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Temporal trends (2006–2019) of metals and nonmetals in livers of great egrets (*Ardea alba*) from the São Paulo metropolitan region

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Abstract

Temporal trends (2006–2019) of metals (Cd, Co, Cs, Cu, Fe, Hg, K, Mg, Mn, Na, Rb and Zn) and nonmetals (Br, Cl and Se) were assessed in livers of great egrets (*Ardea alba*) from São Paulo Metropolitan Region, Brazil. Male and female concentrations were compared and the relation between body mass and contaminant levels was evaluated as well as the risks of contaminant levels for the birds. Large variations were observed for toxic elements (Cd and Hg) over time. Some specimens presented toxic levels of Hg, Cu, Fe and Zn. Females presented lower concentrations of Br, Co, Cs, Rb, Se and Zn, while body mass and Zn were negatively correlated.

Keywords São Paulo metropolitan region · Great egret · Adverse level risk · Toxic element · INAA · AAS

Introduction

The production and release of chemical elements such as metals by anthropogenic sources are one of the major threats to the health of ecosystems [1]. The knowledge of possible temporal trends of contaminants in the environment is important to check if the levels are increasing or decreasing and requires level comparisons for the same species at different time periods [2]. Chemical elements can bioaccumulate and biomagnify in the biosphere and may cause deleterious effects at high trophic level species, including humans [3, 4]. Therefore, there is great interest in monitoring the levels of elements in the biosphere and predatory birds, at the top of the food chain, are commonly chosen as biomonitors [5, 6]. These species can offer important information about the effects of pollutants on these animals and on the human species. Contamination by metals and nonmetals has been associated with a variety of problems such as severe damage to organs, nervous system, and reproductive system, resulting in decrease in reproductive success and survival of birds [3]. In this sense, the use of birds as bioindicators is an import resource for ecological assessment, which can promote the conservation of bird species [7]. In relation to human health, bird monitoring can provide early warning of potential exposure for humans that live in the same ecosystems and have the same diet [8]. Previous studies in Brazil reported bioaccumulation of metals in livers and kidneys [9–12] and in feathers [13, 14] of birds. However, these studies have not examined long time series. Herons and egrets feed on fish, amphibians, reptiles, and invertebrates in various wetlands, including farming areas, streams, and rivers [15]. They occupy the top of the food chain in these ecosystems and are more susceptible to bioaccumulation. Therefore, herons and egrets might be suitable bioindicators for the evaluation of inorganic pollution [15, 16]. Individual differences such as age, sex, location and morphological and physiological factors are important for the absorption and elimination of contaminants, and consequently have effects on bioaccumulation. Adult specimens, due to their longer life, tend to accumulate higher levels of contaminants and can be used to monitor contamination [8, 17]. In the São Paulo Metropolitan Region (SPMR), Brazil, industrial effluents release Fe,

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Mn, Cd, Cu, Hg and Zn into aquatic environments [18]. Dissolved Fe and total Mn may also indicate the intensification of erosive processes resulting in transport and the entrance in water bodies of particulate material coming from the soil, according to the Environmental Company of São Paulo State [18]. Livers of great egrets were considered a suitable bioindicator to evaluate metal and nonmetals contamination in SPMR because this species is resident, occupies the top of the food chain of aquatic environments and presented high levels of chemical elements in liver [12]. Therefore, periodic monitoring of chemicals in these samples could show potential contaminants trends in the region. The main objective this study was to investigate temporal trends of bioaccumulation of metals (Cd, Co, Cs, Cu, Fe, Hg, K, Mg, Mn, Na, Rb and Zn) and nonmetals (Br, Cl and Se) in the period from 2006 to 2019 in livers of adult specimens of great egrets collected in the SPMR. In the present study, it was also evaluated if the levels of the investigated elements represent risks for great egrets in the SPMR, based on known levels of adverse effects for wild birds. In addition, male and female concentration comparisons, and the relation of the mass of birds and levels of contaminants were evaluated.

Experimental

Fifty-two liver samples of adults of both sexes from great egrets (*Ardea alba*) found injured or sick in the SPMR were donated by the Wildlife Division of the Municipality. Biometry of the specimens is shown in Table 1. Arithmetic mean and range of body mass (g) were calculated for two-year periods, except for the end (2017–2019). From a total of fifty-two adult specimens, weight was available for forty-six specimens and the gender was determined in 21 females and 24 males.

