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DETERMINATION OF TOXIC ELEMENTS IN FISH OF THE GENUS ASTYANAX 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*) collected at Billings Dam by fishermen from the Riacho Grande District - (São Bernardo do Campo city/SP) 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 and contributes to the food safety assurance, regarding inorganic contaminants referred by the The Brazilian Health Regulatory Agency (ANVISA).

1. INTRODUCTION

1.1 Toxic elements at the Billings Dam, Riacho Grande, São Bernardo do Campo, SP - 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 Watershed Protection Area (APM). The region also has the presence of great biodiversity and is considered an area 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 Dam are located 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]. For the region of springs and the presence of great biodiversity, Riacho Grande is protected by the State Law nº 1,172 of November 17, 1976.

The presence of the ferries 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 [6]. However, surface waters - such as water sources - are rarely free of contamination, even in places with little or no human presence [7].

Disorganized occupations around the Billings Dam, pollution generated by urban and rural activity, runoff from domestic rains and manure cause changes in 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 this contamination can occur mostly through food, such as the ingestion of fish contaminated with Hg, methylmercury (MeHg), among other elements [8, 9, 10].

However, many fishermen and family members, because they are unaware of the health risks associated with the consumption of fish from contaminated water, 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 fishermen members of Formership Z-17 Orlando Feliciano from São Bernardo do Campo will be investigated. The Formenship is located in Riacho Grande and receives fishermen from São Bernardo do Campo, Santo André and São Caetano do Sul, in order to offer support to artisanal fishermen.

1.2 Billings Dam Fishing Products and Food Security

At the Billings Dam, the most common fish to be found are *Tilapia rendali, Oreochromis niloticus, Astyanax spp, Cyprinus carpio*, *Hoplias malabaricus* and *Geophagus brasilienses* [11]. 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 per 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 dam [11].

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 [5] 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 taken into account: quantity, quality and regularity in access to food [12].

The quality of food consumed is a very important aspect, since the food available for consumption by the population cannot be subjected to any type of contamination risks [12]. 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 metals by ingestion. High concentrations of As and Se, for example, may cause cancer of the skin, lungs, liver and bladder, can result in respiratory tract irritation, bronchitis, difficulty breathing, and stomach pains. Longer-term exposure can cause respiratory irritation, bronchial spasms, and coughing. Other elements may also cause damage to the digestive and respiratory systems [13].

2. METHODOLOGY

2.1 Collection of fish samplings

The lambari (Astyanax genus) samples used in this analysis were obtained through artisanal fishermen from the Billings Dam. Samples were collected in the second half of 2018. Since lambari is a small sample, which measures on average 10 to 15 centimeters, a composite sample formed by the pool of some specimens of the same order 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 As, Fe, Zn among others [14]. The analysis by INAA has been widely used 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, organ tissues [15].

The INAA has high sensitivity, precision and accuracy with no risk of sample contamination after irradiation [16, 17]. 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.

The method can be comparative or absolute. Measurement by the absolute method uses some nuclear factors, such as neutron flux, isotope abundance of the target nucleus, among others [18]. In the comparative method, the sample is irradiated together with a known amount of the element to be determined 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 concentration to be directly calculated from the sample and standard count rates and the mass of both [19]. For this study this comparative method was used.

For the irradiation and measurement of the radioactivity of the elements of interest such as 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 procedure was performed for two certified reference materials (CRM) *Micropogonias furnieri* [20] and NIST SRM 1566b - Oyster Tissue [21]. 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 (IPEN – CNEN/SP) by the pneumatic station for 20 s under thermal neutron flux of approximately 1.9 x 10^{12} cm^{-2} s^{-1}. After irradiation, the elements 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 [22].

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

[a]Expanded uncertainty, $k = 2$

3. RESULTS AND DISCUSSIONS

3.1 Quality Control

The validation of the INAA method was done from the analysis of the CRMs NIST SRM 1566b and *Micropogonias furnieri* TP-1 IPEN by calculating the z score. 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 Equation 1 and 2 below and is shown in Figures 1 and 2.

$$Z = \frac{X_{lab} - X_{ref}}{\sigma} \quad (1)$$

Where X_{lab} is the concentration obtained by the laboratory, X_{ref} is the certified concentration 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 [23].

