levels in the tissues of Great Kiskadees suggest that terrestrial invertebrates and plants probably contain elevated Pb concentrations. Another potential explanation for the observed differences between omnivorous species and the piscivorous birds may be the migratory behavior of some species. Preliminary data that we have collected indicate that the over-wintering population of Neotropic Cormorants decreases to approximately 12.5% of the spring–summer population in the Embalse La Florida ecosystem. Consequently, it is possible that Neotropic Cormorants reside in a less polluted aquatic ecosystem for at least a portion of the year, which may diminish their contamination load.

Pb and Cd in eggs

Although Pb and Cd are thought to accumulate less in eggs when compared to the internal tissues of birds,[11,45,46] we found Pb and Cd levels in Great Grebe eggs to be on par with several tissues in adult birds. In agreement with this finding, Swaileh and Sansur[36] reported similar magnitudes of Pb and Cd in eggs and some tissues of House Sparrow (*Passer domesticus*). They found Pb concentrations (ppm dw) of 3.3 and 1.6 in eggshell and egg contents, respectively, and 2.4 in brain, 1.9 in heart, and 1.4 in muscle tissue; Cd concentration (ppm dw) was 0.02 in egg contents, 0.01 in eggshell, 0.02 in brain, 0.02 in feathers, 0.02 in heart, and 0.03 in both lung and muscle tissue.

Other authors have measured Pb and Cd in bird eggs, and the levels they report were similar in magnitude to what we found here. Gochfeld[12] found that mean Cd levels in eggs of Herring Gulls (*Larus argentatus*) from New York Bight ranged from 123 to 400 (ng/g ww) and mean Pb levels were in the 934 to 4150 range (ng/g ww). In other studies, the levels of Pb and/or Cd were lower than our results here. For instance, Goutner *et al.*[14] found that mean Pb levels ranged from 7 to 83 (ppb ww) and mean Cd levels were between 3 and 7 (ppb ww) in the eggs of four bird species from the wetlands in Greece. Additionally, when Burger *et al.*[47] analyzed the temporal variation (from 1996 to 2001) of metals in eggs of Scrub-Jays from south-central Florida, they found Cd concentrations between 2 and 3 (ppb, dw) and Pb levels that ranged from 3 to 22 (ppb, dw).

Goutner *et al.*[14] stated that eggs can be useful tools for environmental biomonitoring, especially in environments with high concentrations of these metals. The eggshell, provided that it is collected after chicks hatch, can be used as a noninvasive tool for monitoring levels of these metals in the environment. Our results of similar concentrations of Pb and Cd in eggs and in some tissues of Great Grebes gives support to Goutner *et al.* statement,[14] though we must caution readers that we analyzed a limited number of samples (3 eggs and 2 adult individuals).

Inter-sex comparison

Metals found in eggs may come from the mobilization of mother tissues and/or from the diet.[25] In either case, transference of metals to eggs could lessen the burden on females and generate differences between the sexes in contamination load. Unfortunately, we did not have the opportunity to collect female Great Grebes to test this hypothesis since the literature is controversial about this issue.[24,36,37,48–50] In the other two species we studied, Neotropic Cormorant and Great Kiskadee, we did not find differences in Pb or Cd levels between males and females.

Potential adverse effects

Our hazard evaluation for birds of Embalse La Florida is based on a comparison of the levels found in this study and those previously reported as being harmful to other avian species, because there are no species-specific toxicity assays available for the studied species or reports of Pb and Cd dynamics in the studied ecosystem. To our knowledge, the level of contamination with these metals in birds of Argentina has not been studied before. Therefore this is the first attempt to assay heavy metals in birds from midwestern Argentina and provides a starting point for studies examining the impact these metals have on both the wildlife and humans in the region.

Lead. Quantification of Pb in the liver is routinely used to determine the poisoning in birds. A Pb liver burden in birds higher than 10 ppm ww has been associated with clinical signs of Pb poisoning; first signs of this intoxication, however, occur at lower concentrations.[51] Liver Pb concentrations associated with adverse effects in birds have been classified in the following categories: no effects (NE), physiological effects (PE, *e.g.*, delta-aminolevulinic acid dehydratase (ALAD) depression, *etc.*, without overt signs of poisoning), toxic effects (TE, overt signs of poisoning, including muscle wasting, weakness, anemia, abnormal movement coordination), and severe toxic effects (STE, mortality and morbidity).[51,52] For Waterfowl, these liver Pb concentrations (ppm ww) are: NE < 2, PE 2–6, TE 6–15, and STE > 15;[51,53] for birds of prey: NE < 2, PE 2–4, TE > 3, and STE

