Determination of Zn, Fe, Cr and Cu in Marine Fish Commonly Consumed in Senegal

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ABSTRACT

The content of four heavy metals (zinc, iron, chromium and copper) in muscles, liver and gonads has been studied for the five most consumed fish species (*Cephalopholus taeniops*, *Scomber japonicus*, *Lagocephalus laevigatus*, *Pagellus bellottii* and *Pagrus caeruleostictus*) in the Soumbédioune region. The samples were digested by acid digestion. The metal contents were quantified using a PF-11 type photometer. The results obtained show that metals such as zinc, iron and chromium are significantly higher ($p < 0.05$) in the muscles of the four species than in the liver and gonads. However, the levels of heavy metals in the muscles, liver, and gonads of the five species are above the acceptable limit recommended by the FAO and WHO, with the exception of the copper levels in the gonads of the species *Pagellus bellottii*.

Keywords: contamination; metal; fish, Soumbédioune coast.

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I. INTRODUCTION

Fish is one of the most important foods of human diet and nowadays, its demands are rising in the field of consumption due to the current increase of the world population. Considered as a relative cheap source of protein,

fishes are mainly provided by the sea. It's important to mention that the maritime environment plays a key role in the qualities of the fishes and especially in term of pollution. However, in Senegal, many researchs have been showing that the maritime environment is increasingly polluted by much industrial waste waters which are mostly partially treated or

untreated [1].

Dakar, economic capital of Senegal, concentrates almost of the light and heavy industries near the sea. So, Dakar must be faced the issue sea's pollution. In this paper, we focus on Soumbédioune which is located on the west ledge of Dakar. Soumbédioune beach is well known by the residents of Dakar for its tasty grilled fish, accessible to all budgets [2], but it represents also the rallying point of the rainwater evacuated by the channel which crosses several districts and dumps a huge amount of wastewater into the sea [3]. The objective of this channel is diverted by the populations who have finished making it a garbage dump.

According to this alarming situation, it will be interesting to study the evaluation of metal accumulation in fish caught off the Soumbédioune coast [4].

The main purpose of this study is to determine the impact of the marine pollution by the quantification of Zn, Fe, Cr, and Cu contained in muscle, liver and gonads of fish species caught off, sold, and consumed on the coast of Soumbédioune, in order to identify a potential exposure of the population.

II. MATERIAL AND METHODS

A. Study Site

The map (Fig. 1) shows the study area. Soumbédioune is located in the Dakar region, which are between the longitudes of 17°10 and 17°32 West and the latitudes of 14°53 and 14°35 North. To the East it is limited by the region of Thiès and by the Atlantic Ocean in its northern, western, and southern parts, with a population of 3.233.460 inhabitants.

B. Reagents

All chemical reagents used were of high-purity analytical grade. Ultrapure water (Millipore Milli-Q System) was used for solutions preparation and dilutions. All glass-ware were soaked in 1.5% (w/v) HNO₃ (65%) of ultrapure quality for 24 h and then rinsed with ultrapure water before doing the metal analysis. Ultrapure standard solutions of 1000 mg L⁻¹ of the metals (Merck, Darmstadt, Germany) were used for

calibration. Nitric acid (65%) (Merck, Darmstadt, Germany) of ultrapure quality was also used for digestion.

C. Sample Collection and Preparation

Fish samples were collected from Soumbédioune coast located in the Medina district, on the western corniche of Dakar (Fig. 1). It is a locality which receives wastewater coming from the western channel crossing many districts of the region of Dakar.

For the collect of fishes, we have two points. The first is located around hundred meters from the coast. The second site is the Sarpant Island, which is located around hundred meters from the previous point. It is important to mention that Sarpant Island is a particular refuge for a huge varieties of fish. Fishermen caught fish by using beach chains with larges mesh nets. The collect was done in the same day. The species selection criteria are based on the abundance of the species, the sedentary lifestyle, and the ability to accumulate metals in their flesh.

The fishes were transported to the laboratory and then stored in a refrigerator at -20 °C before preparation.

In the laboratory, fishes were thawed at room temperature before dissection and analysis; their weight and total length were measured. Fish samples were dissected to separate the muscle, liver and gonads as recommended by UNEP/FAO/IOC/IAEA, 1984 [5] (Table I). The separated organs were neatly placed in labeled acid washed Petri dishes and dried at 60 °C for two days [4]. Teflon mortar was used for pulverization and homogenization of tissues powder before digestion.

