2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
***ISBN:* 978-85-99141-05-2**

TOXIC METAL, MICRO AND MACRONUTRIENT ASSESSMENT IN FISH MOST CONSUMED BY IGUAPE COMMUNITY, SÃO PAULO STATE, BRAZIL

Déborah I. T. Fávaro[1], Luciana A. Farias[2], André S. Vieira[1], Isabella C.A.C. Bordon[3], Elisabete S. Braga[4]

[1] Neutron Activation Analysis Laboratory, LAN/CRPQ, IPEN/CNEN - SP,
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
defavaro@ipen.br

[2] Federal University of São Paulo, UNIFESP, BRAZIL - UNIFESP
Rua Prof. Artur Riedel, 275 – Jardim Eldorado
09972-270 - SP
lufarias2@yahoo.com.br

[3] Center for Chemical and Environmental Technology, CQMA, IPEN/CNEN-SP
isabella.bordon@gmail.com

[4] Oceanographic Institute, LABNUT – IO/USP
Praça do Oceanográfico, 191, 05508-900 São Paulo, SP
edsbraga@usp.br

ABSTRACT

It is well-known that, fish is an important source of protein for populations around the world. As such, fish consumption has increased because it is a healthy and low cholesterol source of protein and other nutrients. The city of Iguape, in the extreme south of the São Paulo State coast, is located in a well-preserved Atlantic Forest region. During the last two centuries the city suffered from drastic environmental changes, reinforcing the importance of environmental monitoring in this region. In the present study, 23 samples of the three most consumed fish species by the Iguape city population were analyzed. Fish samples were bought at local markets, conditioned in isothermic boxes in crushed ice (-4°C) and then identified before registering the biometric information (total length, total weight and body weight). The following micro and macronutrients As, Br, Ca, Co, Fe, K, Na, Rb, Sc, Se and Zn concentration in muscle from 02 predatory fish species *Macrodon ancylodon (*King weakfish – Pescada) and *Centropomus parallelus* (Fat snook - Robalo peba) and one detritivorus species: *Anchoviella lepidentostole (Broadband anchovy - Manjuba)* were assessed by INAA. Toxic metals Cd, Hg and Pb were also evaluated by AAS. Statistical correlation between element concentrations and fish species were tested.

1. INTRODUCTION

Fish and fishery products are recognized as important food alternatives for human consumption since they provide high quality and easily digestible proteins, as well as lipids, minerals and lipid soluble vitamins. They are also known as good sources of polyunsaturated fatty acids, mainly the Omega-3 family, which is important not only for promoting but maintaining good health [1]. Aside from their nutritional value, fish is considered one of the most important bioindicators in aquatic systems to estimate toxic metal pollution levels. This is possible because fish is able to bioaccumulate these elements and occupy different trophic levels.[1]

The Cananéia/Iguape estuarine lagoon complex is a naturally preserved area and presents pronounced differences between its northern and southern regions. The southern coastal region is comprised of Iguape, Ilha Comprida and Cananéia municipalities. Throughout the area drinking water distribution is precarious and the sewage treatment is insufficient. Urban sewage is released directly into the sea.[2] The quality of the estuarine water of the region is considered good, but the fresh water is predominant in the north part of this system, exactly where Iguape city is located, in function of human intervention by the Valo Grande channel that links the Ribeira rivers to the estuarine system. The region's municipalities have developed touristic activities but industrial activity is practically non-existent.

The purpose of the present study was to present new data about the nutritional and some toxic contents in muscles of the three most consumed fish species from Iguape city: two predatory species, *Macrodon ancylodon (*King weakfish – Pescada) and *Centropomus parallelus* (Fat snook - Robalo peba) and one detritivorus species, *Anchoviella lepidentostole (Broadband anchovy – Manjuba).* Micronutrients (Ca, Fe, K, Na, Se and Zn) and some trace elements (As, Br, Co, Cr, Rb) in fish muscle were determined by means of neutron activation analysis (NAA). The purpose of the present study was also to continue the environmental assessment in the Cananéia/Iguape estuarine lagoon complex in order to understand the bioaccumulation processes of Cd, Hg and Pb in ictiofauna products of the region available for human consumption, level of exposure and possible health risks for the population. Regarding the nutritional content, it is also very important to assess macro and micronutrients in the most consumed fishes considering the high intake due to the great offer in the region.

In Brazil, particularly in coastal cities such as Iguape, the population consumes fish from local markets or local public fisheries. Thus the assessment of nutrients, composition of the most commonly consumed fish species in Iguape as well as the toxic metal levels (Cd, Hg and Pb) can be a valuable contribution for planning and programs to prevent, control and treat dietary deficiencies.

There is at present little information regarding nutritional and chemical constituents of fish species in Brazil and still less evaluating toxic metal levels (Cd, Hg and Pb). It has been observed that element concentration levels vary widely between different species and even between individuals of the same species as a result of time of year, location, habitat, sex, age, etc [3]

Curcho [1] contributed with important data on the nutritional and toxic constituents in muscles of the most consumed fish species from two coastal regions, Cananéia and Cubatão. Proximate composition and fatty acids from the ω-3 and ω-6 families were also evaluated. *Micropogonias furnieri (White mouth croaker - Corvina), Macrodon ancylodon* (King weakfish - *Pescada), Centropomus undecimalis* (Fat snook - Robalo Peba) and *Mugil platanus (Mullet-Tainha) from Cananéia and Micropogonias furnieri, Macrodon ancylodon, Menticirrhus americanus (Perna de moça*), *Sardella b*raziliensis (Sardine-*Sardinha*) and *Mugil liza (Mullet- Tainha)* from Cubatão were assessed.The results for fish from Cananéia has already been published.[4]

Morgano et al [5] evaluated the contaminants occurrence as total As, Cd, Cr, Pb and total inorganic mercury content in tissues of *King weakfish, Mullet, White mouth croaker* and *Sardine* purchased during winter 2009 and summer 2010. The selection of fish samples were done according the sale amount (ton/year) of the CEAGESP/SP, the largest public market of São Paulo, and where the fish samples were purchased. The concentration levels of these contaminants were compared to the Brazilian legislation and compared between the species analyzed.

Some other similar studies were published in the literature [6,7] on toxic metal contents in fish commercialized in the São Paulo State.

2. MATERIAL AND METHODS

2.1 Description of the studied Area

The Cananéia-Iguape estuarine-lagoon complex is one of the world´s most important marine areas. It is an Environmental Protected Area and is composed of four water bodies located between four main islands. In 1999 this system received the UNESCO title of Natural Patrimony of Humanity. The Cananéia region preserves the largest mangrove area in the region. The Iguape region is under a process of changing its native vegetation profile.

The population´s main source of income is tourism and fishing. The region´s population according to the Brazilian census is 28,841 habitants. [8] Due to high organic matter content and phytoplankton production, there is a great abundance of fish and marine life. [9].

