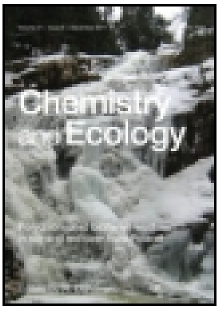

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Tissue distribution of Cd, Cu, Zn, Ni, Cr, Pb and Hg in striated heron, *Butorides striatus* (Aves: Ardeidae), in a fluvial ecosystem

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In this study, the striated heron (*Butorides striatus*), a species that reproduces in the Middle Paraná River floodplain, was examined for lead, cadmium, chromium, zinc, nickel, mercury and copper concentrations, using liver, kidney, muscle, vertebra and feathers. The results showed low exposure to chromium because all tissue samples had concentrations below the limit of detection. Similar results were obtained for nickel and lead, with the exception of vertebra. This might be associated with either long-term exposure to these metals, or it may be that both nesting and wintering areas are non-polluted. Zinc and copper, both essential metals, were found in all tissues and their concentrations were within the ranges reported in the literature. Mercury was also found in all tissues, but at very low concentrations and even at concentrations below those that produce negative effects in several species of birds (e.g. reproductive success, food intake). Studies of this type are needed to interpret the role of organisms within environments impacted by different human activities.

Keywords: heavy metals; herons; *Butorides striatus*; middle Paraná River

1. Introduction

Chemicals such as heavy metals resulting from industrial processes, urban and suburban run-off, agricultural practices, natural erosion and geochemical cycles end up in aquatic ecosystems.[1,2] As a consequence, metals enter the food chain where biomagnification may occur through higher trophic levels.[2] In this context, organisms at the top of the food chain (e.g. birds in aquatic ecosystems) are exposed to higher concentrations of chemicals and usually have higher levels of contaminants.[3,4] In particular, top-level piscivores such as herons accumulate much higher levels of contaminants than birds that are lower in the food chain.[2]

When metals enter the bodies of birds, they can be excreted or be stored and accumulate in tissues. Trace elements can be excreted in urine or faeces or can be removed from the body by being sequestered in feathers,[5–7] by elimination in eggs and eggshells,[3,8] or in some cases, by elimination through the salt gland.[4] However, when exposure to chemicals in these species occurs over time, risks from both lethal and sublethal effects appear as the burden of metals in the

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body increases.[9] Exposure to sublethal concentrations of heavy metals may lead to behavioural changes and reduced growth or may influence body weight, reproduction, embryogenesis and the general health of some birds.[10–13]

In wetland ecosystems, heavy metal pollution not only deteriorates water quality, which has a negative influence on plants and animals, but also leads to a decrease in the range of bird species, with reduced biodiversity in wetlands.[9,14]

The Paraná River has the second largest drainage basin in South America (2.6×10^6 km^2), stretching from ∼15°S to its mouth in the Río de La Plata estuary at 34°S.[15] The middle Paraná is a broad complex floodplain extending 60 km into the heart of the interior lowlands of South America.[16] There is an extensive record of the migrant and resident bird species that use the area for feeding and in some cases, feeding and reproduction.[17–20] Even though several studies have been conducted on the bird species that inhabit this ecosystem, information about heavy metal levels metals is scarce.[21] Keeping in mind that large rivers are usually used to dump industrial and urban wastes, investigations are essential to understand the processes behind the accumulation of toxic substances throughout the ecosystem, as well as their toxicological effects.

Several sources may contribute greatly to the increase in heavy metals concentrations. The main source of heavy metals in the study area is the Salado River, a very important tributary of the Middle Paraná, which is one of the most important basins in Argentina. It receives important inputs of heavy metals, such as Cr, Cu, Pb and Cd, mainly from tanneries and metallurgic industries, thus representing an important segment of the economy.[22] The study area is surrounded by several major cities (Santa Fe, Paraná), industries (electronics, pharmaceuticals, automotive), harbours and extensive lands used for agriculture (including fertilisers, pesticides). Thus, the input of metals may also be via dumping of industrial and urban wastes and terrestrial run-off. Atmospheric deposition may being another source of certain pollutants, like Hg.

