

Determination of Total Hg in fish samples from the Billings Dam, Sao Paulo

**Talita Oliveira¹, Jorge E. S. Sarkis¹, João C. Ulrich¹, Paula Maria Gênova de Castro²,
Lidia Sumile Maruyama², Renata Bazante Yamaguishi¹ e Luciana Carvalho Bezerra de
Menezes²**

¹ Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
taoliveira@ipen.br

² Instituto de Pesca / Secretaria da Agricultura e Abastecimento do Estado de São Paulo (IP / SAA - SP)
Avenida Francisco Matarazzo, 455, Parque da Água Branca,
05001-900 - São Paulo (SP) - Brasil
lcbm@usp.br

ABSTRACT

The Billings reservoir, located in Sao Paulo, Brazil, is used for several purposes such as: water supply, electric generation, fishing and leisure. Although considered an area of environmental protection, in recent years the dam has suffered diverse environmental aggressions including the release of toxic metals. One of the contaminations most well known is related with the mercury present in Rio Grande, one of the main tributaries of the dam. This is a worrying situation considering that the inhabitants of the region use fishes as an important item in their nutrition. In this paper it will be presented a recent study concerning the levels of mercury in the main consumed fish species of the region. The selection of species analyzed was based on the consumption of the population and by trophic level (benthophagous, piscivorous, herbivore and omnivore). The species were collected during winter 2009 and summer 2010. Samples were collected in the main centers between the fishermen of the dam, as follows: Barragem, Borore, and Sao Bernardo do Campo. The determination of total mercury was performed by using technique of atomic absorption spectrophotometry with flow injection system and cold vapor generation (FIA-CV-AAS). The methodology has been validated using material certified reference Dogfish Muscle (DORM-2). The maximum levels of Hg found for individuals of the species *Astyanax ssp* – Lambari (95 individuals), *Tilapia rendalli* – Tilapia (16 individuals), *Geophagus brasiliensis* – Acara (106 individuals) and *Hoplias malabaricus* – Traira (26 individuals) was 162, 114, 108 and 193 $\mu\text{g kg}^{-1}$ respectively. The results show that despite the persistent presence of Hg in the dam, the concentrations in the analyzed individuals were within the limits established by the World Health Organization (WHO) and Brazilian National Health Surveillance Agency being in both criterions, suitable for human consumption.

Keywords: Billings dam, mercury, fish, FIA-CV-AAS.

1. INTRODUCTION

Today one of the main concerns of modern society is the pollution water resources. Water is of fundamental importance in the whole terrestrial ecosystem. For people, in particular, is closely associated with basic activities such as industrial and public supply, sanitation, agricultural irrigation, electricity production, transportation, recreation, among others [1, 2].

Billings reservoir is extremely important due to the fact that it is the largest water reservoirs in South America's. Dam is located within the Metropolitan Region of Sao Paulo – RMSP, Brazil, and occupies an area of approximately 582.8 km². The reservoir produces 12 m³/s of the water that supplies the Great ABC (Santo André, Sao Bernardo do Campo and Sao Caetano do Sul), part of Sao Paulo and the Santos region [3].

The reservoir has been subjected to an intense eutrophication since the decade of 1970. Characterized by environmental impacts caused from illegal settlements, industrial and sewage discharges all of which seriously affect its water quality. One of the major concerns refers to environmental impacts caused by anthropogenic liberation of toxic metals to the environment [1, 3].

During the last decade, the water quality in some areas in the dam were monitored by Society of Environmental Sanitation Technology (CETESB) [4], with the Secretariat of Environment of Sao Paulo state government. Levels of toxic metals were found (lead, copper, aluminum, chromium and mercury) above the requirements recommended by the National Environment Council (CONAMA) [5].

These data are particularly disturbing considering that fishing is an important activity for the inhabitants of the region [6, 7].

In 2005, the Fishing Institute initiated a monitoring program involving the artisan fishing production. However, the concentration of toxic metal as mercury in tissue of these fished was not studied [9]. Most recently, with the collaboration with the Nuclear and Energy Research Institute a systematic evaluation of the presence of this metal in fish tissues was carried out.

Mercury is a major public health concern because of its widespread occurrence in the environment and its toxic effects on humans, mainly through fish ingestion [10, 11, 12].

According to World Health Organization (WHO) [13], several neurological diseases are diagnosed by evaluating the concentration of mercury (Hg) present in the body. The main symptoms are paresthesias, ataxia, neurasthenia, dysarthria, tremors and spasms, reaching, in severe cases, coma and death. Once in the environment Hg can be accumulated through food chains [14, 15].

