TRACE ELEMENTS IN LIVERS OF GREAT EGRET (*ARDEA ALBA*) FROM THE SÃO PAULO METROPOLITAN REGION: A PRELIMINARY ASSESSMENT OF TEMPORAL TRENDS

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ABSTRACT

This study aimed to assess the variation of trace element concentrations present in great egret livers during six years, from 2006 to 2011. The data were obtained in twenty six livers of great egrets from the São Paulo Metropolitan Region (SPMR). The elements Br, Co, Cs, Cu, Fe, K, Mg, Mn, Na, Rb, Se and Zn were determined by using Instrumental Neutron Activation Analysis (INAA) and Cd and Hg by Atomic Absorption Spectrometry (AAS). Arithmetic means and standard deviations of element concentrations of the results obtained in each year were calculated for the samples collected in each year. Analysis of variance (ANOVA; $\alpha = 0.05$) followed by Kruskal Wallis test was applied to examine if there are temporal differences in the element mean concentrations over time. In general, significant differences of element concentrations were not obtained. However, an increase in Cd and Mn concentrations in the recent years was observed which may indicate recent increase to these elements in environment. The preliminary data obtained suggests the continuation of this kind of study to better understand the temporal trends of trace elements in the aquatic environment of SPMR.

1. INTRODUCTION

Contaminants, such as trace elements, can present adverse effects on the health and well-being of organisms, including humans. Thus, there is a great interest to evaluate information on temporal trends of environmental contamination, especially in highly urbanized and industrialized regions [1]. In the São Paulo Metropolitan Region (SPMR after here), intense urbanization and industrialization have resulted in the contamination of the aquatic systems by significant release of untreated domestic and industrial effluents, agricultural activities and the diffuse pollution resulting from anthropogenic activities in the region [2].

In aquatic environments, chemicals can be stored in bottom sediments providing a pool for years [3]. Once in the aquatic environments, different contaminants can enter the food chain in different ways and be distributed among tissues or excreted. With each step in the food chain there is the potential for bioamplification [1]. Therefore, top levels carnivores are exposed to higher levels of contaminants than species that are lower levels, and are often used as bioindicators to study temporal trends [1, 4, 5]. Egrets are at the top of the food chair, forage in aquatic environments and feed on large fish and integrate contaminants over time and space [3], therefore can reveal spatial and temporal trends in contaminants levels [1].

The liver is the detoxification organ of the body, and due to its capacity to accumulate chemicals, it allows to monitor exposure in birds and their ecosystem to trace elements [6]. Although, the collection of liver samples occurs sporadically, it can establish a baseline for comparisons of contaminants in long-term monitoring in the region [7]. However, the low number of samples representing each sampling year must be kept in mind.

Campos [2], that studied the presence of metals in the sediments in the Tiete River basin, the main water resource of the São Paulo State, verified the accumulation of Cd, Cu, Hg and Zn, especially in selected areas of the SPMR. A fact also observed for samples of great egret livers from SPMR indicated that concentrations these trace elements, as well as Fe, Mn and Se were higher in comparison with literature data for ardeids livers from other regions [8, 9]. The aim of this study was to evaluate the variations of concentrations from 2006 to 2011, and also to assess the risk of exposure to contaminants found in great egret livers.

2. EXPERIMENTAL

2.1. Data Sources

To assess temporal changes variations of trace elements concentrations in liver great egret from SPMR during 2006-2011, the data were obtained from previous studies by Silva and Saiki [8] and Silva [9]. Details of the sampling and analytical methods can be found in the cited literature.

2.2. Sampling

Twenty-six sample livers 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).

2.3. Analytical Procedure

The sample livers were ground, lyophilized and homogenized. The concentrations of Br, Co, Cs, Cu, Fe, K, Mg, Mn, Na, Rb, Se and Zn were determined by the Instrumental Neutron Activation Analysis (INAA) 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. The concentrations were determined by the comparative method.

Cd and Hg were determined by Atomic Absorption Spectrometry (AAS) method. 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 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 the element concentrations were calculated for the samples collected per year. Analysis of variance (ANOVA) followed by Kruskal Wallis test were applied to verify if there is difference of trace elements concentrations among the years. The differences were considered significant at level $\alpha < 0.05$. The ANOVA were applied using the Minitab program version 17 and Kruskal Wallis test using SPSS program version 22.