The purpose of this study was to evaluate selected metal concentrations (Cu, Zn, Ni, Pb, Cr, Cd and Hg) and their distribution within tissues/organs of specimens of *Butorides striatus* (striated heron), a migrant species that reproduces in the Middle Paraná River floodplain ecosystem, keeping in mind the potential risk associated with the metal burdens in the body.

2. Materials and methods

Between September 2003 and April 2004, several dead specimens of *B. striatus* (adult males; $n = 8$) were incidentally found on Carabajal Island, Santa Fe Province, Argentina (31°39′S, 60°42′W), which belongs to a geomorphological unit called a bank plain.[23] The island comprises ∼4000 ha with many lentic water bodies, some of which are very extensive.

Although there is limited information available on *B. striatus*, it is known to be a migratory species that nests in the study area and migrates south during the winter; it has not been detected beyond southern Brazil (pers. comm.).

Whole-body samples of birds were stored in polyethylene bags and immediately frozen at −20°C until analysis. Later, specimens were dissected and samples of different organs and tissues were removed including pectoral muscle, liver, kidney, vertebra and feathers. All the equipment used for dissection of the samples had previously been cleaned with diluted nitric acid (0.7% v/v) to prevent contamination. For the analysis of Cu, Cd, Zn, Ni, Pb and Cr, acid digestion of the organ and tissue samples was carried out following the methodology of Marcovecchio and Ferrer.[24] Subsamples between 250 and 500 mg were mineralised with 3 mL of concentrated HNO_3 and 1 mL of concentrated $HClO_4$ and heated in a glycerin bath at 120 ± 10°C. After acid digestion, the residue was transferred to centrifuge tubes and made up to 10 mL with diluted HNO_3 (0.7%). Sample digestion was replicated to ensure the reproducibility of the results, with $< 10\%$ relative standard deviation (RSD). Heavy metal concentrations were determined by atomic absorption spectrophotometry (AAS) with an air–acetylene flame using a Perkin–Elmer AA-2380®. Total Hg contents in all samples were determined using cold vapour flameless atomic absorption spectrophotometry (CV-AAS) after acid digestion, following the methodology of Marcovecchio *et al.*,[25] which is a modification of the method originally described by Uthe *et al.*[26] Samples were treated with a mixture of concentrated HNO_3:H_2SO_4 (1:4 v/v) and heated in a bath at 60°C. Later, the samples were oxidised with a $KMnO_4$ (6% p/v) solution, clarified for 24 h before a ClH_4NO solution was added. A reducer solution of $SnCl_2$ was finally added.

All concentrations are expressed in parts per million ($\mu g\ g^{-1}$) on a wet weight (ww) basis. Percentage moisture was determined for each organ or tissue to facilitate the conversion of dry-mass-based metal concentrations to wet-mass-based metal concentrations. The method detection limit (MDL) was calculated experimentally as the standard deviation (SD) of 12 blank replicates. MDL values, expressed in $\mu g\ g^{-1}$, were as follows: Cu, 0.77; Cd, 0.27; Cr, 0.29; Zn, 0.88; Ni, 1.54; Pb, 0.29; and Hg, 0.02. The RSD of the replicate samples was between 10 and 20%. For analytical quality control, reagent blanks and calibration curve build-up, certified reference materials (mussel tissue flour, Reference material No. 6, National Institute for Environmental Studies, Tsukuba, Japan) and analytical grade reagents (Merck or Baker) were used. The recovery percentages for the three metals in certified reference materials were between 90 and 110%. For samples that were lower than the limit of detection of the applied analytical method, a value of one half the detection limit was assigned, and the sample was included within the data set for statistical treatment.[27]

The Infostat 5.1® for Windows (Grupo Infostat Professional, FCA, Universidad Nacional de Cordoba, Argentina) statistical package was chosen. For Cu, Zn, Hg and Cd, a one-way analysis of variance (ANOVA) was performed to assess possible differences between the five sampled tissues (excluding liver in Cd). Fisher's least significant difference test was used for multiple comparisons. For Pb, Cr and Ni, no statistical analysis was performed because most of the values were below the detection limit.