This study reports the results on the concentration of total mercury (T-Hg) present in fish tissue collected during the winter and summer in the three main fishing centers of the Billings Reservoir: Barragem (BA), Borore (BO) and Sao Bernardo do Campo (SBC).

2. MATERIALS AND METHODS

2.1. Study area

Reservoir Billings (Figure 1) contains 127 km² and an average depth of 10 m. Studies estimate that the concentrations of toxic metals and the amount of nutrients has been changed by the strong presence anthropogenic approximately 800,000 inhabitants, around the reservoir. Daily, tributaries flow into the reservoir containing waste from industrial activities and domestic sewage.

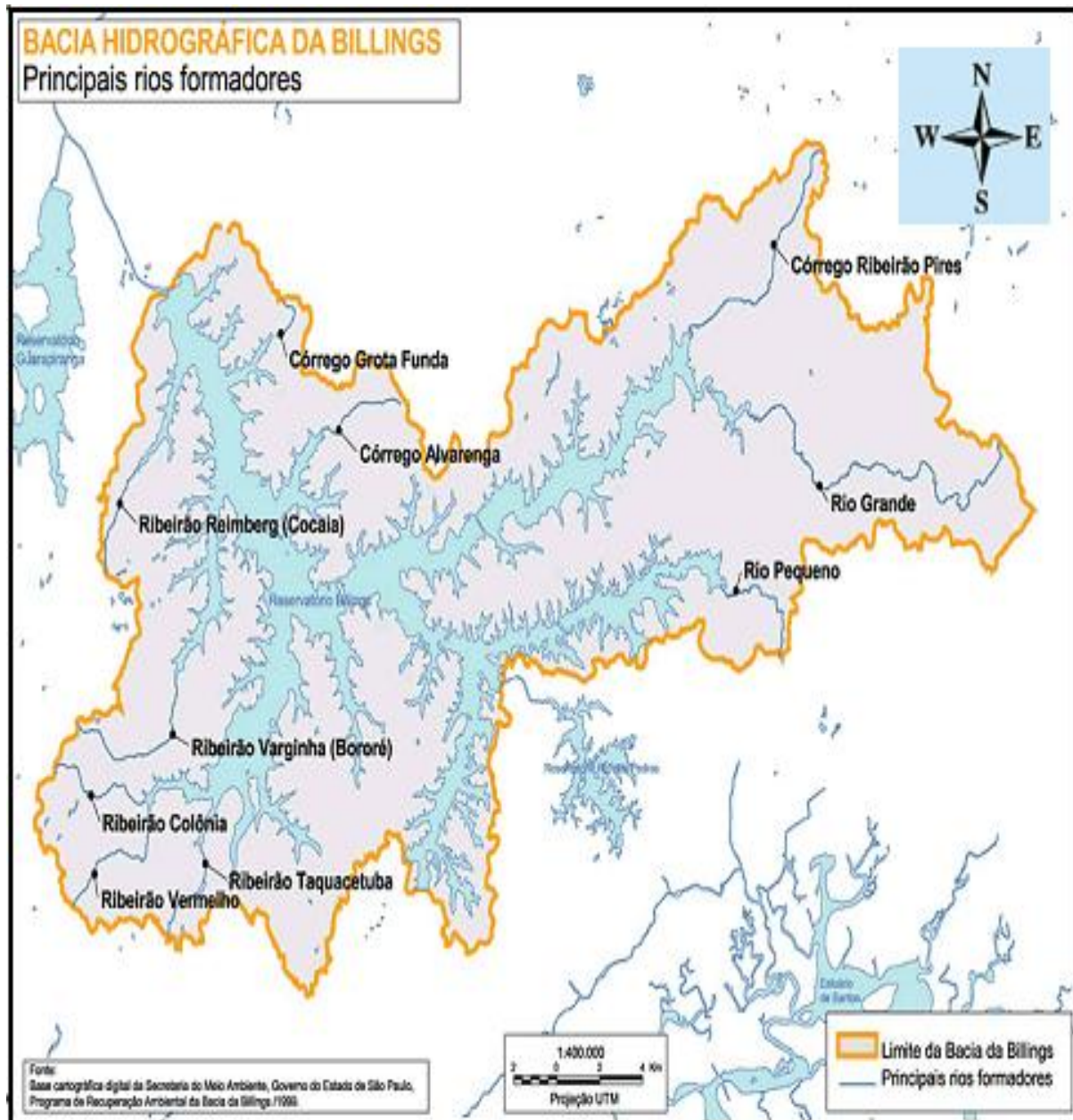


Figure 1. Map showing the Billings Reservoir and Metropolitan Region of São Paulo – RMSP. (Adapted of the http://www.mananciais.org.br/slideshow/albuns/1165258798/ma3_RiosForm.gif)

