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TRACE ELEMENTS IN LIVERS OF GREAT EGRETS (*Ardea alba*) FROM SÃO PAULO METROPOLITAN REGION: TEMPORAL CONSIDERATIONS (2006-2013) AND THE RELATIONS WITH SEX AND MASS OF BIRDS

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ABSTRACT

To evaluate possible variations over time (2006-2013) in trace element levels in livers from adult specimens of great egret from the São Paulo Metropolitan Region (SPMR), recent data and published data have been used. The elements Br, Cl, Co, Cs, Cu, Fe, K, Mg, Mn, Na, Rb, Se and Zn were determined by Neutron Activation Analysis (NAA) and the toxic elements Cd and Hg by Atomic Absorption Spectrometry (AAS). The large inter annual variations observed for several elements showed the importance of expanding the time period for this analysis as the series is considered short (<10 years). However, non-significant increase of Br, Cu, Mn, Fe and Zn and a decrease in Cd, also non- significant, were observed at the end of the series. The concentration relations with sex and mass of the birds were also assessed. Females presented lower concentrations of Br, Co, Cs, Se and Zn and it may be related to metabolic differences, detoxification pathways, and other factors. No relationship between body mass and element levels were observed. Obtained results demonstrated the importance of temporal monitoring of trace elements in livers of great egrets, especially for the results obtained for Cu, Fe and Zn, since the contamination by these elements in the region should be considered. The present study is the first relating contaminant level with conditions of birds in the region, encouraging future studies that evaluate this issue.

1. INTRODUCTION

The production and release of trace elements by anthropogenic sources is one of the major threats to the health of ecosystems [1]. Trace elements can bioaccumulate and biomagnify in the biosphere and may have deleterious effects at high trophic levels, including humans [2, 3]. Therefore, there is great interest in assessing and monitoring trace element concentrations in the biosphere and predatory birds at the top of the food chain are commonly chosen as bioindicators or biomonitors [4, 5].

The knowledge of possible temporal trends of contaminants in the environment requires comparison of levels in the same species at different time periods [6]. Although, there is an abundance of trace element level data for many species of birds, there are relatively few long-term time series for the same locality, especially for species at high trophic levels [7].

Herons and egrets exhibit longevity and occupy the top of the food chain and are more susceptible to bioaccumulation [8]. The liver of great egrets (*Ardea alba*) were considered a suitable bioindicator to evaluate trace element contamination in the São Paulo Metropolitan Region (SPMR) because this species is resident, occupy the top of food chain of aquatic environments and presented high levels of trace elements in liver [9].

Results from previous studies showed the increase of Cd and Mn in the period from 2006 to 2011 in liver samples, possibly related to the disposal in the environment [10]. Besides, the analyzed period may be considered short and the continuity of determination of trace elements in liver samples was recommended.

The archiving of samples and the continuity of sampling of great egret livers has allowed the continuity of the trace element temporal analysis in the SPMR, as well as the study of the levels of Cl from 2011 to 2013.

Thus, the present study examined temporal trends of essential and non-essential elements (Br, Cd, Cl, Co, Cs, Fe, Hg, K, Mg, Mn, Na, Rb, Se and Zn) in the period of 2006 to 2013 in great egret livers. It also been verified if evaluated if the levels of trace elements investigated represent risks for great egrets in the SPMR based on known levels of adverse effects for ardeids. In addition, male and female concentration comparisons and the relationships of the mass of birds and levels of contaminants were determined.

2. EXPERIMENTAL

2.1. Data Sources

To assess temporal variations of trace element concentrations in livers of great egrets from SPMR during the period of 2006 to 2013, the data were obtained from previous studies by Silva and Saiki [11] and Silva et al. [9] and new data were added from the analyses of liver samples, following the same sampling protocol and using the same analytical methods.

2.1.1. Sampling

Forty-nine liver samples of adults of both sexes from great egret found injured or sick in the SPMR were donated by the Technical Division of Veterinary Medicine and Wildlife Management of the city of São Paulo (DEPAVE 3). Samples were collected under license from the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA).

2.2. Analytical Procedure

Liver samples were ground, lyophilized and homogenized. The concentrations of Br, Cl, Co, Cs, Cu, Fe, K, Mg, Mn, Na, Rb, Se and Zn were determined by the Instrumental Neutron Activation Analysis (INAA) comparative method. Aliquots of about 150–200 mg of each liver sample were irradiated together with elemental standards in the nuclear research reactor IEA-R1 (IPEN - CNEN/SP). Gamma ray spectra were acquired using MAESTRO software from Ortec EG &G and processed using VISPECT2 computer program. Cd and Hg were determined by Atomic Absorption Spectrometry (AAS) method. Hg was determined by Cold Vapor Atomic Absorption Spectrometry (CV AAS), using the Perkin Elmer FIMS (Flow Injection System Mercury) spectrometer, while Cd was determined by Electrothermal Atomic Absorption Spectrometry (ET AAS), using the Perkin Elmer Analyst800 Spectrometer. Liver samples were subjected to acid digestion. Approximately 100 mg of each sample was weighed and appropriate standard solutions of Hg and Cd were used for the construction of analytical curves. Certified reference materials, replicate samples and blanks were routinely analyzed in parallel with samples.