D. Mineralization of the Samples

For mineralization, each organ sample was divided in three portions of about 0.5g and the powder are treated with 4 mL HNO₃ (Merck, Darmstadt, Germany) (14 mol.L⁻¹ in glass capsules (Merck, Darmstadt, Germany); then placed in the oven at 80 °C during one hour for tissue digestion and total evaporation of the acid [6]. The product of digestion is recovered with a few mL of distilled water and taken up in a 50 mL volumetric flask by adding distilled water.

Fig. 1. Site of Soumbédioune.

TABLE I: FISH SAMPLES CHARACTERISTICS (LENGTH, WEIGHT, ORGANS)

Fish species	Measurements (cm)			Weighings (g)		Organs			
	LS	LF	LT	PT	Pev		Liver	Muscle	Gonads
Ct	14.5-17.2	-	17-20.2	63.8-120.5	56.9-108.1	Wet weight (g)	-	13.7-30.1	-
						Dry weight (g)	-	4.3-6.2	-
						Water content (%)	-	76.2-80.2	-
Sj	26.3-30.6	28.4-31.5	31.1-39	246.3-399.7	183.5-324.3	Wet weight (g)	3-3.4	47.3-95.5	10-18.1
						Dry weight (g)	1.1-1.4	19.1-39.6	3.1-6.8
						Water content (%)	58.8-63.3	50.8-67.6	62.4-69
Ll	17.9-21.7	20-23.5	20.8-25.2	154.4-275	131.2-224.8	Wet weight (g)	9.6-9.9	29.4-61.4	-
						Dry weight (g)	4.2-5.2	5.4-17.4	-
						Water content (%)	47.5-56.2	68.4-81.6	-
Pb	16-19.9	17.6-21.1	19.9-23.8	112.9-196.3	92.5-179.1	Wet weight (g)	0.8-1.5	32.1-53.2	-
						Dry weight (g)	0.2-0.3	8.7-13.5	-
						Water content (%)	75-80	69.8-79.1	-
Pc	17.6-19.4	20-21.8	23-24.8	191.5-278.9	170.4-253.3	Wet weight (g)	1.3-1.6	48-57.6	-
						Dry weight (g)	0.3-0.4	14.4-26.3	-
						Water content (%)	69.2-81.2	54.2-67.4	-

III. ANALYSIS OF SAMPLES

The solution obtained after mineralization is used for analysis with a PF-11 photometer (Macherey Nagel, France). The analysis of metallic elements (Zn, Fe, Cr, and Cu) in muscle, liver and gonads of fish species was carried out at the Laboratory of Organic Physical Chemistry and Environmental Analysis (LCPOAE) of the Faculty of Sciences and Techniques of Cheikh Anta Diop University of Dakar [4]. The analyses were performed by UV-visible spectrophotometry using the PF-11 photometer (Macherey Nagel). It was done according to colorimetric methods from kits.

A. Preparation of the Blank and the Sample

The blank is constituted of demineralized water or the sample to be analyzed without addition of reagents.

For the preparation of sample, we applied the method of analysis per proportioned addition. We used specific reagents for each metal as a complexant. In fact, 5 or 10 mL are sampled from the solution obtained after mineralization, and then we added some drops of reagents. After we have to wait some minutes for the homogenization of the solution. Finally, we take a measure with the photometer. It's important to know, that the numbers of drops and minutes to wait depend on each metal.

The preparation of the sample to be analyzed consists in adding reagents in 5 or 10 mL of the prepared solution. It is a method of analysis per proportioned addition. It is very significant to respect the reading time prescribes in the protocol of analyses to ensure a homogeneity of the solution to be proportioned.

B. Principle of the Analysis with the Photometer

We chose the suitable wavelength for each element destined to analyze. 5 mL were taken from the solution obtained after mineralization as a sample and then we added reagents as complexants. It was very important to respect the order and time prescribed in the analysis protocol to ensure the reaction of the reagents with the analyte. The zero of the concentration (blank) must be adjusted before each measurement. Blank is done on the sample without addition of reagents. The sample was placed in the measuring cell and the concentration of the sample was read directly on the screen of the apparatus in mg L^{-1} .