Table 1 Body mass (g) and gender (Male = M; Female = F; Not identified = NI) of specimens sampled by period (2006–2013 and 2017–2019) of great egret from São Paulo Metropolitan Region

Period	Body mass (g)	Gender
2006–2007	Mean ± SD = 692 ± 211 Range = 535–1055	M = 04 F = 05
2008–2009	Mean ± SD = 779 ± 202 Range = 545–1220	M = 03 F = 05 NI = 01
2010–2011	Mean ± SD = 709 ± 161 Range = 560–1045	M = 10 F = 05 NI = 01
2012–2013	Mean ± SD = 757 ± 196 Range = 465–1013	M = 06 F = 05 NI = 01
2017–2019	Mean ± SD = 781 ± 175 Range = 490–940	M = 01 F = 01 NI = 04

Metals and nonmetal concentrations from (2006–2013 were obtained from previous studies by Silva and Saiki [19] and Silva et al. [12] and more recent data (2017–2019) were added for this study from the analyses of collected liver samples.

Samples were collected under licenses from the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) and were renewed annually. Livers were stored at − 20 °C after collection, until their treatment for analysis. Each liver sample was first cleaned by removing blood and was cut into small pieces, using a titanium knife. Then, they were freeze-dried and ground in an agate mortar to obtain a fine homogenous powder.

Br, Cl, Co, Cs, Cu, Fe, K, Mg, Mn, Na, Rb, Se and Zn were determined by the comparative method of INAA. Aliquots of about 150 mg of each liver sample were irradiated together with element standards which were prepared using certified standard solutions provided by Spex Certiprep Chemicals, USA. Long irradiations were performed for 16 h under a thermal neutron flux of about $2–4 \times 10^{12}$ cm^{-2} s^{-1} of the IEA-R1 nuclear reactor for determination of elements presenting half-lives longer than 14.96 h (Br, Co, Cs, Fe, Na, Rb, Se and Zn). Three series of measurements were carried out after 7, 15 and 20–25 days of decay times of the standards and samples and using counting times varying from 36,000 to 50,000 s. Short irradiations were used for half-lives shorter than 12.4 h (Cl, Cu, K, Mg and Mn), and the irradiation time was 15 s under thermal neutron flux of 6.69×10^{12} cm^{-2} s^{-1}. In this case, two series of measurements were carried out for decay times of 5 and 90 min and counting times varying from 300 to 600 s. The irradiated samples and standards were measured by HPGe detector Model GEM 20,190 coupled to a gamma spectrometer both from EG & G Ortec.

Hg was determined by Cold Vapor Atomic Absorption Spectrometry (CV AAS), using Perkin Elmer FIMS (Flow Injection System Mercury) spectrometer, while Cd was determined by Electrothermal Atomic Absorption Spectrometry (ET AAS), using Perkin Elmer AAnalyst 800 spectrometer. For CV AAS and ET AAS determination, liver samples were subjected to acid digestion. Approximately 100 mg of each sample were weighed and appropriate standard solutions of Hg (0.3, 1.25, 2.5, 3.8 and 5 ng mL^{-1}) and Cd (1.5, 2.8, 4.1, 5.8 and 6.7 ng mL^{-1}) were used for the construction of calibration curves. Coefficients of correlation for the linear model fitting was higher than 0.998, obtained using the software Winlab 32 for AAS, version 6.2.0.0079 (Perkin Elmer).

For INAA, accuracy was checked by measuring certified reference materials simultaneously irradiated with samples and element standards (3 or more aliquots). Obtained results were considered adequate. Cl, Cu, Fe, K, Mg, Mn, Na, Rb, Se and Zn were determined in NIST SRM 1577b bovine liver (National Institute of Standards & Technology, USA) and recoveries were 100, 111, 108, 95, 108, 94, 107, 104, 95 and 107%, respectively. Br, Co, Cs, Rb and Zn were determined in INCT-TL-1 tea leaves (Institute of Nuclear Chemistry and Technology, Poland) and recoveries were 102, 98, 106, 101 and 106%, respectively. For AAS, certified reference materials were digested in the same batch as for liver samples. Hg was determined in *Perna perna* mussel (Nuclear and Energy Research Institute, Brazil) and recovery was 90% and Cd was determined in NIST SRM 1577b bovine liver (National Institute of Standards & Technology, USA) and recovery was 92%. Reagent blanks were also used as parts of the quality control and quality assurance for the determination of Cd and Hg and for all results. Limits of detection (LOD) and quantification (LOQ) were determined according to Currie [20] and results were:

(A) LOD - Br 0.14, Cd 0.00072, Cl 14, Co 0.01, Cs 0.02, Cu 8.6, Fe 8.0, Hg 0.006, K 0.04, Mg 160, Mn 0.2, Na 13, Rb 0.4, Se 0.26 and Zn 0.4 mg kg^{-1}.
(B) LOQ - Br 0.43, Cd 0.0024, Cl 42.6, Co 0.033, Cs 0.070, Cu 28, Fe 24, Hg 0.02, K 0.136, Mg 533, Mn 0.7, Na 44, Rb 1.4, Se 0.8 and Zn 1.3 mg kg^{-1}.