2.3. Statistical Analysis of the Data

Arithmetic means and standard deviations of element concentrations were calculated for the samples collected per year. The non parametric Kruskal Wallis test was applied to verify if there were differences of trace element concentrations year by year and Mann Whitney test was used for differences between genders. The differences were considered significant at level $\alpha < 0.05$. Correlations between the elements and among the elements and mass of specimens were evaluated calculating Pearson Correlation Matrix ($r = 0.7$) using SPSS version 25.0 program. Results were considered as significant at p-values 0.05 and 0.01.

3. RESULTS AND DISCUSSION

3.1. Temporal Trends of Trace Elements in Great Egrets Livers (2006-2013)

No significant differences were observed in element levels over the years according to the Kruskal Waliis tests ($\alpha < 0.05$). Table 1 shows the result of the descriptive statistics of trace element concentrations year by year.

Table 1: Concentrations of trace elements (mean±SD mg kg^{-1} dw unless indicated) in livers of great egret (*Ardea alba*) from Metropolitan Region of São Paulo (2006 to 2013).

year	n[a]	gender	Br	Cd	Cl	Co	Cs
2006	3	F+M	49±18	0.162±0.012	-	0.108±0.032	0.116±0.086
2007	6	F+M	46 ±26	0.170±0.101	-	0.13±0.03	0.176±0.045
2008	2	NI[b]	59±22	0.188±0.030	-	0.264±0.044	0.279±0.035
2009	7	F+M	37±36	0.352±0.379	-	0.184±0.105	0.288±0.352
2010	4	F+M	27±14	1.56±1.74	-	0.112±0.077	0.163±0.153
2011	11	F+M	30±18	0.356±0.239	4285±1328	0.138±0.036	0.173±0.097
2012	9	F+M	41±6	0.16±0.10	4157±1178	0.139±0.039	0.156±0.122
2013	3	F+M	62±22	0.09±0.07	3687±2030	0.225±0.008	0.273±0.175
year	n[a]	gender	Cu	Fe	Hg	K (%)	Mg
2006	3	F+M	43.5±0.8	2886±2283	0.359±0.090	0.867±0.010	519±69
2007	6	F+M	37±29	3194±1915	2.42±1.96	1.48±0.42	583±338
2008	2	NI[b]	ND[c]	4329±3029	3.07±1.56	ND[c]	597±20
2009	7	F+M	122±90	3247±1756	3.43±4.27	0.748±0.166	496±99
2010	4	F+M	41±28	4195±3839	1.50±1.07	0.885±0.049	532±69
2011	11	F+M	61±64	2875±2081	3.35±3.19	0.795±0.247	491±166
2012	9	F+M	95±70	3515±1940	7.15±6.08	0.995±0.204	547±146
2013	3	F+M	133±190	3897±2584	5.96±3.65	1.01±0.41	568±119
year	n[a]	gender	Mn	Na	Rb	Se	Zn
2006	3	F+M	6.76±1.83	4721±443	42±19	5.2±4.2	150±80
2007	6	F+M	10.6±3.9	4806±1051	48 ±21	3.95±0.69	232±103
2008	2	NI[b]	9.1±0.6	3715±368	ND[c]	3.93±0.28	250±56
2009	7	F+M	9.63±3.67	4310±1670	30±15	4.2±2.4	233±115
2010	4	F+M	12.8±7.5	3893±857	37 ±12	4. 0±2.2	232±178
2011	11	F+M	9.9 ±3.8	4078±1383	38±14	4.0±1.2	248±115
2012	9	F+M	10.7 ±2.6	4212±1503	38 ±12	3.8±1.4	201±79
2013	3	F+M	11.7±1.9	4909±172	42±20	5.5±1.9	340±66

a. Number of samples, b. not identified, c. not determined

It should be noted that the elements that showed more stable levels during the period were the essential elements Cl, K, Mg, Na e Se, which are regulated by normal homeostatic mechanisms [12].

Large variations in annual levels were observed for Br, Cd, Cs, Cu, Fe, Hg, and Mn. These results can be linked to biological factors such as reproduction, moult and gender [10]. However, according to Berglund et al. [7], annual and regional differences in the diversity and density of food items may contribute primarily to variations in exposure, which may lead to a large annual variation in concentrations. In fact, bird diet is the main trace element exposure route, so the concentrations of dietary elements can affect levels in birds [12].

