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STUDY ON TRACE ELEMENT DETERMINATION IN LIVER SAMPLES OF GREAT-WHITE-EGRET *Ardea alba* LINNAEUS, 1758 (ARDEIDAE, AVES) FOR ENVIRONMENTAL CONTAMINATION BIOMONITORING

Rita de Cássia A. Silva and Mitiko Saiki

Instituto de Pesquisas Energéticas e Nucleares, IPEN - CNEN/SP
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP, Brazil
rcsilva@ipen.br, mitiko@ipen.br

ABSTRACT

Predatory birds such as herons have been used as bioindicators of pollution since they are at the top of their food webs. The tissues of these animals are analyzed for assessing environmental pollution caused by toxic elements. In the present study, adequate experimental conditions were established for determination of trace elements concentrations in the liver samples of Great-white-egret (*Ardea alba* Linnaeus, 1758) for further application of this specimens as a bioindicator of environmental contamination. Four liver samples were collected from Great-white-egrets found in the metropolitan region of São Paulo and were they analyzed by the method of neutron activation analysis (NAA). Concentrations of the elements Br, Co, Cs, Fe, Na, Rb, Se and Zn were measured in these liver tissues. The findings of this present study demonstrated that the established procedure for liver sample treatment was appropriate to obtain a homogeneous sample. The method of neutron activation analysis (NAA) was very promising for liver sample analysis for evaluation of environmental contamination.

1. INTRODUCTION

With the increase of environmental contamination in the last years, studies on determination of trace elements in biological systems have been of great interest in order to use them as a bioindicator.

Many studies have reported that trace elements accumulate at high levels in species at higher trophic levels [1-5]. As the birds are ordinarily at the top of the food web, they are valuable species for monitoring environmental contamination [1, 4].

The use of herons to evaluate the environmental contamination is rather indicated for two reasons. The herons being also the first at the top of the food web they tend to accumulate contaminants in their tissues, and its close relationship with the environment. That is, they constantly eat sediments to facilitate digestion [2, 4].

On the other hand, the analysis of bird tissues is also highly relevant in the field of conservation of wildlife. The environmental contamination from chemical pollutants, such as heavy metals and organic substances is a threat to the survival and reproduction of bird populations. Since toxic elements can cause weight loss, organ damage, metabolic disorders and behavioral changes in specimens [4].

Livers of the birds are one of the most important organs of their metabolism and this tissue can concentrate chemical elements at high levels [6, 7]. Unfortunately, available elemental data of this liver analysis are still far from sufficient. It is, therefore, important to establish element concentration ranges of this tissue. The main difficulty to obtain these data is the acquisition of a statistically significant number of samples for the analyses.

From all these considerations, the purpose of this study was to establish adequate experimental conditions to determine of trace element concentrations in the liver samples of Great-white-egret (*Ardea alba* Linnaeus, 1758) by neutron activation analysis (NAA), for further application of this kind as bioindicator of environmental contamination.

2. EXPERIMENTAL

2.1. Sampling and Preparation of Samples

The Great-white-egret (*Ardea alba* Linnaeus, 1758) specimens selected for this study were limited to those found dead or injured in the metropolitan area of São Paulo. Four liver samples of egrets were acquired from the Divisão Técnica de Medicina Veterinária and Manejo da Fauna Silvestre (DEPAVE 3) /SVMA, Prefeitura Municipal de São Paulo. The causes of death for almost of these birds were due to the disease caused by parasites.

Sample preparation for the analyses and the measurements trace elements were performed at the Laboratory of Neutron Activation Analysis, at the Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP, Brazil. The livers were stored at -20º C after collection until their treatment. For the analysis, each liver sample was first cleaned by removing blood and cut in small pieces using titanium knife. Then, it was freeze-dried and ground in an agate mortar to obtain a fine homogenous powder. A loss weight of 73.3% was obtained in this drying process.

