



**DETERMINATION OF HEAVY METALS IN FROZEN FISH IMPORTED BY
FISHERY COMPANIES OF BENIN**

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Received 4th Aug. 2020; Revised 20th Sept. 2020; Accepted 15th Oct. 2020; Available online 1st July 2021

<https://doi.org/10.31032/IJBPAS/2021/10.7.5108>

ABSTRACT

In order to fill its fish deficit, Benin imports more than 45,000 tons of frozen fish each year. According to some authors, this dependence on fish products imports could be a great threat to food security. The objective of this study is to assess the level of heavy metal contamination of frozen fish species consumed by Beninese populations. Heavy metals such as cadmium, lead, copper and zinc were determined by atomic absorption spectrophotometry in fresh, fried and cooked fish. Results showed that cadmium levels in fresh fish ranged from 0 to 0.004 mg / kg, lead 0 to 0.008 mg / kg, copper 0 to 0.31 mg / kg and zinc from 0.1205 to 0.2785 mg / kg. In the same species of cooked fish, cadmium concentrations ranged from 0.003 to 0.006 mg / kg, lead from 0 to 0.0165 mg / kg, copper from 0 to 0.033 mg / kg and zinc from 0.159 to 0, 33 mg / kg. In the same fried fish species, cadmium concentrations ranged from 0.0035 to 0.009 mg / kg, lead from 0 to 0.0025 mg / kg, copper from 0.0065 to 0.058 mg / kg and zinc from 0.109 to 0.222 mg / kg). The levels of heavy metals are all below the standards accepted by FAO / WHO. The consumption of these fish would therefore have no negative effect on the population's health. Depending on the two cooking methods, there is a reduction of heavy metal concentrations in some fish species. Although the concentrations of heavy metals are not worrying, populations must be careful and must vary their diets to guarantee a better health.

Keywords: Heavy metals, imported frozen fish, Cotonou, Benin

1. INTRODUCTION

A healthy population is the first development factor of any nation [1]. The well-being of this population depends on its diet quality [2]. That is why governments often established surveillance rules to ensure food quality and safety for consumers. Nutritional food quality monitoring has greatly increased since the 19th century with the birth of national consumer associations and global standardization bodies [3]. But despite this watch, most of the food consumed in recent years is not free of chemical residues and this has consequences in the short, medium and long term for the human body [4, 5].

In Benin, fish production is provided by rivers and other water bodies. In recent years, population needs for fish have increased and the amount of fish caught has dropped by about 15% from 2003 to 2008 [6]. To fill this gap, Benin imports more than 45,000 tons of frozen fish every year [6]. This dependence on imported fish poses a great threat to food security [7]. Indeed, fish species are bioaccumulative. They are able to absorb and concentrate in their body some chemicals such as heavy metals. Since they occupy a prominent place on Beninese people's plates, their contamination could pose a public health problem. Although several studies showed the contamination of fish caught in the country's rivers and streams [8-13], few

were interested in the imported fish contamination. This study proposes to evaluate the contamination level of heavy metals in frozen fish species consumed by Beninese populations.

2. MATERIALS AND METHODS

2.1-Study area

Five marketing companies of imported frozen fish of Cotonou commonly called "fishmarket".

2.2-METHODS

2.2.1- Fish sampling

Fishes were collected from importing frozen fish sellers. Sampled fish caught were immediately kept cool in cool boxes until they were sent to laboratory for analysis.

2.2.2- Choice of fish species and heavy metals

Fish choice criteria are their availability throughout the year and their appreciation by the population. The species sampled are: *Euthynnus alletteratus*, *Tilapia guineensis*, *Merluccius merluccius*, *Dentex angolensis*, *Pseudotolithus senegalensis*, *Albula vulpes*, *Pseudotolithus typus*, *Cynoglossus canariensis*, *Thrachurus trachurus*. Ten fish were collected per species.

Heavy metals such as lead and cadmium are chosen in this study because they were the most toxic to humans and more frequently found in the environment [14]. Copper and zinc were associated with them for two

reasons: firstly they were not chemically metals and then they were only toxic beyond a certain threshold.

