BJRS

BRAZILIAN JOURNAL
OF
RADIATION SCIENCES
09-01A (2021) 01-16

Determination of toxic elements in fish of the genus 17 consumed by artisanal fishermen of the District of Riacho Grande, São Bernardo do Campo city, Brazil

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ABSTRACT

Toxic elements in contact with the human body cause numerous health problems. The contamination occurs mostly by food consumption, such as the ingestion of fish contaminated with high concentrations of As, Cd, Hg or Pb among other elements. Many fishermen and their family members end up exposing themselves to different toxic elements due to fish-based diet as the main protein nutrient because they are unaware of the health risks associated with the consumption of fish from contaminated waters. In the present study, quantification of the toxic or potentially toxic elements As, Br, Cs, Cr, Co, Fe, K, Na, Sc, Se and Zn in samples of fish of the genus Astyanax (known by the common name of *lambari*). The fish was collected at Billings Reservoir by fishermen from the Riacho Grande District - (São Bernardo do Campo city, São Paulo State, Brazil) is presented. The *lambari* fish had great relevance in this study because it is consumed as a snack, in which the subject feeds on the whole organism of the fish, having a greater risk of direct contact with toxic elements through ingestion. Elements were determined by Instrumental Neutron Activation Analysis (INAA). This study is important in establishing an updated spatiotemporal vision of the contamination by various elements of interest in the region. Additionally, it contributes to the food safety assurance, regarding inorganic contaminants referred by the Brazilian Health Regulatory Agency (ANVISA).

Keywords: **neutron activation analysis, Billings Reservoir, toxic elements, Astyanax genus, lambari.**

ISSN: 2319-0612
Accepted: 2020-07-30

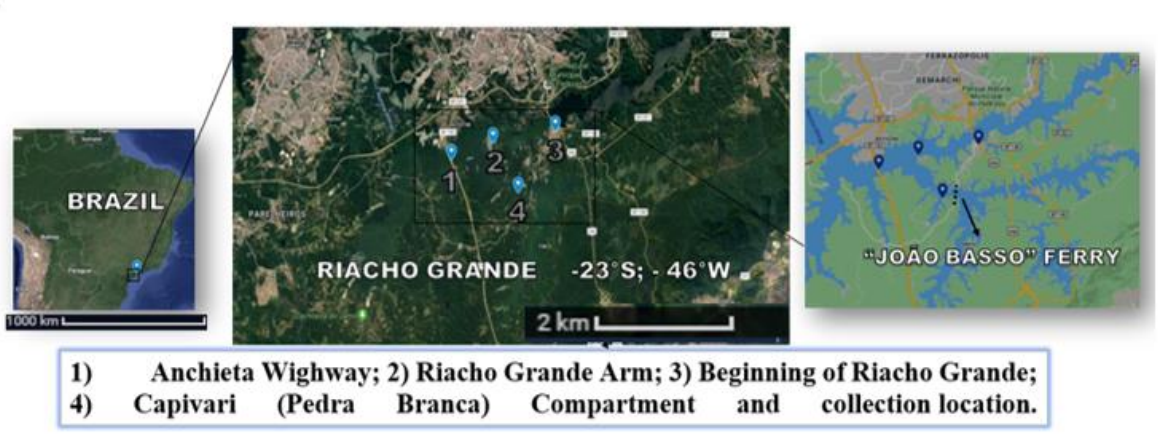
1. INTRODUCTION

1.1 Toxic elements at the Billings Reservoir, Riacho Grande, São Bernardo do Campo, São Paulo State - Brazil

The District of Riacho Grande belongs to the municipality of São Bernardo do Campo (SBC) and has an area of 10.69 km² of urban area and 214.42 km² of rural area [1]. More than half of its territory belongs to the State Watershed Protection Area (WPM). The region also has the presence of great biodiversity and is considered of great importance and with high priority for conservation, sustainable use and sharing of the benefits of Brazilian biodiversity [2]. However, this ecosystem is constantly undergoing alteration due to the anthropic action, mainly from the dumping of commercial and household wastes directly in the water environment and from the process of deforestation of native vegetation from irregular occupations [3,4].

