



# The great egret (*Ardea alba*) as a bioindicator of trace element contamination in the São Paulo Metropolitan Region, Brazil

Rita C. A. Silva<sup>1</sup> · Mitiko Saiki<sup>1</sup> · Edson G. Moreira<sup>1</sup> · Paulo T. M. S. Oliveira<sup>2</sup>

Received: 15 October 2017

© Akadémiai Kiadó, Budapest, Hungary 2018

## Abstract

To investigate great egret as a possible bioindicator of trace element contamination, the concentrations of Br, Cd, Co, Cs, Cu, Fe, Hg, K, Mg, Mn, Na, Rb, Se and Zn were determined in livers of great egrets (*Ardea alba*) by the methods of Instrumental Neutron Activation Analysis and Atomic Absorption Spectrometry. It was observed that the levels of trace elements found in their livers may indicate contamination of the studied region. Thus, the livers of this species can be considered as a suitable bioindicator of contamination of the aquatic systems in the São Paulo Metropolitan Region.

**Keywords** Great egret · Bioindicator · Contamination · Trace elements · Livers

## Introduction

Trace element contamination is a major threat to the quality of the environment and biodiversity [1, 2]. These elements are present into the environment through geological cycles and anthropogenic sources, such as industrialization and urbanization. In general, human activities are more relevant in deposition of contaminants in the environment [2, 3].

In birds, trace element accumulation can be largely affected by the contamination of the surrounding environment [4]. The increasing concentration of toxic elements in the environment makes avifauna, which is declining in most parts of the world, most vulnerable [5].

As some pollutants undergo biomagnification up the food chain, their concentrations have mainly been studied in top level predators [4, 6–9] that are considered valuable bioindicators for environmental monitoring in polluted ecosystems [10]. Other advantages for the preference of

birds as bioindicators are their abundance, wide distribution, long lifespan and sensitivity to anthropogenic pollution [8, 11]. Moreover, the use of wild birds as monitors of environmental contamination offers important information about the effects of pollutants on these animals and on the humans as well [12, 13].

The exposure to some trace elements in birds may cause increase of mortality in nestling and adult as well as sublethal effects as bone deformities and slower growth rates [14–16]. These effects endanger the survival and reproduction of birds.

Reproduction failure may be related to the fact that in females, the transfer of toxic elements to eggs can cause impair development and fitness of bird nestlings [1, 17, 18]. These effects constitute a major threat to the species of higher trophic levels with greatest potential to bioaccumulate [10, 19].

Concerning the study area São Paulo Metropolitan Region (SPMR), its intense urbanization and industrialization have resulted in the contamination of the region aquatic systems with several chemicals (e.g., Cu, Fe, Hg, Mn and Zn), and high concentrations of these contaminants may be associated with the occurrence of toxic effects on aquatic biota [20]. Consequently, there is interest in monitoring the levels of these contaminants to evaluate their effects.

Ardeids have been used as bioindicators of aquatic environmental contamination because they are at the top of

✉ Rita C. A. Silva  
rcsilva@ipen.br

<sup>1</sup> Nuclear and Energy Research Institute, IPEN - CNEN/SP, Av. Prof. Lineu Prestes, 2242, São Paulo, SP 05508-000, Brazil

<sup>2</sup> São Carlos School of Engineering of the University of São Paulo, EESC/STT-USP, Avenida Trabalhador São Carlense, 400, São Carlos, SP 13566-590, Brazil

the food chain and tend to accumulate high concentrations of trace elements in their tissues, reflecting environmental conditions [21]. Besides they are geographically widespread and present longevity and sensitivity to anthropogenic pollution [22]. However, the levels of trace elements in ardeids have been poorly investigated in Brazil, as well as South America.

Different body tissues and organs of ardeids such as feathers, eggs, eggshells and blood have been used to evaluate the accumulation of contaminants [19]. The liver is the detoxification organ and due its capacity to accumulate chemicals, it allows to monitor exposure in birds and their ecosystem to trace elements [3, 8]. However, the low number of representative samples usually available for each study area is small and this fact should be kept in mind.

Great egrets can be considered residents in the SPMR, since they live in this area for breeding, feeding, and are not migratory birds. The accumulation of trace elements may be high in their livers due to longer exposure to the contaminants in the area throughout the entire year. Therefore, great egret liver deserves to be studied to be used as possible bioindicator of environmental contamination [23, 24].

The objectives of this study were (a) to use the great egret liver as a bioindicator to assess the trace element contaminations in the SPMR, (b) to evaluate the risk of exposure to contaminants found in great egret livers, (c) to compare the trace elements levels found in male and female livers, and (d) to contribute to the identification of sources of main contaminants in the SPMR.

## Experimental

### Samples of great egret livers and their treatment for analysis

Livers used in this study were taken mainly from wounded birds brought to rehabilitation centers between 2006 and 2011 collected in three geographical locations of the State of São Paulo, Brazil. Figure 1 shows the location of great egret habitats whose liver samples were analyzed.

Twenty six great egret livers from SPMR analyzed in this study were donated during the period from 2006 to 2011 by the Veterinary Medicine and Wildlife Management Technical Division of the São Paulo City (DEPAVE 3). Samples were collected under license from the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA). Thirteen birds were found with broken bones caused by collisions with obstacles such as trees or vehicles. In some cases, egrets had injuries from kite lines. Five birds presented different diseases and for eight

birds, uninformed cause of death. Some of the birds died under treatment and others were euthanized, because they were untreatable. Only one egret survived at DEPAVE 3 Center for two weeks, the other egrets died in less than seven days.

In addition three liver samples of egrets from the Sorocaba city and one from São Sebastião city were analyzed. Sorocaba city is located in the Southwest region of the State of São Paulo, about 90 km from the SPMR. Sorocaba city occupies smaller area than SPMR, but it is the fifth largest city in economic development of the State of São Paulo and is recognized by the United Nations as a sustainable city. The liver samples from the SPMR (highly urbanized) egrets were compared with the liver samples of great egrets from Sorocaba city.

The samples from Sorocaba city were donated by the municipal zoo. The egrets are not in exposition in the zoo, rather they use the area to feed and rest and are resident in the city [25].