2.2.3 Method of analysis

In order to better assess the risks faced by consumers, it was agreed to analyze the flesh (muscle tissue), which is the portion consumed by the population. Thus, the flesh of 10 fish per species was mixed and divided into 3 parts: the first was analyzed fresh, the second part was boiled with demineralized water and the third fried in oil. They were analyzed. Fish samples were mineralized in a programmable muffle furnace and then analyzed by atomic absorption spectrophotometry with a Spectr AA110 atomic absorption spectrophotometer and its control computer.

2.2.4 Statistical analysis

Heavy metal contents from the analyzed fish species were submitted to a mixed-effect linear model in order to evaluate their variation according to the cooking method. The cooking mode (fresh, cooked and fried) and the species factor were considered fixed and the repetition as random. The mixed-effect linear model was made possible by the lmer function of the lmerTest package [15]. Adjusted averages were obtained by the lsmeans function of the lsmeans package [16]. The ggplot2 package [17] presented these averages graphically.

In order to evaluate the two-to-two correlation between the various heavy metals analyzed, corrplot function of the corrplot package [18] were used with the Pearson method. All analyzes and graphs were done with the R 3.5.1 software.

3. RESULTS AND DISCUSSION

3.1 Heavy metal content in fresh fish, cooked and fried

The results showed the presence of cadmium, lead, copper and zinc in several analyzed fish species according to **Figure 1**.

In fresh fish, the average cadmium content ranged from 0 to 0.004 mg / kg. Lead, copper and zinc ranged from 0 to 0.008 mg / kg, 0 to 0.031 mg / kg and 0.145 to 0.2785 mg / kg, respectively. *Euthynnus alletteratus* species had the highest concentration of cadmium, the highest concentration of lead and copper was found in *Thrachurus trachurus* and *Pseudotolithus senegalensis* species had the most zinc.

In cooked fish, the average levels of cadmium, lead, copper and zinc ranged from 0.003 to 0.006 mg / kg, from 0 to 0.0165 mg / kg, from 0 to 0.033 mg / kg and from 0.159 to 0.33 mg / kg. *Albulavulpes* species contained both cadmium, lead, copper and zinc. As for fried fish, the average cadmium, lead, copper and zinc contents are between 0.0035 and 0.009 mg / kg, respectively, 0

and 0.0025 mg / kg, 0.0065 and 0.058 mg / kg, 0.109 and 0.222 mg / kg. The most elevated concentrations of cadmium, lead, copper and zinc are in *Merluccius merluccius*, *Euthynnus alletteratus*, *Pseudotolithus senegalensis* and *Albula vulpes*, respectively. Heavy metal levels do not exceeds FAO and WHO standards in all of these fish species. The consumption of these fish could therefore have no negative effect on the population's health.

Statistical analysis of these results showed that, with the exception of zinc, the content of other heavy metals such as cadmium, lead and copper varied significantly depending on the method of cooking and the species, as well as following the interaction of these two factors ($p < 0.05$) (**Table 1**). This variation of heavy metal content in imported frozen fish may be due to the phenomenon of uptake, diffusion and excretion at the time of fish capture.

3.2 Effects of cooking methods on the fish heavy metals content

Heavy metals were measured in fresh fish, cooked fish and fried fish. **Table 2** shows

the comparison of heavy metal contents in the different forms of cooking.

It can be seen from this table that the trend in different metal contents varied from one species to another depending on the cooking methods of the fish. In fact, depending on the fish species, there were a decrease or increase of cadmium, lead, copper and zinc content depending on whether the fish is fresh, fried or cooked. It can therefore be deduced that both cooking methods have positive effect on the heavy metal levels reduction in some fish species. In other fish, the effect is negative. This behaviour could be attributed to several parameters such as fish size, oil absorption, water loss and metal evaporation during cooking [19].

Statistical analysis revealed more or less strong correlation between cadmium and lead in fresh fish. For cooked fish, the correlation is strong and positive between lead and copper while for fried fish, the correlation between metals is low or even negative (**Figure 2**).