Two arms of the Billings Reservoir are in Riacho Grande: Rio Grande arm and the Capivari (Pedra Branca) compartment. The Rio Grande arm is formed, to the north, by precarious agglomerations and areas of urban expansion. And to the south lies the isolated core of Riacho Grande, with areas of urban expansion, clubs, farms, and parks. The Anchieta Highway is located at the eastern end of this region. Since the Capivari compartment can only be accessed by a raft and unpaved roads, this site has native forests still preserved, dispersed occupation and predominantly agricultural activities. The Pedra Branca region is cut by the Imigrantes Highway in the north - south direction. The region of Capivari Arm is quite isolated, little urbanized and its population is concentrated to the north. The area is adjacent to the Rio das Pedras reservoir and has preserved forests and rugged relief. [5]. As a region of natural springs, with the presence of great biodiversity, Riacho Grande is protected by the State Law nº 1,172 of November 17, 1976 [6]. Figure 1 shows the location of Riacho Grande, identifying the Anchieta highway, the Capivari and Rio Grande arms region and the collection site.

Figure 1: Riacho Grande localization



1) Anchieta Wighway; 2) Riacho Grande Arm; 3) Beginning of Riacho Grande; 4) Capivari (Pedra Branca) Compartment and collection location.

The presence of ferries, as the João Basso Ferry, makes it difficult for residents of neighboring cities (Diadema, Santo André and São Paulo) to travel to the region due to the lack of public transportation infrastructure and the delay in their crossing. This difficulty delays the intense occupation of the region, preserving it [7]. However, surface waters - such as water sources - are rarely free of contamination, even in places with little or no human presence [8].

Disorganized occupations around the Billings Reservoir, pollution generated by urban and rural activity, runoff from domestic rains and manure cause changes in the water quality and potential harmful effects on different organisms, including fish and humans.

Toxic elements in contact with the human body cause numerous health problems and contamination may occur mostly through food, such as the ingestion of fish contaminated with Hg, methylmercury (MeHg), among other elements [9,10,11].

However, many fishermen and family members in this region, because they are unaware of the health risks associated with the consumption of fish from contaminated waters, end up exposing themselves to different toxic elements due to the fish-based diet as the main protein nutrient. Faced with the possible exposure of fishermen from Riacho Grande to toxic elements, the risk perception of the members of the “Orlando Feliciano” Fishermen Association from São Bernardo do Campo will be investigated. The Association is in Riacho Grande and receives fishermen from São Bernardo do Campo, Santo André and São Caetano do Sul, to offer support to artisanal fishermen.

1.2 Billings Reservoir Fishing Products and Food Security

At the Billings Reservoir, the most common fish to be found are *Tilapia rendali*, *Oreochromis niloticus*, *Astyanax* spp., *Cyprinus carpio*, *Hoplias malabaricus* and *Geophagus brasiliensis* [12]. Hunting and fishing with the use of circular net (fishing net), nets and motorized aluminum boats are prohibited during the closed season. In the closed season, which is usually between November and February, only amateur fishing can be carried out, provided it is by fisherman regularized at the Ministry of Fisheries and Aquaculture and uses only hand line or rod, line and hook. This measure aims to protect the aquatic fauna during the breeding season or its greater growth, guaranteeing the sustainability of the Reservoir stocks [12].

In general, the Watershed Protection and Recovery Area of the Billings Reservoir does not have very adequate living conditions for the fish, as the water quality protection index classifies the entire Billings Reservoir as poor, except for the Rio Grande, considered regular [6]. The consumption of fish from contaminated waters endangers the health of consumers, especially those who have the fish-based diet as the main protein nutrient. According to the concept of Food Security there are three main aspects to be considered: quantity, quality, and regularity in access to food [13].

The quality of food consumed is a particularly important aspect since the food available for consumption by the population cannot be subjected to any type of contamination risks [13]. In larger amounts, the elements As, Br, Co, Cr, Se, Cs, Sc, Rb, Fe and Zn may be dangerous. Generally, humans are exposed to these elements by ingestion. High concentrations of As and Se, for example, may cause cancer of the skin, lung, liver, and bladder, and may also cause problems to the digestive and respiratory systems [14]. In this study, Instrumental Neutron Activation Analysis (INAA) was used to determine essential and toxic elements [15] in Astyanax genus specimens as a contribution to food security for the Billings Reservoir fishing community.

2. MATERIALS AND METHODS

2.1 Fish sampling

The lambari (Astyanax genus) samples used in this study were obtained through artisanal fishermen from the Billings Reservoir. Samples were collected in the second half of 2018 in a single moment. Five adult specimens for the following species were analyzed: *Astyanax fasciatus* and *Astyanax bimaculatus*. Since lambari is a small fish which measures on average 10 to 15 centimeters long, a composite sample formed by the pool of some specimens was necessary to obtain the analysis of the fish viscera.