2.2. Procedure for Neutron Activation Analysis

The synthetic elemental standards were prepared by pippeting 50 µl of the elemental standard element solutions onto sheets of Whatman No. 40 filter paper using an Eppendorf pipetto. These element solutions containing one or more elements were prepared using certified standard solution provided by Spex Certiprep Chemical, USA. The filter sheets were dried at room temperature inside a desiccator and, then they were placed into clean polyethylene involucres. The masses of the elements in 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 sample liver and reference material weighed in clean polyethylene involucres were irradiated together with synthetic elemental standards for 16 h under a thermal neutron flux of about 2.1 $x10^{12}$ n $cm^{-2}s^{-1}$. Three series of measurements were carried after 7, 15 and 20-25 days of decay times using an HGe detector coupled to a gamma ray spectrometer. The gamma ray spectra were obtained using the MAESTRO software from Ortec and they were processed using the VISPECT2 computer program. The radioisotopes were identified according to half-lives and gamma ray energies. The characteristics of

radioisotopes used are listed in Table 1. The concentrations of elements were calculated by comparative method [8].

Table 1. Characteristics of radioisotopes utilized in this study

Element	Radionuclides	Half-life	Gamma ray energy measured (keV)
Br	^{82}Br	35.3 hours	776.52
Co	^{60}Co	5.27 days	1173.24/1332.50
Cs	^{134}Cs	2.06 years	795.85
Fe	^{59}Fe	44.5 days	1099.25/1291.60
Na	^{24}Na	14.96 hours	1368.60
Rb	^{86}Rb	18.66 days	1076.60
Se	^{75}Se	119.77 days	264.66
Zn	^{65}Zn	243.9 days	1115.55

The accuracy and precision of liver analysis results were verified by analyzing certified reference materials NIST 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. These reference materials were analyzed by applying the same experimental conditions used in liver analyses. The element concentrations of reference materials were evaluated on a dry weight basis, as recommended in the certificate. Aliquots of these materials were dried in an oven at 85^0 C until a constant weight is obtained to obtain the percentage of humidity. In this drying process a mean loss of 4.33% was obtained for NIST 1577 b Bovine Liver and of 4.84% for INCT -TL-1 Tea Leaves.

3. RESULTS AND DISCUSSION

In Table 2 are presented the average concentrations of obtained in the analyses of NIST 1577b Bovine Liver reference material together with the values of the certificate. Concentrations of the elements Br, Co, Fe, K, Na, Rb, Se and Zn determined show, in general, a good precision with relative standard deviations lower than 9.2% and good accuracy with relative errors ranged from 0.5 to 13.6%. Thus the results obtained can be considered satisfactory for the determination of these elements.

Table 2. Mean concentrations of elements obtained in the analysis of certified reference material NIST 1577b Bovine Liver (in mg kg^{-1} unless indicated)

Element	This Study			Values of the certificate [9]
	Mean ±SD (n)[a]	RSD[b], %	RE[c], %	
Br	9.38±0.72 (4)	7.7	-	9.7[d]
Co	0.24±0.02 (4)	6.4	-	0.25[d]
Fe	181±4 (4)	2.0	0.5	184±15
Na (%)	0.20±0.013(4)	6.2	13.6	0.242±0.006
Rb	13.4±0.7 (4)	5.3	2.3	13.7±1.1
Se	0.74±0.07 (4)	9.2	1.4	0.73±0.06
Zn	119.1±5.8 (4)	5.0	6.2	127±16

a. Arithmetic mean and standard deviation, n indicates number of determinations; b. Relative standard deviation; c. Relative error; d. Informative values

Table 3. Mean concentrations of elements obtained in the analysis of certified reference material INCT-TL-1 Tea Leaves (in mg kg^{-1} unless indicated)