> 5; for Doves and Pigeons: NE < 2, PE 2–6, TE > 6, and STE > 20; and for Quail and Pheasant: NE > 2, PE 2–6, TE > 6, and STE > 15.[51,52,54]

Pb concentrations in the liver of Great Kiskadees were within the range reported for passerines from areas thought to be unpolluted to areas that are known to be severely contaminated.[36,42,43,55,56] Considering the above classification, the highest liver level of Pb (4.69 ppm ww) we detected in Great Kiskadees was comparable to those associated with toxic effects in birds, and Pb concentrations found in the liver of two females and two males (2.07 to 2.32 ppm ww) were also similar to those that could generate negative physiological effects in others species. Also, these values were higher than those liver levels (<1.0–2.9 ppm ww) used as controls in some studies on Pb in passerines.[43] Mean Pb liver levels found in Great Kiskadees were comparable or greater to those mean levels reported in livers of Song Sparrows (*Genus species*) from the Pb contaminated floodplains in the Coeur d'Alene River Basin, Idaho, and this species had a mean ALAD inhibition of 51% compared to that in its reference site.[55] The maximum level of Pb in the liver of Great Kiskadees was similar to those (mean values) reported in the liver of Northern Cardinals (*Cardinalis cardinalis*) from the Pb, Zn, and Cd contaminated areas of the Tri State Mining District (Oklahoma, Kansas and Missouri).[56] In these contaminated areas, a 60% inhibition of ALAD activity was observed when compared to the reference site.[56] In Neotropic Cormorants and Great Grebes, the Pb liver concentrations we detected were greater than those previously published for conspecifics or congenerics (*Phalacrocorax and Podiceps*) from the other parts of the world;[37,57,58,59] these concentrations were, however, lower than the risk levels mentioned earlier. The elevated Pb levels found in the tissues of Great Kiskadee (liver, gonad, bone, and brain) and in some tissues (gonad and brain) of Neotropic Cormorants and Great Grebes examined in this study suggest that Pb is a problem in Embalse La Florida and needs to be closely monitored.

Cadmium. Cd has both teratogenic and carcinogenic properties, and is thought to be deleterious to wildlife.[3] Concentrations of Cd in liver (Table 3) of all birds in this study were below the levels assumed as a background (<3 ppm dw) for freshwater waterfowls.[60,61] These levels are far lower than those concentrations considered adverse in other bird species. In birds, poisoning may occur when Cd concentrations in the liver reach approximately 40 ppm ww.[56] Based on the concentrations we detected in tissues of birds from Embalse La Florida, it seems unlikely that these species are at risk from Cd exposure. Additional work is needed, however, to support this conclusion. The low Cd levels we report in bird tissues here may be explained by the mean concentration range of this metal (0.29–1.05 ppb) in the water of Embalse La Florida,[10,32] which is below that reported (>10 ppb) to produce adverse effects (mortality, reduced growth rates, reduction or inhibition of reproduction) on freshwater biota.[3]

Additionally, all species assayed are resident to the study area[62] and are present there in high numbers, are nonmigratory sedentary birds, not endangered, and have a wide geographic distribution in Argentina and the Americas.[63] These characteristics make these species suitable candidates for use as biomonitors for metal pollution.

Conclusions

We found that birds from the Embalse La Florida ecosystem are exposed to high Pb and low Cd contamination. These metals have been detected in all examined sample tissues of three bird species.

The omnivorous species (Great Kiskadee) exhibited higher Pb and Cd burdens in bone, liver, and brain than the piscivorous ones (Neotropic Cormorant and Great Grebe). Interestingly, however, Great Kiskadees are positioned at a lower trophic level than both the Neotropic Cormorant and Great Grebe.

No difference was found between males and females for Cd or Pb.

Differences were observed in the distribution patterns of Pb and Cd between tissues belonging to the same species. Gonad tissues were found to contain the highest Pb levels compared with other tissues. High concentrations of Cd were detected in the liver and gonad tissues of Neotropic Cormorants and Great Kiskadees.

Pb contaminations detected in the liver of Great Kiskadees of Embalse La Florida were greater than those levels that produce adverse effects in other avian species.

Based on our findings, additional work is needed to examine how Pb and Cd levels in the Embalse La Florida ecosystem change over time and the possible impact these environmental pollutants are having on both the wildlife and humans that rely on this ecosystem.

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