Prior to performing any step for the quantification of toxic elements of interest in the fish samples, they were washed with demineralized water and subsequently lyophilized for removal of water from the tissues.

2.2 Instrumental Neutron Activation Analysis (INAA)

Instrumental Neutron Activation Analysis is used for the determination of several elements such As, Fe, Zn among others [16]. The analysis by INAA has been widely used for biological materials because it has many advantages over other analytical methods and has great applicability, as examples, in the use in the analysis of hair, nails, blood, urine and organ tissues [17].

INAA has high sensitivity, accuracy (trueness and precision) with no risk of sample contamination after irradiation [18,19]. INAA is a technique that provides a simultaneous and non-destructive multielement analysis, being an advantageous method and one of the most important of the techniques of qualitative and quantitative analysis of trace elements, as it generates high levels of precision compared to other analytical techniques.

In the comparative method of INAA, the sample is irradiated together with a known amount of the element to be determined in a standard, at the same time and neutron flux. After irradiation, both the standard and the sample are measured using the same system for gamma spectrometry, allowing the unknown mass fraction to be directly calculated from the sample and standard count rates. [20,21]. For this study, this comparative method was used.

For the irradiation and measurement of the radioactivity of the elements of interest such As, Na, K, Br, Co, Cr, Se, Cs, Sc, Rb, Fe and Zn through INAA, approximately 200 mg of the powdered samples were weighted in analytical balance (Shimadzu AEM-5200) in previously decontaminated 1.8 x 1.8 cm polyethylene bag (24 h in 10% v/v Merck HNO_3) and sealed (Selapack). A similar pro-

cedure was performed on two certified reference materials (CRMs) [22]. Elemental standard solutions (Spex CertiPrep) were pipetted into filter papers strips (Whatman 40) using Eppendorf pipettes with previously checked nominal volumes. For some elements, it was necessary to dilute the standard solutions in a 10 mL volumetric flask before pipetting them. The papers strips were dried at room temperature in a laminar flow hood then were folded and placed in polyethylene bags of the same sample size. Each irradiation batch consisted of a sample or CRM and elemental standards. They were irradiated in the IEA-R1 Nuclear Reactor (Nuclear and Energy Research Institute (IPEN) of the Brazilian Nuclear Energy Commission (CNEN) by the pneumatic station for a period of 20 s under thermal neutron fluence rate of approximately 1.9 x 10^{12} cm^{-2} s^{-1}[22]. After irradiation, the element mass fractions were determined by gamma spectrometry of radionuclides of these elements, performed in a CANBERRA HPGe detector (model GC2018) coupled to CANBERRA DSA 1000 digital spectral analyzer. Further information about the pipetted standards and radionuclides used for the element determinations is show in Table 1.

Table 1: Parameters of radionuclides and pipetted standards used in comparative INAA [17]

Element	Radionuclide	Half-Life	Photopeak energy, keV	Element mass in pipetted standard, µg[a]
As	^{76}As	26.32 h	559.1	2.453 ± 0.076
Br	^{82}Br	17.68 min	776.52	1.219 ± 0.038
Co	^{60}Co	5.27 y	1773.24	2.454 ± 0.035
Cr	^{51}Cr	27.7 d	320.08	2.392 ± 0.046
Cs	^{134}Cs	2.06 y	795.85	0.1226 ± 0.0016
Fe	^{59}Fe	44.5 d	1099.25	499.4 ± 2.0
K	^{42}K	12.36 h	1524.58	1497.4 ± 5.0
Na	^{24}Na	14.96 h	1368.6	498 ± 2
Rb	^{86}Rb	18.66 d	1076.60	9.71 ± 0.34
Sc	^{46}Sc	83.81 d	889.28	0.2450 ± 0.0016
Se	^{75}Se	119.77 d	264.66	2.453 ± 0.038
Zn	^{65}Zn	243.9 d	1115.55	24.52 ± 0.31

[a]Expanded uncertainty, $k = 2$

3. RESULTS AND DISCUSSION

3.1 Quality Control

The validation of the INAA method was done from the analysis of the CRMs NIST SRM 1566b, Oyster Tissue and IPEN TP-1 Fish Tissue by calculating z-scores [23-24]. Table 2 shows the obtained values and certified values in parenthesis (with the standard deviation for n = 2). The z-score for each reference material was calculated according to Equations 1 and 2 below and is shown in Figure 2.

