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# PRELIMINARY STUDY ON ELEMENT MASS FRACTION DETERMINATION ON CATFISH SAMPLES FROM PARAGUAY

**Edson G. Moreira[1], Sixto A. Frutos[2], Mario S. Insaurralde[2], Marilia G. M. Catharino[1], Marina B. A. Vasconcellos[1]**

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
emoreira@ipen.br
mbvascon@ipen.br
mariliasemmler@uol.com.br

[2] Departamento de Pesca y Acuicultura
Facultad de Ciencias Veterinarias de la Universidad Nacional de Asunción
Campus Universitario, San Lorenzo
Mariscal Estigarribia Km 11 (910) Paraguay
tony8013@hotmail.com
insaurraldemar9@hotmail.com

## ABSTRACT

South American catfish (*Pseudoplatystoma*), commonly known in Spanish as *atigrado* or *surubí* and in Portuguese as *surubim* or *pintado* is a large fish that typically reaches 1 m long and weighs 60 kg to 80 kg and may be found at the basins of the Amazon, the São Francisco and de la Plata rivers, usually in riverbeds and deep wells. Being a much appreciated fish for human consumption, it is quite sought after by fishermen who have been contributing to the reduction of the stocks. This fact attracted the attention of the Paraguayan authorities to the point of imposing restrictions to free fishing and commercialization. This study aims to assist the conservation efforts towards this fish by investigating its exposure to possible pollutants. Preliminary results on element determination on six samples of catfish from Paraguayan rivers are presented. Cs, Co, Fe, Se and Zn were determined by applying an Instrumental Neutron Activation Analysis method. While these element levels were lower than the legislation for human consumption, the elements As, Cr e La were not detected in the samples as they are below the detection limit of the method employed. Atomic Absorption Spectrometry was used to investigate the presence of Cd, Hg and Pb in the samples. Hg was detected in the samples while Cd and Pb were below the detection limit of the method.

## 1. INTRODUCTION

Catfishes of the genus *Pseudoplatystoma* Bleeker (Siluriformes; Pimelodidae) are large, neotropical migratory predators that inhabit the major river basins of South America, such as Paraná, Amazon, São Francisco and Orinoco [1, 2]. These long-whiskered catfishes are familiar due to their marked color pattern and are found in diverse habitats including rivers, lakes, and flooded forests [1]. Because of their large size, they are important food fish in South America and many species have been hybridized in an effort to get better growth performance in aquaculture, even though it has been established that hybridization between species is one of the primary causes for biodiversity loss [3-5]. These catfish also play

important ecological role in their environments due to their voracious predatory behaviour [6, 7].

*Pseudoplatystoma corruscans* (Spix & Agassiz, 1829), popularly known as spotted sorubim in English, *surubim* or *pintado* in Portuguese and *atigrado* or *surubí* in Spanish, represents an important fisheries resource, reaching up to 145 cm in size and weighing up to 120 kg. It is a native species of high economic value in South American fisheries, and its cultivation is spreading rapidly. This species presents characteristics that meet the current and future preferences of the market and make the meat a product of interest in national and international markets. It is also a major target of both professional and amateur fishing. The commercial importance of this species is due mainly to the high quality of its meat, high market value and its remarkable participation in commercial fishing. For this reason the species is suffering sharply from the fishing effort in the various regions in which it occurs [8]. *Pseudoplatystoma fasciatum* (Linnaeus, 1766), known as barred sorubim in English and as *cachara* in Portuguese, reaches a maximum length of 104 cm and weighs up to 20 kg. It is also much appreciated as a fishing resource [9].

The decline of fish stocks is a well-known problem worldwide and it is not different for South American catfish species. Uncontrolled pollution of water sources, construction and operation of hydroelectric dams, deforestation and inadequate fishery management policies among other human activities that impact the environment, have compromised the global stocks of fish [10, 11]. In the freshwater environments of South America, migratory fish are being decimated at an accelerated pace without the establishment of proper techniques of exploitation [8]. Spotted sorubim is considered critically endangered in São Paulo state in Brazil as dams block its reproductive migration, but also due to overfishing [12]. In Brazil, fisheries management and regulations are based on classic methods such as closed seasons during spawning migrations, limits on mesh size, minimum catch sizes and prohibition of certain kinds of gear [10]. Overexploitation also attracted the attention of the Paraguayan authorities to the point of imposing restrictions to free fishing and commercialization.

