J Radioanal Nucl Chem (2012) 291:119–122
DOI 10.1007/s10967-011-1298-1

Concentrations of trace elements in livers of the Great Egret (*Ardea alba*) from the metropolitan region of São Paulo, SP, Brazil

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Received: 10 June 2011 / Published online: 5 July 2011

Abstract The determinations of trace elements in tissues of herons are of great interest in order to evaluate environmental contamination. As the herons are at the top of the food chain they tend to accumulate high concentrations of contaminants in their tissues. Besides, the effects of pollution are also severe for this species, endangering the survival and reproduction of bird populations. In the present study, concentrations of the trace elements Br, Co, Cs, Fe, Rb, Se, and Zn were determined in livers from Great Egret (*Ardea alba*) species. The liver samples were those obtained from the adult specimens found dead in the metropolitan region of São Paulo, SP, Brazil. The liver samples were ground, freeze-dried and the elements were determined by the method of neutron activation analysis. The elemental concentration obtained in livers of these species showed wide variations depending on the element. Comparisons made between our results with literature data indicated that element concentrations obtained for herons from metropolitan region of São Paulo are higher or at the same order of magnitude of those obtained for specimens from polluted areas. Comparisons made between the results obtained for different genders of herons by applying non-parametric Mann–Whitney *U* test at the significance level of 0.05 indicated that female herons present Co and Se concentrations lower than those from males.

Keywords Liver tissue · Great Egret · NAA · Environmental contamination

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Introduction

The metropolitan region of São Paulo is the largest center of national wealth, concentrating the most important industrial, commercial and financial enterprises of the country. As in other urban centers of the world, together with its development the region presents in increasing serious problem of pollution. Consequently determinations of trace elements in animal tissues such as the herons are of great interest in order to evaluate environmental contamination of the region. As the herons are at the top of the food chain they tend to accumulate high concentrations of contaminants in their tissues [1–3].

Analyses of liver of birds have been widely used in the diagnosis of environmental contamination due to its high potential to accumulate toxic elements. Besides, a liver sample provides sufficient tissue to enable its analysis by different analytical techniques [1, 4, 5].

In addition, the determination of trace elements in bird tissues is not only used for the evaluation of environmental contamination, but also to contribute in the preservation of the species, since environmental pollution is a risk for the survival and the reproduction of birds [1–3]. Toxic elements can cause weight loss, organ damage, metabolic disorders and behavioral changes in individuals living in contaminated areas [3].

The levels of chemical pollutants accumulated in tissues of herons have been associated with ecological and individual factors including differences between the genders [1, 3, 4, 6]. Fasola et al. [2] determined heavy metals and other contaminants in eggs of herons, reporting that reproductive failure of this species may be related to the transfer of toxic elements to the eggs as there is a decrease in size and weight egg, eggshell thinning, increased mortality of embryos, among other anomalies [7–9].

On the other hand, studies on concentrations of toxic elements among the birds in regard to gender are very scarce. According to Gochfeld and Burger [7], the main difficulty of this kind of investigation is the visual identification of sex in birds, except the cases where sexual dimorphism occurs.

In the present study, elemental concentrations of Br, Co, Cs, Fe, Na, Rb, Se, and Zn were determined by the experimental conditions adopted, for the first time, in livers from the Great Egret (*Ardea alba*) of RMSP, because pollution caused by toxic metals and organic materials is more severe for species of higher trophic level [3]. This study also includes evaluation of environmental contamination and determination of elemental concentration differences between male and female birds.

Experimental

Samples of heron livers and its preparation for analysis

The number of Great Egret (*Ardea alba*) liver specimens obtained for this study was limited to those found dead or injured in the metropolitan region of São Paulo. Eleven liver samples from egrets were acquired from the Divisão Técnica de Medicina Veterinária and Manejo da Fauna Silvestre (DEPAVE 3)/SVMA, Prefeitura Municipal de São Paulo. The cause of death for almost of these birds was disease caused by parasites or collisions with obstacles like trees or vehicles. Samples were collected under license from the Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis (IBAMA).

The livers were stored at −20 °C after collection until their treatment for analysis. Each liver sample was first cleaned by removing blood and cut in small pieces using a titanium knife. Then, it was freeze-dried and ground in an agate mortar to obtain a fine homogenous powder. A weight loss of about 73.3% was obtained in this preparation process.