$$Z = \frac{X_{lab} - X_{ref}}{\sigma} \tag{1}$$

Where X_{lab} is the mass fraction obtained by the laboratory, X_{ref} is the certified mass fraction value and σ is the target range. In this study, the modified Horwitz equation was used to estimate the reproducibility standard deviation of the method (SR) that was used as the target range, according to Equation 2 [25].

$$S_R = \begin{Bmatrix} 0.22\,c \\ 0.02\,c^{0.895} \\ 0.01\,c^{0.5} \end{Bmatrix} \qquad \begin{pmatrix} If\ c < 1.2\,x\,10^{-7} \\ If\ 1.2\,x\,10^{-7} \leq c \leq 0.138 \\ If\ c > 0.138 \end{pmatrix} \tag{2}$$

*The mass fraction c is expressed in g/g.

The results for z-scores are presented in Table 2. IPEN TP-1 has no certified and reference value for Co e Rb. Arsenic could not be measured for NIST SRM 1566b as the arsenic standard was damaged by error in sample handling.

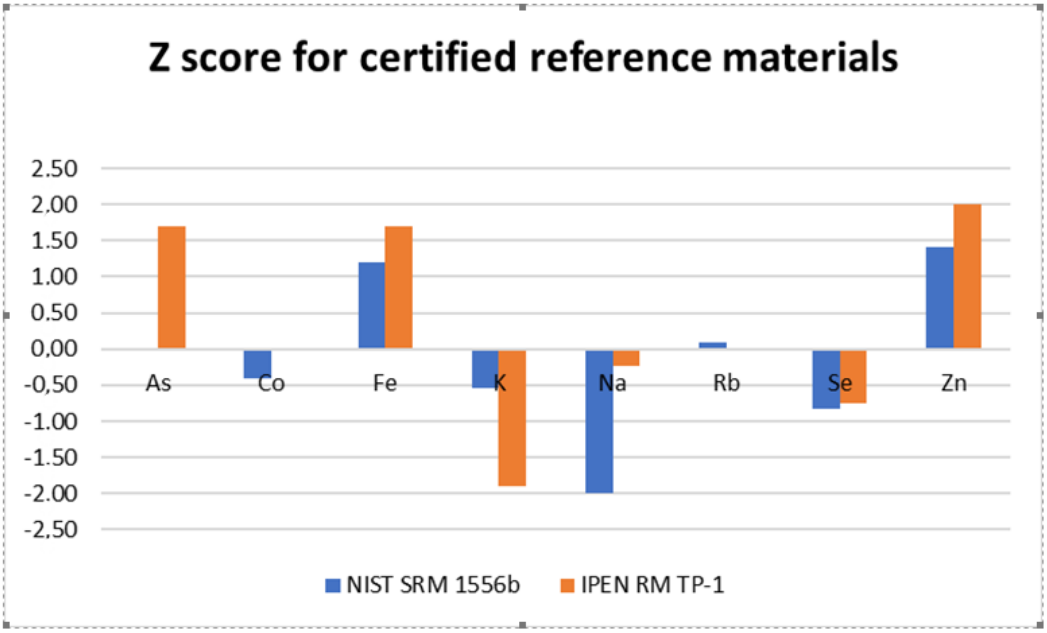
Table 2: Mass fractions obtained by comparative INAA (mean values and expanded uncertainties, k = 2, dry weight) and the certified values of reference materials.

Element	CRM			
	NIST SRM 1556b		IPEN RM TP-1	
	Obtained value (certified value)	z-score	Obtained value (certified value)	z-score
As (mg kg^{-1})	not analyzed (7.65 ± 0.65)	-	7.14 ± 0.25 (5.9 ± 0.27)	1.7
Co (mg kg^{-1})	0.340 ± 0.051	-0.41	0.168 ± 0.021	-