2.2. Sampling

The species chosen were those considered the most consumed in the diet of the population, they are: *Hoplias malabaricus* (Traíra) with 26 samples, representing trophic level of ichthyophagous, *Geophagus brasiliensis* (Acara) with 106 samples being classified as benthophagous, *Tilapia rendalli* (Tilapia), pertencent to class of the planktivorous with 16 samples, and representing the class omnivorous was chosen *Astyanax spp* (Lambari) with 95 fishes.

All samples were weighed and measured (total length and standard), then were stored in plastic bags, identified and frozen until digestion preceding the analysis. In a total of 243 samples of muscle tissue were analyzed total mercury by the technique of atomic absorption spectrophotometry with flow injection system and cold vapor generation (FIA-CV-AAS).

2.3. Analysis of total mercury (T-Hg)

2.3.1. Materials and reagents

The analysis of samples was performed by instrument atomic absorption spectrophotometer manufactured by Varian, AA220-FS model.

Seeking to ensure the reliability of the measurements, we used fractions of samples of a certified reference material (MRC), the DORM-2 - "Dogfish" certified reference materials for trace metals" provided by the National Research Council Canada - NRCC.

To ensure the reproducibility and repeatability of the method was selected a sample Quality Assurance Quality Control (QAQC). The selected sample belongs to the species *Hoplias malabaricus* (Traira), was chosen because it has a concentration close to the other samples and for having sufficient amount of sample required for all digestions.

All samples were prepared using reagents with low T-Hg concentrations. The dilutions were made with demineralized water obtained from the DPL model demineralizer 10000-FB (Deion, Equipment and Processes Ltd.). The whole process of sample preparation was carried out based on weight, with a balance properly calibrated. The solutions for the preparation of standards for instrument calibration were prepared according to standard operating procedures by the Quality Management System implemented in the laboratory.

2.3.2 Sample preparation

The Laboratory of Chemical and Isotopic Characterization has the ABNT ISO / IEC 17025:2005 [16] for analysis of T-Hg, and is accredited by the National Institute of Metrology and Quality Technology (INMETRO) is encoded in the laboratory with a number 0903.001-010.

The methodology was based on the work of Akagi, Lima and Ulrich [10, 11, 17]. Some of the collected individuals presented high levels of fat, forming plaques in solution. For them, it INAC 2011, Belo Horizonte, MG, Brazil.

was necessary to perform additional tests involving the following parameters: time and temperature of digestion, the sample weight and volume of acids. Fig 2 shows the procedure adopted for all samples.