3. Results and discussion

The concentrations and tissue distribution of Cd, Cu, Zn, Ni, Cr, Pb and Hg in *B. striatus* from the Middle Paraná River floodplain ecosystem were determined.

Table 1 summarises the range and mean concentrations of heavy metals for each tissue of *B. striatus*. Where possible, statistical differences between the tissues are displayed in the same table. A moisture content of between 75 and 80% was established for soft tissues (liver, kidney and muscle); for bones and feathers the value was 15–20%. These percentages coincide with those reported in the literature.[28,29]

Cr was below the detection limit in all tissue samples; whereas Ni was only detected in all vertebra samples and in 22.2% of the feather samples. For this reason, mean Ni concentration could only be calculated for vertebra (Table 1).

Pb was detected in all vertebra samples and in 44% of the kidney and feathers samples. In liver and muscle, all samples showed levels below the detection limit. Again, vertebra was the only tissue which allowed us to calculate mean Pb concentration, whereas for the other two tissues minimum and maximum concentrations were presented instead (Table 1). In this study, vertebra represented 56% of the total distribution of Pb in the analysed tissues, followed by feathers at 31.7% and kidney at 12.3%. This metal is a widespread environmental contaminant which has long been recognised as a poison to living organisms, with negative effects on general health, reproduction and behaviour, potentially leading to death.[30,31] On entering the blood, Pb is distributed throughout the body and accumulates in bone.[32] Approximately 95% of the total

Table 1. Ranges and mean metal concentrations ($\mu g\,g^{-1}$ wet wt; $n = 8$) in the organs of specimens of *Butorides striatus*.

	Tissue									
	Liver		Kidney		Muscle		Feathers		Vertebra	
Metal	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Cd	–	nd to 0.61	0.80 ± 0.73^{ab}	nd to 2.09	0.34 ± 0.20^{a}	nd to 0.71	0.39 ± 0.33^{a}	nd–1.04	1.27 ± 0.52^{a}	0.53–1.92
Pb	–	nd	–	nd to 4.11	–	nd	–	nd–11.76	15.94 ± 4.42	7.21–20.81
Cu	5.40 ± 1.60^{b}	3.78–8.37	5.30 ± 2.34^{b}	3.10–9.24	6.12 ± 2.11^{b}	3.36–8.72	11.36 ± 2.99^{a}	5.95–14.90	4.49 ± 0.97^{b}	3.39–5.67
Zn	41.02 ± 9.31^{b}	30.32–57.09	42.54 ± 13.77^{b}	21.49–62.50	18.69 ± 4.04^{c}	11.64–24.81	121.30 ± 22.05^{a}	76.14–153.81	107.45 ± 17.00^{a}	80.92–134.58
Cr	–	nd	–	nd	–	nd	–	nd	–	nd
Ni	–	nd	–	nd	–	nd	–	nd to 3.12	6.26 ± 2.98	4.20–13.94
Hg	1.35 ± 0.50^{b}	0.74–2.04	0.85 ± 0.56^{ab}	0.19–1.73	0.53 ± 0.22^{a}	0.21–0.73	1.38 ± 0.93^{b}	0.76–2.97	0.83 ± 0.29^{ab}	0.44–1.27

Note: nd, not detected. When more than 50% of the samples were below the detection limit, the mean was not calculated. Means in the same line with different superscripts were significantly different among tissues ($p < 0.05$).

