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Total Mercury and Methylmercury Distribution in *Paguellus bellottii* Fish from Soumbedioune Beach, Senegal

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Abstract

Paguellus bellottii fish proceeding from Soumbedioune beach (Senegal) were analyzed to evaluate their contents of total mercury and methylmercury. Simplified analytical procedures (microwave digestion and ultrasonic assisted extraction) were used for sample preparation. The total mercury content in fish varied between 0.0626-0.3542 µg/g, dry weight. The ANOVA analysis allows to conclude that significant differences ($p < 0.05$) were not found between *Paguellus bellottii* fish from Soumbedioune beach. However, the mercury levels obtained were always lower than the European legislation limits for fish. The ratio methylmercury/total mercury varied between 42.3-42.8 % in fish tissues. A satisfactory correlation ($p < 0.0001$; $r = 1$) was found between total mercury and methylmercury results.

Introduction

Mercury is an important pollutant of concern and one of the most studied, although the factors that control bioconcentration and accumulation are not completely understood. Mercury enters the aquatic ecosystem in the form of inorganic mercury [1].

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Microbacteria in the water and sediments convert the inorganic mercury into highly toxic methylmercury. Methylmercury binds to protein in aquatic organisms and is passed up the food chain where the methylmercury can reach dangerous levels in certain fish species. Therefore, the consumption of fish, marine bivalves and other seafood is considered one of the main ways of methylmercury intake in humans and, consequently, several organizations have published their own regulations in order to establish the maximum mercury levels in food and the tolerable weekly intake, in order to preserve the human health [2, 3].

The main aim of this work is to evaluate the content of total mercury and methylmercury in *Paguellus bellottii* fish proceeding from Soumbedioune beach, which are commonly used for human consumption. It permits to evaluate the pollution levels of the studied coastal ecosystem and extract conclusions about the safety of seafood products.

Materials and Methods

Instrumentation and reagents

Mercury determination was carried out by (CV-AAS) using a NIC-RA-3000 System at 253.7 nm and $SnCl_2$ as reducing agent. A high intensity ultrasonic processor (Sonopuls, HD 2200) equipped with titanium probes was used as ultrasound energy source. A domestic microwave oven (Moulinex, 900 W power) was used for microwave heating. A 45 mL capacity Parr reactor (model 4782) was used for acid digestion. A ball-mill (Retsh, model S100) equipped with a 250 mL capacity agate cup was used for a fast reduction of sample particle size. A nylon sieve (70 µm) was used to homogenize the solid samples. A centrifuge (Kubota 5100) was used for a complete separation of the extracts. All reagents employed were of high-quality analytical reagent grade and all of them supplied by Merck. High purity water (Millipore Milli-Q System) was used throughout.

All chemicals used were of analytical-reagent grade from Merck (Darmstadt, Germany). Ultrapure water (Millipore Milli-Q System) was used throughout all the work. The calibration standards were prepared from the stock solution of mercury with a concentration of 1000 µg/mL. All glass and plastic containers and materials were soaked in a 1.5 % w/v HNO_3 solution for 24 h and rinsed with ultrapure water three times before use.

Study area

The study area is located in the coast of Dakar, country of Senegal (Africa). It is important to indicate that the sampling zone is characterized by intensive fish activity and several swimming zones; however, also receives both domestic and industrial wastes from the Dakar city and the surrounding industrial areas. In Figure 1 are shown the sampling point for fish species from Soumbedioune beach. This site is a place of landing fishery products and receives numerous urban wastewater discharges through the western channel [4]. Located on the ledge, Soumbedioune is a traditional village, a fishing village. This is where hundreds of canoes leave every morning in search of the fish that will supply the markets in Dakar. Soumbedioune beach is a place for landing fishery products and receives numerous discharges of urban wastewater through the western canal [5, 6]. With a craft village in 1961 on the occasion of the World Festival of Negro Arts, Soumbedioune became a tourist hotspot. However, fishing remains the main activity of this locality.