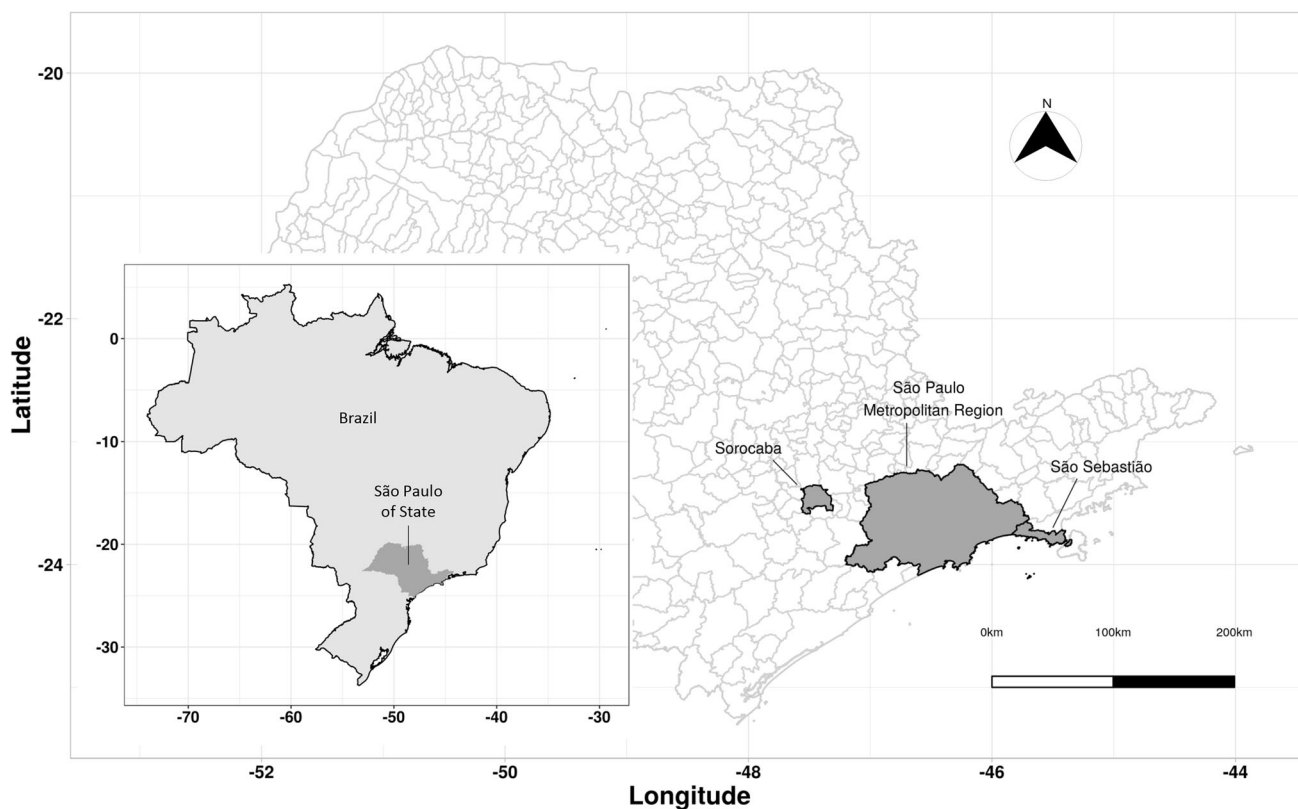
Only one specimen was acquired from São Sebastião city, a seashore tourist resort, considered contaminated due to its harbor activities. This sample was donated by the Wild Animals Screening Center (CETAS). In Sorocaba and São Sebastião cities the number of samples analyzed was small due to difficulties of findings the injured birds, unable to live, as well as specialized personnel for collection of the liver. The specimens of great egret were sexed by gonadal examination [26], in their original locations.

Livers were stored at  $-20\text{ }^{\circ}\text{C}$  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, each sample was freeze-dried and ground in an agate mortar to obtain a fine homogenous powder. A weight loss of about 72% was obtained in the drying process by freeze-drying.

### Analytical procedure

#### Neutron activation analysis

The elements Br, Co, Cs, Cu, Fe, K, Mg, Mn, Na, Rb, Se and Zn were determined by the comparative method of instrumental neutron activation analysis (INAA). In this method, samples and standards are irradiated simultaneously with neutrons under the same conditions. Elemental concentrations are calculated by comparison of the activities of gamma-rays from the sample and standards [27]. The synthetic element standards were prepared by pipetting 50  $\mu\text{L}$  of diluted element standard solutions onto small sheets of Whatman No. 40 filter paper using an Eppendorf pipette. These element standard solutions containing one or more elements were prepared using certified standard



**Fig. 1** Location of sampling sites of great egrets; São Paulo Metropolitan Region, Sorocaba, and São Sebastião

solutions 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. In these standards the quantities of each element in  $\mu\text{g}$  (in parentheses) were the following: Br (4.985), Co (0.1503), Cs (0.60), Cu (240.336), Fe (360.324), K (500.05), Mg (498.95), Mn (3.992), Na (200.2), Rb (9.99), Se (8.003) and Zn (36.0391).

Aliquots of about 150–200 mg of each liver sample weighed in polyethylene envelopes were irradiated together with synthetic element standards. Long irradiations were performed for 16 h under a thermal neutron flux of about  $2\text{--}4 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$  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 36000 to 50000 s. Short irradiations were used for half lives shorter than 12.4 h (Cu, K, Mg and Mn), and the irradiation time was 15 s under thermal neutron flux of  $6.6 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$ . 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 HPGc detector Model GEM

20190 coupled to a gamma spectrometer both from EG & G Ortec. The resolution (FWHM) of the system was 1.07 for 122 keV gamma-ray peak of  $^{57}\text{Co}$  and 2.14 for 1332 keV gamma-ray peak of  $^{60}\text{Co}$ . The gamma spectra were acquired using MAESTRO MCA software from EG & G Ortec and were processed using VISPECT software developed in our laboratory by Dennis Piccot from Saclay, France.

The radionuclides use were  $^{82}\text{Br}$ ,  $^{60}\text{Co}$ ,  $^{134}\text{Cs}$ ,  $^{59}\text{Fe}$ ,  $^{24}\text{Na}$ ,  $^{86}\text{Rb}$ ,  $^{75}\text{Se}$  and  $^{65}\text{Zn}$  (for long irradiations) and  $^{66}\text{Cu}$ ,  $^{42}\text{K}$ ,  $^{27}\text{Mg}$  and  $^{56}\text{Mn}$  (for short irradiations).

#### Atomic absorption spectrometry

The elements 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 Analyst 800 spectrometer. Liver samples were subjected to acid digestion. Approximately 100 mg of each sample were weighed and appropriate standard solutions of Hg and Cd were used for the construction of analytical curves. All samples were analyzed in replicate. When the values of Relative Standard Deviation

(RSD) of replicates were above 25%, data were not included for statistical treatment.

### Quality assurance and statistical treatment

For quality control of the results, the certified reference materials INCT-TL-1 (Tea Leaves) [28] and NIST SRM 1577b (Bovine Liver) [29] were analyzed. We analyzed INCT-TL-1 (Tea Leaves) since the element Cs is not certified in the NIST SRM 1577b (Bovine Liver). Cadmium and mercury were determined by AAS in the certified reference material NIST SRM 2976 (Mussel Tissue) [30]. Values of detection limits of elements in livers were also obtained according to [31].

Arithmetic means and standard deviations of the liver element concentrations were calculated. Comparisons of the results between locations and between genders of great egrets were evaluated using the nonparametric Mann–Whitney  $U$  test. The significance level was set at 0.05 and SPSS for Windows 22.0 program was used for statistical analysis of these data. Correlations between the elements were evaluated calculating Pearson Correlation Matrix ( $r = 0.7$ ) using Minitab 17.0 program. Results were considered as significant at  $p$ -values  $< 0.05$  and  $< 0.01$ .

## Results and discussion

### Certified reference materials analyses

For accuracy evaluation, relative errors (RE) of the results were calculated and they ranged between 0.12 and 13.3% for elements (Table 1). The precision of results was evaluated calculating relative standard deviations (RSD), and they were lower than 10% except for Na (16.4%, INCT-TL-1 Tea Leaves [28]) (Table 1).

The RE obtained for Cd and Hg were 6.0 and 22.8%, respectively and RSD were 3.7 and 17.0%, respectively in the mussel reference material.

### Element concentrations in livers of great egrets

Results obtained in the analyses of 30 liver samples of adult specimens are presented in Table 2. In this table, arithmetic means, standard deviations and the ranges of element concentrations obtained are presented. Comparison of results obtained for egrets from the SPMR and from Sorocaba city (Mann–Whitney  $U$  test  $p < 0.05$ ) are also shown in the table. Concentrations of trace elements in liver of egrets from São Sebastião were not included in the statistical analysis for comparison since there was only one sample.

