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Arsenic Content in Trahira (*Hoplias Malabaricus)* from São Paulo, Brazil, Determined by INAA

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1. Introduction

Fish consumption is of great importance for human health as fish is an excellent source of nutrients [1] as essential elements, high-quality protein, vitamins, and omega-3 acids [2]. However inorganic contaminants are also present in the aquatic environment, including toxic elements such as mercury, lead and arsenic, coming from natural and anthropogenic sources [3]. Concern about these contaminants occurs as inorganic contaminants are present in bioaccumulation and biomagnification processes associated to the food web [4] and human dietary exposure to contaminants may occur when eating fish [5].
Therefore, the determination of toxic and potentially toxic elements in fish edible tissues is very important. Inorganic arsenic, is a well-known human carcinogen and Reservoir with fish contaminated has led to human exposure, mainly artisanal fishermen who can consume fish more frequently. In this study, the mass fraction of arsenic (As) in tissues of trahira (*Hoplias malabaricus* (Bloch, 1794)) was determined by the comparative method of Instrumental Neutron Activation Analysis (INAA). Twenty specimens of trahira - one of the most consumed fish by Billings Reservoir artisan fishermen – were analyzed. Results obtained for As under the same irradiation conditions for Certified Reference Materials (CRM) are in agreement with their certificate values, which indicate that the performed analyses were appropriate.

2. Methodology

Twenty individuals of the species trahira (*Hoplias malabaricus*) were purchased from fishermen at the Billings Reservoir in the southeast of São Paulo State: (23° 49' 27" S; 46° 32' 26" W). The fish was collected in October, 2019. After collection, they are transported between layers of ice to the Neutron Activation Analysis Laboratory (IPEN/CNEN).
The fish remained in refrigerators until the time of their preparation. They were weighed, washed with purified water (Milli-Q), the tissues commonly eaten by Brazilians were freeze-dried (Thermo Savant Modulyo D, Thermo Electron Corporation), milled, homogenized and stored in decontaminated flasks. The residual humidity content was measured before the analysis by the oven drying method until constant mass.
Approximately 200 mg of the powdered samples and 150 mg of each certified reference materials CRM were weighted in analytical balance (Shimadzu AEM-5200) in previously decontaminated 1.8 x 1.8cm polyethylene bag (24 h in 10% v/v Merck HNO3) and sealed (Selapack). (NIST SRM 1556b - *Oyster tissue* and NRCC DORM-4 - *fish protein*) were utilized [6, 7]. Elemental standard solution (Spex CertiPrep) were pipetted into filter paper strips (Whatman 40) using Eppendorf pipette with previously checked nominal volume. The paper 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 sample, CRM and elemental standard. They were simultaneously irradiated in the IEA-R1 Nuclear Reactor (IPEN/CNEEN) for

8 h under thermal neutron flux of approximately $5x10^{12}$ cm^{-2} s^{-1}. After irradiation, As was determined by gamma spectrometry in a CANBERRA HPGe detector (model GC2018) coupled to a CANBERRADSA 1000 digital spectral analyzer. Further information about the pipetted standard and radionuclide used for the element determinations is shown in Table I.

Table I: Parameters of radionuclide and pipetted standard used in the INAA comparative method [22].

Element	Radionuclide	Energy, keV	Half-Life, h	Element mass in pipetted standard, µg[a]
As	^{76}As	559.1	26.32	2.422 ± 0.026

[a]Expanded uncertainty, $k = 2$

After a seven-day decay period, induced gamma activities were measured for ^{76}As radionuclides for a period of 7200 s. Element mass fraction calculations were carried out using a Microsoft Excel spreadsheet.

3. Results and Discussion

Table II presents experimental mass fraction values obtained for the CRMs using INAA method, certified values as well as calculated z-scores. In this study, z-scores were calculated using the modified Horwitz equation as criterion to approval values $|z| < 3$, which means that the CRM should be in the approximately 99% confidence interval of the certified value [8].

Table II: As mass fractions obtained by comparative INAA (mean values and expanded uncertainties, $k = 2$, dry weight, $n = 5$) and the certified values of reference materials.

Element	NRCC DORM-4		NIST SRM 1556b	
	This study (certified value)	z-score	This study (certified value)	z-score
As (mg kg^{-1})	6.96 ± 0.95 (6.80 ± 0.64)	0.21	7.03 ± 0.72 (7.65 ± 0.65)	-0.69

It is considered criteria to approval values $|z| < 3$, which means that the CRM result should be in the approximately 99% confidence interval of the certified value [9]. Values of z-score varied between ± 1, which indicates good accuracy for determination of the mass fraction of As in the simple fish under the same analysis conditions. Table III shows the mean mass fraction of As for the twenty specimens of trahira.

Table III: Element mass fraction in wet weight in fish samples, n= 20 (mean ± SD, range in parenthesis).

Element	*Hoplias malabaricus* (n= 20)
As, mg kg^{-1}	0,44 ± 0,22 (0,03-0,74)

Trahira is a predatory fish that feeds on small fish such as lambari (*Astyanax spp*). Results obtained in this study for the As element in *Hoplias malabaricus* were lower than the values permitted by the Brazilian legislation and international legislations [10, 11, 12, 13].

Results obtained in other study, in areas of gold mining in the region of Alto Guaporé in Mato Grosso do Sul, state of southwestern Brazil the level of As was also under the values permitted by the Brazilian

legislation (0,022 ± 0,011) mg kg^{-1} for trahira fish [14]. But other study from Taim wetlands, a Ramsar site in southern Brazil, was higher the legal limit (13,1 ± 3,2) mg kg^{-1} for this species [15].

4. Conclusions

The results obtained in this study offer information about As mass fraction in the edible fish tissue of the species trahira (*Hoplias malabaricus*), one the consumed fish species by fisherman of Billings Reservoir in São Paulo state. The procedure for the characterization of edible fish tissues by INAA was adequate, with satisfactory z-score results for the used CRMs under the same irradiation conditions. The As content determined in trahira samples were lower than the values permitted by the Brazilian legislation and international legislations. These data are important in view of toxicology; food safety and environmental protection are in accordance with previous studies. More accurate conclusions regarding food safety require further analyses, such As speciation.

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