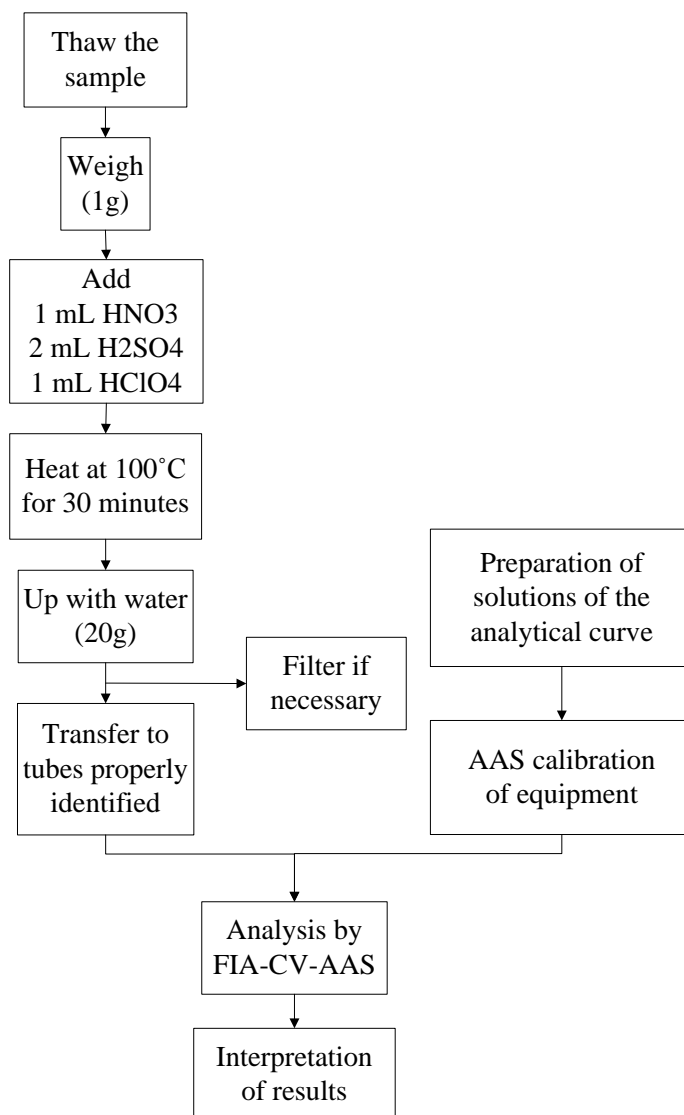


Figure 2: Representation of the process for analysis of T-Hg.

3. RESULTS AND DISCUSSION

3.1. Analysis of certified reference materials

The validation of analytical methodology was carried out with the certified reference material (MRC), DORM-2, which is certified for T-Hg $4.64 \pm 0.26 \mu\text{g kg}^{-1}$. The expanded uncertainty in concentration obtained for DOMR-2 was $0.52 \mu\text{g kg}^{-1}$, equivalent to 12.5%.

QAQC samples was digested and analyzed during the preparations of the samples. The expanded uncertainty of the sampling method is 13.2% for QAQC sample, the equivalent INAC 2011, Belo Horizonte, MG, Brazil.

concentration of 14.5 mg kg^{-1} . In Figure 3 shows that the values are within the ranges of uncertainty of the method.

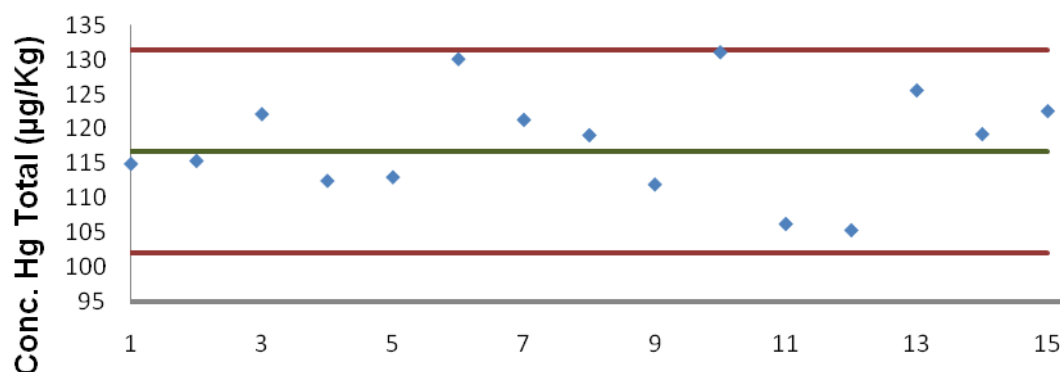


Figure 3. QAQC results: period corresponding to April/2010 February/2011

Analyzing the Table 1, it can be observed that the highest concentrations of T-Hg are present in Acaras collected in Sao Bernardo do Campo. According to Figure 4, the samples collected at the Barragem and Borore show concentrations near the median values, with greater uniformity among the samples. The samples collected in Sao Bernardo do Campo indicate that concentrations of T-Hg increase in summer and the values are farther from the median.

Table 1: Concentration of T-Hg ($\mu\text{g kg}^{-1}$) found in muscle of species *Geophagus brasiliensis*

Collection Point	Sao Bernardo do Campo (SBC)		Barragem (BA)		Borore (BO)	
Season	Winter	Summer	Winter	Summer	Winter	Summer
Median ($\mu\text{g kg}^{-1}$)	37,838	53,385	----	8,565	21,568	----
Average ($\mu\text{g kg}^{-1}$)	42,133	54,940	----	11,623	22,262	----
Deviation between samples ($\mu\text{g kg}^{-1}$)	27,672	21,912	----	12,871	5,788	----
Maximum ($\mu\text{g kg}^{-1}$)	108,448	90,375	----	42,143	37,849	----
Minimum ($\mu\text{g kg}^{-1}$)	11,436	7,382	----	< 2	10,014	----
Number of sample (unit)	20	15	----	7	64	----

(----) No sample.

There was no case of samples exceeding the limits established by national legislation [18] and international [19], this which is more restrictive, determines the maximum concentration of 0.5 mg kg^{-1} for predatory fish species or non-predatory.