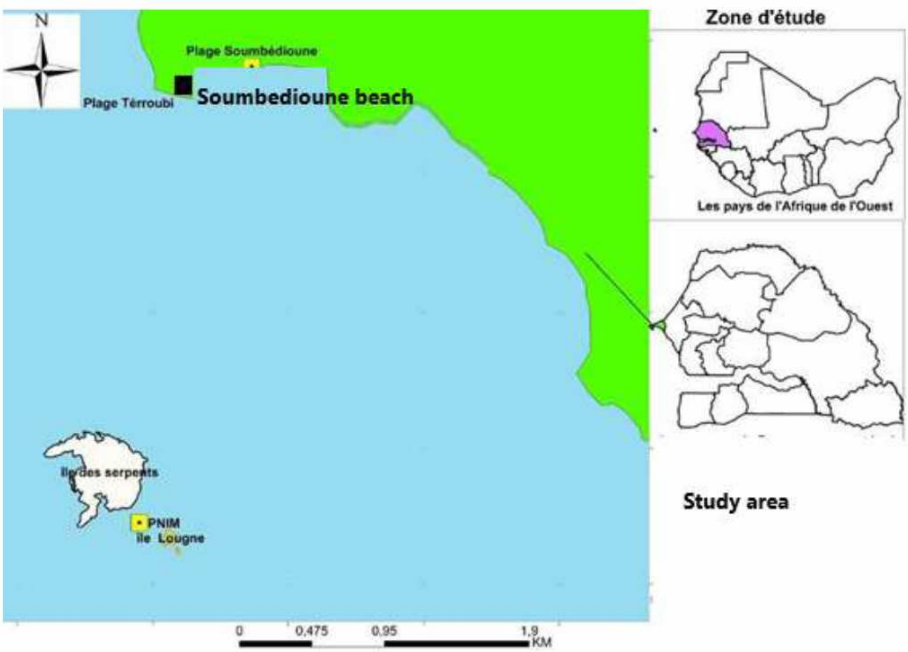

Figure 1. Location of Soumbedioune beach in the Dakar coast (Senegal).

Sample collection and preparation

From the sampling point above indicated (Figure 1) and during the same period, fish of the same species, commonly traded in this area were collected and transported. In the laboratory, the size (between 16-31 cm) and weight (between 50-475 g) of the fish were measured. The muscle of the selected fish was cut into small pieces and homogenized using an agate mortar. Then the samples were lyophilized and processed. After freeze-drying, the humidity percentages of the fish were measured with values ranged from 70 to 79%.

Analysis of total mercury content

The mineralization of the solid samples for total mercury determination was performed by acid digestion using a microwave procedure. A portion of dry fish sample (about 0.15 g) was weighted and placed in the PFA vessel of the Parr reactor and 4 mL of nitric acid (65 %) were added. The vessel was closed and heated in the microwave oven during 2 minutes at 450 W of power. After cooling, the reactor was opened and 1 mL of hydrogen peroxide (30 %) was added to complete the sample decomposition by heating again during 1 minute at the same power. Finally, the resultant solution was quantitatively transferred into a 25 mL volumetric flask and diluted to volume with ultrapure water. The final solution was stored, at 4ºC, in stoppered glass bottles until analysis by CV-AAS. The corresponding blank solution was prepared in a similar way but without any sample added [7].

Analysis of methylmercury content

The extraction of methylmercury from the samples was made by means of a high intensity sonicator equipped with a titanium probe, operating at 25 % of amplitude. A small portion of dry fish sample (about 0.2 g) was placed into a glass tube of 50 mL capacity and 15 mL of hydrochloric acid solution 10 % (w/v) were added. Then, the mixture was sonicated at room temperature during 3 minutes at 25 % of amplitude. After sonication, the supernatant liquid was separated from the solid phase by centrifugation during 6 min a 1200 rpm. This solution was quantitatively transferred into a 25 mL volumetric flask and diluted to volume with ultrapure water. The final solution was submitted to UV radiation for 15 min in order to transform the methylmercury in Hg (II) to be quantified by CV-AAS using $SnCl_2$ as reducing agent. The corresponding blank solution was prepared in a similar way but without any sample added [8].

Results and Discussion

Distribution of methylmercury and total mercury

Fish samples studied come from the locality of Soumbedioune. This area is a place of landing and sale of fishery products where several families of the Dakar population come to get their supplies. The analytical results obtained for total mercury and methylmercury in fish species are summarized in Table 1 and all of them are expressed in µg/g dry weight. All values are given as mean of four separated determinations and their standard deviation. Total mercury content in fish species from Soumbedioune vary between 0.0626 and 0.3542 µg/g dry weight. In addition, some authors, Kehrig et al. [9] 2002; Mormede and Davis, 2001, concluded that parameters such as diet, life style of the species, size, reproductive maturity and season can influence the accumulation of mercury [9, 10].

In fact, the observed results can be correlated to the influence of certain factors such as: (i) size: the larger size species, therefore its mercury content is considerably higher. (ii) weight linked to size: fish species like *Belloti Paguellus*, with varying weights between 50 and 475 g, sizes between 16-31 cm, have significant levels of total mercury, ranging between 0.0626-0.3542 µg/g dry weight. This can be attributed to their different absorption of metals.