Procedure for neutron activation analysis (NAA)

The synthetic elemental standards were prepared by dosing 50 μL of the elemental standard solutions onto small sheets of Whatman No. 40 filter paper using an Eppendorf pipette. These elemental standard solutions containing one or more elements were prepared using certified standard solution provided by Spex Certiprep Chemicals, USA. The filter sheets with the aliquot of standard solution were dried at room temperature inside a desiccator and, then placed into clean polyethylene envelopes. The masses of various elements per 50 μL aliquot of the synthetic elemental standards used in this study were (in μg): Br = 5.0, Co = 0.15, Cs = 0.60, Fe = 349.685, Na = 99.98, Rb = 9.99, Se = 8.004, and Zn = 35.0.

Aliquots of about 150–200 mg of each liver sample and reference material weighed into clean polyethylene envelopes were irradiated together with the standards for 16 h under a thermal neutron flux of about 2.1×10^{12} $cm^{-2}s^{-1}$ at IEA-R1 research reactor. Three series of measurements were carried out after 7, 15 and 20–25 days of decay times using an HPGe detector coupled to a multichannel pulse height analyzer. The gamma ray spectra were acquired using the MAESTRO software from Ortec EGG and processed using the VISPECT2 computer program. The radioisotopes formed were identified according to their half-lives and gamma ray energies and the element concentrations were calculated by the comparative method.

Quality control of the results

The accuracy and precision of the results were verified by analyzing certified reference materials NIST SRM 1577b bovine liver provided by the National Institute of Standards & Technology, USA and INCT-TL-1 tea leaves provided by the Institute of Nuclear Chemistry and Technology, Poland. Results obtained in these analyses showed a good precision with relative standard deviations lower than 9.7% and good accuracy with relative errors lower than 10%.

Results and discussion

Results obtained in the analyses of eleven liver samples are presented in Table 1. The table includes the arithmetic mean, standard deviation and the range of concentrations obtained on the dry basis for the elements studied.

As it can be seen from the table, the results show wide variations. These variations may be associated with different levels of contamination throughout the habitats of the metropolitan region of São Paulo and also with individual factors [3, 6, 10].

Data obtained for the Great Egret in this study were compared with published data for ardeids. The values found for Co are within the concentration ranges obtained for ardeids in Japan (0.0377–0.197 mg kg^{-1} dry weight). These range values for Co are maintained by normal homeostatic mechanisms, being an essential element [6, 10].

The range of concentrations for the elements on a dry basis for Cs (0.0541–1.80 mg kg^{-1}), Fe (539–2170 mg kg^{-1}), Rb (19.8–75.7 mg kg^{-1}), Se (6.14–29.3 mg kg^{-1}), and Zn (88.3–268.0 mg kg^{-1}) for *Ardea cinerea* heron species from Hodaka Japan presented by Horai et al. [3], may be considered lower or equivalent to the results found for Great Egret (*Ardea alba*) species in this study.

Table 1 Mean, standard deviation and range of element concentrations in livers of Great Egret (*Ardea alba*) species from the metropolitan region of São Paulo. Results in mg kg^{-1} dry weight

Code of samples	Br	Co	Cs	Fe	Na	Rb	Se	Zn
G1	37.7[a]	0.144	0.214	5511.5	5010.2	62.66	10.03	242.0
	1.9[b]	0.013	0.005	110.9	160.9	1.14	0.13	4.0
G2	69.7	0.094	0.071	1778.9	4942	23.9	2.94	97.0
	3.9	0.004	0.002	30.3	149	0.3	0.04	0.7
G3	39.8	0.085	0.058	1366.8	3892	39.2	2.49	112.6
	3.2	0.003	0.008	4.7	567	1.3	0.17	3.7
G4	22.2	0.112	0.235	1404.0	3685.8	46.6	3.48	102.0
	1.4	0.019	0.006	51.0	16.5	2.2	0.07	6.5
G5	64.0	0.183	0.207	4341.7	5320.9	86.4	4.6	259.6
	2.5	0.003	0.014	59.5	600.7	1.0	0.2	4.1
G6	35.8	0.121	0.106	1319.5	4884	28.7	4.45	203.9
	4.3	0.001	0.005	18.4	366	0.7	0.14	3.6
G7	92.9	0.147	0.174	6361.0	6662	34.8	3.99	382.3
	8.8	0.003	0.014	473.2	5	4.7	0.09	34.4
G8	26.6	0.094	0.154	2878.6	4067	38.9	2.84	144.0
	0.6	0.004	0.001	69.6	457	1.6	0.38	6.0
G9	37.9	0.128	0.179	2835.3	4730.2	53.3	4.24	299.4
	1.0	0.003	0.017	14.1	544.3	0.2	0.37	2.3
G10	42.9	0.199	0.269	6512	3976.4	47.3	4.13	294.3
	2.5	0.012	0.056	101	66.5	1.3	0.36	12.4
G11	74.6	0.234	0.255	2187	3455	46.7	3.74	210.3
	3.1	0.019	0.011	63	114	1.5	0.13	3.4
General mean[c]	43.3	0.148	0.169	3316.7	4570	46.3	4.34	213.4
SD	22.3	0.066	0.065	2021.6	899	17.3	2.26	93.0
Min	22.2	0.085	0.058	1319.5	3455	23.9	2.50	97.0
Max	92.9	0.295	0.269	6512.0	6662	86.4	10.0	382.3