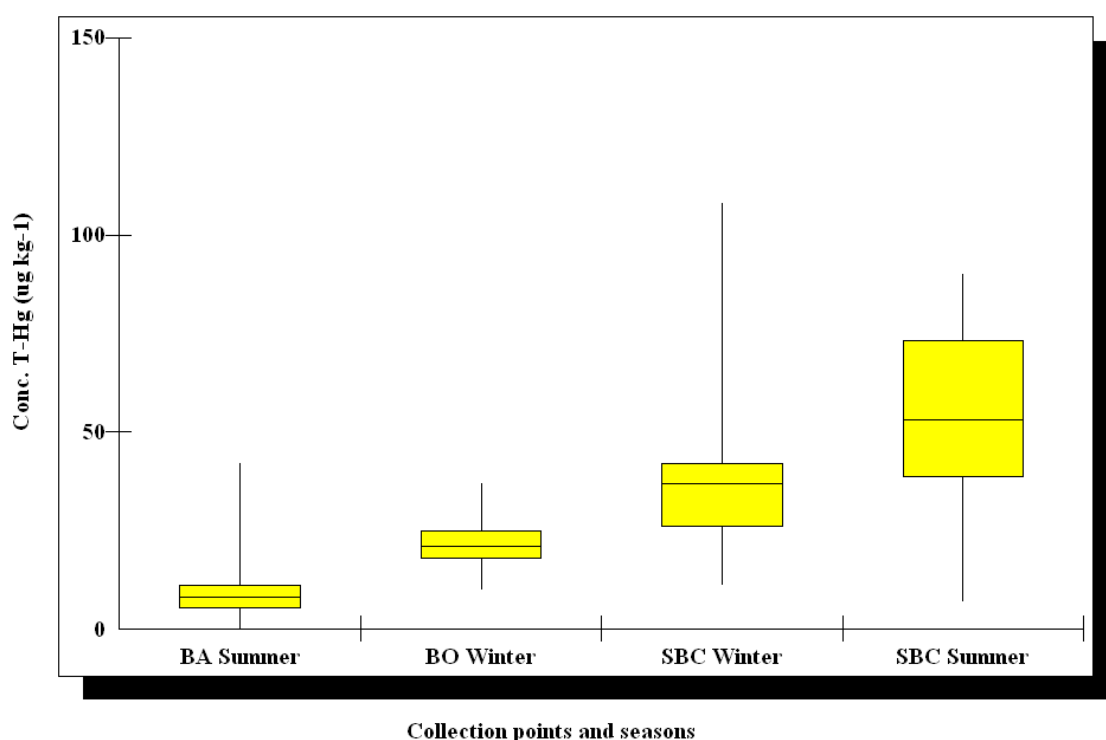


Figure 4. Median concentrations of T-Hg found in the species *Geophagus brasiliensis*

Table 2 shows the difference in concentrations from Lambaris, the maximum and minimum values are up to six times more concentrated in some samples.

Table 2: Concentration of T-Hg ($\mu\text{g kg}^{-1}$) found in muscle of fish *Astyanax spp*

Collection point	Sao Bernardo do Campo (SBC)		Barragem (BA)		Borore (BO)	
Season	Winter	Summer	Winter	Summer	Winter	Summer
Median ($\mu\text{g kg}^{-1}$)	52,177	65,827	----	----	----	78,587
Average ($\mu\text{g kg}^{-1}$)	59,625	72,388	----	----	----	79,637
Deviation between samples ($\mu\text{g kg}^{-1}$)	32,649	33,710	----	----	----	29,772
Maximum ($\mu\text{g kg}^{-1}$)	162,151	130,330	----	----	----	132,470
Minimum ($\mu\text{g kg}^{-1}$)	18,707	21,520	----	----	----	26,170
Number of sample (unit)	53	19	----	----	----	23

(----) No sample.

Evaluating Figure 5, it is clear that comparison between winter and summer can only be performed for *Astyanax spp* in Sao Bernardo do Campo and, once again, were found higher concentrations during the summer. The samples collected in summer in Borore presented T-Hg concentrations at the same order the magnitude. All samples showed T-Hg concentrations within the established legal limits for consumption.