Below we resumed the protocol of preparation of the

samples for each metal.

Determination of chromium VI: 5 mL of the sample is taken into a test tube in which are added 5 drops of reagent 1 (Cr^{6+}), stirred for a few seconds. Then we add 5 drops of reagent 2 (Cr^{6+}) and stir again. Finally, wait for 3 minutes before taking the reading in Visocolor Eco mode.

Determination of iron: 5 mL sample is placed in a test tube with 4 drops of reagent 1 (Fe), stirred for a few seconds. Then add a spoonful of reagent 2 (Fe) and stir again. Then wait 7 minutes before taking the reading in Visocolor Eco mode.

Determination of zinc (II): 5 mL sample is placed in a test tube in which are added 5 drops of reagent 1 (Zn^{2+}), stirred for a few seconds. Then 5 drops of reagent 2 (Zn^{2+}) are added, the mixture is stirred again. Finally, 10 drops of reagent 3 (Zn^{2+}) are added while homogenizing. Wait 1 minute before taking the reading in Visocolor Eco mode.

Determination of copper: 5 mL sample is placed in a test tube in which are added 5 drops of reagent 1 (Cu^{2+}), stirred for a few seconds. Then 5 drops of reagent 2 (Cu^{2+}) are added, the mixture is stirred again. Finally, wait 10 minutes before taking the reading in Visocolor Eco mode.

C. Statistical Analysis

The datas obtained were subjected to analysis of variance (ANOVA) in order to quantify the different metal concentration in the five fish species and organs. Duncan Multiple Range Test was used to separate differences between average of measure Statistical significance level was set at $p < 0.05$ using SPSS version 17.

IV. RESULTS

The results of the analysis of metals in the organs of *Cephalopholus taeniops*, *Scomber japonicus*, *Lagocephalus laevigatus*, *Pagellus bellottii*, *Pagrus caeruleostictus*, for a serie of three measurements are shown in Table II.

The concentration of zinc (Zn) in fish muscles varied from 10 mg kg^{-1} in *C. taeniops* (Ct) (Fig. 2), to 100 mg kg^{-1} in *S. japonicus* (Sj) as shown in Figure 3. Zinc was not detected in the liver and in the gonads of *C. taeniops* (Ct). This is due to the inactive nature of the muscle, which favors the accumulation of heavy metals.

These results are in agreement with some previous studies. The content of Zn observed in the muscles of *Cyprinus carpio* (12.5 mg kg^{-1} wet weight) [7] confirm that. However, higher

Zn values (13.8-187.6 mg kg⁻¹ dry weight) were noted in the muscle of *Labeo umbratus* from the Olifants river [8]. Moreover, some studies have proved the contrary [9] because in the fish muscle of the Danube river, the Zn content is low (7.2 mg kg⁻¹ in *Leuciscus cephalus*). Likewise, in *Chondrostoma regium* (7.9 mg kg⁻¹ wet weight) and *Capoetta trutta* (5.3 mg kg⁻¹ wet weight) from Lake Ataturk [10] and from Lake Peninsula de Taimyr [11]. Even more, low values were reported in the muscle of *Lisa aurata*, *Anguilla anguilla* and *Solea vulgaris* (3.10-8.41, 10.1-13 and 4.17-8.52 mg kg⁻¹ wet weight, respectively) from the southern Atlantic coast of Spain [12].

For the animals Fe is an important trace element, which is essential for cellular respiration of the animals because it facilitates the transport of oxygen. It is a powerful catalyst for certain biochemical reactions [4]. The concentration of iron recorded in the muscles varies from 13 mg kg⁻¹ in *P. bellottii* (Pb) (Fig. 5) to 82.3 mg kg⁻¹ in *C. taeniops* (Ct). The iron content in *L. laevigatus* (Figure 4) varies from 55-85 mg kg⁻¹ in the liver.

