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Determination of some Metal-ions in the Bodies of Black-bass (*Micropterus salmoides*) and Tench (*Tinca tinca*), and from Water Reservoirs Close to Border of Portugal/Spain

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ABSTRACT

The heavy metal (Zn, Cu, Mn, Fe, Pb, Cd) concentrations of muscle tissues of *Micropterus salmoides* and *Tinca tinca* from fresh water reservoirs and Fish Production Units situated in Portuguese and Spanish regions close to the border Portugal/Spain, were evaluated by atomic spectrophotometer absorption. Dried powders from these fishes were used to be calcinated, followed by acid digestion and atomic spectrophotometric metal analyses. Mean values in mg kg^{-1} wet weight of *M. salmoides* from ESA and Urra reservoirs were respectively the following: Cd (0.03 & 0.03), Cu (3.23 & 2.41), Fe (5.03 & 4.90), Mn (0.58 & 0.49), Pb (0.46 & 0.63) and Zn (6.73 & 7.01). The mean contents in mg kg^{-1} wet weight in *T. tinca* from Casillas and Olivenza are, respectively the following: Cd (0.02 & 0.04), Cu (1.30 & 1.47), Fe (3.92 & 6.41), Mn (0.26 & 0.59), Pb (0.29 & 0.99) and Zn (6.27 & 8.96). It was concluded that these metallic concentrations are below the maximum permissible for a safety utilization of theses fishes in human nutrition.

Key Words: Heavy metals; Zn; Cu; Mn; Fe; Pb; Cd; Fishes; Black bass; Tench

INTRODUCTION

The interest in the determination of heavy metals in food is increasing due to the health problems, which may cause for human beings, when fishes are contaminated by relatively high concentration of those elements. In fact, when these metal concentrations increase above the permissible limits, the food becomes toxic to organisms (Von Schiruding *et al.*, 1991; Ipinmoroti *et al*., 1997). Concerning metal pollution in waters, research has been focused on the distribution of trace metals in fresh waters, as well as in seawaters (Ashraf *et al*., 1997). Metal accumulation in fish tissues has been recognized to be mainly found in liver and gills, but in muscle such accumulation is not so severe (Dabeka *et al*., 1985).

In certain regions of Portugal and Spain namely some, which are close to the border of these two countries, there are water natural courses with good fish not yet commercially explored to be routinely consumed, particularly the species *Micropterus salmoides* (black bass) and *Tinca tinca* (tench), much appreciated due to their very good flavour properties. On the other hand those fishes are often consumed without any information about their metallic content. In fact, as far as we know, there are not published data concerning this question. Obviously, it is very important to know if those fishes are in safety conditions to be consumed by human beings.

With this in mind we decided to determine the concentration of some heavy metals (Cd, Cu, Fe, Mn, Pb & Zn) in the muscle of those fishes, from fresh waters situated close to the border of Portugal (Beira Baixa & Alto Alentejo) and Spain (Estremadura), in a preliminary study of a more ambitious future project, covering many more fresh water reservoirs and more elements. It should be recalled that this study is very important to assure that these fishes are safe to be consumed by local populations.

MATERIALS AND METHODS

Present research reflects a concern presented to the authors by the Portalegre (Portugal) Council Municipal authorities regarding the quality and heavy metal contamination of fishpond water, which are in a radius of close to 150 km from the Spanish Nuclear Central of Almaraz.

After fishing, fishes were stored at -20ºC. In sampling operations they were slowly un-frozen, washed, fine cut with plastic knife and dried on petriplates at 105ºC for 24 h or longer, until constant weight. The dried matter was triturated to get a fine powder for calcinations. Two grams of powder samples in porcelain crucibles were calcinated in triplicate at 550ºC for 15 h with accompanying blank tests. After stabilization to room temperature, digestion with 10 mL of HCl: HNO_3: H_2O (1: 1: 8) at about 180ºC for 3 min, was performed, followed by quantitative filtration to a final volume of 50 mL. Those extracts were kept in amber glass containers to be used for espectrophotometric readings. Samples of black bass from the ESA reservoir (Castelo

Branco, Portugal) and from Urra reservoir (Portalegre, Portugal) were prepared for spectrophotometric analysis, as well as tench samples from Casillas and Olivenza reservoirs (these from Spain). A Shimadzu atomic spectrophotometer model 6501, with automatic sampler and deuterium lamp for background corrections, was used. Lamps from Hamamatsu Photonics K.K. were utilized and metal atomization was performed with air-acetilene mixtures (temperatures between 2600 & 2800ºC). The analysis conditions for metal readings were according the Instructions Manual. Standards were prepared by diluting the stock solutions (1 g L^{-1}) to appropriate concentrations. Calibration was performed by analysing five standard solutions and two reagent blank samples; each point of the calibration curve was obtained with the auto-dilution feature of the auto sampler. Dog fish (DORM-2) was used as a certified material to validate the EAA method. Analysis for Cd, Cu, Fe, Mn, Pb and Zn have presented results with an error less than 5% relatively to the certified values.