2.2 Fish selection and preparation for analysis

Tabulated data from this study identified the 3 most consumed fish species in Iguape which were: two predatory fish species *Macrodon ancylodon* (King weakfish – Pescada) and *Centropomus parallelus* (Fat snook - Robalo peba) and one detritivorus species: *Anchoviella lepidentostole* (Broadband anchovy – Manjuba). The families that took part in this study informed that they usually bought fish from the local markets, except for fishing families.
The fish samples were bought directly from local street markets in Iguape during March of 2010 and in a municipal fish market, at Iguape city. All samples were assessed for quality indicators and external organoleptic characteristics (eyes, gills and scales). Fish were conditioned in isothermic boxes in crushed ice (-4°C) and then identified before registering the biometric information (total length, total weight and body weight). In the laboratory, the samples of fish muscle were obtained from 23 fishes, were dried at 40° C in a ventilated oven until constant weight. Dried samples were ground, homogenized and prepared for INAA and AAS determinations.

2.3. Total Hg determination

Total Mercury determination was performed by Cold Vapor Atomic Absorption Spectrometry (CV AAS), using a FIMS (Flow Injection Mercury System) from Perkin Elmer. The experimental procedure applied in fish samples have already been described in previous papers. [10,11] The methodology validation for total Hg determination in fish samples was carried out using certified reference materials Oyster Tissue (NIST SRM 1566b), Dogfish

Muscle Certified Reference Material for Trace Metals (DORM-2, NRCC) and Fish Homogenate (IAEA 407). The detection limit (DL) for total Hg determination is 0.001 mg kg^{-1} and quantification limit (QL), 0.005 mg kg^{-1} [10].

All analyses were performed at the Neutron Activation Analysis Laboratory in Nuclear and Energy Research Institute – LAN/IPEN, SP-Brazil.

2.4 Cd and Pb determinations by GF AAS

Cd and Pb were determined in the fish muscles by GF AAS (Graphite Furnace Atomic Absorption Spectrometry) using a Perkin Elmer AAnalyst 800 equipment. About 300 mg of samples and SRMs were dissolved by adding Merck concentrated HNO_3 and left standing for a period of 6 hours, after which 30% H_2O_2 was added. The flasks were stirred and left again for about 15 hours. To finalize digestion, the closed flasks were put in an aluminum block at 90^0C, for 3 hours. Details of methodology were described in a previous paper. [12]

2.5 INAA determination for some macro and micronutrients

For multielemental analysis, approximately 200 mg of fish (duplicate samples) and about 150 mg of reference materials were accurately weighed and sealed in pre-cleaned double polyethylene bags, for irradiation. Single and multi-element synthetic standards were prepared by pipetting convenient aliquots of standard solutions from Assurance® Multi-Element Solution Standards (SPEX CERTIPREP, USA), onto small sheets of Whatman no 41 filter paper. Fish samples, reference materials and synthetic standards were irradiated for 8 hours, under a thermal neutron flux of 10^{12} cm^{-2} s^{-1} in the IEA-R1 nuclear research reactor at IPEN. The elements determined by INAA were As, Br, Ca, Co, Fe, K, Na, Rb, Sc, Se and Zn. The precision and accuracy of the method were verified by measuring the reference materials Peach Leaves (PL) (NIST SRM 1547) and DORM-2 (Dogfish Muscle Certified Reference Material for Trace Metals, NRCC). Details of the analytical methodology have already been described in a previous paper. [13]

2.6 Statistical Analysis

In order to statistically evaluate the concentration variations observed and if these were significant or not, Kruskal-Wallis test was applied to the data for median comparison. Multivariate statistical technique, discriminate analysis was used, to identify sample groups with similar chemical composition for the determined elements. The statistical analyzes were performed using the Statistica Package, Statistica Student Version 6.0. (USA).

3. RESULTS AND DISCUSSION

Fish samples were submitted to a biometric procedure and applied to each individual before the muscle sampling. Table 1 presents the total body length, total weight, feeding habits, humidity (%) in the different fish species analyzed from Iguape city.

Table 1 – Parametric data and interval, humidity (%) and feeding habits in the different fish species analyzed

	Fish samples	**Total lenght (cm)**	**Total weight (g)**	**Muscle (g) (wet weight)**	**Humidity (%)**
	P-01	36.8	394	163	78.0 ± 0.2
Macrodon ancylodon (King weakfish – Pescada) (predatory species) (n=10)	**P-02**	36.4	371	129	79.5 ± 0.1
	P-03	38.5	533	229	77.9 ± 0.5
	P-04	40.2	687	279	75.9 ±0.4
	P-05	40.1	644	225	77.9 ± 0.1
	P-06	39.9	671	285	76.2 ± 0.3
	P-07	41.5	579	258	75.1 ± 0.1
	P-08	34.9	355	198	75.6 ±0.4
	P-09	36.8	341	210	75.9 ± 0.2
	P-10	43.5	700	320	75.7 ± 0.3
Mean ÷ standard deviation (humidity,%)	**interval**	**34.9 - 43.5**	**341 - 700**	**163 - 320**	**76.5 ± 1.9**
	M-01	12.6	19.6	244	71.5 ± 0.3
Anchoviella lepidentostole (Broadband anchovy – Manjuba) (detritivorus species) (n=8)	**M-02**	12.5	21.6	215	72.1 ± 0.1
	M-03	12.4	19.9	205	72.4 ± 0.3
	M-04	12.7	20.3	184	71.9 ± 0.1
	M-05	12.0	18.4	228	72.7 ± 0.4
	M-06	13.0	21. 7	232	70.8 ± 0.1
	M-07	12.0	17.0	243	72.1 ± 0.2
	M-08	13.3	22.4	271	77.2 ± 0.3
Mean ÷ standard deviation (humidity,%)	**interval**	**12.0 -13.3**	**17.0 - 22.4**	**184 - 271**	**72.1 ± 1.3**
Centropomus parallelus (Fat snook – Robalo peba) (predatory species) (n=5)	**R-01**	40.5	637	195	76.6 ± 0.2
	R-02	39.9	800	274	75.6 ± 0.1
	R-03	43.3	829	279	77.1 ± 0.2
	R-04	47.7	776	224	75.6 ± 0.5
	R-05	44.7	746	248	75.3 ± 0.3
Mean ÷ standard deviation (humidity,%)	**interval**	**39.9 - 47.7**	**637 - 829**	**195 - 279**	**76.0 ± 0.7**

n- number of determinations

3.1 Total mercury, Cd and Pb methodologies validation

The precision and accuracy of the method for total mercury were verified by means of the reference material analysis with different concentration levels: Oyster Tissue (NIST SRM 1566b, [Hg]= 37.1 ± 1.3 µg kg^{-1}), Fish Homogenate (IAEA 407, [Hg]= 222 ± 24 µg kg^{-1}) and Dog Fish Muscle (DORM-2, NRCC, [Hg]= 4640 ± 260 mg kg^{-1}). The results obtained showed good precision and accuracy with relative standard deviation ranging from 0.5 to 2.4% and relative error from 5.7 to 8.3 %.