Values of detection limits were calculated for the egret liver samples and these results in  $\text{mg kg}^{-1}$  dry weight, were the following: Br (0.14), Co (0.01), Cs (0.02), Cu (8.6), Fe (8), K (40), Mg (160), Mn (0.47), Na (13), Rb (0.37), Se (0.26), Zn (0.35), and in  $\mu\text{g kg}^{-1}$  dry weight Cd (0.72) and Hg (6.1).

The elements found in livers are ubiquitous since they were determined in all samples, or in most of them for the three studied areas. Cu was found frequently in concentration lower than its detection limits.

In general, high concentrations of elements K, Na and Mg, associated with natural origin [19], were measured in egret livers. Besides, high concentrations of Fe and Zn were also found and Cu in some samples. These elements are essential, with many important biological functions, however they may also become toxic if excessively high concentrations are accumulated in tissues [8, 32].

The ranges of trace element concentrations measured in livers from SPMR egrets indicated variability between the individuals. Several physiological and biological processes, such as feeding habits, gender, growth or age, reproduction and molting [4, 17, 22, 33], may influence these variations observed for toxic elements and essential elements. On the other hand, these element concentration differences may be associated with the levels of contaminations throughout the habitats of great egrets [10, 34]. The fact that some egrets have exhibited concentrations of Cd, Cs, Cu, Fe, Hg and Zn markedly higher than their mean levels may indicate contamination of these individuals and consequently of their habitats [3].

### Differences between the element concentrations results obtained for egrets from the SPMR and Sorocaba city

Although the small number of samples for egrets from Sorocaba city, comparisons between the results obtained in this city and those obtained in the SPMR were made. The Mann–Whitney  $U$  test showed significant differences only for Cs ( $p = 0.0037$ ) (Table 2), with higher levels at the SPMR. Although this difference, it is not possible to infer that great egret suffer contamination by Cs in the SPMR because very little is known about Cs in birds. Also, little information is available for comparison of other trace elements in livers of great egrets, apart from the toxic elements Hg and Cd and the essential element Se. Although the differences between the two areas were not very expressive, the present study can provide baseline data on the elemental levels in this species.

**Table 1** Mean concentrations of elements obtained in the analysis of certified reference materials NIST 1577b Bovine Liver and INCT-TL-1 Tea Leaves (in mg kg<sup>-1</sup> unless indicated)

Element	NIST 1577b Bovine liver				INCT-TL-1 Tea leaves			
	Values of the certificate	This study			Values of the certificate	This study		
		Mean ± SD (n) <sup>a</sup>	RSD <sup>b</sup> , %	RE <sup>c</sup> , %		Mean ± SD (n) <sup>a</sup>	RSD <sup>b</sup> , %	RE <sup>c</sup> , %
Br	9.7 <sup>d</sup>	9.6 ± 0.7 (13)	7.0	–	12.3 ± 1.0	12.6 ± 1.2 (16)	9.2	2.4
Co	0.25 <sup>d</sup>	0.25 ± 0.02 (3)	8.0	–	0.387 ± 0.042	0.39 ± 0.03 (16)	7.7	0
Cu	160 ± 8	173.6 ± 13.9 (13)	8.0	8.5	20.4 ± 1.5	–	–	–
Cs	–	–	–	–	3.61 ± 0.37	3.60 ± 0.22 (14)	6.0	0.3
Fe	184 ± 15	186.5 ± 10 (17)	5.5	3.6	432 <sup>d</sup>	470.6 ± 27.6 (11)	6.0	–
K (%)	0.994 ± 0.002	1.02 ± 0.09 (8)	9.0	3.0	–	–	–	–
Mg	601 ± 28	638.8 ± 38.2 (4)	6.0	6.3	(0.224 ± 0.07) × 10 <sup>-4</sup>	–	–	–
Mn	10.5 ± 1.7	11.0 ± 0.4 (4)	3.6	4.8	1570 ± 110	–	–	–
Na	2420 ± 60	2250 ± 230 (15)	6.5	5.0	24.7 ± 3.2	27.9 ± 4.6 (15)	16.4	13.3
Rb	13.7 ± 1.1	13.01 ± 0.85 (17)	6.5	5.0	81.5 ± 6.5	81.4 ± 6.5 (15)	5.3	0.12
Se	0.73 ± 0.06	0.76 ± 0.079 (16)	9.2	4.0	0.076 <sup>d</sup>	–	–	–
Zn	127 ± 16	121.2 ± 10.9 (15)	9.0	4.6	34.7 ± 2.7	37.0 ± 3.0 (17)	8.0	6.6

<sup>a</sup>Arithmetic mean and standard deviation; n indicates number of determinations

<sup>b</sup>Relative standard deviation

<sup>c</sup>Relative error

<sup>d</sup>Informative values

## Relevancy of concentration and potential effects

To understand about the SPMR exposure and the potential effects of trace elements on egrets, concentrations of trace elements in livers of great egret from the SPMR were compared with levels in livers of the ardeids from others geographical areas and level indicative of poisoning and toxicological thresholds that can affect wild birds. However, it is difficult to assess the contaminants effects in the SPMR on great egrets, because sensitivity to contaminants may differ among species and populations [31, 35], and toxicological thresholds have not yet been established for several ardeid species [35]. There are few published data on the element levels in the livers of ardeid species. Most published data are for feathers and eggs. The reported data for livers of ardeids species used for comparison are presented in Table 3.

## Essential elements

Concentration data of the essential elements K and Na in livers of ardeids were not found in the literature for comparison with our results. The obtained range of Mg concentrations is lower than that published for grey herons from Hodaka, in Japan [10] and they are higher than the reported values for grey heron from East Poland [8]. Little is known about the Mg level in birds, however it is an

essential element that can be toxic at high levels [36]. For Br, no published data for livers from ardeids were found in the literature. Br is considered an essential element, but it is still unknown whether it has functions on living organisms [37].

The mean concentrations found for Co in livers determined in the current study are higher than the ones for grey herons from Japan [10]. Cobalt is normally present in vitamin B12. Although essential, its body burden is low [26]. As Cd and Hg, it is considered as immunosuppressive chemical [6].

The mean Cu concentrations determined in livers of great egret were lower than the ones reported for livers of egrets from Japan [10] and in little egrets from Tamil Nadu in India. However, they are higher than the reported values for ardeids from Poland [40], cattle egrets and pond herons from India [38], *Ardea alba modesta* from Korea [33], *Butorides striatus* from Argentina [14] and grey heron from East Poland [8]. Furthermore, high Cu concentrations were determined (190 ± 12, 196 ± 14 and 209 ± 4 mg kg<sup>-1</sup>, dry basis) in three specimens, which may be lethal for other wetland birds, according to [40].