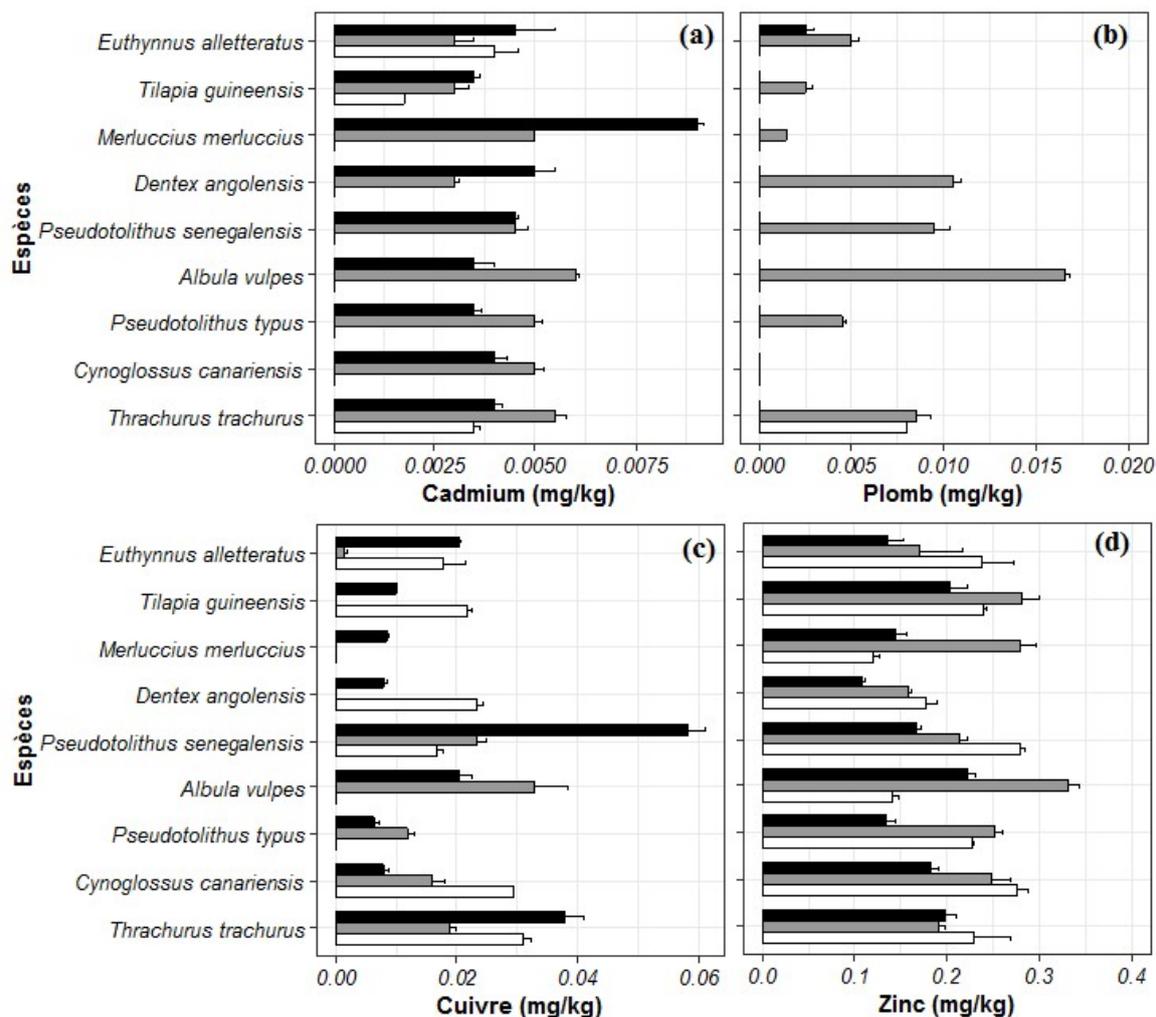


Figure 1 : Variation in Heavy Metal Levels in Imported Frozen Fish

Table 1 : Results of the mixed-effect linear model on the variation of heavy metal contents

| | Cadmium | | | Plomb | | Cuivre | | Zinc | |
|----------------|---------|------|--------|-------|--------|--------|--------|------|-------|
| | ddl | F | Prob | F | Prob | F | Prob | F | Prob |
| MC | 2 | 2,25 | 0,116 | 29,73 | <0,001 | 3,74 | 0,030 | 0,23 | 0,792 |
| E | 8 | 5,66 | <0,001 | 9,29 | <0,001 | 6,44 | <0,001 | 1,79 | 0,099 |
| MC : E | 16 | 5,65 | <0,001 | 14,75 | <0,001 | 6,36 | <0,001 | 1,43 | 0,164 |
| ICC Répétition | | | 0,00 | | 0,00 | | 0,96 | | 0,88 |