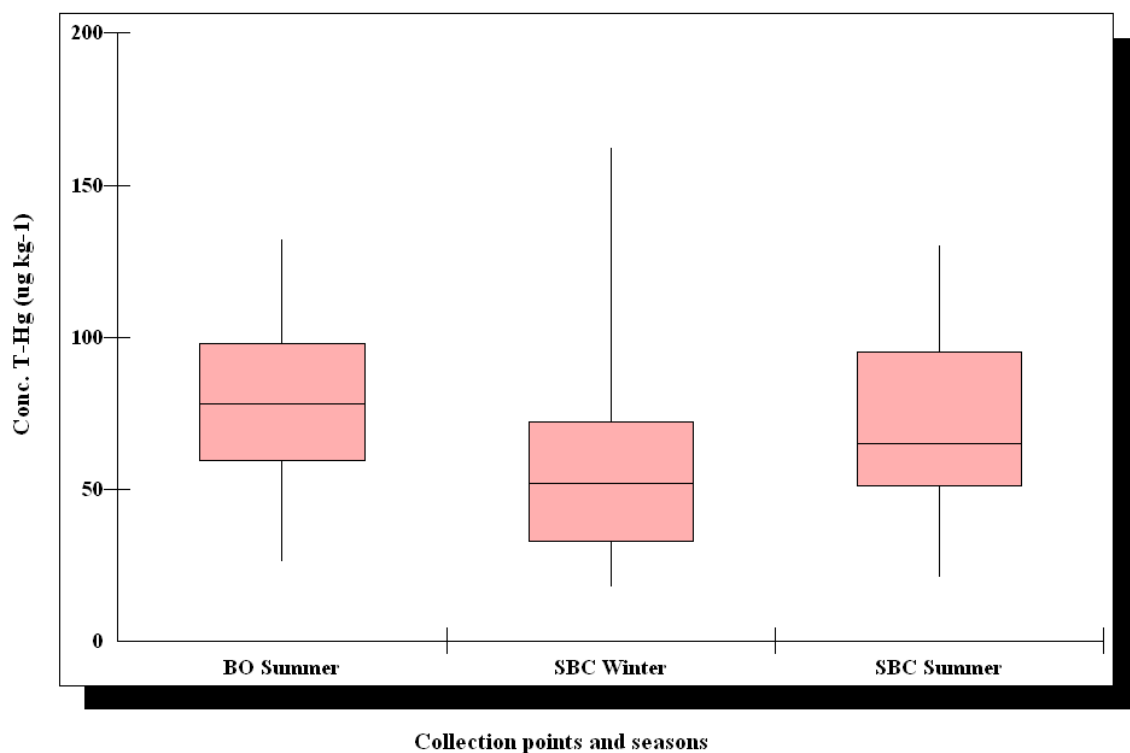


Figure 5. Median concentrations obtained in *Astyanax spp*

Table 3 presents the results obtained with the specie *Tilapia rendalli*. As it can be observed there is a minor differences between the seasons and, in some cases, the samples collected during the winter presented higher concentrations than those collected during the summer time. A single sample was collected in Sao Bernardo do Campo during the winter, however it presents a level of mercury twice higher in comparison with the samples collected in the summer, however the number of samples is too small for any detailed evaluation.

Table 3: Concentration of T-Hg ($\mu\text{g kg}^{-1}$) found in muscle of fish *Tilapia rendalli*

Collection point	Sao Bernardo do Campo (SBC)		Barragem (BA)		Borore (BO)	
	Winter	Summer	Winter	Summer	Winter	Summer
Median ($\mu\text{g kg}^{-1}$)	45,021	16,003	----	31,790	38,330	27,092
Average ($\mu\text{g kg}^{-1}$)	45,021	16,471	----	29,922	39,246	19,088
Deviation between samples ($\mu\text{g kg}^{-1}$)	0,000	4,688	----	27,120	9,992	19,117
Maximum ($\mu\text{g kg}^{-1}$)	45,021	21,376	----	58,093	49,665	31,531
Minimum ($\mu\text{g kg}^{-1}$)	45,021	12,036	----	<2	29,744	<2
Number of sample (unit)	1	3	----	4	4	4

(----) No sample.

The collection held at the Barragem during the summer, Figure 6, indicates that most of the results are below the median. In the Borore site, it was observed higher level of mercury INAC 2011, Belo Horizonte, MG, Brazil.

during the winter in comparison with the samples obtained in summer. Again, all individuals are within the parameters set by legislation.