Determined concentrations were above LOD and most of the liver samples presented quantifiable concentrations of the elements.

Sample aliquots were analyzed in replicates (2 or 3) and when the values of Relative Standard Deviation (RSD) of replicates were above 25%, the analyses were repeated. All concentrations are expressed in mg kg^{-1} on a dry weight basis. Arithmetic means and standard deviations of element concentrations were calculated for the samples collected for two-year periods to evaluate temporal trends.

To compare the chemical element concentrations between males and females and the relation between body mass and contaminant levels, firstly, the normality of the data was tested using the Anderson–Darling test on Minitab. As the results did not show a normal distribution, the non-parametric Kruskal Wallis test was applied to verify if there were differences on element concentrations year by year and Mann Whitney test was used for differences between genders. The differences were considered significant at level < 0.05. Correlations between elements and among elements and mass of specimens were evaluated calculating Pearson's correlations (r) using SPSS version 25.0 program. Results were considered significant at p-values 0.05 and 0.01.

Results and discussion

Table 2 shows the number of samples, arithmetic means, and standard deviations of element concentrations determined over the years in livers of great egrets from SPMR. Due to the small number of samples, which does not allow

Table 2 Dry weight concentrations of metals and nonmetals (mean ± SD, mg kg^{-1}, unless indicated) in livers of great egrets (*Ardea alba*) from São Paulo Metropolitan Region (2006–2013 and 2017–2019)

Element	2006–2007	2008–2009	2010–2011	2012–2013	2017–2019	p value
Br	47.1 ± 23.0(9)	41.8 ± 33.3(9)	29.0 ± 13.6(15)	46.0 ± 20.5(12)	44.6 ± 21.6(6)	0.099
Cd	0.168 ± 0.085(8)	0.334 ± 0.355(9)	0.73 ± 1.13(13)	0.144 ± 0.100(12)	0.128 ± 0.070(6)	0.287
Cl	ND	ND	4285 ± 1329(7)	4040 ± 1343(12)	3949 ± 523(6)	0.943
Co	0.124 ± 0.031(9)	0.202 ± 0.098(9)	0.188 ± 0.229(16)	0.160 ± 0.051(12)	0.132 ± 0.048(6)	0.560
Cs	0.156 ± 0.063(9)	0.286 ± 0.297(8)	0.167 ± 0.105(16)	0.195 ± 0.143(9)	0.115 ± 0.071(6)	0.454
Cu	39.0 ± 23.1(6)	122 ± 90(4)	80.4 ± 93.8(10)	109 ± 116(8)	15.9 ± 1.9 (2)	0.275
Fe	3091 ± 1903(9)	3487 ± 1920(9)	3302 ± 2503(16)	3611 ± 1942(12)	2436 ± 1217(6)	0.855
Hg*	1.90 ± 1.91(8)	3.34 ± 3.66(8)	2.79 ± 2.78(16)	6.85 ± 5.44(12)	0.541 ± 0.404(5)	0.009
K (%)	1.20 ± 0.45 (8)	0.78 ± 0.17(7)	0.82 ± 0.23(12)	0.99 ± 0.24(12)	0.58 ± 0.08(6)	0.119
Mg	559 ± 360(8)	513 ± 98(6)	500 ± 148(13)	553 ± 135(12)	237 ± 95(5)	0.054
Mn	9.16 ± 3.70(8)	9.54 ± 3.29(6)	10.5 ± 4.1(13)	10.9 ± 2.4(14)	6.99 ± 1.92(5)	0.163
Na	4777 ± 861(9)	4178 ± 1475(9)	4105 ± 1233(16)	4386 ± 1322(12)	3853 ± 784(6)	0.530
Rb	46.0 ± 19.3(9)	33.5 ± 15.1(9)	37.5 ± 12.5(16)	39.1 ± 13.5(12)	40.6 ± 6.2(6)	0.614
Se	4.35 ± 2.26(9)	4.11 ± 2.05 (9)	3.94 ± 1.42(16)	4.19 ± 1.65(12)	2.71 ± 0.55(6)	0.437
Zn	205 ± 99(9)	236 ± 102(9)	246 ± 124(16)	236 ± 96(12)	191 ± 54(6)	0.991

Data 2006–2009 from Silva and Saiki [19], 2010–2013 from Silva et al. [12] and 2017–2019 present study. Sample size is in parentheses, ND, not determined

*Significant variation among periods, $p < 0.05$

obtaining statistically significant results [21], the data were grouped in two-year periods from 2006 to 2013.