For Cd and Pb, the reference materials analyzed were Mussel Tissue (SRM 2976),with certified values for Cd (820 ± 160 µg kg^{-1}) and Pb (1190 ± 180 µg kg^{-1}) and Oyster Tissue (NIST SRM 1566b), with 2480 ± 80 µg kg^{-1} for Cd and 308 ± 9 µg kg^{-1}, for Pb. The results obtained showed good precision and accuracy with relative standard deviation ranging from 1.6 to 3.8% and relative error from 1.0 to 3.2% for Cd and from 1.6 to 3.5% and 1.0 to 10% for Pb, respectively.

3.2 INAA methodology validation

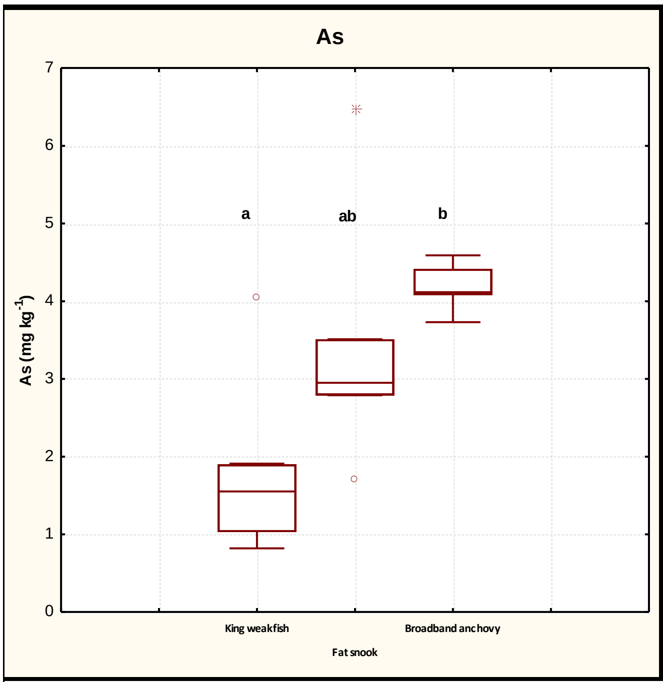
The precision and accuracy of the method were verified by measuring the reference materials: Peach Leaves (PL) (NIST SRM 1547) and DORM-2 (Dogfish Muscle Certified Reference Material for Trace Metals, NRCC). The Z-score criterion was applied to the results and all values were within the $/Z/ \leq 3$ interval, showing good precision and accuracy.

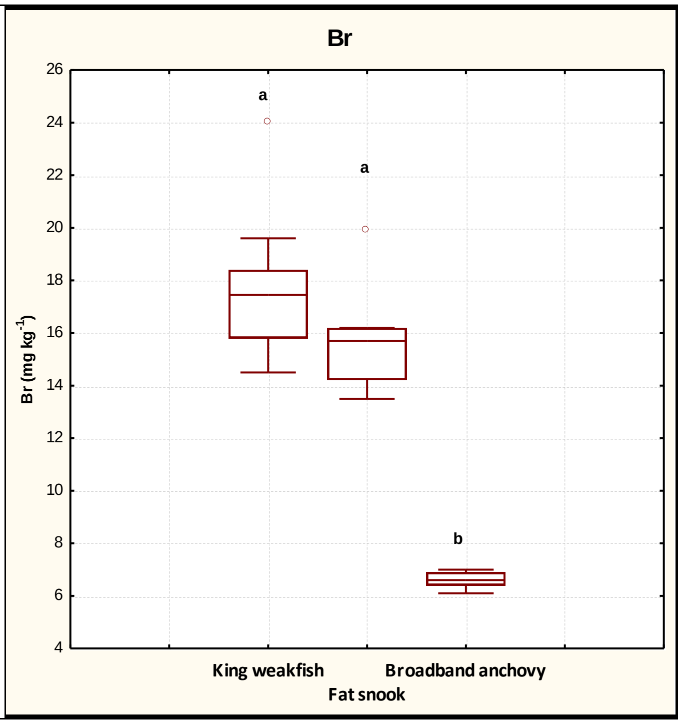
3.2.1 INAA fish muscle results

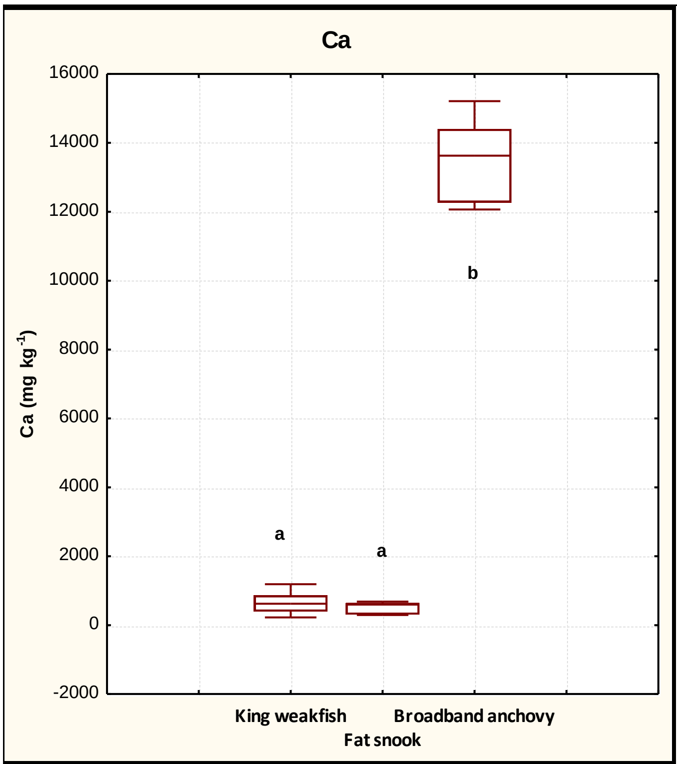
Figure 2 shows the box plot (median) for the results obtained for the determination of the As, Br, Ca, Cd, Co, Fe, Hg, K, Na, Pb, Rb, Sc, Se and Zn concentrations (in dry weight) in muscle from 02 predatory fish species Macrodon ancylodon (King weakfish – Pescada) and Centropomus parallelus (Fat snook - Robalo peba) and one detritivorus species: Anchoviella lepidentostole (Broadband anchovy - Manjuba).