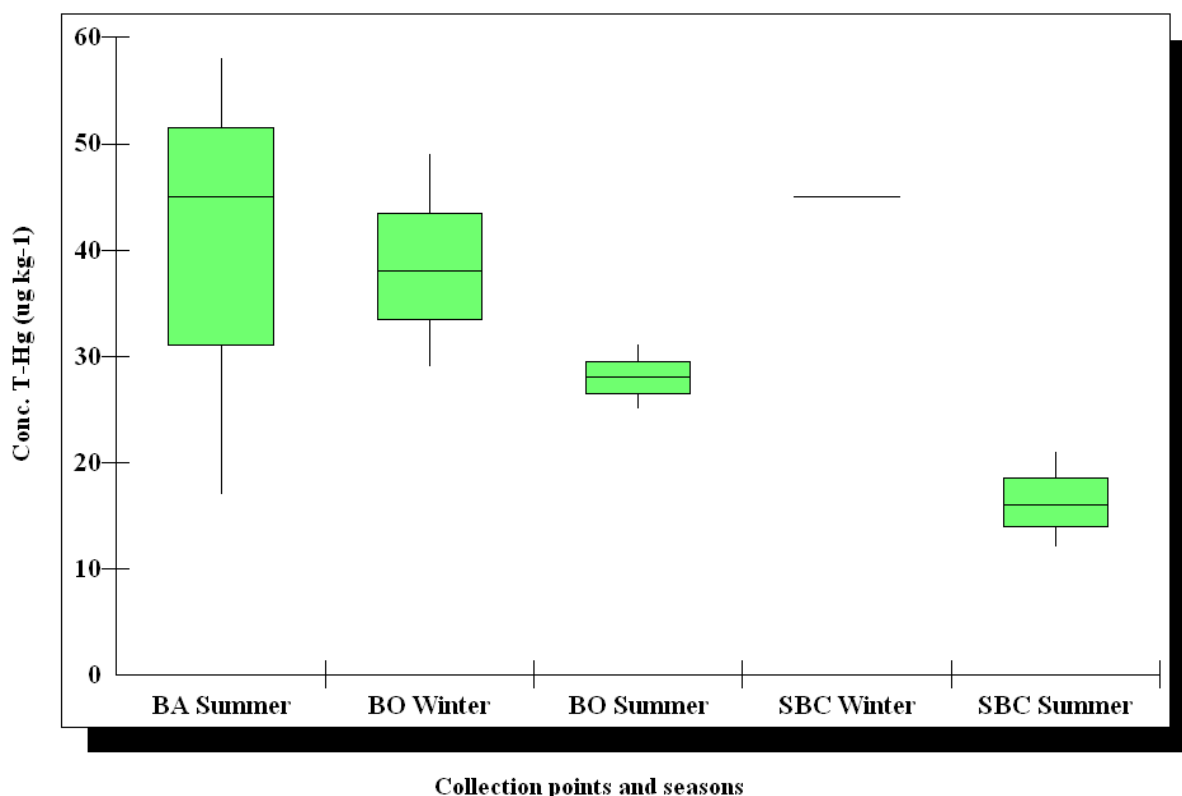


Figure 6. Median concentrations obtained in the species *Tilapia rendalli*

The *Hoplias malabaricus* is classified as piscivorous species and, as expected, presented the higher concentration in comparison with others collected species.

The collection in Sao Bernardo do Campo site was realized during the winter season. The results are presented in the Table 4. As can be noticed they present a higher variation among individuals collected at the same period, some of them presenting concentrations below the detection limit (2 mg kg^{-1}) and others reaching 50 mg kg^{-1} , however. Only a few of them presented T-Hg concentrations exceeding to 100 mg kg^{-1} .

The samples collected at the Barragem and the Borore, also showed wide variation between the extreme values, regardless of the season, indicating less uniformity among the species.

Table 4: Concentration of T-Hg ($\mu\text{g kg}^{-1}$) found in muscle of fish *Hoplias malabaricus*

Collection point	Sao Bernardo do Campo (SBC)		Barragem (BA)		Borore (BO)	
Season	Winter	Summer	Winter	Summer	Winter	Summer
Median ($\mu\text{g kg}^{-1}$)	4,210	----	----	33,898	51,005	79,566
Average ($\mu\text{g kg}^{-1}$)	26,353	----	----	67,176	52,685	92,662
Deviation between samples ($\mu\text{g kg}^{-1}$)	45,783	----	----	60,559	24,897	49,643
Maximum ($\mu\text{g kg}^{-1}$)	132,722	----	----	184,623	93,999	192,999
Minimum ($\mu\text{g kg}^{-1}$)	< 2	----	----	3,198	6,860	35,088
Number of sample (unit)	13	----	----	15	20	9

(----) No sample.

Observing Figure 7 and Table 4, it can be concluded that the results obtained for *Hoplias malabaricus* are superior to those of other species. This result is expected because this species belongs to the class of carnivores. The individuals collected in summer season in Borore site presented greater levels T-Hg, in comparison it those collected at the Barragem.