[a] Mean of three or four determinations in each sample

[b] Standard deviation of determination in each sample

[c] Arithmetic mean of the analysis of 11 samples; *SD* standard deviation of the results obtained in samples

However, the value found for Fe in this study (872.8 mg kg^{-1} wet weight) is far greater than that found in Korea (230 mg kg^{-1} wet weight) by Honda et al. [11] for Great Egret (*Ardea alba*). According to the literature there is a close correlation between the element concentrations present in tissues of herons and those present in the sediments of their habitats. In other words, when the population of herons presents high levels of chemicals in their tissues, these birds are exposed to these elements in the environment [2, 3].

The concentration range of Se (2.5–10.03 mg kg^{-1}) found in this work is of the same order of magnitude from that obtained for the species Great Egret (*Ardea alba*) in the industrial area of Haneda (4.56–14.4 mg kg^{-1}), considered polluted in Japan [3]. It is important to note that Se also can be toxic for some birds. High levels of this element have been associated with mortality and malformations in embryos of several species of birds. There are few studies about this phenomenon; however, for ardeids it has not been observed so far [12].

The element Zn in liver samples at the concentrations above 122 mg kg^{-1} dry weight are considered high as reported by Gómez et al. [5]. The range of concentrations of Zn (97.0–382.3 mg kg^{-1} dry weight approximately 25.5–100.6 mg kg^{-1} wet weight), obtained in this work is above the values found in livers of ardeids from contaminated regions in Europe, Korea and Japan [1, 3, 11]. The high concentrations obtained for Zn can be probably related to the increased concentrations in the environment of metropolitan region of São Paulo. According to the environmental company of São Paulo (CETESB), the levels of this element in metropolitan region of São Paulo have grown in recent years due to the release of industrial effluents [13].

Table 2 Concentrations of trace elements (mean ± SD, mg kg^{-1} dry weight) and associated statistical parameters p obtained in livers of males ($n = 5$) and females ($n = 5$) of the Great Egret (*Ardea alba*)

Elements	Males Mean ± SD	Females Mean ± SD	p
Br	60.7 ± 24.4	39.2 ± 18.6	0.310
Co	0.166 ± 0.044	0.101 ± 0.016	0.016*
Cs	0.190 ± 0.056	0.140 ± 0.075	0.310
Fe	3944 ± 2147	2050 ± 748	0.310
Na	4995 ± 1136	4264 ± 545	0.222
Rb	59 ± 23	41 ± 11	0.548
Se	5.4 ± 2.6	3.2 ± 0.7	0.032*
Zn	560 ± 72	151 ± 85	0.095

*$p < 0.05$ significant

The results obtained for Co, Cs, Fe, Rb, Se, and Zn in the livers of Great Egret (*Ardea alba*) were higher or they are within the range of values presented in the literature [3, 5, 11] for birds from polluted regions.

Comparisons were made between the concentrations of the elements Br, Co, Cs, Fe, Na, Rb, Se, and Zn obtained for male and female Great Egret species. Since these results did not show a normal distribution, a nonparametric statistical Mann–Whitney *U* test [14] was applied at significance level lower than <0.05 to test for differences in elemental concentration between genders. Statistical analysis of the data was conducted using SPSS for Windows version 17.0 [15]. In Table 2 results of this comparison are presented.

The elements Co and Se showed significant differences. These findings may be related to physiological or ecological differences between the genders [7, 9]. There are several mechanisms for removing elements trace from the organisms of birds, such as direct elimination through feces or transfer to the feathers. Also, the female birds can also transfer trace elements to the eggs reducing levels of some elements in the tissues. The diet may also vary because males and females may have differing feeding habits. [7].

Our preliminary results obtained in this study encourage us to continue research to obtain additional data which compare element concentrations in livers from egrets from in regions of different levels of pollution.

Acknowledgment The authors acknowledge the assistance and support from Divisão Técnica de Medicina Veterinária e Manejo da Fauna Silvestre (DEPAVE 3)/SVMA, Prefeitura Municipal de São Paulo, for the liver samples analyzed in this study.

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