This study aims to assist the conservation efforts towards this fish by investigating its exposure to possible pollutants. It is part of a collaborative scientific study supported by the International Atomic Agency (IAEA) in the framework of a Latin America and Caribbean Regional Project (ARCAL) dealing with seafood safety and biomonitoring of contaminants in molluscs and fish [13]. Preliminary results on element determination on six samples of catfish from Paraguayan rivers are presented.

## 2. EXPERIMENTAL

### 2.1. Study area and sample pre-treatment

A spotted sorubim specimen (*Pseudoplatystoma corruscans* (Spix & Agassiz, 1829)) was collected at Remanso site of Paraguay River, close to Asunción, while other 4 specimens of spotted sorubim and one specimen of barred sorubim (*Pseudoplatystoma fasciatum* (Linnaeus, 1766)) were collected at the Tebicuary River, a tributary of Paraguay River, located at the southwestern part of the country.

Table 1 lists the catfish samples analyzed in this study. Catfish individuals were collected in January 2011, beheaded, gutted and kept under refrigeration until transport to the UNA Veterinary Faculty. At Veterinary laboratories, representative muscle tissues were collected at the dorsal part of the fish samples. Tissues were frozen before transporting to IPEN – CNEN/SP. Figure 1 presents the typical aspect of the collected samples. In this study, recommendations from the IAEA ARCAL project for sampling and sample pre-treatment were followed [14].

**Table 1: List of catfish samples analyzed in this study**

| Sample | Species | Sampling site | Mass[a], kg | Moisture[b], % |
|---|---|---|---|---|
| 1 | *Pseudoplatystoma corruscans* | Tebicuary River | 4.30 | 81.2 |
| 2 | *Pseudoplatystoma fasciatum* | Tebicuary River | 4.85 | 80.2 |
| 3 | *Pseudoplatystoma corruscans* | Tebicuary River | 5.00 | 81.0 |
| 4 | *Pseudoplatystoma corruscans* | Paraguay River | 5.44 | 83.0 |
| 5 | *Pseudoplatystoma corruscans* | Tebicuary River | 5.65 | 80.7 |
| 6 | *Pseudoplatystoma corruscans* | Tebicuary River | 5.65 | 80.7 |
| 7 | *Pseudoplatystoma corruscans* | Tebicuary River | 7.75 | 79.2 |

[a] gutted fish mass;
[b] mass loss due to freeze drying process;

**Figure 1: Typical aspect of collected samples.**

After sample collection and transport to IPEN-CNEN/SP, fish tissues were properly treated prior to analysis. Samples were removed from the freezer and allowed to thaw at room temperature. After thawing, the outer parts of the tissues were removed with a titanium knife. The remaining parts were freeze-dried in a Thermo Savant Modulyo D freeze-drier (Thermo Electron Corporation) and the mass loss was calculated. After freeze-drying, the samples

were ground in a mill provided with glass balls (IKA Ultra-Turrax) and kept in freezer until the time of analysis. Residual moisture contents of the freeze-dried samples were determined in an oven at 85$^{o}$C for 24 h using subsamples of approximately 0.5 g.

Element mass fractions were determined in triplicate. For the validation of the methods the following certified reference materials (CRM) were used: “dogfish muscle” (NRCC DORM-2) and “fish tissue” (IAEA-407) for the INAA analysis an “oyster tissue” (NIST SRM 1566b) and “mussel tissue” (NIST SRM 2976) for AAS analysis.