body Pb burden is stored in the skeleton [33], indicating that bone tissue has a high capacity to accumulate and store Pb. The residence time of this metal in the blood is ∼4–6 weeks, so blood Pb reflects recent exposure; however, the residence time in bone ranges from several years to decades.[34] Although blood Pb remains the best and most widely used indicator of recent exposure, bone Pb is considered a good biomarker of long-term or cumulative exposure.[35,36] In addition, Pb shows strong kinetics of incorporation/elimination through the gastrointestinal system (basically the liver), and its residues are usually deposited within bone in a carbonate complexed way.[37] Birds may accumulate a certain amount of this metal in the bone over their lifetimes due to chronic and non-lethal acute exposure, with few signs of toxicity.[38,39] Vertebra of *B. striatus* showed the highest percentage of the total distribution of Pb, followed by feathers and kidney. Taking into account that all the samples corresponded to adult individuals, these results may be associated with long-term exposure to this metal. Scheuhammer [40] suggested that Pb levels above 5 $\mu g\,g^{-1}$ dry weight (dw) in bones of adult birds would be indicative of some degree of increased environmental exposure to Pb. By contrast, in waterfowl, bone Pb concentrations of $>20\,\mu g\,g^{-1}$ dw are considered to be associated with excessive exposure to Pb; moreover, birds that have died of Pb poisoning often have bone Pb concentrations in excess of 20–30 $\mu g\,g^{-1}$.[41] The results of this study showed Pb concentrations in bones of *B. striatus* between undetectable and 20.81 $\mu g\,g^{-1}$ ww (∼17 $\mu g\,g^{-1}$ dw). Therefore, although the values indicate a certain environmental exposure to Pb, the potential risk associated with this metal may be quite low for this species.

Levels of the remaining metals (Cd, Cu, Zn and Hg), were detectable in >50% of the samples for all tissues of *B. striatus*, with the exception of Cd in liver (22.2% of the samples). For this reason, Table 1 shows a range of Cd concentrations in this tissue.

Like Pb, the greatest percentage of total Cd in the analysed samples was found in vertebra tissue, with 45.3%, followed by kidney (28.7%), feathers (13.9%) and muscle (12.1%). Significant differences in Cd concentrations were found between tissues (one-way ANOVA, $p < 0.0009$; Table 1). The statistics showed that mean Cd concentrations in vertebra were significantly greater than in feathers and muscle, but were similar to kidney concentrations. Because most of the liver samples were below the limit of detection, this tissue was not included in this analysis. According to Scheuhammer,[40] the Cd concentration in liver tissue is probably the best measure of the body burden of this metal because liver accumulates approximately half of the body burden of Cd; moreover, the Cd content of the liver plus kidneys usually accounts for ∼90% of the total body burden. Nevertheless, the results of this study did not coincide with this state; moreover, low Cd concentrations in liver have also been recorded for other bird species.[34,41–43] Scheuhammer,[40] suggested that the low detection of Cd in the liver might be associated with a low-level exposure to this metal, or, conversely, to the toxic effects of elevated exposure to Cd. Scheuhammer also stated that in low Cd exposure, the concentrations of this metal in kidney would be higher than in liver. For this reason, liver/kidney concentration ratios are useful to determine acute or chronic exposure to Cd. Liver/kidney concentration ratios >1 indicate acute exposure to relatively high doses of Cd, whereas liver/kidney ratios of <1 are more indicative of chronic, low-level exposure. Taking this into account, the ratios were then calculated in this study. It is worth noting that in those liver samples with concentrations below the detection limit, a value of one half the detection limit was assigned (0.135 $\mu g\,g^{-1}$ for Cd). The liver/kidney concentration ratios in this study were on average <1, suggesting that *B. striatus* may be exposed to a chronic low level of Cd. Moreover, the presence of ∼50% of the total tissue distribution of this metal in vertebra also reinforces this, considering that this tissue is usually associated with chronic long-term exposure (see above). Based on laboratory studies, Eisler [44] estimated that a kidney or liver concentration of ∼10 ppm (ww) was associated with adverse effects. The concentrations of Cd found in liver and kidney samples of *B. striatus* in this study were below this value. Therefore, the potential risk associated with this metal may be quite low for *B. striatus*.