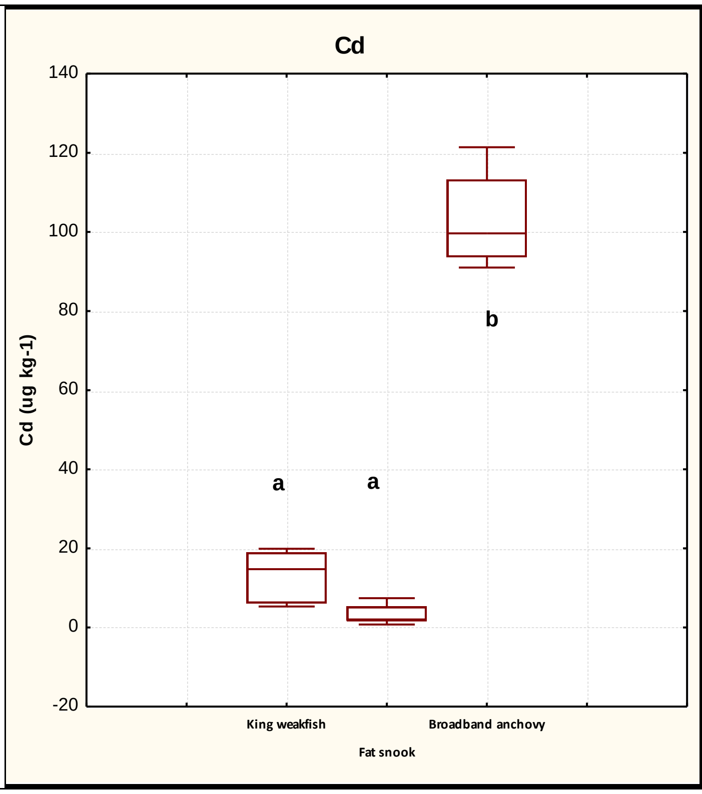
In order to statistically evaluate the variations observed and if they were significant or not, Kruskal-Wallis test was applied to the data for median comparison because data distribution was not normal, the data for each fish species did not have the same number of determinations (n) and did not show variance equality. In the box plot graphics, different letters means that the medians are statistically different, for a 95% significance level ($p<0.05$).

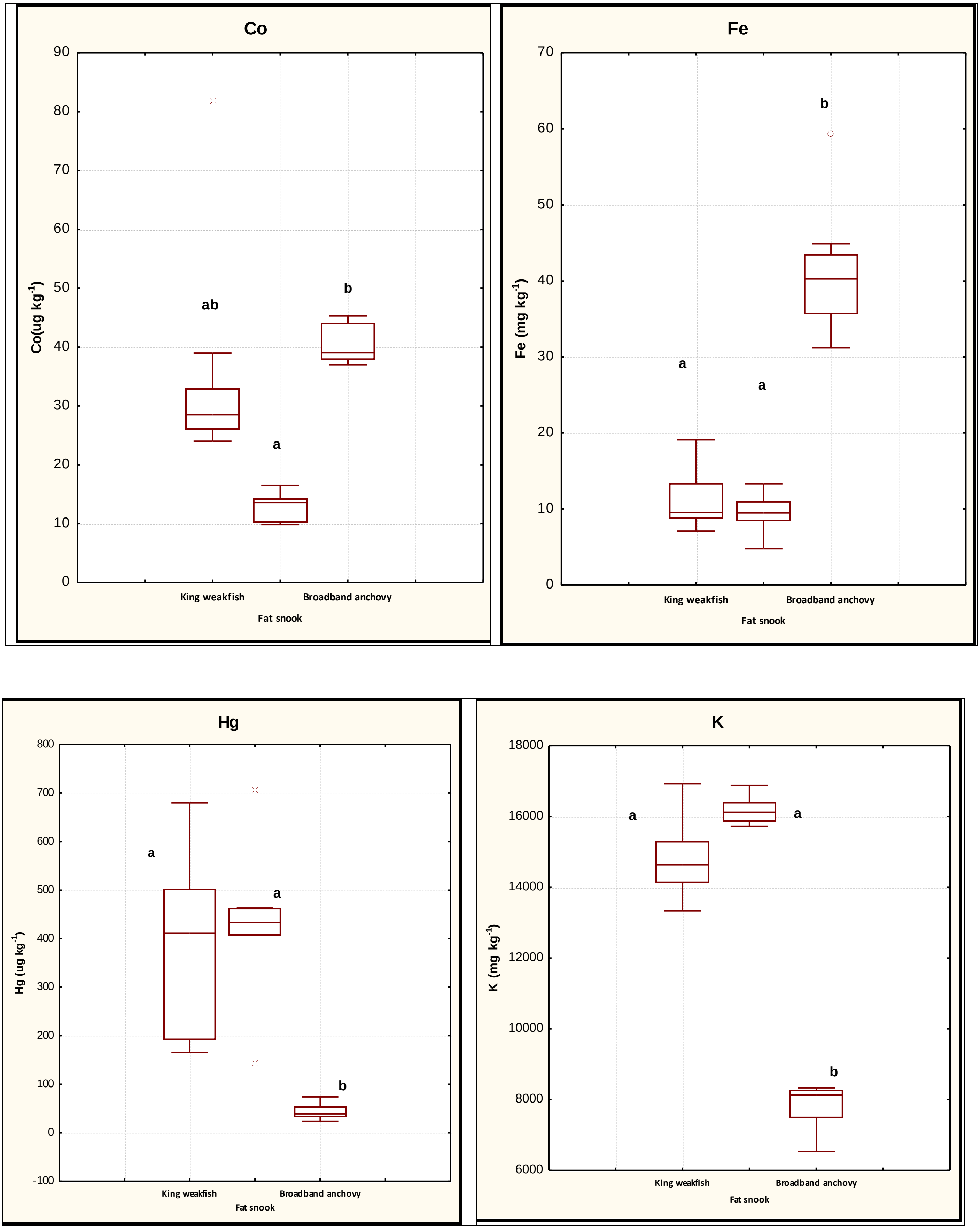
Discriminate analysis was used for the values for toxic metal, macro and micronutrient contents for all fish species analyzed in order to identify samples of similar chemical composition, respective food habits and salt and fresh water habitats (Figure 3). The discriminate model was statistically significant ($p<0.0000$) as well as the two obtained functions (Functions 1 and 2 = $p<0.0000$) The first function provided the separation of Anchoviella group (Manjuba) from the other species with the contribution of Ca, Cd, Na, As, Br and Hg coefficients. The second function enable the identification of *Macrodon* (Pescada) and *Centropomus* (Robalo) groups due to the contribution of Br, As, Cd, K and Hg coefficients. The three species analyzed showed a distribution where *Macrodon* and *Centropomus* (carnivorous species) were different but situated near in the graphic, confirming that the chemical composition of the 3 fish species is different.