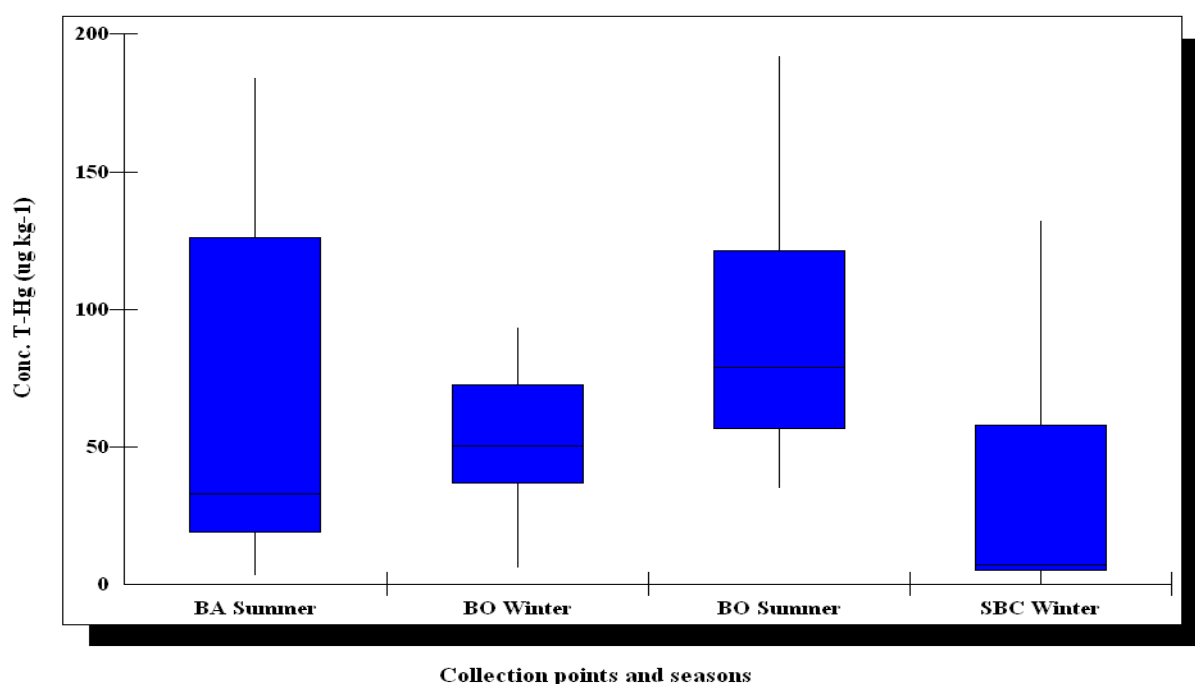
**Figure 7. Median concentrations in samples obtained in the specie *Hoplias malabaricus***

Figure 7 shows that the species *Hoplias malabaricus*, despite being classified as piscivorous its highest concentration does not exceed 200 mg kg^{-1} and is again within the limits established by legislation.

The species Traira, Lambari and Acara presented high concentrations during the summer. However, the Tilapia was the only specie in this studies, that presented high levels of T-Hg during the winter season.

4. CONCLUSIONS

All individuals analyzed in this study do not present high concentrations of total mercury, regardless of eating habits (predator or no predator) and sample season. All results were within the limits established by Brazilian legislation and even by the European Community.

Then, there is no risk to human health caused by consuming the studied species, for instance Acara, Lambari, Tilapia and Traira, collected in the main reservoir fisheries: Barragem, Borore and Sao Bernardo do Campo.

However, it was observed that there is an increase in mercury levels during the summer season for the species *Geophagus brasiliensis*, *Astyanax spp* and *Hoplias malabaricus*. The specie *Tilapia rendalli*, showed higher concentration during winter, however the sample number is insufficient for a definitive conclusion.

As expected, the species *Hoplias malabaricus* (Traira) showed the highest concentration due to dietary habits. The average concentration of other species can be considered at the same order the magnitude and even with different feeding habits have lower concentrations.

It is important to know that all individual analysed presented levels out the risks acceptable limits for consumption. However considering T-Hg is potentially toxic and hazards it's important to studies a continuous monitoring in order in the maintain and control the all this metal in the dam.

ACKNOWLEDGMENTS

The authors are grateful to the Institute of Energy and Nuclear Research - IPEN / CNEN-SP for technical support, the Institute of Fisheries / Department of Agriculture and Food Supply of Sao Paulo (IP / SAA - SP) for their support in collecting samples and the Foundation for Research Support of São Paulo - FAPESP for financial support.

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