The levels of essential metals, such as Cu and Zn are metabolically regulated in seabird tissues.[45] Cu was detected in all *B. striatus* tissue samples. The mean concentrations of Cu in vertebra, muscle, liver and kidney were quite similar (Table 1). Feathers showed the greatest Cu concentrations, representing 34.78% of the total tissue distribution of Cu. Mean Cu concentrations in feathers were significantly greater than in the remaining tissues (one-way ANOVA, $p < 0.0001$; Table 1). These results coincide with a previous study on *Jacana jacana* from the same study site in which Beltzer *et al.* [21] found the greatest Cu concentrations in feathers ($270.9 \pm 121.4\,\mu\text{g g}^{-1}$ ww), representing 65.19% of the total tissue distribution of Cu.

Zn, the other essential metal, was also detected in all tissue samples. Muscle of *B. striatus* showed the lowest mean concentrations of Zn, representing 5.65% of the total tissue distribution. Meanwhile, liver and kidney showed similar mean Zn concentrations (aprroximately twice that of muscle, Table 1), representing 12.39 and 12.25% of the total tissue distribution, respectively. Finally, vertebra and feathers showed the greatest levels of Zn, with percentages very similar to the total tissue distribution (32.46 and 36.64%, respectively). Statistical analysis showed significant differences between tissues (one-way ANOVA, $p < 0.0001$; Table 1). In this case, mean Zn concentrations in vertebra and feathers were not significantly different (mean: $114.37 \pm 20.38\,\mu\text{g g}^{-1}$ ww; Table 1) and were significantly greater than concentrations in the other tissues. However, mean Zn concentrations in liver and kidney were not significantly different (mean: $41.78 \pm 11.43\,\mu\text{g g}^{-1}$ ww). Beltzer *et al.* [21] also found the lowest levels of Zn in the pectoral muscle of *J. jacana* ($12.60 \pm 6.58\,\mu\text{g g}^{-1}$ ww), whereas feathers samples had the greatest Zn concentrations ($127.20 \pm 122.8\,\mu\text{g g}^{-1}$ ww). The high concentration of Zn in feathers might be associated with pigmentation in which Zn is used.[28] Because this metal has an important role in many metabolic processes, especially the activation of enzymes and the regulation of gene expression,[45] it is not surprising to find a high concentration of Zn in *B. striatus* tissues.

The concentrations of Cu and Zn reported here are in close agreement with those found in studies in several aquatic bird species.[46–48]

Hg, the last metal analysed in this study, was detected in all tissue samples, like Cu and Zn. The lowest mean Hg concentrations were observed in *B. striatus* muscle, representing 10.65% of the total tissue distribution. However, mean Hg concentrations in kidney and vertebra samples were quite similar (Table 1), representing 17.24 and 16.83% of the total tissue distribution of Hg, respectively. Liver and feathers also showed similar mean Hg concentrations (Table 1), with both tissues together representing >50% of the total tissue distribution (27.34 and 27.94%, respectively).

The statistics showed significant differences between some tissues (one-way ANOVA, $p = 0.010$; Table 1). Thus, the results indicated that mean Hg concentrations in liver and feathers were significantly greater than in muscle (1.35 and 1.38 vs $0.53\,\mu\text{g g}^{-1}$ ww, respectively). In wild bird species living in environments receiving little or no industrial Hg contamination, levels of Hg in liver tissue range from 1 to $10\,\mu\text{g g}^{-1}$ ww, the highest values being associated with scavengers and piscivores.[39] *Butorides striatus* is a migrant species that reproduces in the Paraná River valley floodplain. Specimens arrive in the area in September and stay until April.[19] This species can be considered to be a top-level piscivore because it feeds mainly on fish (62.4% of total prey items), followed by insects (33.4%), arachnids (2.36%), crustaceans (1.39%) and amphibians (0.43%).[24] Because liver and kidney concentrations are primarily considered to indicate recent exposure,[39] the range of Hg concentrations in liver of *B. striatus* found in this study (Table 1) suggests that the levels of Hg in this environment may be quite low or even background values.