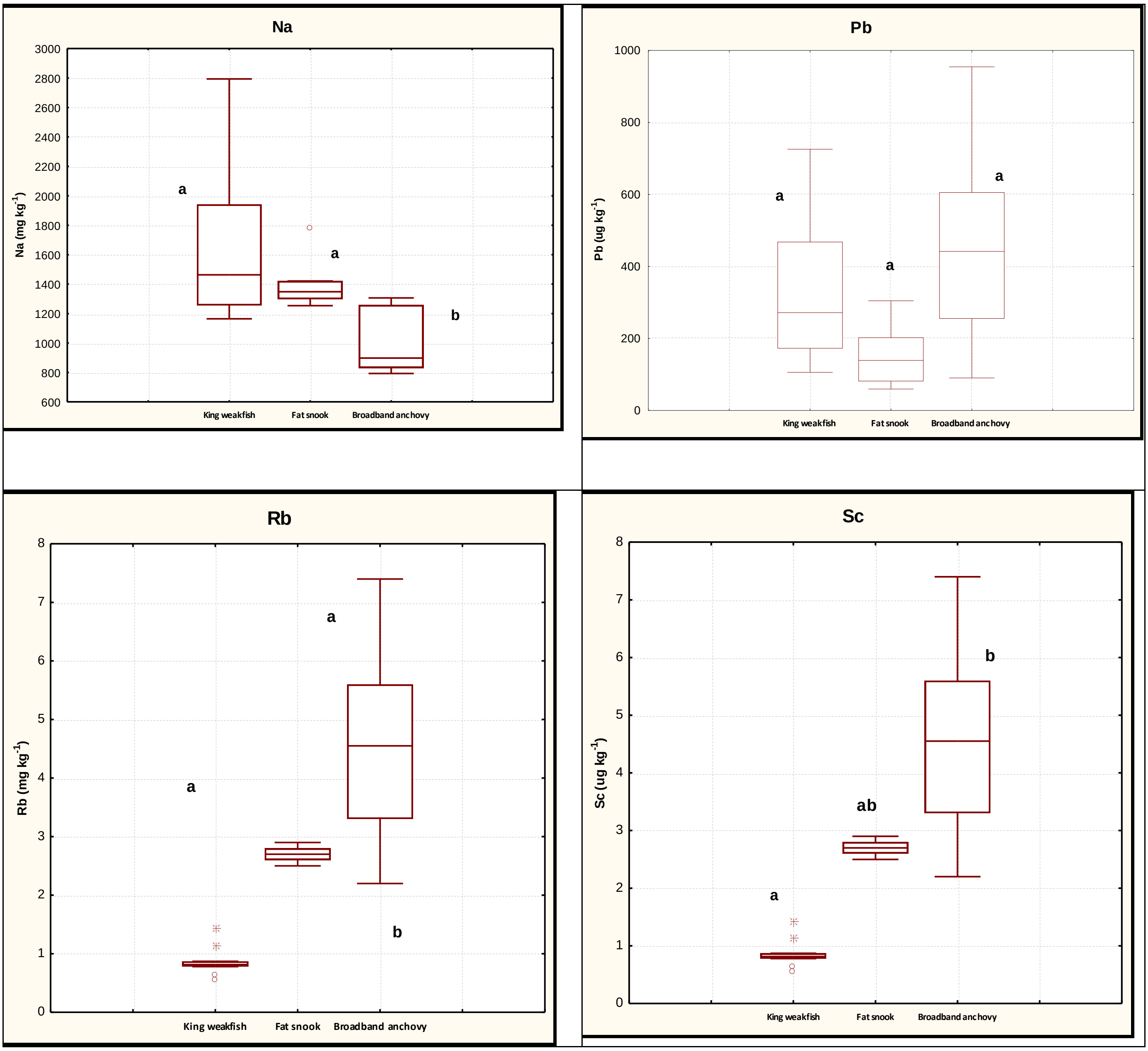

As
As (mg kg-1)
7
6
5
4
3
2
1
0
a
ab
b
King weakfish
Broadband anchovy
Fat snook


Br
Br (mg kg-1)
26
24
22
20
18
16
14
12
10
8
6
4
a
a
b
King weakfish
Broadband anchovy
Fat snook


Ca
Ca (mg kg-1)
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
b
a
a
King weakfish
Broadband anchovy
Fat snook


Cd
Cd (ug kg-1)
140
120
100
80
60
40
20
0
-20
b
a
a
King weakfish
Broadband anchovy
Fat snook


Co
Co(ug kg-1)
90
80
70
60
50
40
30
20
10
0
ab
b
a
King weakfish
Broadband anchovy
Fat snook
Fe
Fe (mg kg-1)
70
60
50
40
30
20
10
0
b
a
a
King weakfish
Broadband anchovy
Fat snook
Hg
Hg (ug kg-1)
800
700
600
500
400
300
200
100
0
-100
a
a
b
King weakfish
Broadband anchovy
Fat snook
K
K (mg kg-1)
18000
16000
14000
12000
10000
8000
6000
a
a
b
King weakfish
Broadband anchovy
Fat snook



Na
Na (mg kg-1)
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
a
a
b
King weakfish
Fat snook
Broadband anchovy
Pb
Pb (ug kg-1)
1000
800
600
400
200
0
a
a
a
King weakfish
Fat snook
Broadband anchovy
Rb
Rb (mg kg-1)
8
7
6
5
4
3
2
1
0
a
a
b
King weakfish
Fat snook
Broadband anchovy
Sc
Sc (ug kg-1)
8
7
6
5
4
3
2
1
0
b
ab
a
King weakfish
Fat snook
Broadband anchovy