CM: Cooking mode; E: Species; ddl: degree of freedom; F: Fischer statistic; Prob: Probability of significance

Table 2 : Comparison of the contents of heavy metals in the different forms of cooking

| Fish Species | Cadmium (mg/kg) | | | Plomb (mg/kg) | | | Cuivre (mg/kg) | | | Zinc (mg/kg) | | |
|------------------------------------|-----------------|-------|--------|---------------|--------|--------|----------------|--------|------|--------------|------|------|
| | Frais | Cuit | Frit | Frais | Cuit | Frit | Frais | Cuit | Frit | Frais | Cuit | Frit |
| <i>Thrachurus trachurus</i> | 0,004 | 0,006 | <0,001 | 0,01 | 0,009 | <0,001 | 0,031 | 0,019 | 0,04 | 0,23 | 0,19 | 0,2 |
| <i>Cynoglossus canariensis</i> | <0,001 | 0,005 | <0,001 | <0,001 | <0,001 | <0,001 | 0,03 | 0,016 | 0,01 | 0,28 | 0,25 | 0,18 |
| <i>Pseudotolithus typus</i> | <0,001 | 0,005 | <0,001 | <0,001 | 0,005 | <0,001 | <0,001 | 0,012 | 0,01 | 0,23 | 0,25 | 0,14 |
| <i>Albula vulpes</i> | <0,001 | 0,006 | <0,001 | <0,001 | 0,017 | <0,001 | <0,001 | 0,033 | 0,02 | 0,14 | 0,33 | 0,22 |
| <i>Pseudotolithus senegalensis</i> | <0,001 | 0,005 | 0,01 | <0,001 | 0,01 | <0,001 | 0,017 | 0,024 | 0,06 | 0,28 | 0,22 | 0,17 |
| <i>Dentex angolensis</i> | <0,001 | 0,003 | 0,01 | <0,001 | 0,011 | <0,001 | 0,024 | <0,001 | 0,01 | 0,18 | 0,16 | 0,11 |
| <i>Merluccius merluccius</i> | <0,001 | 0,005 | 0,01 | <0,001 | 0,002 | <0,001 | <0,001 | <0,001 | 0,01 | 0,12 | 0,28 | 0,15 |
| <i>Tilapia guineensis</i> | 0,002 | 0,003 | <0,001 | <0,001 | 0,003 | <0,001 | 0,022 | <0,001 | 0,01 | 0,24 | 0,28 | 0,2 |
| <i>Euthynnus alletteratus</i> | 0,004 | 0,003 | 0,01 | <0,001 | 0,005 | 0,003 | 0,018 | 0,002 | 0,02 | 0,24 | 0,17 | 0,14 |