	(0.371 ± 0.009)		(not available on certificate)	
Fe (mg kg^{-1})	223 ± 52 (205.8 ± 6.8)	1.2	16.0 ± 2.3 (13.55 ± 1.05)	1.7
K (٪)	6.4 ± 1.6 (6.52 ± 0.09)	-0.55	11.6 ± 1.7 (12.55 ± 0.49)	-1.9
Na (٪)	2.99 ± 0.15 (3.297 ± 0.053)	-2.0	4.72 ± 0.25 (4.775 ± 0.145)	-0.23
Rb (mg kg^{-1})	2.681 ± 0.061 (3.262 ± 0.145)	0.091	2.59 ± 0.69 (not available on certificate)	-
Se (mg kg^{-1})	1.81 ± 0.12 (2.06 ± 0.15)	-0.83	2.65 ± 0.31 (2.95 ± 0.14)	-0.75
Zn (mg kg^{-1})	1530 ± 379 (1424 ± 46)	1.4	20.1 ± 2.2 (16.65 ± 0.50)	2.0

It is considered approval criteria values |z| < 3, which means that the CRM results should be in the approximately 99% confidence interval of the certified value [26].

Figure 2: Z-score CRM Oyster Tissue NIST SRM 1566b and Fish Tissue IPEN RM TP-1



Values of z-score varied between ± 2, which indicate good accuracy for determination of the mass fraction of As, Co, Fe, K, Na, Rb, Se and Zn in the fish tissues under the same analysis conditions.

3.2. Mass Fraction Determination in Fish Samples

Table 3 shows the mean mass fractions of Br, Co, Cs, Fe, K, Na, Rb, Sc, Se and Zn of specimens of each species. It was decided to report the results with the standard deviation as the natural variation of the specimens is higher than the uncertainty associated with the respective input quantities into account in this study.

Table 3: Element mass fraction in wet weight in fish samples (mean ± SD, n = 5) and range in parentheses.

Element	*Astyanax fasciatus*		*Astyanax bimaculatus*	
	Tissue (mean value)	**Visceras (pool)**	**Tissue (mean value)**	**Visceras (pool)**
As mg kg^{-1}	not analyzed	0.080 ± 0.010	0.025 ± 0.020 (0.010 – 0.050)	0.081 ± 0.011
Br mg kg^{-1}	13.5 ± 2.8 (11.0 – 17.1)	18.11 ± 0.22	11.6 ± 1.4 (10.6 – 12.6)	13.51 ± 0.17
Cs mg kg^{-1}	0.250 ± 0.050 (0.220 – 0.330)	0.1400 ± 0.0041	0.280 ± 0.040 (0.250 – 0.320)	0.1231 ± 0.0041
Co mg kg^{-1}	0.010 ± 0.029 (0.040 – 0.020)	0.0400 ± 0.0031	0.0410 ± 0.0031 (0.0360 – 0.0400)	0.0400 ± 0.0031
Cr mg kg^{-1}	< 0.035*	0.050 ± 0.020	< 0.029*	0.031 ± 0.030
Fe mg kg^{-1}	34.5 ± 5.2 (30.1 – 40.2)	128.4 ± 2.6	35.2 ± 4.6 (30.1 – 40.2)	97.6 ± 2.4
K %	10.6 ± 1.3 (8.6 – 11.4)	5.76 ± 0.33	10.92 ± 0.36 (10.66 – 11.17)	5.31 ± 0.30
Na	1.67 ± 0.24	1.460 ± 0.010	1.95 ± 0.17	1.1900 ± 0.0041

%	(1.46 – 2.02)		(1.83 - 2.07)	
Sc g kg^{-1}	3.14 ± 0.10 (2.11 – 4.51)	12.30 ± 0.20	3.15 ± 0.13 (3.30 – 3.70)	5.10 ± 0.10
Se mg kg^{-1}	1.03 ± 0.06 (0.92 – 1.05)	2.130 ± 0.051	0.80 ± 0.17 (0.68 – 0.92)	1.431 ± 0.091
Rb mg kg^{-1}	12.3 ± 2.9 (9.4 – 16.2)	8.10 ± 0.19	14.21 ± 0.46 (13.9 – 14.5)	7.89 ± 0.19
Zn mg kg^{-1}	103.0 ± 1.7 (101.1 – 105.1)	130.0 ± 1.3	62.3 ± 2.5 (60.5 – 64.0)	106.1 ± 1.0

* LD - less than the detection limit

Similar results were obtained for Zn at the same research area, in Riacho Grande Region, Billings Reservoir, since it was the element with the highest concentration for the *Astyanax* spp. tissues in a study by Oliveira [27]. In this study and in a study by Rocha et al. [28] it was also verified that Cr presented samples with values below the detection limit (1.0 μg kg^{-1}). The other elements (As, Br, Cs, Co, Fe, K, Na, Sc, Se and Rb) were not analyzed in both studies [27, 28].