At the end of the period, an increase in levels of Br, Cu, Fe, Mn and Zn was observed, which may be related to environmental deposition. Because the habitat is also a source of contamination [13] and the contaminant levels in birds are related to the contamination of their ecosystems [3].

In the SPMR, industrial effluents release Fe, Mn, Cd, Cu, Hg and Zn in the aquatic environments. Dissolved Fe and total Mn may also indicate the intensification of erosive processes resulting in transport and the entrance in water bodies of the particulate material coming from the soil. These sources are difficult to control [14] and cause exposure to great egrets and their prey in the region. Cd presented decrease in the last 2 years, but this result cannot yet be explained. Other stressors such as temperature and precipitation may affect the accumulation of metals and cause annual differences in results [7].

The fact is that the observed large variations between years in the concentrations demonstrated the importance of long periods of monitoring (10-20 years) in order to clearly discern contaminant trends [7, 15].

3.1. Significance of Trace Element Levels

3.1.1. Cd and Hg

We compared the concentrations of trace elements in great egret livers from SPMR with the levels found for great egrets, grey herons and intermediate egrets from Haneda and grey herons from Hodaka, both areas from Japan which also receive industrial discharges [16].

Cd mean of great egret livers (0.359±0.649 mg kg^{-1} dw) from SPMR is above of great egrets (0.187±0.206 mg kg^{-1} dw) and grey herons (0.190±0.129 mg kg^{-1} dw and 0.273±0.407 mg kg^{-1}dw) from Japan [16]. But, the obtained results for Cd are within the natural background in livers (< 3 mg kg^{-1} dw) of ardeids [12], except for one sample (4.04 mg kg^{-1}dw). For the observed Cd levels we can conclude that there is no threat to physiological processes in great egret from the SPMR.

Although Cd levels in great egrets livers from SPMR are below levels associated with negative effects, Cd is of great concern because it is common, non-essential and toxic [17] and an increasing number of studies suggest that physiological stress occurs for many species at low background levels [18].

Hg levels (3.84±4.09 mg kg^{-1} dw) are above of great egrets, grey herons and intermediate egrets (2.31±2.41, 1.28±1.52, 2.02±1.86 mg kg^{-1} dw, respectively) from Haneda, Japan [16], but are similar to grey herons (3.78±7.67mg kg^{-1} dw) from Hodaka also in Japan [16].

Hepatic Hg levels from 4 to 40 µg/g dw can affect growth, individual development, reproduction, metabolism and behavior in birds [3, 19]. In this study, 14 specimens (31%) presented concentrations within this range. These results reinforce the importance of continuing to monitor Hg and Cd in the in great egrets livers from SPMR.

3.1.2. Cu, Fe, Mn, Se and Zn

In the birds, chronic exposure to these elements from dietary pathway routes can result in elevated deposits within organs and tissues, which are the most common pathway for adverse biological effects to occur, although toxicity thresholds vary among species and tissues [12, 20, 21].

Although Cu mean 81.5±89.2 mg kg^{-1} dw found here is well below of great egrets (173±209 mg kg^{-1} dw), grey herons (791±1260 mg kg^{-1} dw) and intermediate egrets (787±739 mg kg^{-1} dw) from Haneda, Japan, whose results were associated with contamination local by Cu [16], six specimens (21.4%) presented Cu levels within the range (187-323 mg kg^{-1} dw) of acute Cu poisoning as described for Canada gooses. The effects of these levels can damage the gizzard and proventriculus [22, 23].

The mean value for Fe was elevated (3550.6±2112.3 mg kg^{-1} dw) and is far above the Fe mean (1190±467 mg kg^{-1} dw) for grey herons from Hodaka, Japan [16]. The Fe values varied greatly (498-7910 mg kg^{-1}dw), but 6 specimens showed Fe hyperaccumulation (> 6000 mg kg^{-1}dw).

High concentrations of Fe in the liver are symptoms of advanced inflammatory states and exaggerated immunological responses and may be associated with Cd, Pb, or Zn poisoning. However, birds strongly infested with nematodes or fungi may also accumulate large amounts of Fe [24, 25].

From the clinical data received with the specimens, 3 specimens presented Fe hyperaccumulation identified 1 specimen (7139.8 mg kg^{-1} dw) with ectoparasite mites, 1 specimen (7163.5 mg kg^{-1} dw) with moderate helminthes infestation and for 1 specimen (7079 mg kg^{-1} dw), the tests for protozoa and helminthes were negatives. Therefore, some cases can be explained by this possibility, but not all.