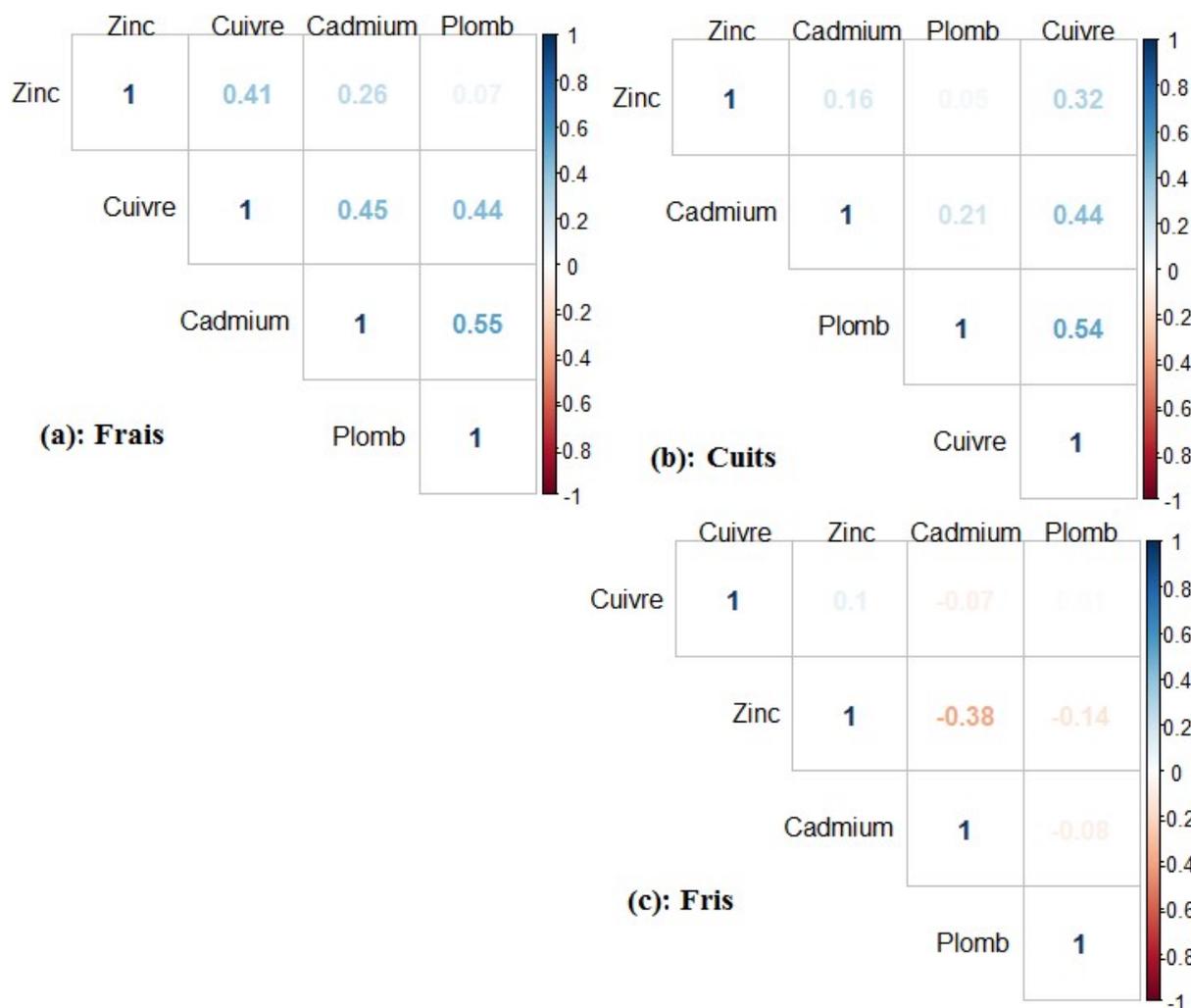


Figure 2 : Correlation between heavy metals found in imported frozen fish

3.3 DISCUSSION

In this study, heavy metal values obtained in fresh fish analyzed are lower than those recorded in fresh fish sampled in Saudi Arabian markets by Alturiqi and Albedair in 2012 [20] in which concentrations levels varied between 1.17 and 4.25 $\mu\text{g} / \text{g}$ for cadmium, between 3.24 and 9.17 $\mu\text{g} / \text{g}$ for lead, between 2.3 and 12.05 $\mu\text{g} / \text{g}$ for copper and between 16.79 and 49.43 $\mu\text{g} / \text{g}$ for zinc. Our results are significantly lower than those obtained in *Scomber scombrus*, frozen in Nigeria, where cadmium and lead

levels are 2.192 mg / kg and 15.13 mg / kg respectively [21]. According to Oluyemi and Olabanji [22], the lead (0.42-0.49 $\mu\text{g} / \text{g}$) and cadmium (0.04-0.07 $\mu\text{g} / \text{g}$) levels obtained in frozen fish (*Scomber scombrus*, *Trachurus trachurus*, *Salmo solar*, *Clupea harengus* and *Sardinella tawilis*) imported to Nigeria were higher than those in this study. The average lead and cadmium concentrations recorded by these authors in *Trachurus trachurus* are respectively 46 and 15 times higher than those obtained in the same species of fish in this study. The

dissimilarity between these results and those recorded in this study may be related to the origin of the fish, their ecological zone and their habitat.