3. RESULTS AND DISCUSSION

3.1. Temporal Variation of Element Concentrations in Great Egret Livers

The concentrations of trace elements in livers of the great egret from SPMR (2006-2011) are presented in Table 1. The trace element concentrations showed large variations. Evaluation of the interaction between year and gender was not possible due to few samples. Generally, there were no significant differences during the period from 2006 to 2011(Table 2)

Several factors affect the levels of trace element concentrations in livers of birds. In special growth stage or age of birds is one the causes of large variations [10]. Generally, high concentrations in the liver are found in adults. Honda et al. [10] observed higher concentrations Cd, Cu, Fe, Hg, Mn and Zn in adult livers than in chicks livers of *Ardea alba modesta*. All great egrets sampled in SPMR were adults. Although the age of the egrets is not known, the concentration of liver generally indicates a recent contamination of the individuals [11, 12]. Others factors could be feeding habitats, gender, reproduction and molting [10].

According to Table 1 concentrations of Co, Mg and Rb presented less variation between the periods and this fact may indicate that these elements have remained more stable in the environment, as the accumulation of the essential elements in birds was related to the degree of metabolic need and bioavailability [5].

The elements Br and Cs presented decrease in their concentrations in the last two years while Cu and Zn concentrations increased in 2011. In this year, particularly ecological and physiological differences between genders may have influenced the results to Cu and Zn, since only males were sampled. Males and females may feed in different habitats or eat different foods [13]. Females can transfer elements to eggs, thus they tend to present lower levels of elements in their tissues [14], and genders may differ in their production of various metalloproteins which play fundamental roles in the transport, storage and excretion of elements [13].

Table 1: Mean concentrations and standard deviation (mg kg^{-1} dry basis) of trace elements in great egret (*Ardea alba*) livers from SPMR (2006-2011).

Year	n	Gender	Br	Cd	Co	Cs	Cu	Fe	Hg	K	Mg	Mn	Na	Rb	Se	Zn
2006	3	F+M	48.8±18.11	0.162±0.012	0.108±0.032	0.116±0.086	43.5±0.78	2886±2283	0.359±0.090	0.867±0.096	519±69	6.76±1.83	4721±443	41.8±19.5	5.15±4.23	150±80
2007	6	F+M	46.2±26.7	0.170±0.101	0.132±0.030	0.176±0.045	36.9±29.5	3194±1915	2.42±1.96	1.48±0.42	583±338	10.59±3.92	4806±1051	48.0±20.7	3.95±0.69	232±103
2008	2	[a]NI	58.7±22.5	0.188±0.030	0.264±0.044	0.279±0.035	[b]ND	4329±3029	3.07±1.56	0.963±0.004	597±20	9.10±0.58	3715±368	46.8±0.2	3.93±0.28	250±56
2009	7	F+M	37.0±35.6	0.352±0.379	0.184±0.105	0.288±0.352	122.05±89.8	3247±1756	3.43±4.27	0.748±0.166	496±99	9.63±3.67	4310±1670	29.7±15.0	4.16±2.37	233±115
2010	4	F+M	26.7±14.0	1.56±1.74	0.112±0.077	0.163±0.153	40.7±28.4	4195±3839	1.50±1.07	0.885±0.049	532±69	12.80±7.50	3893±857	37.0±11.6	4.10±2.16	232±178
2011	4	M	39.8±18.0	0.509±0.397	0.152±0.028	0.167±0.070	138.2±81.7	3443±2183	2.09±1.07	0.980±0.160	570±58	14.03±0.37	5005±1263	40.7±5.9	3.70±1.02	348±70

n indicates number of samples; a. not identified; b. not determined

Tabela 2: Results of statistical tests for means of trace elements (2006-2011) in great egret livers of SPMR.

Element	period	significance
Br	2006-2011	0.728
Co	2006-2011	0.123
Cs	2006-2011	0.779
Cu	2006-2011	0.215
Fe	2006-2011	0.964
K	**2006-2011**	**0.008***
Mg	2006-2011	0.980
Mn	2006-2011	0.290
Na	2006-2011	0.682
Rb	2006-2011	0.407
Se	2006-2011	0.964
Zn	2006-2011	0.386
Cd	2006-2011	0.145
Hg	2006-2011	0.718

Year bold significament different (Kruskal-Wallis), *denotes significance at 0.05 level.