TABLE II: THE MINIMUM, MAXIMUM VALUES OF METAL CONTENT (MG/KG) IN FISH SPECIES

Fish species	Organs	Zn	Fe	Cr	Cu
		Min-Max	Min-Max	Min-Max	Min-Max
<i>Cephalopholus taeniops</i> (Ct)	Muscle	20-230	17-120	31-80	n.d.
	Liver	n.d.	n.d.	n.d.	n.d.
	Gonads	n.d.	n.d.	n.d.	n.d.
<i>Scomber japonicus</i> (Sj)	Muscle	20-180	66-124	3-54	n.d.
	Liver	n.d.	n.d.	n.d.	n.d.
	Gonads	n.d.	n.d.	9-11	n.d.
<i>Lagocephalus laevigatus</i> (Ll)	Muscle	20-90	4-64	14-42	n.d.
	Liver	n.d.	55-85	n.d.	n.d.
	Gonads	n.d.	n.d.	n.d.	n.d.
<i>Pagellus bellottii</i> (Pb)	Muscle	0-10	8-15	9-39	n.d.
	Liver	n.d.	n.d.	n.d.	n.d.
	Gonads	n.d.	18	37	40
<i>Pagrus caeruleostictus</i> (Pc)	Muscle	20-30	14-48	15-36	n.d.
	Liver	n.d.	n.d.	n.d.	n.d.
	Gonads	n.d.	n.d.	n.d.	n.d.

Few amounts of Fe were found in muscle from *Lisa abu* and *Silurus triostegus* (6.88 and 6.38 mg kg⁻¹ wet weight, respectively) from Lake Ataturk [8]. We noted the same trend in the muscles of *Leuciscus cephalus* (7.67 mg kg⁻¹), *Rutilus rutilus* (5.90 mg kg⁻¹) and *Perca fluviatilis* (5.05 mg kg⁻¹) fresh weight from the Nitra river in Slovakia [13]. It is the same for the muscle of *Lisa aurata*, *Anguilla anguilla* and *Solea vulgaris* (4.11-7.13; 4.11-5.89 and 2.13-5.01 mg kg⁻¹ wet weight, respectively) from the southern Atlantic coast of Spain [12]. In contrast, high Fe concentrations have been reported in the muscles of *Labeo umbratus* in the Olifants River [14].

Chromium (Cr) is a metallic element, which can be present in foodstuffs. The Fishes ingest foods contaminated by chromium, that passe through the gastrointestinal tract and are distributed, accumulated, or detoxified by the liver [15]. The concentration of chromium in the muscles varies from 18.5 in *P. bellottii* (Pb) to 43.6 mg kg⁻¹ in *C. taeniops* (Ct). This metal is absorbed by the gills and transported by the blood to the liver where it is detoxified and excreted under the form of bile before reaching the flesh and leading to the lower toxicity of heavy metals in the flesh [16]. The

chromium in the liver was not detected in the different species of fish studied. However, the gonads of the species *S. japonicus* (Sj) (Fig. 3) and *P. bellottii* (Pb) show that the chromium contents vary between 9 and 37 mg kg⁻¹ (Fig. 5).

Low quantities of chrome were reported in muscles of *Lisa aurata*, *Solea vulgaris* and *Anguilla anguilla* (0.029-0.038, 0.033-0.045 and 0.143-0.368 mg kg⁻¹ wet weight, respectively) from the southern Atlantic coast of Spain [12]. As well, very high concentrations of chrome (5.57-197.12 mg kg⁻¹ dry weight) have been detected in the muscles of *Labeo umbratus* upstream of the Olifants River [9].

Copper (Cu) is an essential element, which improves the enzymatic activity of the body [17]. The concentration of copper in the muscle and liver of the different fish species was not detected (Figure 4). However, a content of 20 mg kg⁻¹ of copper dry weight was found in the species *P. bellottii* (Pb). The value obtained in this study is lower than the safety recommended limit value which is 30 mg kg⁻¹, dry weight [18].

A High amount of Cu was found in the muscle of *Labeo umbratus* (1.53 to 13.08 mg kg⁻¹ dry weight) [7]. At the same time, a low content of Cu was noted in the muscle of *Lisa aurata*, *Anguilla anguilla* and *Solea vulgaris* (0.2-0.6, 0.5-1.5 and 0.4-0.5 mg kg⁻¹ of body weight fresh, respectively) from the southern Atlantic coast of Spain [10]. It is the same for the *Pagellus erythrinus* (0.15-0.23 mg kg⁻¹ wet weight) from the eastern Aegean Sea of Turkey [19] and in muscles fish from Lake Ataturk [20]. Similar results to the previous ones were observed in the muscles of fish species (*Prochilodus lineatus*, *Cyprinus carpio* and *Mugil cephalus*) from the river Rio de la Plata [7].