Feathers of several avian species generally carry up to 70% of the total Hg burden, indicating that this tissue reflects the storage of Hg during moulting as an excretory pathway.[28,49] In general, once metals enter the bodies of birds, they may be excreted or may accumulate in tissues. Excretion can be through urine or faeces, by being sequestered in feathers, by elimination in eggs or by elimination in the salt gland.[4] In particular, metal concentrations in feathers partially reflect the extent of pollution at the birds' location during feather formation.[5] Metals enter feathers during the 2–3 weeks it takes for them to grow, after which the blood supply atrophies and there is no further uptake of metals. Thus, feathers are an archive of metal exposure during their formation weeks or months earlier.[50] Moulting in *B. striatus* individuals usually occurs every year after breeding and before they leave the site of reproduction. Therefore, the Hg burden in these individuals would reflect metal uptake between breeding seasons and across the species' wintering range. The detectable levels of Hg in feathers of this species found here (Table 1) suggest the availability of this metal in the environment. Several studies have found that metal levels (including Hg) in feathers accurately reflect levels in the blood when the feathers are formed and that concentrations in the feathers are stable and inert after formation.[5,51,52] It is widely known that atmospheric deposition is the dominant source of Hg over most of the landscape. Once in the atmosphere, Hg is widely transported and disseminated as gaseous elemental mercury Hg(0), which has a lifetime of ∼1 year in the troposphere,[53] accounting for its widespread distribution. Nevertheless, under laboratory conditions, the levels of Hg associated with toxic effects in birds are 5–40 $\mu g\,g^{-1}$ dw in feathers,[2] values that exceeded those obtained in this study. For this reason, the potential risk associated with this metal for *B. striatus* may be quite low.

4. Conclusions

The concentrations of metals evaluated in *B. striatus* (striated heron) inhabiting the Middle Paraná River floodplain ecosystem provide useful information because there are no previous data for this species. In addition, data are scarce for these types of inorganic contaminants in birds in this area. For these reasons, the values reported for the heavy metals analysed in different tissues of *B. striatus* might serve as baseline data for future comparative studies. Differential distribution between the tissues/organs for each heavy metal evaluated shows that feathers and vertebra carry a greater percentage of the total body burden of metals. The highest mean concentrations of almost all the metals analysed (except Cr, which was always undetectable) were found in those tissues.

The metal levels recorded in this study indicated it is not an extremely polluted environment; even though most of the studied elements had accumulated – at different rates – in the tissues of the considered bird species. This demonstrated that, even in the case of low environmental metal concentrations, accumulation occurs with mechanisms following kinetics and accumulation trends governed by both physiological and environmental factors. In particular, the Hg distribution between organs is of great importance because it is not an essential element and in birds it is associated with numerous adverse effects. Detectable concentrations of Hg in the five organs studied indicate that further work is needed in future, in addition to tracking over time other metals like Pb that showed very high concentrations in the vertebra.

Butorides striatus could be utilised as a bioindicator of pollution (local or regional) because, by virtue of their position at the top of the trophic chain, they may successfully accumulate and concentrate heavy metals in their tissues (as the results for vertebra and feathers indicated). In this aspect, they are the most vulnerable components of ecosystems. The observed decrease in the body burden of some metals (i.e. Zn), might indicate that those metals were excreted via the feathers during moulting. Consequently, is necessary consider the growth stage of organs and tissues to understand the bioaccumulation process. Finally, this work reinforces the idea that the birds are good for the biomonitoring of metals in aquatic environments, and that the feathers are a non-destructive tool with which to evaluate metal levels in birds.

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