RESULTS AND DISCUSSION

Results for metals content for both fish species can be observed in Tables I to IV. To our knowledge, there are no studies concerning the fishes we have studied from the mentioned reservoirs. Thus, the presented mean values are compared with some literature data dealing with other species of fishes, from Portugal and other different regions of the world.

Cadmium median concentration (mg kg^{-1} wet weight) was low for both species (0.03 & 0.03) for ESA and Urra black bass, respectively and (0.02 & 0.04 for Olivenza & Casillas tench respectively). Those values are much lower than the maximum permissible (0.1 mg kg^{-1}) (Tarley *et al.*, 2001). In some other studies the reported mean value for Cd in fish was 0.02 mg kg^{-1} and the highest concentration was found in shellfish (0.2 – 1.0 mg kg^{-1}) (Galal-Gorchev, 1991). Cadmium is one of the most toxic metals for human beings. However, contamination by Cd is not a concern in fish (Sharif *et al*., 1993). In fact, Cd content in fish muscle tissue is generally very low, being preferentially accumulated in kidney and liver, which should not be consumed.

Relatively Cu content was 3.23 and 2.41 for ESA and Urra black bass respectively, and 1.30 and 1.47 for Casillas and Olivenza-tench respectively. Copper is an essential micronutrient, important in association with proteins (hemocyanins, tyrosinases, plastocyanins, cytochrome oxidase) being very toxic if consumed above 30 mg day^{-1} (WHO, 1996). Indeed, elevated concentration of copper in aquatic food is not common, except when the waters are contaminated by waste liquids from mines. So, the values found for both species allow safety utilization as food. Studies with other fishes of different marine species have shown values between 0.1 and 4.7 mg kg^{-1} wet weight in muscle and 10.1 mg kg^{-1} wet weight in the anglerfish

Table I. Metal Concentration (mg kg^{-1} wet weight) in ESA black bass

Element	Mean	Lower	Higher	Maximum Tolerable
Cd	0,03 ± 0,01	0,01	0,06	0,1
Cu	3,23 ± 0,44	2,06	4,26	20
Fe	5,03 ± 0,38	2,27	10,8	50
Mn	0,58 ± 0,09	0,23	0,96	4
Pb	0,46 ± 0,20	0,44	0,47	1
Zn	6,73 ± 0,23	4,87	8,51	50

Note: The values are means ± s.d. of 12 samples

Table II. Metal Concentration (mg kg^{-1} wet weight) in Urra black bass

Element	Mean	Lower	Higher	Maximum Tolerable
Cd	0,03 ± 0,02	0,02	0,04	0,1
Cu	2,41 ± 0,72	1,54	3,61	20
Fe	4,90 ± 0,40	4,25	6,14	50
Mn	0,49 ± 0,03	0,38	0,55	4
Pb	0,63 ± 0,15	0,30	0,91	1
Zn	7,01± 0,13	6,40	7,97	50

Note: The values are means ± s.d. of 9 samples

Table III. Metal Concentration (mg g^{-1} wet weight) in *Casillas tench*

Element	Mean	Lower	Higher	Maximum Tolerable
Cd	0,02 ± 0,00	0,00	0,01	0,1
Cu	1,30 ± 0,25	0,78	1,81	20
Fe	3,92 ± 0,10	2,53	6,10	50
Mn	0,26 ± 0,03	0,17	0,39	4
Pb	0,29 ± 0,14	0,00	0,50	1
Zn	6,27 ± 0,71	5,31	7,93	50

Note: The values are means ± s.d. of 15 samples

Table IV. Metal Concentration (mg g^{-1} wet weight) in *Olivenza tench*

Element	Mean	Lower	Higher	Maximum Tolerable
Cd	0,04 ± 0,03	0,03	0,04	0,1
Cu	1,47 ± 0,08	1,45	1,49	20
Fe	6,41 ± 0,01	6,12	6,70	50
Mn	0,59 ± 0,02	0,54	0,64	4
Pb	0,99 ± 0,25	0,51	1,46	1
Zn	8,96 ± 0,12	8,45	9,47	50

Note: The values are means ± s.d. of 9 samples

(*Lophius piscatorius*), liver (Assunção *et al*., 2003), which is a normal accumulator of Cu and other metals. In Brazilian canned sardines mean copper values ranged from 1.31 to 2.25 mg kg^{-1} wet weight (Tarley *et al*., 2001). So, the obtained values for these other fishes species are close to the ones we have found for black bass and tench. However, It has been referred that the average copper concentration in fish muscle is 0.2 - 0.5 mg kg^{-1} wet weight, with high accumulation in organs and other tissues (in decreasing order: liver, scales, spleen, kidney, gills). Comparing median values for Cu (Table V), the content differences

between the two fishes were significant.