The mean Fe concentrations in the livers obtained in this study were higher than the ones obtained for grey herons from Hodaka, Japan [10], and *Ardea alba modesta* from Korea [33]. Some individuals exhibited concentrations of

**Table 2** Concentrations of trace elements in livers of great egrets

Element	SPMR Mean $\pm$ SD <sup>a</sup> (n) <sup>b</sup> Range	Sorocaba Mean $\pm$ SD (n) Range	São Sebastião Mean $\pm$ SD (n) –	Differences between SPMR and Sorocaba ( $p < 0.05$ ) <sup>a</sup>
Br	40.68 $\pm$ 24.48 (26) 7.29–107.8	22.33 $\pm$ 13.47 (3) 16–37.88	31.8 $\pm$ 2.9 (1) –	NS
Cd	0.480 $\pm$ 0.837 (23) ND–4.04	0.233 $\pm$ 0.063 (3) ND–0.233	0.322 $\pm$ 0.015 (1) –	NS
Co	0.186 $\pm$ 0.184 (26) 0.035–0.412	0.1387 $\pm$ 0.044 (3) 0.110–0.190	0.136 $\pm$ 0.007 (1) –	NS
Cs	0.198 $\pm$ 0.182 (26) 0.013–0.964	0.0587 $\pm$ 0.017 (3) 0.047–0.079	0.074 $\pm$ 0.005 (1) –	0.037
Cu	77.0 $\pm$ 68.6 (15) ND–209	23.40 $\pm$ 0.04 (3) 20.40–28.0	ND	NS
Fe	3561 $\pm$ 2125 (26) 498–7910	1431 $\pm$ 623 (3) 940–2132	1763 $\pm$ 52 (1) –	NS
Hg	2.20 $\pm$ 2.37 (25) ND–11.83	0.545 $\pm$ 0.386 (3) 0.117–0.865	2.20 $\pm$ 0.21 (1) –	NS
K (%)	1.01 $\pm$ 0.37 (20) ND–1.91	0.89 $\pm$ 0.06 (3) 0.830–0.940	1.03 $\pm$ 1.0 (1) –	NS
Mg	537 $\pm$ 175 (20) ND–1162	516.3 $\pm$ 64.3 (3) 444–567	663 $\pm$ 47 (1) –	NS
Mn	10.02 $\pm$ 4.23 (20) ND–18.11	10.62 $\pm$ 0.93 (3) 9.70–11.57	10.83 $\pm$ 0.63 (1) –	NS
Na	4498 $\pm$ 1160 (26) 2958.7–7049.8	3883 $\pm$ 1030 (3) 3213–5069	3340 $\pm$ 295 (1) –	NS
Rb	39.32 $\pm$ 15.30 (26) 13.5–86.7	33.93 $\pm$ 1.75 (3) 32–35.4	8.48 $\pm$ 0.44 (1) –	NS
Se	4.17 $\pm$ 1.90 (26) 1.65–10.03	2.92 $\pm$ 1.479 (3) 1.72–4.57	8.42 $\pm$ 0.22 (1) –	NS
Zn	243.6 $\pm$ 112.9 (26) 42–489	135.9 $\pm$ 26.8 (3) 117–166.6	186.7 $\pm$ 6.8 (1) –	NS

Results in mg kg<sup>-1</sup> dry weight, unless otherwise indicated and Mann–Whitney *U* test comparisons of the trace element concentrations in great egret livers from SPMR and Sorocaba

ND not detected, NS non-significant, SD standard deviation, *n* = number of samples

<sup>a</sup>*p* values < 0.05 are considered statistically significant

Fe that are markedly higher than levels for contaminated areas of mallards (5917.0 mg kg<sup>-1</sup>) [41].

These obtained high levels of Fe can be attributed to the erosion caused by intense rains and the decrease of riparian vegetation. Fe present in the soil from the SPMR can be carried to the water bodies, and levels of Fe in these environments are not in accordance with Brazilian legislation [20]. The results presented here seem to confirm this problem.

The mean Mn concentrations in livers of great egrets from the SPMR were of the same order of magnitude of egrets from Japan, which is considered high [10] and grey heron from East Poland [8]. They are higher than the results

for ardeids from Poland [40]. However, the concentrations are lower than *Ardea alba modesta*, from Korea [33]. According to [41], birds are good indicators of contamination by Mn. Despite this fact, it is an essential element, its presence in tissues of birds is not high. It is known that Mn reduces growth and disrupts behavior, such as pecking and feeding, balance, and thermoregulation in birds [7]. However, potential effects of Mn on ardeids are not known [19].

The Se concentrations found in this study were lower than the ones published for egrets from Japan [10]. Our several data for Se are within the range of concentrations of Se obtained for specimens of grey heron, in

**Table 3** Trace element concentrations (arithmetic mean with standard deviation and range in mg kg<sup>-1</sup> dry weight) in livers of ardeids obtained in this study and reported values

Locations	Species	Cd	Co	Cs	Cu	Fe	Hg	Mg	Mn	Rb	Se	Zn
SPMR,	Great Egret ( <i>Ardea</i>	0.480 ± 0.837	0.186 ± 0.184	0.198 ± 0.182	77.0 ± 68.6	3561 ± 2125	2.20 ± 2.37	537 ± 175	10.02 ± 4.23	39.32 ± 15.30	4.17 ± 1.90	243.6 ± 112.9
This study	<i>alba</i> )	ND-4.04	0.035-0.412	0.013-0.964	ND-209	498-7910	ND-11.83	ND-1162	ND-18.11	13.5-86.7	1.65-10.03	42-489
		0.133 ± 0.232 <sup>a</sup>	0.052 ± 0.051 <sup>a</sup>	0.055 ± 0.050 <sup>a</sup>	21.38 ± 19.05 <sup>a</sup>	989 ± 590 <sup>a</sup>	0.61 ± 0.66 <sup>a</sup>	149.2 ± 48.6 <sup>a</sup>	2.78 ± 1.17 <sup>a</sup>	10.92 ± 4.25 <sup>a</sup>	1.16 ± 0.53 <sup>a</sup>	67.7 ± 31.4 <sup>a</sup>
		ND-1.12 <sup>a</sup>	0.0097-0.114 <sup>a</sup>	0.008-0.269 <sup>a</sup>	ND-58.52 <sup>a</sup>	139.4-2214.8 <sup>a</sup>	ND-3.10 <sup>a</sup>	ND-325.4 <sup>a</sup>	ND-5.07 <sup>a</sup>	3.75-24.25 <sup>a</sup>	0.458-2.79 <sup>a</sup>	11.7-135.8 <sup>a</sup>
Haneda,	Great Egret ( <i>Ardea</i>	0.187 ± 0.206	-	-	173 ± 209	-	2.31 ± 2.41	-	9.85 ± 2.34	-	8.60 ± 2.68	96.3 ± 28.7
Japan	<i>alba</i> )	0.0280-0.983	-	-	14.7-727	-	0.270-12.1	-	6.15-17.1	-	4.56-14.4	52.0-154
[10]		0.190 ± 0.129	-	-	791 ± 1260	-	1.28 ± 1.52	-	13.4 ± 3.33	-	9.80 ± 8.10	236 ± 332
	Grey Heron ( <i>Ardea</i>	0.0323-0.605	-	-	71.0-4970	-	0.0902-6.80	-	9.22-21.0	-	3.73-39.7	79.3-1890
	<i>cinerea</i> )	0.412 ± 0.342	-	-	787 ± 739	-	2.02 ± 1.86	-	12.0 ± 1.90	-	17.0 ± 12.7	146 ± 61.4
	Intermediate Egret	0.0729-1.14	-	-	26.9-2420	-	0.0283-6.25	-	8.88-15.8	-	2.90-41.0	79.6-272
	( <i>Egretta</i>											
	<i>intermedia</i> )											
Hodaka,	Grey Heron ( <i>Ardea</i>	0.273 ± 0.407	0.0859 ± 0.0394	0.437 ± 0.477	127 ± 87.4	1190 ± 467	3.78 ± 7.67	1850 ± 219	13.1 ± 4.28	39.4 ± 13.8	13.4 ± 5.98	133 ± 45.9
Japan	<i>cinerea</i> )	0.0684-1.92	0.0377-0.197	0.0541-1.80	18.9-341	539-2170	0.096-31.7	1560-2390	7.57-24.1	19.8-75.7	6.14-29.3	88.3-268
[10]												
India [38]	Litte Egret	0.11 ± 0.06 <sup>a</sup>	-	-	53.31 ± 23.19 <sup>a</sup>	-	-	-	-	-	-	8.50 ± 3.30 <sup>a</sup>
	( <i>Bulbucus ibis</i> )											
	Cattle Egret	0.03 ± 0.03 <sup>a</sup>	-	-	16.27 ± 9.83 <sup>a</sup>	-	-	-	-	-	-	2.10 ± 1.99 <sup>a</sup>
	( <i>Egretta garzetta</i> )											
	Pond Heron	0.07 ± 0.04 <sup>a</sup>	-	-	9.32 ± 3.75 <sup>a</sup>	-	-	-	-	-	-	4.74 ± 1.58 <sup>a</sup>
	( <i>Ardeola grayii</i> )											
Korea [33]	<i>Ardea alba modesta</i>	-	-	-	7.05 ± 2.96 <sup>a</sup>	230 ± 39.8 <sup>a</sup>	-	-	3.47 ± 0.29 <sup>a</sup>	-	-	33.8 ± 3.26 <sup>a</sup>
UK [16]	Grey Heron ( <i>Ardea</i>	0.004-2.85 <sup>a</sup>	-	-	-	-	0.26-16.02 <sup>a</sup>	-	-	-	1.0-37.9 <sup>a</sup>	30-123 <sup>a</sup>
	<i>cinerea</i> )											
East Poland	Grey Heron ( <i>Ardea</i>	0.27-0.35	-	-	25.32-69.30	-	7.53-18.14	578.50-643.35	11.13-19.93	-	5.91-32.31	217.92-350.22
[8]	<i>cinerea</i> )											
Argentina	<i>Butorides striatus</i>	-	-	-	5.40 ± 1.60 <sup>a</sup>	-	1.35 ± 0.50 <sup>a</sup>	-	-	-	-	41.02 ± 9.31 <sup>a</sup>
[14]					3.78-8.37 <sup>a</sup>	-	0.74-2.04 <sup>a</sup>	-	-	-	-	30.32-57.09 <sup>a</sup>
USA [39]	Hérons Louisianaia	0.078 ± 0.010 <sup>a</sup>	-	-	-	-	-	-	-	-	-	-
		0.041-0.125	-	-	16.91	-	-	-	3.23	-	-	124.89
Poland [40]	Great bittern	0.50	-	-	-	-	-	-	-	-	-	-
	( <i>Botaurus stellalis</i> )											
	Grey Heron ( <i>Ardea</i>	0.45 ± 0.16	-	-	21.07 ± 12.64	-	-	-	6.43 ± 3.71	-	-	115.28 ± 34.59
	<i>cinerea</i> )	0.23-0.59	-	-	10.77-47.31	-	-	-	1.38-11.92	-	-	73.70-177.08