High Fe levels in liver of great egrets may be associated with a response to Zn poisoning because a strong correlation was obtained between Fe and Zn (r = 0.60031; p =0.01) and the Zn mean (234.5±106.8) in great egrets is high compared to great egrets and intermediate egrets (96.3±28.7, 146±61.4 mg kg^{-1}dw, respectively) from Haneda, Japan [16]. Additionally, in the present study 18 specimens (39%) exceeded 280 mg kg^{-1} dw in liver, which may be responsible for sub lethal effects for adults [25].

In this study it was not possible to explain these results more clearly, firstly because Pb was not determined in these specimens and the parasitological evaluation was incipient. It is suggested that direct studies be done on the relationship of parasitic infections with levels of contaminants, in addition to clinical verification of possible symptoms associated with toxic levels described here.

Mn mean concentrations (10.20±3.37 mg kg^{-1} dw) in the present study is on the same order of magnitude of great egrets (9.85±2.3mg kg^{-1} dw), grey herons (13.4±3.33 mg kg^{-1} dw) and intermediate egrets (12.9±1.90, 13.4±3.33 mg kg^{-1} dw) from Japan [16]. These values are at an acceptable range of normal concentrations for herons and egrets [12].

The levels of Se are considered low (mean= 4.19±1.70 mg kg^{-1}dw) when compared to great egrets (8.60±2.68 mg kg^{-1}dw), grey herons (9.80±8.10 and 13.4±5.98 mg kg^{-1}dw) and intermediate egrets (17.0±12.7 mg kg^{-1}dw) from Japan [16].

Concentrations of Se >10 mg/kg dw in liver may cause reproductive impairments [4] it was detected in one great egret specimen. But, this study revealed statistically significant interactions between the hepatic concentrations of Se and Hg (r= 0.61685 p= 0.001), which may be linked to the Se protection capacities against the toxicity of Hg [21]. But, this requires further examination because our Se levels are low.

Very little is known about the adverse effects of the other elements to wild birds, but the results presented here indicate the importance of monitoring trace elements in great egret livers.

3.2. Comparison between Male and Female Concentrations

In this study mean element concentrations in livers from 23 males and 20 females great egrets were compared (Table 2). Concentrations for Br, Co, Cs, Rb, Se and Zn presented significant differences between males and females. Females had lower concentrations than males.
The causes of differences in concentrations between genders are complicated to be established because of their multifactorial nature. Metabolic differences, detoxification pathways, diet, longevity among other factors may be involved [26]. Anyway, this study as previous studies [9, 27], alert to the possibility of transfer of Se to eggs which is an ecotoxicological concern due to the possibility of teratogenesis [28].

Table 2: Concentrations of trace elements (mean±SD mg kg $^{-1}$dw) in males and females of great egret livers from São Paulo Metroplitan Region (SPMR).

Element	Males Mean±SD(n= 23)	Females Mean±SD(n=20)
Br	46.7±5.2	30.3±4.4[a]
Cd	0.314 ±0.083	0.243±0.049
Cl	4483±539	3723±184
Co	0.198 ±0.039	0.135±0.017[a]
Cs	0.235 ±0.039	0.140±0.029[a]
Cu	96 ±28	63±17
Fe	3285±387	3574±505
Hg	4.12 ±0.81	3.57±1.09
K (%)	0.918±0.068	0.969±0.085
Mg	477±30	586 ±43
Mn	10.69±0.86	9.80±0.73
Na	4528±277	4198±260
Rb	42.3±3.1	33.4±3.2
Se	4.69±0.38	3.38±0.31[a]
Zn	269±21	201 ±23[a]

a-indicates significant differences in concentrations by applying the non-parametric Mann-Whitney U test to p<0.05

3.3. Relation between Body Mass and Trace Element Concentrations

In this study, no correlations were observed between the mass of specimens and the level of the elements in great egret livers. In contaminated ecosystems negative correlations between morphometric measures and element levels are expected [20] and inversely correlated concentrations with body mass suggest potential negative effects on bird health [5]. Although, this study did not indicate that trace element contamination in the region is affecting the health of great egret, it is the first to address this issue.

3. CONCLUSIONS

The elements Cu, Fe, Mn and Zn had a non-significant increase at the end of the analyzed period, which may be associated with disposal in the environment, and Cd a decrease that cannot be explained. The great variability in the concentrations for several elements over the years did not allow the observation of clear trends, being necessary the continuity of the series. Some specimens showed Cu, Fe, Hg and Zn toxic levels for ardeids or others wide birds and females presented lower Se levels than males, which may be related to the transfer of Se to eggs. No correlations were observed between the body masses and element levels. But, because this is the first analysis of this naturein in the region, further studies on the effects of contamination on bird health are encouraged.

In summary, obtained results indicate a combination of the continuity of trace element monitoring in great egret livers and new studies on the effects of contaminants on the breeding and health of great egrets from SPMR.

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