## 2.2. Instrumental Neutron Activation Analysis

The comparative method of Instrumental Neutron Activation Analysis (INAA) was used for the determination of Co, Cs, Fe, Se and Zn in the catfish samples as well as in certified reference materials (CRM) used as control samples [15]. The presence of As, Cr and La were also investigated, but these elements were below the respective detection limits for the used method. Some of these investigated elements may not be considered pollutants to the Paraguay River basin environment but their determination was promptly available from the used method and their results may be used for future comparisons.

### 2.2.1. Sample and elemental standards preparation

Subsamples of approximately 0.2 g were weighed in properly cleaned polyethylene bags using a Shimadzu AEM-5200 analytical balance. Elemental standards were prepared by pipetting Spex standard element solutions onto Whatman paper filters, using variable volume pipettes (Eppendorf). For some elements, the original solution was diluted in volumetric flasks prior to pipetting. After drying, paper filters were kept in polyethylene vials with the same geometry as for the samples. Catfish sample analyses were performed in triplicate.

### 2.2.2. Irradiation and element determination

Catfish subsamples, CRM subsamples and elemental standards were simultaneously irradiated for 8 hours at $10^{12}$ cm$^{-2}$ s$^{-1}$ thermal neutron flux of the IEA-R1 Nuclear Research Reactor at IPEN-CNEN/SP. $^{60}$Co, $^{134}$Cs, $^{59}$Fe, $^{75}$Se and $^{65}$Zn radionuclides were measured for 10 hours, after a 15-day decay period. Gamma ray measurements were performed using a GC2018 Canberra HPGe detector coupled to a Canberra DSA-1000 multichannel analyzer. Gamma ray spectra were collected and processed using a Canberra Genie 2000 version 3.1 spectroscopy software. Element content calculations were carried out using a Microsoft Excel spreadsheet for suitable radionuclide photopeak energies.

## 2.3. Atomic Absorption Spectrometry

Hg was determined in the samples by Cold Vapor Atomic Absorption Spectrometry (CV AAS) using a Perkin Elmer FIMS spectrometer. It was observed that the concentrations

of Cd and Pb were below the detection limits of the Electrothermal Atomic Absorption Spectrometry (ET AAS) methods used, using a Perkin Elmer AAnalyst 800 spectrometer.

### 2.3.1. Sample digestion and elemental standards preparation

Catfish and CRM subsamples with approximately 0.35 g were weighed into perfluoroalkoxy vials (Savillex). 4 mL of P. A. nitric acid (Merck) and 1 mL of 20 % (v/v) hydrogen peroxide (Merck) were added to the subsamples and the mixtures were allowed to stand overnight for approximately 15h. Subsequently, the vials were placed in a digestion block at 90°C for 3 h. After completion of the digestion, digests were allowed to cool to room temperature and the volume of the vials was adjusted to 25 mL [16].

Hg standard solutions with nominal concentrations of 0.5, 1.4, 2.3, 3.3 and 4.0 ng $mL^{-1}$ for construction of the analytical curve were prepared from a 0.1 mg $mL^{-1}$ Hg stock solution. Reagent blanks were also prepared, consisting of the same volume of reagents for digestion. Standard solutions and reagent blanks went through the same process of digestion of the samples. To use the FIMS spectrometer, a 3% (v/v) solution of P. A. HCl (Merck) was used as the carrier solution and a 3 % (w/v) solution of P. A. $SnCl_2 \cdot 2H_2O$ (Merck) was used as the reducing agent solution. Catfish samples analyses were performed in triplicate.

Cd standard solutions with nominal concentrations of 1.3, 2.6, 4.0, 5.3 and 7.0 ng $mL^{-1}$ were prepared by dilution of a 7.0 ng $mL^{-1}$ stock solution by the AS-800 autosampler for construction of the analytical curve. The autosampler also diluted a 15.0 ng $mL^{-1}$ Pb stock solution to prepare standard solutions with nominal concentrations of 3.0, 6.0, 9.0, 12 and 15 ng $mL^{-1}$. Both stock solutions were prepared daily from 1000 µg $mL^{-1}$ standard solutions provided by Perkin Elmer.