ND not detected

<sup>a</sup>Wet weight basis data

Nottinghamshire, UK. Nevertheless the range for Se concentrations in the present study was considered normal for livers of birds [16]. Concentration of Se higher than  $10 \text{ mg kg}^{-1}$  dry weight, which may cause reproductive impairments, were detected in one great egret specimen [8].

Zn concentrations found in this study are somewhat higher than those reported from egrets from Haneda and Hodaka, both regions of Japan [10], ardeids from Poland [40], grey heron from East Poland [8] and *Butorides striatus* from Argentina [14] and waterfowls of uncontaminated sites [42]. Zn concentration on wet basis reported here are also higher than those for herons from India [38]. In the present study, Zn concentrations of 39% of these specimens were similar to Zn poisoning level for wild birds ( $280\text{--}2900 \text{ mg kg}^{-1}$  dry basis) reported by [2, 8].

Fish consumption, accompanied by a relatively high body mass, may favor the hepatic accumulation of Zn and also Cu in liver of great egrets [8]. Despite important biochemical functions of Zn, severe degenerative abnormalities in the pancreas associated with high concentrations this element in livers, kidneys and blood were demonstrated in birds from Oklahoma, Kansas and Missouri states, USA [42].

High levels of Zn found in this study may probably be associated with increased environmental contamination by this element in the SPMR, in recent years. Moreover, high Zn concentrations interfere with the absorption of Cd, acting as a possible protection against Cd high toxicity [43]. Yet, our results for Cd could not show this relationship since no correlation between these two elements was verified.

### Toxic elements

According to [19] toxic elements are important factors in the formation of free radicals that cause oxidative stress, inhibiting the DNA damage repair that may cause high rates of mutations in birds.

The results obtained for Cd in this study are within the natural background in livers of birds ( $< 3 \text{ mg kg}^{-1}$  dry basis), [8, 35], except for a sample that presented the concentration of  $4.04 \pm 0.28 \text{ mg kg}^{-1}$  dry basis. For the found Cd levels we can conclude that there is no threat of this element to physiological processes of great egrets from the SPMR.

Our results of Hg in livers of great egrets are of the same magnitude to those reported for great egrets and intermediate egrets from Haneda, Japan [10], but they are higher than the observed ones to grey heron too from Haneda, Japan, and they are lower than those found in grey heron from Hodaka in Japan [10], grey heron from East Poland [8] and *Butorides striatus* from Argentina [14].

Hg levels in livers reported in the current study were lower than the estimated threshold ( $6 \text{ mg kg}^{-1}$  wet weight) that were correlated with mortality from chronic diseases in livers of great white herons (*Ardea herodias occidentalis*) [44]. Nevertheless, sublethal effects are likely to occur at concentrations far lower than those at which pathological effects become pronounced [45]. Therefore, it is important to reevaluate the risks to bird populations at low level Hg exposures [6].

Moreover, only the analysis of the Hg content is not sufficient to evaluate its effect, since lethal and sub-lethal effects of Hg is Se concentration dependent. Adult snowy egrets which contained high total mercury (THg) concentrations in their livers (geometric mean  $43.7 \mu\text{g g}^{-1}$  wet weight) showed an apparent tolerance to these concentrations due in part to sequestration of the inorganic mercury by selenium [46]. In this study, a strong positive correlation between Hg and Se ( $r = 0.636$ ,  $p < 0.05$ ) was established and it may be associated to the important function of Se for Hg detoxification.

Overall, the trace elements levels in livers of great egrets were similar or higher than level reported from other geographic areas. This suggests that trace elements contamination in the SPMR is at least as high, or is higher than in ardeids from other countries. This study also clearly showed that some great egrets are in the range of concentrations of Cu and Zn associated with poisoning levels in wild birds.

### Other elements: Cs and Rb

The mean concentrations of Rb and Cs obtained in the present study showed similar for Rb and lower for Cs when compared with results obtained for grey herons from Hodaka, in Japan [10].