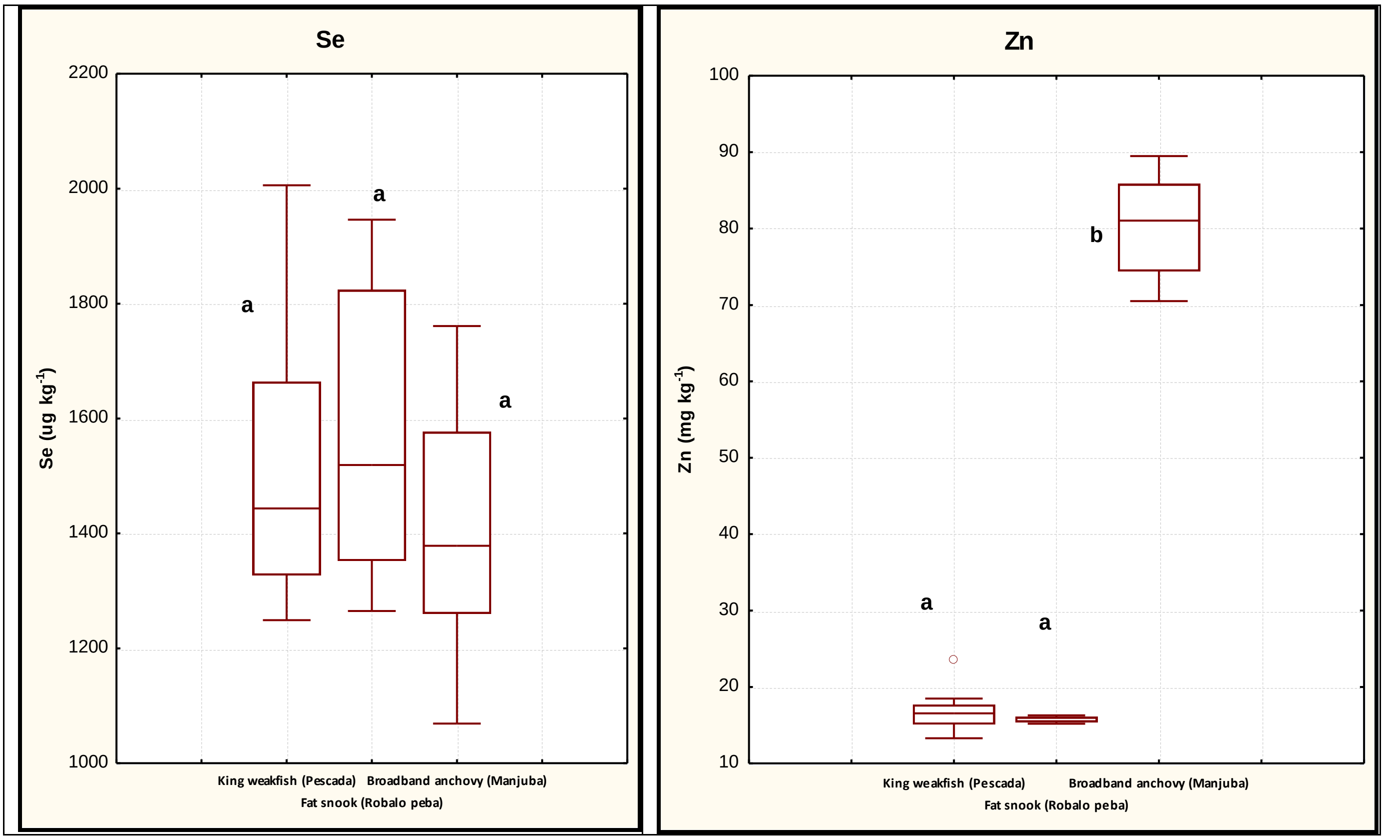


Figure 2 – Box plot (median) for the elements determined by INAA (dry weight) and for AAS (Cd, Hg and Pb) (dry weight) in muscle of analyzed fish species

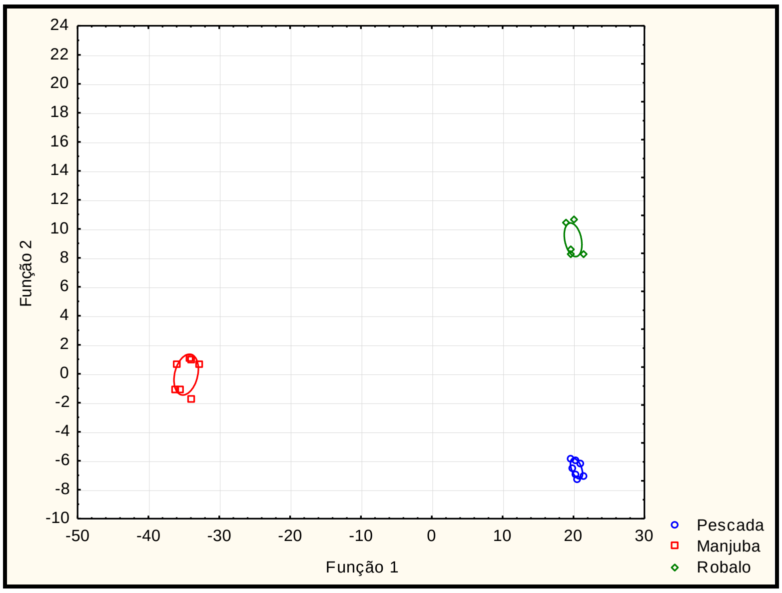


Figure 3 .Discriminate analyses for the chemical data for the 3 fish species

Calcium is directly involved in the development and maintenance of the skeletal system and important for several physiological processes [14]. In vertebrates, Ca is complexed with P in hydroxyapatite to form the principal crystalline material of bone. The Ca requirement of fish is affected by water chemistry and species differentiation. In the present study, Ca concentrations varied widely (226.0 mg kg^{-1} (Pescada) to 15,210 mg kg^{-1}(Manjuba) showing the highest levels for Manjuba (12,067 – 15,210 mg kg^{-1}), detritivorous species. The Ca concentration levels in the predatory fish species were very similar (Pescada – 226 to 1,189 mg kg^{-1} and 296 to 682 mg kg^{-1}- Robalo) from salt and saline water species, respectively confirmed by statistical analysis (Figure 2).

Potassium, as sodium, is also essential for normal body functions. In this study, the maximum K concentrations (16,925 and 16,880 mg kg^{-1}) were found in Pescada and Robalo, and 8,334 mg kg^{-1} in Manjuba, detritivorous species. Na exhibits maximum concentrations of 2,794 and 1,775 (predatory species) and 1,306 mg kg^{-1} in Manjuba, detritivorous species, respectively.

Regarding the micronutrients Co, Fe and Zn, the detritivorous species (Manjuba) showed higher concentration levels (Figure 2). The concentration levels for Fe were low for most fish species analyzed, mainly if compared with the Fe content present in the Amazonian fish species such as Tambaqui (5,340 mg kg^{-1}).[15]. In the present study Fe levels ranged from 31.2 to 59.3 mg kg^{-1} for Manjuba, 7.1 to 19.1 mg kg^{-1} for Pescada and 4.8 to 13.3 mg kg^{-1}, for Robalo. Information on absorption and metabolism of iron in fish is scarce, but the process is generally the same as in other vertebrates. [16]

Muscle tissue Zn levels (from 13.3 to 23.5 mg kg^{-1} for Pescada and 15.2 to 16.3 mg kg^{-1} for Robalo, predatory fish) and from 70.5 to 89.5 mg kg^{-1} for detritivorous species (Manjuba), showed the highest levels. Fish can obtain zinc from dietary sources as well as from water. The gills and gastrointestinal tract are involved in the uptake of this element. [16] Zinc is one of the most important environmental toxicants, yet it is essential in a wide range of biological processes.[17] It plays an important role in the growth, development of all living cells. It is also an essential micronutrient found in abundance in fish. It is a co-factor in metaloenzimes and regulatory proteins, including biosynthesis and DNA and RNA repair. [18]

The content of Selenium ranged from 1,249 to 2,006 µg kg^{-1} (Pescada), from 1,265 to 1,946 µg kg^{-1} (Robalo) and 1,069 to 1,761 µg kg^{-1} (Manjuba). Figure 2 and statistical analysis showed that the three fish species present similar Se concentration levels. Selenium is essential for both humans and animals, including fish. Preliminary studies have verified the effect of feeding habitats and trophic level in Se accumulation. [19-21]

For As, Cd, Hg and Pb the concentration levels in dry weight were transformed to wet weight by using the % humidity for each fish species (Table 1). These levels were compared to the Brazilian legislation limits for these elements (wet weight) [22] (Table 2). For As only one Robalo individual surpassed the limit of 1.0 mg kg^{-1}. However all the results for Manjuba species were higher than the limit. Most As found in fish, and in marine animals in general, is arsenobetaine, often constituting more than 80% of the total As. Arsenobetaine is an organic As compound, which is regarded as non-toxic and does not pose a problem for seafood consumption safety. Marine organisms, such as shrimp, mussels and fish, contain naturally high concentration of this element, typically ranging from 1 to 100 mg As kg^{-1} wet weight. [23]

For Cd and Pb results, all the individuals analyzed present concentration levels much lower than the limits of 1,000 µg kg^{-1} for Cd and 2,000 µg kg^{-1} for Pb. However, a great concentration variation was observed for the same fish species as well as between the species analyzed (Table 2). The higher concentration levels for Cd and Pb were found for the detritivorous species Manjuba, means of 29 ± 3 µg kg^{-1} and 127 ± 78 µg kg^{-1}, respectively.

For Hg, all the individuals of the 2 carnivorous species presented concentration levels much lower than the limit (1,000 µg kg^{-1}). For the non-carnivorous species (Manjuba) the results were below the limit of 500 µg kg^{-1} (mean of 12.1 ± 4.6 µg kg^{-1}) and much lower than the concentration levels found for the carnivorous species Pescada and Robalo, mean of 92 ± 40 µg kg^{-1} and 104 ± 48 µg kg^{-1}, respectively.