The results found in this study are consistent with the reported data. Gochfeld and Burger [13] have also determined low levels of these elements in livers from female ducks. In relation to Br and Cs very little is known of their concentrations in birds.

Se decreased their levels up to 2008, but increased in 2009; however, a decrease occurred in the last year. Due to the increase and decrease of Se concentrations in the period, there is not a time trend in great egret livers from SPMR for this element.

Concentrations of Na varied greatly during the period, and for K there was, a significant increase in the year 2007 and in the three last years of this study. However, these results are difficult to interpret because very little is known about these elements in birds.

The results presented for Fe indicated large variation between the years. Possibly, because the Fe absorption in birds may vary depending on the individual's age, health status, integrity of the gastrointestinal tract and Fe chemistry form available [15].

The Mn increased from 2008 up to end of the period. Although accumulation of metals in birds can be related to biological factors during sampling as could be reproduction and molting [10]. However, the increase of the Mn found may be related to the deposition of the element in the environment. Horai et al. [16] reported that Mn is emitted into the environment through the combustion of fossil fuels, which is one of the main anthropogenic activities in the SPMR. From the results obtained suggest more studies concerning this element to verify the increase of Mn in aquatic environments from SPMR.

Cd levels increased up to 2010, but decreased in 2011. Therefore, the concentrations are high compared to the beginning of the series. Cd accumulates mainly in the liver and its concentration in birds is largely affected by environmental contamination [5]. Although, these data cover a time span of only 6 years and year-to-year variation is to be expected [17], it may give indications of recent changes in Cd concentrations in biota.

The concentrations Hg increased until 2009, but the Hg levels decreased in 2010, increasing again in 2011. No temporal trend in Hg levels was observed in liver of great egret in the region.

In birds mercury levels vary with age, foods, molt, because Hg is transferred to the feathers by internal tissue blood pathways [5, 10], reproductive stage and year, location, physiological condition and clutch are also influential [3]. These factors can be associated with variability in the results for the Hg levels in great egret livers from SPMR.

3.2 Effects of contamination

The results obtained for Cd are within the natural background in livers of ardeids (< 3 mg kg^{-1} dry basis) [18, 19]. For the observed Cd levels we can conclude that there is no threat to physiological processes in great egret from the SPMR.

In great white herons (*Ardea herodius ocidentalis*) threshold of 6 mg kg^{-1} dry weight in liver were correlated with mortality from chronic diseases [20]. In this study, the mean Hg levels in liver of great egret were below this effects level.

Cd and Hg are of great concern because they are common, non-essential and toxic [3]. Although, Cd and Hg levels in great egret from SPMR are below levels associated with negative effects. An increasing number of studies suggest that physiological stress occurs for many species at low background levels [7]. Therefore, it is highly recommended to continue monitoring Hg and Cd in the aquatic biota of SPMR.

Very little is known about the adverse effects of other elements to ardeids. However, many authors have reported the damages caused by high concentrations of essential elements in birds. Manganese reduces growth and disrupts behavior, such as pecking and feeding, balance, and thermoregulation in birds [1]. Selenium induced reproductive failure of aquatic birds. Silva et al. [21] verified that females of great egret from SPMR present lower Se concentrations than males. There is the possibility of transfer of selenium to the eggs, which could affect reproductive success. Severe degenerative abnormalities of pancreas in birds were associated with high Zn concentrations in livers, kidneys and blood [22]. Acute intoxication by Cu may cause damage the gizzard and proventriculus [23]. Fe is an essential element, but at high concentrations can become toxic [24]. Therefore, it is also important to maintain data series of essential elements in specimens from SPMR due o the risks that could lead to the aquatic biota, and by the contamination trace elements in aquatic environments from SPMR.

4. CONCLUSIONS

From the results, it can be concluded that biological and ecological factors during the period of sampling, such as reproduction, feed habitats and molting may have influenced the element concentrations in livers of great egret from SPMR. The concentrations of most elements showed no statistical temporal variations, excepting in the case of Cd and Mn. The increase of the concentrations of these two elements in recent years probably is due to deposition in the environment. However, the analyzed time of this study may be considered short and the continuity of the determinations of trace elements in aquatic biota of SPMR is required for better understanding trace element status in the aquatic environments of SPMR.
Hg and Cd levels in liver of great egret were below effect level. However, contamination by these metals in the region requires great attention due to their toxicities.

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