### 2.3.2. Element determination

Analytical curve fitting was performed by the Winlab 32 for AAS software using the least squares method for the determination of Hg, Cd and Pb. For Hg determination, peak height mode was used at the 254 nm wavelength of the Hg lamp. For Cd, an electrodeless discharge lamp at 228.8 nm was used while for Pb an electrodeless discharge lamp at 283.3 nm was used. In both cases, peak area mode and default equipment parameters were used. $NH_4H_2PO_4$ 0.5 % (m/v) and $Mg(NO_3)_2$ 0.03 % (m/v) were added to the graphite tube as chemical modifier prior to every atomization.

## 3. RESULTS AND DISCUSSION

### 3.1. Certified reference materials

Table 2 presents the element mass fractions obtained by INAA for NRCC DORM-2 and IAEA-407. La was not detected in the CRMs and there are no certified values for this element in the analyzed CRMs. Table 3 presents mercury results obtained by CV AAS for

NIST SRM 1566b and NIST SRM 2976 CRMs. The Horwitz modified function was used to estimate target reproducibility standard deviations for *z* score calculations [15].

**Table 2: Mass fraction, mg kg$^{-1}$, obtained by INAA for NRCC DORM-2 and IAEA-407 Certified Reference Materials**

| Element | Certified Reference Material | | | | | |
|---|---|---|---|---|---|---|
| | NRCC DORM-2 | | | IAEA-407 | | |
| | This study[a] | Certified Value | *z* score | This study[a] | Certified Value | *z* score |
| As | 15.66 ± 0.44 | 18 ± 1.1 | -1.3 | 11.40 ± 0.35 | 12.6 ± 0.3 | -0.87 |
| Cr | 32.0 ± 3.8 | 34.7 ± 5.5 | -1.6 | 0.53 ± 0.08 | 0.73 ± 0.06 | -1.6 |
| Cs | 0.223 ± 0.040 | - | - | 0.070 ± 0.016 | - | - |
| Co | 0.192 ± 0.024 | - | - | 0.0789 ± 0.0091 | 0.1± 0.01 | 0.96 |
| Fe | 137 ± 10 | 142 ± 10 | -0.49 | 137 ± 23 | 146 ± 3 | -0.80 |
| Se | 1.26 ± 0.11 | 1.4 ± 0.9 | -0.64 | 2.37 ± 0.10 | 2.83 ± 0.13 | -1.2 |
| Zn | 23.3 ± 2.3 | 25.6 ± 2.3 | -0.92 | 66.0 ± 2.9 | 67.1 ± 0.8 | -0.19 |

[a]Mean and standard deviation, $n = 3$.

**Table 3: Hg Mass fraction, µg kg$^{-1}$, obtained by CV AAS for NIST SRM 1566b and NIST SRM 2976 Certified Reference Materials**

| Element | Certified Reference Material | | | | | |
|---|---|---|---|---|---|---|
| | NIST SRM 1566b | | | NIST SRM 2976 | | |
| | This study[a] | Certified Value | *z* score | This study[a] | Certified Value | *z* score |
| Hg | 31.7 ± 5.7 | 37.1 ± 1.3 | -0.67 | 61.0 ± 3.6 | 67.1 ± 6.0 | 0.46 |

[a]Mean and standard deviation, $n = 3$.

Obtained *z* scores results for element mass fractions in the analyzed CRMs were all in the range $-2 \leq z \leq 2$, suggesting that the INAA and AAS methods are satisfactory for the analysis of the investigated elements.

### 3.2. Element determination in catfish samples

Table 4 presents element mass fraction results obtained in wet weight for the catfish samples, using INAA and AAS techniques. The mass fraction of the elements Co and Cs were calculated from the gamma rays spectra obtained for the samples. However, reported concentrations are of the same order of magnitude of the respective detection limits.