Concerning iron, its concentration range (mg kg^{-1} wet weight) was 5.03 and 4.90 for ESA and Urra black bass, respectively 3.92 and 6.41 for Casillas and Olivenza tench, respectively. This metal is also an essential nutrient, also associated with proteins (hemoglobin, myoglobin, c-type cytochromes, cytochrome oxidase). Its accepted value for human nutrition is between 10 and 50 mg kg^{-1} wet weight (WHO, 1996). Hence its content in the studied species can be accepted as good to be consumed by humans. Studies in marine species have shown values from 6.43 to 109 mg kg^{-1} wet weight (Santiago *et al.*, 2002) and from 9.52 to 32.4 (Tuzen, 2003) and from 21 to 89 mg kg^{-1} wet weight in canned sardines from Brazil (Tarley *et al.*, 2001). As can be suggested those so different Fe contents should reflect the fish species, the water (sea or fresh water) as well as their quality. Also it may be due to different locations of sampling area or to different depth (Zehra *et al.*, 2003).

Manganese determinations have shown 0.58 and 0.49 in mg kg^{-1} wet weight for ESA and Urra black bass, respectively and 0.26 and 0.59 mg kg^{-1} wet weight for Casillas and Olivenza tincas, respectively. This element is also essential for humans, being important in many enzymatic systems, particularly in enzymes involved in free radical elimination. In Portugal, the maximum permissible has not yet been established, but it can be presumed that values below 4 mg kg^{-1} wet weight are good for health and these fishes are appropriate to be consumed. In marine species, as Brasilian sardines (Tarley *et al.*, 2001), these values were 1.53 - 17.55 mg kg^{-1} wet weight.

Concerning Pb the mean concentration was 0.46 and 0.63 in mg kg^{-1} wet weight respectively for ESA and Urra black bass and 0.29 and 0.99 in mg kg^{-1} wet weight for Casillas and Olivenza tench. In fish from different locations around the world Pb concentrations are in the range 0.05 to 2.50 mg kg^{-1} wet weight (Sharif *et al*., 1993). This element is not essential, being toxic in low concentrations (above 1, 0 mg kg^{-1} wet weight). So, concentration values in black bass and tench are included in the limits for fishes collected in different areas and below the maximum accepted to be consumed. High Pb contents in muscle tissue can be found in waters far from areas with intensive industrial and agricultural activities and/or receiving discharges of un-treated municipal and/or industrial waste waters. Water reservoirs isolated from these discharges but very close to intense traffic can also be progressively contaminated, with the residues containing Pb from gasoline combustion.

Zinc mean concentration in mg kg^{-1} wet weight was 6.73 and 7.01 for ESA and Urra black bass respectively and 6.27 and 8.96 for Casillas and Olivenza tench respectively. Zinc is an essential element for humans, essential to many enzymes activity and in the spatial organization of certain proteins related with DNA biochemistry. Thus many marine organisms rich in proteins have high content of this metal (10 to 50 mg kg^{-1} wet weight) (WHO, 1996) but the normal average Zn content in marine organisms is 3 - 5 mg kg^{-1} wet

Table V. Comparison of elements content in both fishes

Elements	Fishes	Mean (mg/kg wet weight)	P-Value (> 0,05)
Cd	Black bass	0,03	0,78
	Tench	0,04	
Cu	Black bass	2,88	0,01*
	Tench	1,52	
Fe	Black bass	5,04	0,74
	Tench	4,65	
Mn	Black bass	0,54	0,06
	Tench	0,33	
Pb	Black bass	0,53	0,83
	Tench	0,50	
Zn	Black bass	6,85	0,62
	Tench	7,29	

Note: p – Significant value; * The difference is significant for $p < 0,05$

body weight. Taking this into account and the metallic concentrations for black bass and tench, it can be concluded that Zn content was below the maximum permissible, so these fishes can be used safely in human nutrition. Indeed, no reports of concentrations in the edible parts of food fish, to be prejudice to human health have been presented.

Until now the reservoirs whose fishes we have studied have not been contaminated with the heavy metal elements we have analysed, taking into consideration the discussed results. Nevertheless, a good program of heavy metals control, over those and other fishes should be applied with a certain frequency by Municipal Council authorities, to prevent future eventual contamination of fishes and the natural consequences to human health.

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