### Comparative study of element levels found in the livers from specimens of different genders

In this study, livers from 12 males and 12 females great egrets were analyzed and their mean element concentrations were compared (Table 4). Concentrations for Br, Co, Cs, Rb, Se and Zn differed significantly between males and females. Females had lower concentrations than males. These findings may be related to physiological differences, since the ability to remove chemical elements may vary between genders. Females can transfer elements to eggs [1, 17] thus, they tend to present lower levels of elements in their tissues. This is considered an effective process to maintain low levels of contaminants in the body of female egrets [1]. However, these differences may even be associated with ecological factors, such as diet, which can vary

**Table 4** Concentrations of trace elements (mean values and standard deviation (SD) in mg kg<sup>-1</sup> dry weight) in livers of male and female great egrets

Element	Male Mean ± SD	Female Mean ± SD
Br	53.6 ± 27.2	28.7 ± 18.0 <sup>a</sup>
Cd	0.452 ± 0.476	0.264 ± 0.262
Co	0.155 ± 0.038	0.133 ± 0.094 <sup>a</sup>
Cs	0.276 ± 0.233	0.116 ± 0.077 <sup>a</sup>
Cu	92.8 ± 75.2	61.5 ± 66.6
Fe	3864.4 ± 2046.5	3034.7 ± 2180.6
Hg	3.06 ± 3.07	1.38 ± 1.49
K (%)	1.04 ± 0.24	0.98 ± 0.45
Mg	503 ± 102	588 ± 217
Mn	12.0 ± 3.8	8.4 ± 4.0
Na	4663 ± 1136	4372 ± 1314
Rb	47 ± 16	30.8 ± 12.0 <sup>a</sup>
Se	5.0 ± 2.2	3.04 ± 0.87 <sup>a</sup>
Zn	310 ± 91	181 ± 104 <sup>a</sup>

<sup>a</sup>Indicates that element showed significant concentration differences using nonparametric Mann–Whitney *U* test ( $p < 0.05$ )

between genders because male and female birds may feed in different habitats and eat different foods [17, 26].

The differences of Se concentrations presented here are of primary concern. Se contamination is a serious problem for bird populations in various parts of the world due to the effect of mortality and failure in reproduction. The excessive concentration of Se can produce toxic and teratogenic effects on embryos, resulting in malformations and increased mortality [47]. Besides, the results are consistent with previous studies [34, 48], in which female great egrets from the SPMR also presented lower Se levels than those of males. This suggests that Se levels in livers of great egrets from the SPMR are of great concern and require a thorough study.

### Correlation between element concentrations

The evaluation of the correlation between element concentrations is of interest since the elements may present synergism or antagonism from one to another that can cause effects on their concentrations [8, 26].

Moreover, correlation coefficients among element concentrations increase with the contamination levels and also indicate that the birds have accumulated the correlating elements from the same source [19, 49].

In the current study, the results of Pearson correlation analysis ( $r = 7$ ,  $p < 0.01$  and  $p < 0.05$ ) indicated positive correlations between the elements Cd and Se, Hg and Se,

Cd and Cu, Hg and Mn, Hg and Zn, and Mn and Zn as presented in Table 5.

Selenium and zinc are known as elements that have the propriety of reducing toxic effects of Cd and Hg [10, 22, 46]. Therefore, the elevated levels of Cd and Hg lead to the increase of Se and Zn levels, as a protection mechanism. The incidence of high concentrations of selenium in the soil is reported in several parts of the world. However, in regions of the São Paulo State, selenium shortage in grasses is related to deficiency in soil [50]. Further studies are required to investigate if there are sources of input of Se in the SPMR.

Significantly positive correlations among Cd–Cu, Fe–Zn, Hg–Mn, Hg–Zn and Mn–Zn found in this study may indicate similar sources of input of these metals according to [19]. Probably these elements derived mainly from anthropogenic sources, since their levels have increased in urban areas, caused by human activities [42]. According to [51], in the SPMR there is the discharge of untreated industrial and domestic effluents, that release Zn, Cd and Hg. Cu concentrations are high in water bodies which supply cities (Guarapiranga and Rio Grande Reservoirs), due to the use of Cu compounds (copper sulfate) for controlling algae growth [20]. Mn is emitted into environment through the combustion of fossil fuels, which is one of the main anthropogenic activities [10]. Besides, soil erosion in the study area can also contribute to the Mn contamination of aquatic environments, apart from Fe [20].

The results of the present study are consistent with a number of studies reporting the input of Cd, Cu, Hg, Mn and Zn from similar sources and their sources can be mainly related anthropogenic inputs [19, 42], and it reinforces the idea that high concentrations of several trace elements reflected in the livers of great egret can be due to local environmental exposure.

Although little is known about the elements Cs and Rb in bird tissues, these two elements may act as nutritional substitutes for K [4]. However, in this study no correlation was observed between these two elements. At present, no satisfactory interpretation was found to explain the correlations between Br, Cs, K, Na, and Rb and other trace elements (Table 5).

### Conclusions

The results obtained in this study, as well as the comparison with literature data indicated that concentrations of some trace elements (mainly Co, Cs, Cu, Fe, Mn and Zn) were high in the livers of great egrets from the SPMR as expected. As great egrets are residents and top predators of the local aquatic food chain, these levels can reflect environmental conditions and the livers of this species can

**Table 5** Pearson correlation coefficients between element concentrations in the livers of great egrets from SPMR

	Br	Cd	Co	Cs	Cu	Fe	Hg	K	Mg	Mn	Ni	Rb	Se	Zn
Br	-	NS	NS	0.658**	NS	NS	0.608**	NS	NS	0.378*	0.398*	0.430*	0.395*	0.457**
Cd	NS	-	NS	NS	0.635*	NS	NS	NS	NS	NS	NS	NS	0.364*	NS
Co	NS	NS	-	NS	NS	NS	NS	NS	NS	NS	NS	0.348*	NS	NS
Cs	0.658**	NS	NS	-	NS	NS	0.823**	NS	NS	NS	NS	0.387*	0.519**	0.369*
Cu	NS	0.635*	NS	NS	-	NS	NS	NS	NS	NS	NS	NS	NS	NS
Fe	NS	NS	NS	NS	NS	-	NS	NS	NS	NS	0.425*	NS	NS	0.617**
Hg	0.608*	NS	NS	0.823**	NS	NS	-	NS	NS	0.545*	NS	0.491*	0.636**	0.389*
K	NS	NS	NS	NS	NS	NS	NS	-	0.524**	NS	NS	NS	NS	NS
Mg	NS	NS	NS	NS	NS	NS	NS	0.524**	-	NS	NS	NS	NS	NS
Mn	0.378*	NS	NS	NS	NS	NS	0.545*	NS	NS	-	NS	NS	NS	0.584**
Na	0.398*	NS	NS	NS	NS	0.425*	NS	NS	NS	NS	-	NS	NS	0.531**
Rb	0.430*	NS	0.348*	0.387*	NS	NS	0.491*	NS	NS	NS	NS	-	0.354*	0.395*
Se	0.395*	0.364*	NS	0.519**	NS	NS	0.636**	NS	NS	NS	NS	0.354*	-	NS
Zn	0.457**	NS	NS	0.369*	NS	0.617**	0.389*	NS	NS	0.584**	0.531**	0.395*	NS	-

NS not significant

\*Significant at  $p < 0.01$  and \*\*significant at  $p < 0.05$

be a suitable bioindicator of contamination of the aquatic systems in the SPMR.

Although Cd, Hg and Se levels in the livers of great egrets were within the known background level for wild birds, Zn and Cu poisoning indicative levels were detected in livers specimens from the SPMR.

Female egrets presented lower concentrations of Br, Co, Cs, Rb, Se and Zn than males, probably due to physiological and ecological differences.

Correlations among Cd, Cu, Fe, Hg, Mn and Zn found in this study may indicate similar sources of input of these elements in the region, mainly from several anthropogenic sources. These results also suggest that high concentrations of several trace elements in great egret livers may be due local exposure.