In the last period (2017–2019) there was a drastic reduction in the number of obtained samples. This fact can be explained by a yellow fever epidemic from December 2016 to March 2018 in the country. Parks were closed to avoid contact of people with the genus Aedes mosquitoes, which are transmitters of the disease [22]. The Wildlife Division-Anhanguera Unity (CeMaCAS), which provided the liver samples for this study, was also closed during the epidemic. In this period, it was observed an apparent decrease on the levels of Cu, Fe, Hg, Mg, Mn, Se and Zn. However, the Kruskal Wallis tests ($\alpha < 0.05$) indicated a significant decrease only for Hg levels. Anyway, Hg did not present any decreasing trend in previous periods and the low number of samples may relate to this result.

Large variation levels were observed for toxic elements (Cd and Hg) over time that can be linked to factors, such as food habitats, gender, age, reproduction, and molting [23, 24]. However, as diet is the main source of non-essential elements in birds [15], periodic variations of Cd and Hg concentrations may be related to annual and regional differences in the diversity and density of food items [25].

The results also suggested that Cd levels have been suffering a non-significant reduction in concentrations in great egret livers from SPMR in recent years. Globally, Cd concentrations are declining due to reduction of anthropogenic sources, especially batteries [26]. However, a possible environmental reduction of Cd at the regional level has not yet been investigated [27].

Until 2013, the essential elements Co, Fe, Mg, Mn, Na, Se and Zn were observed in quite stable levels, which may indicate that these elements have remained more stable in the environment, because the accumulation of the essential elements in birds is known to be related to bioavailability [23]. Another phenomenon that can contribute to less difference over the years is that essential elements are generally regulated by homeostatic processes that maintain an internal balance [15, 24]. In recent years, efforts for more effective control on influential industries in the region were implemented [28]. As the great egret occupies a high trophic level, it is susceptible to the bioaccumulation and up to now results did not show significant changes in the levels and it is very likely that the risk of bioaccumulation for these elements remains. The suggestion is that in the future these levels are analyzed again. The results presented here can confirm the hypothesis that periodic monitoring of great egret livers can indicate trends in bioaccumulation of contaminants. However, it is necessary to continue the analysis over time, as trends are not clear for toxic elements. Several studies in the literature have indicated a difference in metal concentrations between male and female birds [8, 29–31]. In this study, concentrations for Br, Co, Cs, Rb, Se and Zn were significantly higher in males than in females of great egrets. These differences may be associated with ecological factors, such as diet, which can vary between genders as male and female birds may feed in different habitats and eat different foods or for presenting different metal dynamics [8, 29]. There are several mechanisms for removing elements from the organisms of birds, such as direct elimination through feces or transfer to the feathers. Female birds can also transfer elements to the eggs [8, 17]. Toxic elements and Se can cause a variety of problems such teratogenic activity on embryonic development, which in turn translates into fewer chicks hatching [15, 32]. Therefore, the differences of Se concentrations presented here are interesting and require a thorough study as well as Co and Zn that also become toxic at high levels [33]. The results for Br, Cs and Rb are difficult to interpret due to few studies on the toxicity of these elements in birds.

The calculation of correlations between the body mass of birds and the levels of metals and Se are important because toxic levels of these elements can reduce this biological parameter [34] and in contaminated areas this adverse effect of contamination can be verified by negative correlations [33]. To designate correlation rates in an increasing order, the following criteria were used: weak ($r = 0.0$–0.4), moderate ($r = 0.5$), strong ($r = 0.6$–0.8), and exceptionally strong ($r = 0.9$–1.0) [35]. According to these criteria Pearson's correlations (r) results analysis showed strong negative correlation between body mass and the level of Zn ($r = -0.649$, $p < 0.01$), indicating that birds with smaller masses had the highest Zn concentrations. Only few studies have associated weight loss with high Zn concentrations in wild birds. Therefore, Sileo et al. [36] diagnosed weight loss in geese with poisoning levels of Zn and a negative relation between bird weight and Zn levels in feathers was observed in Japanese quail [37]. Between body mass and Fe there was a significant negative correlation, but weak ($r = -0.473$, $p < 0.01$). Therefore, it was not considered relevant.

Cd and Hg levels were determined in great egret livers from the Haneda industrial region in Japan where heavy metal pollution occurs [38]. Hg levels for great egret livers from SPMR (3.49 ± 4.00 mg kg^{-1}) are above those from that region (2.31 ± 2.41 mg kg^{-1}).