As a general trend, low element mass fractions were obtained for the catfish samples regardless of species or site of collection. Even for Hg, obtained values were much lower than the maximum limit for human consumption of predatory fish, according to Brazilian legislation (1 mg kg$^{-1}$) [18]. These observations lead to the conclusion that the investigated sites are still pristine regarding the presence of toxic elements. However, this conclusion should be treated with care as the number of analyzed specimens is small, the analyzed

species present migratory behaviour and also because due to their relative low weight, they cannot be considered adult individuals which could possibly present extended bioaccumulated toxic elements during their lifespan.

**Table 4: Element mass fraction, mg kg$^{-1}$, in catfish samples (wet weight basis)[a]**

| Element | Sample | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| As | < 0.29 | < 0.29 | < 0.29 | < 0.29 | < 0.29 | < 0.29 |
| Cd, µg kg$^{-1}$ | < 0.16 | < 0.16 | < 0.16 | < 0.16 | < 0.16 | < 0.16 |
| Cr | < 0.011 | < 0.011 | < 0.011 | < 0.011 | < 0.011 | < 0.011 |
| Cs | 0.0085±0.0021 | 0.00560±0.00070 | 0.0072±0.0012 | 0.0069±0.0011 | 0.0099±0.0032 | 0.0239±0.0038 |
| Co | 0.0035±0.0012 | 0.0062±0.0013 | 0.0036±0.0012 | 0.00257±0.00022 | 0.00438±0.00063 | 0.0030±0.0013 |
| Fe | 1.50±0.33 | 1.4 ± 0.2 | 2.38 ± 0.49 | 1.01 ± 0.23 | 1.07 ± 0.23 | 1.82 ± 0.38 |
| Hg, µg kg$^{-1}$ | 33.1±1.3 | 72.8 ± 1.7 | 38.0 ± 3.2 | 27.9 ± 2.7 | 41.1 ± 4.2 | 103.8 ± 5.3 |
| La | < 0.37 | < 0.37 | < 0.37 | < 0.37 | < 0.37 | < 0.37 |
| Pb, µg kg$^{-1}$ | < 4.1 | < 4.1 | < 4.1 | < 4.1 | < 4.1 | < 4.1 |
| Se | 0.1249±0.0049 | 0.1524±0.0013 | 0.1195±0.0066 | 0.1247±0.0050 | 0.0884±0.0024 | 0.1853±0.0091 |
| Zn | 3.48 ± 0.28 | 3.73 ± 0.28 | 4.03 ± 0.28 | 2.76 ± 0.25 | 3.49 ± 0.29 | 3.78 ± 0.34 |

[a]Mean and standard deviation, $n = 3$.

## 4. CONCLUSIONS

Instrumental Neutron Activation Analysis and Atomic Absorption Spectrometry were successfully applied for the investigation and determination of mass fractions of As, Cd, Cr, Cs, Co, Fe, Hg, La, Pb, Se and Zn in catfish samples from Paraguayan rivers. Samples were analyzed in the context of an International Atomic Energy Agency regional program for Latin America. Low element contents were obtained for these elements suggesting that the investigated areas present small pollution impact. For a better understanding of the pollution status of the investigated areas, further collection, at different times of the year are suggested as future working possibilities. Analytical methods could also be optimized to obtain lower detection limits for some elements.

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## REFERENCES

1. U. A. Buitrago-Suárez, B. M. Burr, “Taxonomy of the catfish genus *Pseudoplatystoma* Bleeker (Siluriformes: Pimelodidae) with recognition of eight species”, *Zootaxa,* **1512**, pp. 1–38 (2007).
2. D. C. Carvalho, D. A. A. Oliveira, L. B. Beheregaray, R. A. Torres, “Hidden genetic diversity and distinct evolutionarily significant units in an commercially important

Neotropical apex predator, the catfish *Pseudoplatystoma corruscans*", *Conserv. Genet.* **13**, pp. 1671–1675 (2012).