The results for the Macrodon ancylodon (King weakfish – Pescada) (wet weight) obtained by Morgano et al [5] were: As (<0.1 to 1.7 mg kg^{-1}), Cd (<0.01-0.019 mg kg^{-1}), Pb (<0.02-0.44 mg kg^{-1}) and Hg (0.014-0.042 mg kg^{-1}) for fish tissue, purchased at the CEAGESP/SP central receiving and distribution food market. The results obtained in the present study for this fish species were lower for As and Cd and higher for Hg and Pb.

Curcho [1] analyzed muscle tissues of Macrodon ancylodon (pescada) and Centropomus parallelus (Robalo) fish species purchased in Cananeia and the results obtained were: As (0.54 – 2.50 mg kg^{-1}), Cd (<0.005 – 0.018 mg kg^{-1}), Hg (0.012- 0.100 mg kg^{-1}) and Pb (0.015-0.47 mg kg^{-1}) for Macrodon ancylodon (pescada). For Centropomus parallelus (Robalo) the results were: As (0.030 –0.760 mg kg^{-1}), Cd (<0.005 – 0.010 mg kg^{-1}), Hg (0.015- 0.178 mg kg^{-1}) and Pb (0.015-0.034 mg kg^{-1}). The results for the Macrodon ancylodon (pescada) purchased in Cubatão were: As (0.47 – 1.00 mg kg^{-1}), Cd (<0.005 mg kg^{-1}), Hg (0.012- 0.062 mg kg^{-1}) and Pb (0.025-0.324 mg kg^{-1}). These results were, in general, higher than those of the present study.

Table 2 . Concentration levels for As, Cd, Hg and Pb in the fish species analyzed, dry and wet weights, and Brazilian legislation limits [22]

		As (mg kg^{-1})		Cd (µg kg^{-1})		Hg (µg kg^{-1})		Pb (µg kg^{-1})	
Fish species		**Dry weight**	**Wet weight**	**Dry weight**	**Wet weight**	**Dry weight**	**Wet weight**	**Dry weight**	**Wet weight**
Macrodon ancylodon (King weakfish – Pescada) (predatory species)	**P-01**	**0.82**	**0.19**	**< 7.0**	**< 7.0**	**191**	**44.9**	**661**	**155.4**
	P-02	**4.03**	**0.95**	**< 7.0**	**< 7.0**	**380**	**89.3**	**285**	**67.0**
	P-03	**1.74**	**0.41**	**11.9**	**2.8**	**503**	**118.2**	**173**	**40.6**
	P-04	**1.49**	**0.35**	**17.4**	**4.1**	**680**	**159.8**	**314**	**73.7**
	P-05	**1.62**	**0.38**	**< 7.0**	**< 7.0**	**411**	**96.6**	**468**	**110.0**
	P-06	**1.33**	**0.31**	**18.6**	**4.4**	**434**	**102.0**	**258**	**60.6**
	P-07	**1.9**	**0.45**	**12.1**	**2.8**	**165**	**38.8**	**114**	**26.8**
	P-08	**1.03**	**0.24**	**19.8**	**4.7**	**176**	**41.4**	**230**	**54.0**
	P-09	**0.97**	**0.23**	**19.9**	**4.7**	**411**	**96.6**	**106**	**24.9**
	P-10	**1.91**	**0.45**	**19.0**	**4.5**	**551**	**129.5**	**726**	**170.5**
Mean ± sd		**1.7 ± 0.9**	**0.40 ± 0.21**			**390 ± 171**	**92 ± 40**	**333 ± 217**	**78 ± 51**
***Centropomus parallelus* (Fat snook – Robalo peba) (predatory species)**	**R-01**	**2.79**	**0.67**	**7.4**	**1.8**	**143.4**	**34.6**	**202.1**	**48.7**
	R-02	**3.51**	**0.85**	**< 7.0**	**< 7.0**	**463.0**	**111.6**	**59.5**	**14.3**
	R-03	**6.50**	**1.57**	**< 7.0**	**< 7.0**	**406.7**	**98.0**	**139.3**	**33.6**
	R-04	**1.69**	**0.41**	**< 7.0**	**< 7.0**	**432.9**	**104.3**	**82.0**	**19.8**
	R-05	**2.95**	**0.71**	**< 7.0**	**< 7.0**	**708.1**	**170.7**	**304.7**	**73.4**
Mean ± sd		**3.5 ± 1.8**	**0.84 ± 0.44**			**431 ± 201**	**104 ± 48**	**158 ± 99**	**38 ± 24**
***Anchoviella lepidentostole* (Broadband anchovy – Manjuba) (detritivorus species)**	**M-01**	**4.10**	**1.14**	**121.4**	**33.9**	**40.5**	**11.3**	**279.9**	**78.1**
	M-02	**4.30**	**1.20**	**92.4**	**25.8**	**23.5**	**6.6**	**954.7**	**266.4**
	M-03	**4.53**	**1.26**	**91.0**	**25.4**	**34.2**	**9.5**	**579.6**	**161.7**
	M-04	**3.73**	**1.04**	**99.9**	**27.9**	**36.7**	**10.2**	**590.6**	**164.8**
	M-05	**4.09**	**1.14**	**99.4**	**27.7**	**28.9**	**8.1**	**90.5**	**25.2**
	M-06	**4.07**	**1.14**	**118.0**	**32.9**	**56.8**	**15.8**	**620.5**	**173.1**
	M-07	**4.59**	**1.28**	**94.8**	**26.4**	**73.7**	**20.6**	**231.4**	**64.6**
	M-08	**4.13**	**1.15**	**108.5**	**30.3**	**51.3**	**14.3**	**303.7**	**84.7**
Mean ± sd		**4.2 ± 0.3**	**1.17 ± 0.08**	**103 ± 12**	**29 ± 3**	**43 ± 16**	**12.1 ± 4.6**	**456 ± 280**	**127 ± 78**
Brazilian Limits			**1.00**		**1000**		**500a;1000b**		**2000**

a-Non-carnivorous species; b- carnivorous species; QL (Cd) = 7 µg kg^{-1}, QL(Pb) = 15 µg kg^{-1}, QL (Hg) = 5 µg kg^{-1}

4. CONCLUSIONS

Both analytical techniques used in this study (INAA and AAS) showed good precision, accuracy and enough sensitivity to determine toxic metal, macro and micronutrients in fish samples. For the macro and micronutrients analyzed the detritivorous species Manjuba present the higher concentration values for Ca, Co, Fe, Zn and for the trace elements Rb and Sc. Higher concentration levels for K, Na and Br were found for the carnivorous species Pescada and Robalo. Concentration levels for Se were similar for the three fish species analyzed. For As and toxic metals Cd and Pb higher concentration levels were found for the detritivorous species Manjuba, surpassing the Brazilian limit in all individuals analyzed only for As (1.00 mg kg^{-1}). Carnivorous species Pescada and Robalo showed the higher concentration levels for Hg but lower than legislation limits for this fish species (1,000 µg kg^{-1}). From the nutritional point of view it was very important to assess the content of some macro and micronutrients in the fish most consumed in Iguape city considering the high intake due to the great fish offer in the region. Finally, the environmental assessment in order to understand the bioaccumulation processes of Cd, Hg and Pb in the fish samples proved that fish products of the region available for human consumption, did not show any concentration levels above the legislation limits and there seems to be no risks of these toxic metals to the health of the population in this region.