Hepatic Hg levels from 4 to 40 mg kg^{-1} dry weight can affect growth, individual development, reproduction, metabolism, and behavior in birds [4, 39]. In this study, 13 specimens (26.5%) presented concentrations within this range.

Cd levels (0.330 ± 0.612 mg kg^{-1} dry weight) were also above those from great egrets from Haneda in Japan (0.187 ± 0.206 mg kg^{-1} dry weight). However, except for one sample (4.04 mg kg^{-1} dry weight), Cd levels in great egrets' livers from SPMR are below levels associated with negative effects (< 3 mg kg^{-1} dry weight, according to Kim and Oh [40]).

Cu, Mn, Se and Zn levels were also compared to great egrets from the Haneda industrial region. The Cu mean value (81 ± 90 mg kg^{-1} dry weight) of great egret from SPMR were below the ones (173 ± 209 mg kg^{-1} dry weight) of that region [38], which were much higher than in the other species and were attributed to the background levels of their habitat.

The Cu levels within the range (187–323 mg kg^{-1} dry weight) are of acute Cu poisoning as described for Canada geese. The effects of these levels can cause damage to the gizzard and the proventriculus [30, 41]. In the present study 6 specimens (20%) presented Cu results within this range.

Mn levels (9.78 ± 3.38 mg kg^{-1} dry weight) were like those of great egret from Haneda (9.85 ± 2.34 mg kg^{-1} dry weight). These values are at an acceptable range of normal concentrations for herons and egrets [40].

The Se levels were considered low (3.56 ± 1.70 mg kg^{-1} dry weight) when compared to great egrets (8.60 ± 2.68 mg kg^{-1} dry weight) from Haneda, Japan [38]. Concentrations of Se > 10 mg kg^{-1} dry weight in liver may cause reproductive impairments [5] and it was detected in one great egret specimen. However, the observed concentrations of Se and Hg were clearly correlated ($r = 0.61685$; $p < 0.001$). High exposure to Hg and Se can be individually toxic, but their accumulations reduce the toxicity of each other [5, 42]. As the levels of Se in this study can be considered low, this relation may be linked to the Se protection capacities against the toxicity of Hg.

Zn mean value (229 ± 102 mg kg^{-1} dry weight) in great egrets was high compared to great egrets (96.3 ± 28.7 mg kg^{-1} dry weight) from Haneda [38]. The increase in Zn concentrations causes renal toxicity [21]. Zn levels of about 200 mg kg^{-1} dry weight in livers is considered a threshold value of physiological relevance in seabird species [43] and Zn levels above 280 mg kg^{-1} dry weight may be responsible for sublethal effects for adults [44]. In the present study 17 specimens (32.7%) presented such high levels.

The mean value for Fe presented here (3269 ± 2018 mg kg^{-1} dry weight) was far above the Fe mean (1190 ± 467 mg kg^{-1} dry weight) for grey herons from Hodaka, Japan [38] and six specimens of great egret from SPMR showed very high concentrations of Fe (> 6100 mg kg^{-1} dry weight, [45]). The pathological accumulation of Fe in liver is a phenomenon called iron storage disease or hemosiderosis, being however poorly understood. There might be two explanations for the results in this study: Firstly, it may be due to Zn poisoning [44, 45], because this phenomenon can be observed by the strong positive correlation between Fe and Zn ($r = 0.60031$, $p < 0.01$). Secondly, the high accumulation of Fe may also be indicative of strong bacterial and helminthological infections [45]. The necropsy performed on nineteen specimens of great egrets from SPMR verified that two specimens with very high levels of Fe (7140 and 7163 mg kg^{-1} dry weight) presented nematodes. However, two specimens with unidentified parasitic cysts had Fe levels well below the average (1553 and 1767 mg kg^{-1} dry weight) and two specimens without helminth infections presented high Fe concentrations (7079 and 7910 mg kg^{-1} dry weight). In conclusion, the observed results can support both hypotheses. In the future it would be interesting to include a clinical verification of possible symptoms associated with Hg, Cu, Fe and Zn toxic levels described here.

Conclusions

In this study, it was not possible to observe clear time trends for toxic elements (Cd and Hg). However it was possible to verify that the essential elements Fe and Zn, whose levels are considered high and can cause adverse effects, remained stable in the bird samples and possibly in the environment. The results also showed that Cu and Hg are also a threat to the great egrets in the region. Thus, it is recommended the continuation of the study of time series of chemical elements in samples of great egret livers in order to assist in the control of toxic element contamination in São Paulo Metropolitan Region and enhance biomonitoring programs with birds.

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Declarations

Conflicts of interests All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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