Element	This Study			Values of the certificate [10]
	Mean ±SD (n)[a]	RSD[b], %	RE[c], %	
Br	13.2±1.2(9)	8.8	7.2	12.3±1.0
Co	0,39 ±0,03(8)	7,7	0.0	0,39±0,04
Cs	3.72±0.129(7)	2.7	3.2	3.61±0.37
Fe	464±25(5)	5.3	-	432[d]
Na	25.6±5.7(4)	22.3	3.5	24.7±3.2
Rb	82.7±3.5(10)	4.2	1.5	81.5±6.5
Zn	37.0±2.5(10)	6.8	7.0	34.7±2.7

a. Arithmetic mean and standard deviation, n indicates number of determination; b. Relative standard deviation; c. Relative error; d. Informative values

In Table 3 are presented results obtained in analysis of INCT-TL-1 Tea Leaves reference material. A good precision expressed as relative standard deviations varying from 2.7 to 8.8% was obtained for most of elements. The less precise result was obtained for the element Na, due to low statistical counting of the measurements. The relative errors obtained were lower

than 7.2%, indicating that the results obtained are in good agreement with their respective certified values.

Results obtained in the four replicate analyses of two different samples of liver are presented in Table 4. Results obtained for the elements Br, Co, Cs, Fe, Na, Rb, Se and Zn in these samples indicate a good precision. The relative standard deviations ranged from 1.3% to 9.0% in sample 1, and from 0.7% to 5.4% in sample 2, indicating that the results present good reproducibility. These data indicate that these samples can be considered homogeneous for the determination of these elements.

Table 4. Mean concentrations of elements (in mg kg^{-1}) obtained in replicate analyses of two different liver samples

Element	Sample 1		Sample 2	
	Mean ±SD(n)[a]	RSD[b], %	Mean ±SD(n)[a]	RSD[b], %
Br	37.7±1.9(4)	5.0	69.7±3.9(4)	5.4
Co	0.144±0.013(4)	9.0	0.094±0.004(3)	4.3
Cs	0.214±0.005(4)	2.3	0.072±0.002(4)	2.7
Fe	5511.5±128.05(4)	2.3	1778.9±30.3(4)	1.7
Na	5010.2 ±185.8(4)	3.7	4942±149(4)	3.0
Rb	62.66±1.14(4)	1.8	23.9±0.3(4)	1.5
Se	10.03±0.13(4)	1.3	2.94±0.04(4)	1.4
Zn	242±4(4)	1.6	97.0±0.7(4)	0.7

a. Arithmetic mean and standard deviation, n indicates number of determination of each sample; b. Relative standard deviation

In Table 5 are presented results of mean concentrations, standard deviations and ranges (in mg kg^{-1} on dry weight basis) of elements obtained in the four liver samples of Great-white-egret (*Ardea alba* Linnaeus, 1758). As can be seen in this Table, the elements Br, Co, Cs, Fe, Na, Rb, Se and Zn were detected in all samples analyzed. The concentrations of these elements showed large variations, between the samples mainly for the elements Cs, Fe and Zn. These variations in the element concentrations probably may be associated with environmental changes as in water quality of their habitats. This finding suggests future study related to the effect of environmental contamination in the element accumulation in liver of this species.

Table 5. Arithmetic mean, standard deviations, and ranges of concentrations (in mg kg^{-1} on dry weight basis) of trace elements in livers of Great-white-egret (*Ardea alba* Linnaeus, 1758) (number of livers analyzed = 4).

Element	Mean ±SD[a]	Range
Br	42.4±19.8	22.2 – 69.7
Co	0.106±0.026	0.085 –0.144
Cs	0.145±0.094	0.058 – 0.238
Fe	2515 ±2006	1367 – 5512
Na	4383± 691	3686 – 5010
Rb	43±16	23.9 – 62.7
Se	4.7±3.6	2.49 – 10.03
Zn	138.4±69.4	97 – 242

a. Arithmetic mean and standard deviation

4. CONCLUSION

The results obtained in the present study demonstrated that the established procedure for liver sample treatment was appropriate to obtain a homogeneous sample. The experimental procedure of NAA established was very promising for liver sample analysis since the analysis of certificate reference materials presented good precision and accuracy.

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