According to cooked fish, the results of this work are inferior to those obtained by Huque et al. [23] in *Pampus argenteus* fish samples boiled and taken along Bengali Bay in Bengali, where average recorded concentrations are 6.03 mg / kg, 15.34 mg / kg, 3.55 mg / kg and 24.82 mg / kg for cadmium, lead, copper and zinc, respectively. Obodai et al. study in Ghana [24] on boiled *Sarotherodon melanotheron* also gave average concentrations of cadmium (0.364 mg / kg) and lead (12.154 mg / kg) that were higher than those found in all the fish analyzed in this study. However, high levels of lead in food would lead to kidney failure and damage to the human liver. Also, fatal ingestions of cadmium exceeding 350 mg could produce shock and renal failure [20].

Concerning fried fish, results of this study are much lower than those obtained by Tawfik [25] in fried *Oreochromis niloticus*, *Mugilcephalus* and *Sardinops sagax* where the values recorded for cadmium are 0.021 µg / g, 0.360 µg / g and 0.821 µg / g; As for lead, the values are respectively 0.011 µg / g, 0.011 µg / g and 0.022 µg / g. For copper, the values obtained are respectively 0.48 µg / g, 0.89 µg / g and 1.13 µg / g and finally those of zinc 6.45 µg / g, 8.97 µg / g and 13,

86 µg / g respectively. Our values are also lower than the concentrations recorded by Devi and Sarojnalini [26] in *Amblypharyngodon mola* for copper (0.023 µg / g) and zinc (0.96 µg / g). Copper is an integral part of many enzymes and is needed for hemoglobin synthesis [23]. Zinc is an essential trace element that is a component of more than 300 enzymes needed to play a role in many biological functions of the human body. However, they become toxic when they exceed maximum limits [27].

The results of the effects of cooking methods on heavy metal concentrations obtained in fish are similar to those recorded by Ersoy and al. [28] on *Dicentrarchus labrax* species which have positive and negative effects on the heavy metal reduction according to cooking methods. On this same fish species, frying and microwave cooking showed an increase in cadmium and arsenic, while grilling and boiling showed a decrease in lead. Results obtained are also similar to those recorded by Bassey et al. [19] on three fish species analyzed in Nigeria. The cooking methods had a significant impact on cadmium levels in *Cynoglossus senegalensis*. In *Polydactylus quadratifilis*, boiling and frying led to an increase of cadmium concentration by 100%, while grilling gave seven times the concentration of cadmium in fresh fish. In addition, the concentration

of cadmium in *Chrysichthys nigrodigitatus* increased. The increase in cadmium during grilling and frying was obviously a result of water loss [29]. As for lead, its concentration in *Polydactylus quadratifilis* increased by 5.9% for boiling, 70.6% for grilling and 41.2% for frying. Boiling caused 32.6% decrease in lead concentration in *Chrysichthys nigrodigitatus*, while frying and grilling showed 2.2% and 4.2% increase in lead concentrations, respectively. On the other hand, the different cooking methods caused a decrease in the concentration of lead in *Cynoglossus senegalensis*. In fact, the levels of lead in boiled, grilled and fried *Cynoglossus senegalensis* decreased around 20.8%, 22.6% and 20.8% respectively.

4. CONCLUSION

In sum, this study assessed heavy metal contamination in imported frozen fish. The concentrations recorded in the various imported frozen fish species indicate that none of them exceeds the standards accepted by FAO and WHO. However, frequent consumption and the number of fish consumed per meal can increase the level of heavy metals in the body and could induce chronic toxicity for the consumer. As for the two cooking methods, they have both a positive and a negative effect on the reduction of heavy metal concentrations depending on fish species. Although the concentrations of heavy metals are not

worrying, vigilance of the populations and variation of diets are important to guarantee a better health.

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