**Acknowledgements** We would like to thank the Technical Division of Veterinary Medicine and Wildlife Management (DEPAVE 3), Municipal Zoo Quinzinho de Barros and Wild Animals Screening Center (CETAS) by the provision of samples. We also thank to the Research Reactor Center (CRPq—IPEN—CNEN/SP) which enabled analyses of liver samples.

## References

- Deng H, Zhang Z, Chang C, Wang Y (2007) Trace metal concentration in Great Tit (*Parus major*) and Greenfinch (*Carduelis sinica*) at the Western Mountains of Beijing China. *Environ Pollut*. <https://doi.org/10.1016/j.envpol.2006.11.012>
- Kim J, Oh JM (2016) Assessment of trace element concentrations in birds of prey in Korea. *Arch Environ Contam Toxicol*. <https://doi.org/10.1007/s00244-015-0247-3>
- Naccari C, Cristani M, Cimino F, Arcoraci T, Trombetta D (2009) Common buzzards (*Buteo buteo*) bio-indicators of heavy metal pollution in Sicily (Italy). *Environ Int*. <https://doi.org/10.1016/j.envint.2008.11.002>
- Nam DH, Anan Y, Ikemoto T, Okabe Y, Kim EY, Subramanian A, Saeki K, Tanabe S (2005) Specific accumulation of 20 trace elements in great cormorants (*Phalacrocorax carbo*) from Japan. *Environ Pollut*. <https://doi.org/10.1016/j.envpol.2004.09.003>
- Abbasi NA, Jaspers VL, Chaudhry MJ, Ali S, Malik RN (2015) Influence of taxa, trophic level, and location on bioaccumulation of toxic metals in bird's feathers: a preliminary biomonitoring study using multiple bird species from Pakistan. *Chemosphere*. <https://doi.org/10.1016/j.chemosphere.2014.08.054>
- Movalli PA (2000) Heavy metal and other residues feathers of laggar falcon *Falco biarmicus jugger* from six districts of Pakistan. *Environ Pollut*. [https://doi.org/10.1016/S0269-7491\(99\)00258-4](https://doi.org/10.1016/S0269-7491(99)00258-4)
- Burger J, Gochfeld M (2004) Metal levels in eggs of common terns (*Sterna hirundo*) in New Jersey: temporal trends from 1971 to 2002. *Environ Res*. [https://doi.org/10.1016/S0013-9351\(03\)00081-1](https://doi.org/10.1016/S0013-9351(03)00081-1)
- Kitowski I, Wiacek D, Sujak A, Komosa A, Świetlicki M (2017) Factors affecting trace element accumulation in livers of avian species from East Poland. *Turk J Zool*. <https://doi.org/10.3906/zoo-1606-43>
- Zhilong M, Qiang W, Zhongsheng Z, Xuehong Z (2017) Mercury distribution along the food chain of a wetland ecosystem at Sanjiang Plain, Northeast China. *Bull Environ Contam Toxicol*. <https://doi.org/10.1007/s00128-016-2005-2>
- Horai S, Watanabe I, Takada H, Iwamizu Y, Hayashi T, Tanabe S, Kuno K (2007) Trace element accumulations in 13 avian species collected from the Kanto area, Japan. *Sci Total Environ*. <https://doi.org/10.1016/j.scitotenv.2006.10.010>
- Kwok CK, Liang Y, Wang H, Dong YH, Leung SY, Wong MH (2014) Bioaccumulation of heavy metals in fish and Ardeid at Pearl River. *Ecotoxicol Environ Saf*. <https://doi.org/10.1016/j.ecoenv.2014.04.016>
- Pérez-López M, Mendoza MH, Beceiro AL, Rodríguez FS (2008) Heavy metal (Cd, Pb, Zn) and metalloid (As) content in raptor species from Galicia (NW Spain). *Ecotoxicol Environ Saf*. <https://doi.org/10.1016/j.ecoenv.2007.04.016>
- Carneiro M, Colaço B, Brandão R, Ferreira C, Santos N, Soeiro V, Colaço A, Pires MJ, Oliveira PA, Lavín S (2014) Biomonitoring of heavy metals (Cd, Hg, and Pb) and metalloid (As) with the Portuguese common buzzard (*Buteo buteo*). *Environ Monit Assess*. <https://doi.org/10.1007/s10661-014-3906-3>
- Simonetti P, Botté SE, Beltzer AH, Marcovecchio JE (2014) Tissue distribution of Cd, Cu, Zn, Ni, Cr, Pb and Hg in striated heron, *Butorides striatus* (Aves: Ardeidae), in a fluvial ecosystem. *Chem Ecol*. <https://doi.org/10.1080/02757540.2013.856890>
- Spahn SA, Sherry TW (1999) Cadmium and lead exposure associated with reduced growth rates, poorer fledging success of little blue heron chicks (*Egretta cerulean*) in South Louisiana wetlands. *Arch Environ Contam Toxicol*. <https://doi.org/10.1007/s002449900528>
- Thompson HM, Fernandes A, Rose M, White S, Blackburnn A (2006) Possible chemical of skeletal deformities in grey heron nestling (*Ardea cinerea*). *Chemosphere*. <https://doi.org/10.1016/j.chemosphere.2006.02.007>
- Ayas Z (2007) Trace element residues in eggshells of grey heron (*Ardea cinerea*) and black-crowned night heron (*Nycticorax nycticorax*) from Nallihan Bird Paradise, Ankara-Turkey. *Ecotoxicology*. <https://doi.org/10.1007/s10646-007-0132-6>
- Champoux L, Boily M, Fitzgerald G (2017) Thyroid Hormones, Retinol and Clinical Parameters in Relation to Mercury and Organohalogen Contaminants in Great Blue Heron (*Ardea herodias*) Nestlings from the St. Lawrence River, Québec, Canada. *Arch Environ Contam Toxicol*. <https://doi.org/10.1007/s00244-017-0364-2>
- Malik RN, Zeb N (2009) Assessment of environmental contamination using feathers of *Bubulcus ibis* L., as a biomonitor of heavy metal pollution Pakistan. *Ecotoxicology*. <https://doi.org/10.1007/s10646-009-0310-9>
- Companhia Ambiental do Estado de São Paulo, Relatório de Qualidade das Águas Interiores do Estado de São Paulo. <http://www.cetesb.sp.gov.br/Agua/rios/publicações.asp> Accessed 06 Jul 2013
- Abdennadher A, Ramírez F, Romdhane MS, Ruiz X, Jover L, Sanpera C (2011) Lithe Egret (*Egretta garzetta*) as a bioindicator of trace element pollution in Tunisian aquatic ecosystems. *Environ Monit Assess*. <https://doi.org/10.1007/s10661-010-1560-y>
- Burger J (2013) Temporal trends (1989-2011) in levels of mercury and other heavy metals in feathers of fledgling great egrets nesting in Barnegat Bay, NJ. *Environ Res*. <https://doi.org/10.1016/j.envres.2013.01.003>
- Matarazzo-Neuberger WM (1994) Guilds, community organization and structure: birdlife analysis of billings. Ph. D. Thesis, University of São Paulo (in Portuguese)
- Silva RCA (2013) Study of the Great Egret *Ardea alba* (Linnaeus, 1758) species, as an environmental contamination bioindicator, in the Metropolitan Region of São Paulo. Ph. D. Thesis, University of São Paulo (in Portuguese). <http://www.teses.usp.br/teses/>