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

*The concentration *c* is expressed in g/g.

For results obtained experimentally by the comparative method of INAA for the Oyster Tissue 1556b, and Micropogonias furnieri TP-1 IPEN, their certified values and calculation of z-scores are presented in Table 2. The z-score was obtained using the modified Horvitz equation. The *Micropogonias furnieri* has no certified and reference value for Co e Rb. Arsenic could not be measured 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.

	CRM			
	NIST SRM 1556b		**IPEN RM TP-1**	
Element	**Obtained value (certified value)**	**z-score**	**Obtained value (certified value)**	**z-score**
As (mg kg^{-1})	not obtained experimentally (7.65 ± 0.65)	-	7.14 ± 0.25 (5.9 ± 0.27)	1.7
Co (mg kg^{-1})	0.340 ± 0.051 (0.371 ± 0.009)	-0.41	0.168 ± 0.021 (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 [24]. Z-score values are presented in Table 1 and in Figures 1 and 2.

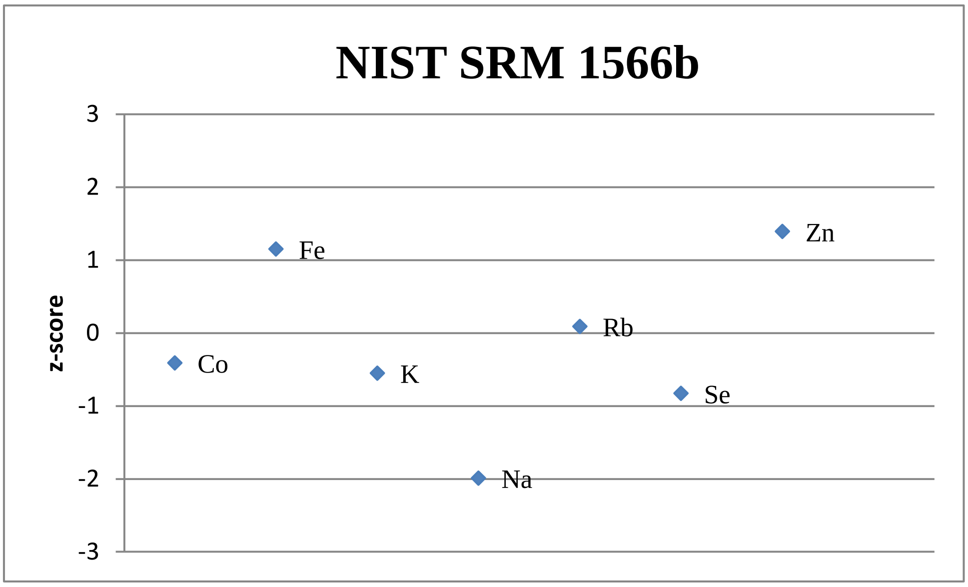

Figure 1: Z-score for Oyster Tissue reference material - NIST SRM 1566b

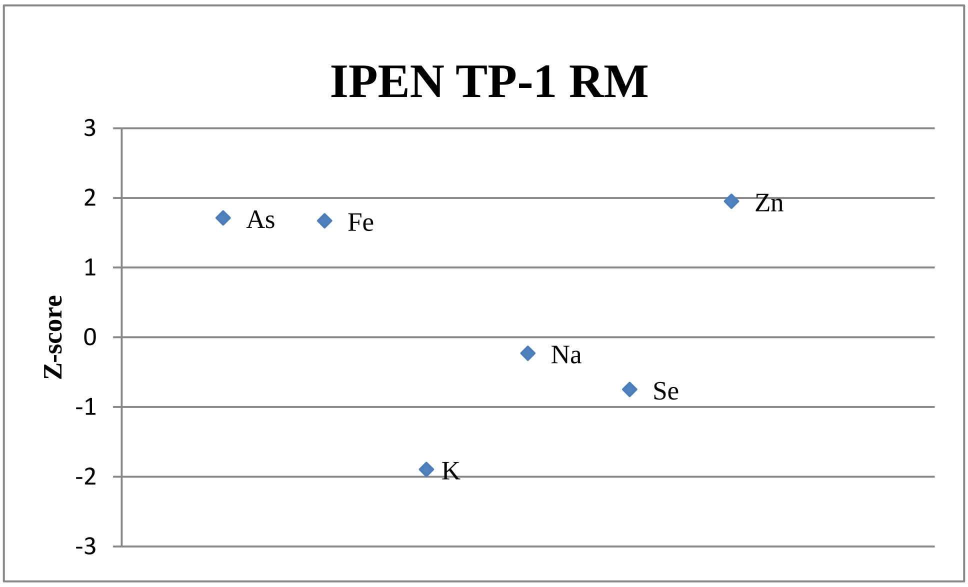

Figure 2: Z-score for *micropogonias furnieri* reference material – IPEN RM TP-1

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 fraction of Na. K. As. Br. Fe. Zn. Co. Se. Cs. Sc and Rb of specimens of each species. It was decided to reports the results with the standard deviation. as the natural variation of the specimens is higher than the imprecision associated with the respective input quantities into account in this study.

Table 3: Element mass fraction in wet weight in fish samples (mean ± SD) and range in parenthesis).

Element	Fish species			
	Astyanax fasciatus		*Astyanax bimaculatus*	
	Tissue (mean value)	**Visceras (pool)**	**Tissue (mean value)**	**Visceras (pool)**
As mg kg^{-1}	not obtained experimentally	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)	18.1 ± 0.22	11.6 ± 1.4 (10.6 – 12.6)	13.5 ± 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 (1.46 – 2.02)	1.460 ± 0.010	1.95 ± 0.17 (1.83 - 2.07)	1.1900 ± 0.0041
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 ± 0.061 (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	14.2 ± 0.46 (13.9 – 14.5)	7.89 ± 0.19