ACKNOWLEDGMENTS

The authors would like to thank the Iguape Secretaria Municipal de Educação for its help and support, the USP Oceanographic Institute South Base for its support and also financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process 552437/2007-7).

REFERENCES

1. M.R.S.M. Curcho. Avaliação de micro e macroelementos, elementos tóxicos (Cd, Hg e Pb) e ácidos graxos, em peixes disponíveis comercialmente para consumo em Cananéia e Cubatão, estado de São Paulo. Dissertação de Mestrado, Instituto de Pesquisas Energéticas e Nucleares, 204p., 2009.
2. CETESB, 2001 CETESB. Sistema Estuarino de Santos São Vicente, Relatório Técnico, São Paulo/SP, Brasil, 2001.
3. E.L. Maia, C.C.S. Oliveira, A.P. Santiago. "Composição química e classes de lipídios em peixe de doce curimatã comum, Prochilodus cearenses". *Ciência e Tecnologia dos Alimentos*, **19(3)**, pp. 433-437 (1999).
4. M.R.S.M. Curcho, L.A. Farias, S.R. Baggio, B.C. Fonseca, S.M. Nascimento, M.C. Bortoli, E.S. Braga, D.I.T. Fávaro. "Mercury and methylmercury content, fatty acids profile, and proximate composition of consumed fish in Cananéia, São Paulo, Brazil". *Rev Inst Adolfo Lutz*, **68(3)**, pp.442-50 (2009).
5. M.A. Morgano, A.P.F. Oliveira, L.C. Rabonato, F. Milani, J.P. Vasconcellos, C.N. Martins, A.L. Citti, E.O. Telles, S.C. Balian. "Avaliação de contaminantes inorgânicos (As, Cd, Cr, Hg e Pb) em espécies de peixes". *Rev. Inst Adolfo Lutz***,** **70(4)**, pp. 497-506 (2011).

6. M.A. Morgano, A.C.A. Perez, R.F. Milani, D.M.B. Mantovani, C.R.P. Neiva, E.F. Furlan et al. “Mercúrio total em pescado da cadeia produtiva da Baixada Santista, estado de São Paulo, Brasil”. *Rev. Inst Adolfo Lutz*, **66(2)**, pp.165-172 (2007).
7. W.R. Pereira Filho, E.F. Ribeira, F.S. Lizarte Neto. “Elementos metálicos em pescados comercializados na cidade de São Paulo”. ConScientiae Saúde, 2, pp. 61-65 (2003).
8. Instituto Brasileiro de Geografia e Estatística. Pesquisa de Orçamentos Familiares 2008/2009, Brasil, 2010.
9. T. R. Garcia. Impactos da implantação de uma cooperativa de produção de ostras junto a comunidades extrativistas caiçaras do litoral sul/SP: um estudo de caso [Dissertação de Mestrado]. São Paulo: Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo; 2005.
10. L.A. Farias, D.I.T. Favaro, M.B.A. Vasconcellos. “Determinação de mercúrio e metilmercúrio em amostras de cabelo e peixes”. *Rev. Inst Adolfo Lutz*, **68(3)**, pp.451-460 (2009).
11. L.A. Farias, N.R. Santos, D.I.T. Favaro, E.S. Braga. “Mercúrio total em cabelo de crianças de uma população costeira, Cananéia, São Paulo, Brasil. *Cad. Saúde Pública*, Rio de Janeiro, **24(10)**, pp.2249-2256 (2008).
12. M.G.M. Catharino, M.B.A. Vasconcellos, E.C.P.M. Sousa, E.G. Moreira, C.D.S. Pereira. Biomonitoring of Hg, Cd, Pb and other elements in coastal regions of São Paulo State, Brazil, using the transplanted mussel *Perna perna* (Linnaeus, 1758). *J. Radioanal. Nuclear Chem*., **278(3)**, 547-551 (2008).
13. D.I.T. Favaro, C. Afonso, M.B.A. Vasconcellos, S.M.F. Cozzolino. “Determinação de Elementos Minerais e Traços por Ativação Neutrônica, em Refeições Servidas no Restaurante da Faculdade de Saúde Pública/USP”. *Revista Ciência e Tecnologia de Alimentos*, **20 (2)**, pp.176-182 (2000).
14. NRC, 1993. Nutrient Requirements of Fish. National Academic Press, (National Research Council), Washington, DC, 200 pp.
15. L. A. Farias, D.I.T. Fávaro, J. O. Santos, M. B. A. Vasconcellos, A. Pessôa, J.P.L. Aguiar, L.K. Yuyama. “Cooking process evaluation on mercury content in fish” *Acta Amazônica*, **40(4)**, pp. 741-748 (2010)
16. T. Watanabe, V. Kiron, S. Satoh, S. “Trace minerals in fish nutrition”. *Aquaculture*, **151**, pp.185-207 (1997)
17. C.N. Glover, C. Hogstrand. 2003. “Effects of dissolved metals and other hydrominerals on in vivo intestinal zinc uptake in freshwater rainsbow trout”. *Aquatic Toxicology*, **62**, pp.281–293 (2003).
18. E.S. Brabo, E.O. Santos, I.M. Jesus, A.F. Mascarenhas, K.F. Faial. “Níveis de mercúrio em peixes consumidos pela comunidade indígena de Sai Cinza na Reserva Munduruku, Município de Jacareacanga, Estado do Pará, Brasil”. *Cad. Saúde Pública*, **15(2)**, pp. 325-331 (1999).
19. J. Burger, K.F. Gainesc, S. Boring, W.L. Stephen, J. Snodgrassd, M. Gochfelde. “Mercury and selenium in fish from the Savannah river: species, trophic level, and locational differences”. *Environmental Research*, **87**, pp.100-108 (2001).
20. M.. Barwick & W. Maher. “Biotransference and biomagnification of selenium, copper, cadmium, zinc, arsenic and lead in a temperate seagras community from lake Macquarie, NSW, Australia”. *Marine Environmental Research*, **56**, pp.465-471 (2003).
21. B. Bhattacharya, S.K. Sarkar, R. Das. “Seasonal variations and inherent variability of selenium in marine biota of a tropical wetland ecosystem: implications for bioindicator species”. *Ecological Indicators*, **2**, pp.360-367 (2003).

22. Brasil, Leis, Decretos. Divisão Nacional de Vigilância Sanitária de alimentos DINAL – Portaria nº 685 de 27 de agosto de 1998. Diário Oficial da União. Brasília. Séc. 1, pt 1, p. 1415-1437, 24 set., 1998.
23. National Institute of Nutrition and Seafood Research (NIFES), http://www.nifes.no