- disponiveis/85/85131/tde-14102013-135313/pt-br.php. Accessed 17 Apr 2017
25. Cruz BB, Piratelli AJ (2011) Avifauna associada a um trecho urbano do Rio Sorocaba, Sudeste do Brasil. *Biota Neotrop.* <https://doi.org/10.1590/S1676-06032011000400022>
  26. Gochfeld M, Burger J (1987) Heavy metal concentrations in the liver of three duck species: influence of species and sex. *Environ Pollut.* [https://doi.org/10.1016/0269-7491\(87\)90012-1](https://doi.org/10.1016/0269-7491(87)90012-1)
  27. Mariano DB, Figueiredo AMG, Semmler R (2014) Implementation of the  $k_0$  standardization method for analysis of geological samples at the Neutron Activation Analysis Laboratory. *J Radioanal Nucl Chem, São Paulo.* <https://doi.org/10.1007/s10967-013-2696-3>
  28. Institute of Nuclear Chemistry and Technology, Poland (2002) Polish Certified Reference Material for Multielement Trace Analysis Tea Leaves (INCT-TL-1)
  29. National Institute of Standards and Technology (1991) Certificate of Analysis Standard Reference Material 1577b Bovine Liver
  30. National Institute of Standards and Technology (1998) Certificate of analysis standard reference material SRM 2976, Mussel tissue (trace elements and methylmercury)
  31. Currie LA (1999) Nomenclature in evaluation of analytical methods including detection and quantification capabilities (IUPAC Recommendations 1995). *Anal Chim Acta.* [https://doi.org/10.1016/S0003-2670\(99\)00104-X](https://doi.org/10.1016/S0003-2670(99)00104-X)
  32. Hargreaves AL, Whiteside DP, Gilchrist G (2011) Concentrations of 17 elements, including mercury, in the tissues, food and abiotic environment of Arctic shorebirds. *Sci Total Environ.* <https://doi.org/10.1016/j.scitotenv.2011.06.043>
  33. Honda K, Min BY, Tatsukawa R (1986) Distribution of heavy metals and their related changes in the Eastern Great White Egret, *Egretta alba modesta*, in Korea. *Arch Environ Contam Toxicol.* <https://doi.org/10.1007/BF01059967>
  34. Silva RCA, Saiki M (2012) Concentrations of trace elements in livers of the Great Egret (*Ardea alba*) from the metropolitan region of São Paulo. *J Radioanal Nucl Chem, SP, Brazil.* <https://doi.org/10.1007/s10967-011-1298-1>
  35. De Luca-Abbott SB, Wong BS, Peakall DB, Lam PK, Young L, Lam MH, Richardson BJ (2001) Review of effects of water pollution on the breeding success of waterbirds, with particular reference to ardeids in Hong Kong. *Ecotoxicology.* <https://doi.org/10.1023/A:1012288811808>
  36. Boncompagni E, Muhammad A, Jabeen R, Orvini E, Gandini C, Sanpera C, Ruiz X, Fasola M (2003) Egrets as monitors of trace-metal contamination in Wetlands of Pakistan. *Arch Environ Contam Toxicol.* <https://doi.org/10.1007/s00244-003-0198-y>
  37. Aguiar RO (2009) Determinação de Elementos em Sangue de Hamster Dourado Usando AAN. Master Dissertation, University of São Paulo (in Portuguese). <http://www.teses.usp.br/teses/disponiveis/85/85131/tde-02062009-172318/pt-br.php>. Accessed 07 Apr 2013
  38. Jayakumar R, Muralidharan S (2011) Metal contamination in select species of birds in Nilgiris District, Tamil Nadu, India. *Bull Environ Contam Toxicol.* <https://doi.org/10.1007/s00128-011-0323-y>
  39. Cheney MA, Hacker CS, Schroder GD (1981) Bioaccumulation of lead and cadmium in the Louisiana Heron (*Hydranasa tricolor*) and the Cattle Egret (*Bubucus ibis*). *Ecotoxicol Environ Saf.* [https://doi.org/10.1016/0147-6513\(81\)90036-1](https://doi.org/10.1016/0147-6513(81)90036-1)
  40. Komosa A, Kitowski I, Komosa Z (2012) Essential trace (Zn, Cu, Mn) and toxic (Cd, Pb, Cr) elements in the liver of birds from Eastern Poland. *Acta Vet.* <https://doi.org/10.2298/avb1206579k>
  41. Kalisinska E, Salicki W, Myslek P, Kavetka KM, Jackowski A (2004) Using the mallard to biomonitor heavy metal contamination of wetlands in north-western Poland. *Sci Total Environ.* <https://doi.org/10.1016/j.scitotenv.2003.08.014>
  42. Beyer WN, Dalgarn J, Dudding S, French JB, Mateo R, Miesner J, Sileo L, Spann J (2004) Zinc and lead poisoning in wild birds in tri-state mining district (Oklahoma, Kansas, and Missouri). *Arch Environ Contam Toxicol.* <https://doi.org/10.1007/s00244-004-0010-7>
  43. Metcheva R, Yrukova L, Teodorova S, Nikolova E (2006) The penguin feathers as bioindicator of Antarctica environmental state. *Sci Total Environ.* <https://doi.org/10.1016/j.scitotenv.2005.05.008>
  44. Spalding MG, Bjork RD, Power GVN, Sundlof SF (1994) Mercury and cause of death in great white herons. *J Wildl Manage.* <https://doi.org/10.2307/3809688>
  45. Scheuhammer MA (1987) The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: a review. *Environ Pollut.* [https://doi.org/10.1016/0269-7491\(87\)90173-4](https://doi.org/10.1016/0269-7491(87)90173-4)
  46. Henny CJ, Hill EF, Grove RA, Chelgren ND, Haggerty PK (2017) Mercury and drought along the lower Carson River, Nevada: IV Snowy egret post-fledging dispersal, timing of migration and survival, 2002–2004. *Ecotoxicol Environ Saf.* <https://doi.org/10.1016/j.ecoenv.2016.10.002>
  47. Ramirez F, Abdennadher A, Sanpera C, Jover L, Hobson KA, Wassenaar LI (2011) Tracing waterbird exposure to total mercury and selenium: a case study at the solar saltworks of Thyna (Sfax, Tunisia). *Environ Sci Technol.* <https://doi.org/10.1021/es200026w>
  48. Silva RCA, Saiki M, Oliveira PTMS (2015) Selenium levels in liver of Great White Egret (*Ardea alba*) from São Paulo metropolitan region, Brazil. *J Heron Biol Conserv.* <http://www.heronconservation.org/journal-of-heron-biology-and-conservation>
  49. Goutner V, Becker PH, Liordos V (2011) Organochlorines and mercury in livers of great cormorants (*Phalacrocorax carbo sinensis*) wintering in northeastern Mediterranean wetlands in relation to area, bird age, and gender. *Sci Total Environ.* <https://doi.org/10.1016/j.scitotenv.2010.10.043>
  50. Faria LA (2009) Selenium quantity in Brazilian soils and plants, and selenium application in different forages. Master Dissertation, University of São Paulo (in Portuguese). <http://www.teses.usp.br/teses/disponiveis/74/74131/tde-13042009-094603/pt-br.php>. Accessed 07 Apr 2013
  51. Campos APS (2012) Presence of metals and organic chemical compounds in surface waters and sediments of the Tiete River. Ph. D. thesis, University of São Paulo, São Paulo (in Portuguese). <http://www.teses.usp.br/teses/disponiveis/6/6134/tde-11092012-203210/pt-br.php>. Accessed 17 Apr 2017