Zn mg kg^{-1}	103.0 ± 1.7 (101.1 – 105.1)	13.0 ± 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 dam, since it was the element with the highest concentration for the *Astyanax* spp. [25]. In this study and in the study of [26] it was also verified that Cr presented samples with values below the LQ (1.0 μg kg^{-1}). The other elements (As, Br, Cs, Co, Fe, K, Na, Sc, Se and Rb) were not analyzed in these studies [25, 26].

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 Dam [26]. The other elements (As, Br, Cs, Co, Fe, K, Na, Sc, Se and Rb) were not analyzed in this study.

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.

The variation in Zn content was 101.10 to 105.10 μg/g in the musculature and 130 μg/g in the viscera. In Brazilian legislation, resolution 269, September 22 of 2005 is recommended by ANVISA the daily intake of an adult of 7 mg of Zn [27]. 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 [26]. In the future, fishermen will be surveyed on the amount of daily fish intake and their relationship with food safety for zinc and other elements.

For both species, *Astyanax fasciatus* and *Astyanax bimaculatus,* the visceral values are higher than in the musculature for the Br, Fe, Se and Zn elements, which indicates that for these elements the highest concentration in the viscera of the fish.

The daily Fe intake is 14 mg. For Se the daily recommendation is 34 μg. And the recommended daily intake of Cr is 35 μg [27]. Other elements (As, Br, Cs, Co, K, Na, Sc and Rb) are not regulated by this resolution. Due to the low incidence of studies on these elements in the literature in the context of this work, the data could not be compared with other studies.

Although no significant amounts of As were found in the analyzed fish, a study carried out in the region indicates that water and sediments have the concentration of As in the range of 13 to 22 mg kg^{-1}[28]. There were no studies related to As and fish in Billings dam in the literature.

4. CONCLUSIONS

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 elements were found to have a higher concentration 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. 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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