Fig. 2. Average metal content and Standard deviations of organs of the species *C. taeniops*.

Fig. 3. Average metal content and Standard deviations of organs of the species *S. japonicus*.

Fig. 4. Average metal content and Standard deviations of organs of the species *L. laevis*.

Fig. 5. Average metal content and Standard deviations of organs of the species *P. bellottii*.

Fig. 6. Average metal content and Standard deviations of organs of the species *P. caeruleostictus*.

V. DISCUSSION

The contents obtained for fish are higher than the standards established by the OMS/FAO for the zinc which is 70 mg kg^{-1} and for the chromium which is 5.5 mg kg^{-1} according to the CEE/R n466/2001 [21]. For iron the reference value is 20 mg kg^{-1} , which corresponds to the threshold tolerated in the foods destined to the consumption [22].

The differences noted on the metal concentrations between the species can be explained or depend on the living environment, the food, the mobility of fish or on other characters.

The accumulation of the four metals in the fish species *C.*

taeniops, *S. japonicus*, *L. laevis*, *P. bellottii*, *P. caeruleostictus* (Fig. 2) varies from one organ to another. The highest contents were given by the species *C. taeniops* (Ct) (Fig. 2) and *S. japonicus* (Fig. 3). However, the lowest amounts of metals were detected in the fish *P. bellottii* (Pb) (Fig. 5), *P. caeruleostictus* (Fig. 6). It is important to mention that, in general, the muscles are able to accumulate metals more than the other organs such as liver or gonads. The contents of heavy metals in the muscles, liver and gonads of the five species are above the acceptable limit recommended by the FAO and WHO but the amount of copper in the gonads of the species *P. bellottii* is considered as the only exception.

The differences related about concentrations of metals between organs vary by order of magnitude according to the organs. These variations might come from the physiological functions of the different organs. Thus, the skin, an external organ, which is in direct contact with water, plays an essential role in the phenomena respiratory systems of fish. So, in case of pollution, the respiratory processes may be disturbed dramatically. After the skin, muscle is another organ that needs to be analyzed because it is the part of fish consumed by humans and involves many risks for health [23]. The liver seems to be more sensitive to environmental conditions, but his sensitivity depends on the nature and the type of the metals. Zn also plays an important role in human health and ecosystems because it constitutes an essential element (in the state of $<0.01\%$) for the proper functioning of the metabolism of living organisms [24]. Indeed, it acts as a cofactor in metallo-enzymes by catalyzing the enzymatic reaction or by providing a structure three-dimensional active enzyme. It is also an essential element for a good functioning of any living organism. It is involved in the activity of many enzymes, and it is essential for a large number of functions or physiological situations, such as growth and cell multiplication, bone metabolism, wound healing, reproduction and fertility, immunity and taste, functioning brain and protection against free radicals [25].

The conditions of the coast of Soumbédioune are getting more and more dynamic and difficult for the fishes, because they live in rocks exposed to extreme weather conditions. Another possible reason may come from the diet, which commonly constituted by contaminated food and then it facilitates the absorption of the heavy metals. In addition to this, we can mention the permanent discharges of urban and industrial wastewater onto the coast of soumbédioune that eventually expose to the metals. It is therefore reasonable to conclude that anthropogenic activities are responsible of the contamination of metals of the fish living in the Dakar coast.

VI. CONCLUSION

This study shows that the contents of metals in the muscles, liver and gonads of the five species (*C. taeniops*, *S. japonicus*, *L. laevis*, *P. bellottii*, and *P. caeruleostictus*) are above the acceptable limit recommended by the FAO and WHO, except the level of copper in the gonads of the species *P. bellottii*. This accumulation of heavy metals, particularly in the muscles of fish, is one of the top concerns for human health. It has therefore become important for the government to control the importation of frozen fish more effectively and to educate public about the impact of the accumulation of

heavy metals in food and their consequences on human health.

The high amounts of metals found in the organs of the fish, revealed the real danger that this part of the Dakar coast presents, in term of fishing, and in conclusion, the consumption of fish from this area can cause a real public health problem.

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