3. P. H. Mello, B. C. Araújo, R. L. L. Venturieri, R. G. Moreira, "Fatty acids as a tool to compare cachara (*Pseudoplatystoma reticulatum*) (Siluriformes: Pimelodidae) and hybrid (*Pseudoplatystoma corruscans* x *Pseudoplatystoma reticulatum*) larvae during early development", *Aquacult. Int*., **20**, pp. 1139–1160 (2012).
4. Vieira F., Pompeu P. "Peixamentos, uma alternativa eficiente?" *Ciência Hoje*, **175**, pp. 29–33 (2001) apud 2.
5. F. D. Prado, D. T. Hashimoto, J. A. Senhorini, F. Foresti, F. Porto-Foresti, "Detection of hybrids and genetic introgression in wild stocks of two catfish species (Siluriformes: Pimelodidae): The impact of hatcheries in Brazil", *Fisheries Research*, **125–126,** pp. 300– 305 (2012).
6. L. H. G. Pereira, F. Foresti, C. Oliveira, "Genetic structure of the migratory catfish *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae) suggests homing behavior", *Ecol. Fresh. Fish*, **18**, pp. 215–225 (2009).
7. M. M. Abreu, L. H. G. Pereira, V. B. Vila, F. Foresti, C. Oliveira, "Genetic variability of two populations of Pseudoplatystoma reticulatum from the Upper Paraguay River Basin", *Gen. Molec. Biol.*, 32, **4**, pp. 868-873 (2009)
8. R.V.R. dos Santos, S.G.F. Martins, P.S. Pompeu, "An individual-based model for evolutionary effects of selective fishing applied to *Pseudoplatystoma corruscans*", *Physica*, **A 391,** pp. 5112–5120 (2012).
9. E. A. Adriano, M. M. Carriero, A. A. M. Maia, M. R. M. Silva, J. Naldoni, P. S. Ceccarelli, S. Arana, "Phylogenetic and host–parasite relationship analysis of *Henneguya multiplasmodialis* n. sp. infecting P*seudoplatystoma* spp. in Brazilian Pantanal wetland", *Vet. Parasit.*, **185**, pp. 110– 120 (2012).
10. A. A. Souza, S. G. F. Martins, P. S. Pompeu, "An individual-based model applied to the study of different fishing strategies of pintado *Pseudoplatystoma corruscans* (Agassiz, 1829)", *BioSystems* **110**, pp. 51– 59 (2012).
11. P. H. Mello, R. L. L. Venturieri, R. M. Honji, R. G. Moreira, "Threatened fishes of the world: *Pseudoplatystoma corruscans* (Agassiz, 1829) (Siluriformes: Pimelodidae)", *Environ. Biol. Fish*, **85**, pp. 359–360 (2009).
12. "Hidrelétricas podem afetar sistema hidrológico do Pantanal", Agência FAPESP 23/04/2013, http://agencia.fapesp.br/17168.html (2013).
13. International Atomic Energy Agency, "Ensuring seafood safety in Latin America and the Caribbean through a regional programme for the biomonitoring of contaminants in molluscs and fish (Project Number RLA/5/054, ARCAL CIII), http://www.iaea.org/programmes/aqcs/rla5054/index.html (2010).
14. J. L. Sericano, V. Besada, "Recomendaciones para la toma y preparación de muestra de bivalvos y peces - Preparado para el Programa Regional para el Biomonitoreo de Contaminantes en Moluscos y Peces RLA/5/054 (ARCAL)" (2009).
15. E. G. Moreira, M. B. A. Vasconcellos, M. Saiki. "Uncertainty assessment in instrumental neutron activation analysis of biological materials". *J. Radioanal. Nucl. Chem.* **269**, p.p. 377-382 (2006).

16. MESL, IAEA Marine Environment Studies Laboratories. “Mineralization procedures for sediment and biological materials. Standard operating procedures”, IAEA, Monaco, (1998).

17. M. Thompson, “Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing”, *Analyst*, **125**, pp. 385-386 (2000).

18. Brasil, Ministério da Saúde, Secretaria de Vigilância Sanitária, “Portaria n$^{o}$ 685, de 27 de agosto de 1988, Fixa limites máximos de tolerância de contaminantes químicos em alimentos”, *Diário Oficial da República Federativa do Brasil*, Brasília, 24/09/1998, S. 1, P. 1, pp. 1414-1437 (1998).