In this study, it was verified that the Zn concentrations were higher in the viscera than in the muscle of the *Astyanax* spp fish. Similar result was also obtained by a study done in the Billings Reservoir about Zn [29].

As *Astyanax* spp. are small fishes, often little or no evisceration is carried out by the fisherman and, together with the musculature, end up being consumed large portions of the contaminated digestive tract. Therefore, it is important to compare the results of the viscera and muscle.

The variation in Zn content was 60.5 to 105.10 mg kg^{-1} in the musculature and 106 to 130 mg/kg^{-1} in the viscera. In Brazilian legislation, the Resolution 269, September 22 of 2005 recommended by ANVISA the daily intake of an adult of 7 mg of Zn [29]. Zinc is an essential and useful element in metabolism, but in cases of food poisoning, it can cause lung problems, fever, chills, gastroenteritis, drowsiness, nausea, dehydration, and muscular incoordination [27]. For both species, *Astyanax fasciatus* and *Astyanax bimaculatus*, the visceral element mass content values are higher than in the musculature for the Br, Fe, Se and Zn.

The daily Fe intake is 14 mg and for Se the daily recommendation is 34 μg, for the recommended daily intake of Cr is 35 μg [30]. Other elements (As, Br, Cs, Co, K, Na, Sc and Rb)

are not regulated by this resolution. In an ongoing study, the authors observed that fishermen in the region consume in average at least 2 to 3 servings of fish per week. By the concentration obtained and considering a 200 mg portion of fish, the elements Fe, Se, Cr and Zn are may be within the recommended limit. But for a conclusion one should make a daily assessment of the entire diet of the fisherman of the region.

Although no significant amounts of As element was found in the analyzed fish, a study carried out in the region indicates that sediments have the concentration of As in the range of 13 to 22 mg kg^{-1}[30]. Arsenic is toxic even at very low concentrations; therefore, it is particularly worrisome for health [31].

The element Sc is in the same group in the periodic table as element Ca and therefore its chemical properties are similar to those of calcium. For this reason, strontium behaves like calcium in the body. Calcium is important in bones and there are cellular mechanisms to hijack calcium in the bone cells from which it is established to form the structure of the bone. Strontium is sequestered and established in bone by the same system, and although there is no legislation to the amount ingested, it can cause problems when in excess [32]. Regarding bromine, it is considered nonessential to human health and it can be combined with hemoglobin causing hematologic diseases [33]. For Sc and Br, the concentration was higher in the viscera compared to muscle tissue.

The elements K and Na are considered essential elements. It is lack leads to metabolic functions mediated by inadequate enzymes and results in organ defects, chronic diseases and, ultimately, death. Therefore, regular intake of these elements through food intake is necessary [34, 35]. Co is essential trace element, having a complex role in many body functions, but it is toxic in large amount [36]. The element potassium had higher concentration in the tissue compared to viscera. Na and Co had low concentration in both.

Few studies can be found in the literature that study rubidium and cesium in fish. Rb and Cs have similar physical and chemical properties, hence behaving similarly when accumulating within fish tissues. Rb and Cs are commonly considered to be biochemical analogs of K and to possess similar physiological behaviors [37]. Rubidium is generally listed as having a low degree of toxicity [38]. The obvious manifestations of toxicity in Cs e Rb are chiefly in the neuromuscular sphere, in test with the animals usually becoming progressively more irritable and finally dying in convulsions [39]. The variation in Rb was high in musculature as comparation the viscera. The musculature

content was 9.4 to 16.2 mg kg-1 in the musculature and 8 mg kg-1 in the viscera. As for Cs, its concentration was low in all the fish parts considered.

4. CONCLUSION

The procedure for the characterization of edible fish tissues by INAA was adequate, with satisfactory z-scores for the used CRMs under the same irradiation conditions. Br, Fe, Se and Zn were present at higher concentrations in the guts compared to the musculature tissues. The analysis of this accumulation difference is important for this case, since the *lambari* fish is eaten as a whole, without removing the viscera by many fishermen. There are no studies in the literature related to the elements Br, Cs, Co, K, Na, Sc and Rb in the region of Riacho Grande, but it is considered important to analyze these elements for future correlations and comparisons related to food security. Although food safety has been discussed in this paper, conclusions in this direction require further analysis. New analyzes and investigations will be carried out in a near future.

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