Table 1. Total mercury and methylmercury contents in fish from Soumbedioune.

Fish species	Fish weight (g)	Fish length (cm)	Hg Total (µg/g)	MeHg+ (µg/g)	Ratio MeHg+/Hg total
Paguellus bellottii	50	16	0.0626 ± 0.0061	0.0265 ± 0.0023	42.3 %
Paguellus bellottii	86	18	0.0906 ± 0.0038	0.0386 ± 0.0021	42.6 %
Paguellus bellottii	130	20	0.1153 ± 0.0064	0.0488 ± 0.0021	42.3 %
Paguellus bellottii	154	22	0.1326 ± 0.0031	0.0568 ± 0.0049	42.8 %
Paguellus bellottii	219	25	0.2367 ± 0.0102	0.1008 ± 0.0054	42.4 %
Paguellus bellottii	475	31	0.3542 ± 0.0144	0.1506 ± 0.0072	42.5 %

Thus, total mercury contents, of the order of 0.50 µg/g dry weight, have also been found in marine fish consumed in Malaysia, such as short-body mackerel (*Rastrelliger brachsoma*), and tuna long tail (*Thunus tonggol*) [11]. However, higher levels of total mercury, varying between 0.324-1.740 µg/g dry weight, were obtained in the muscle tissue of fish (*Symphodus melops*) from the Gulf of Taranto (Italy). These concentrations have even exceeded the limits set by European legislation [12]. The presence of mercury in fish can lead to possible contamination of consumers [13, 14]. Several decisions have been made to measure the level of mercury in fish tissues in order to assess its toxicity to humans [15, 16]. The results obtained in this work show that the total mercury levels found in fish samples from Soumbedioune are considerably lower than the permissible levels. Indeed, the European Union has fixed the mercury content, of 0.5 µg/g wet weight for fish and 1 µg/g wet weight for predatory fish. For seafood, the limit is 0.5 µg/g wet weight [2].

Consequently, the use of the analyzed fishery products in the diet does not constitute a potential risk of toxicity. However, their accumulation can, in the long term, become a public health problem. Thus, the taking over of the control of marine resources by the authorities can be materialized by the creation of a bio-surveillance network along the coasts of the city of Dakar, in particular the locality of Soumbedioune. Paguellus bellottii species concentrates high levels of methylmercury, depending on the size and weight. Hence the role of bioaccumulator of this type of species. However, the content of methylmercury present in all the fish species analyzed is considerably lower than the content of total mercury (Figure 2).

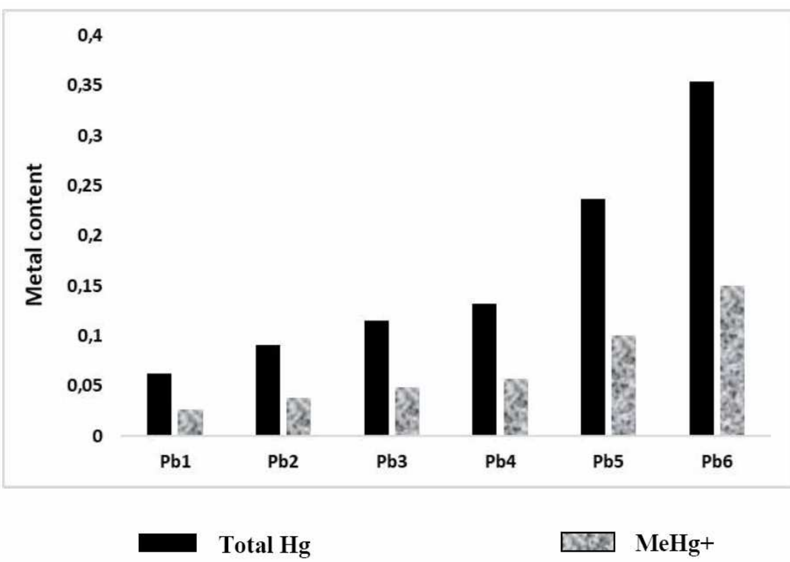

Figure 2. Distribution of methylmercury and total mercury in fish.

The percentages of methylmercury in relation to total mercury range from 42.3% to 42.8%. According to some authors, Kehrig et al. 2002 and Spada et al. 2012, this difference can be linked to the trophic level of aquatic organisms, their physiology, metabolism or even to the distribution of metals in proteins [9, 12]. In addition, studies have shown higher percentages, with values ranging from 70 to 83%, for marine fish species available on local markets in Malaysia and southwestern Germany [15, 16]. However, commercial fish, from the Adriatic Sea and Guanabara Bay (Brazil), have ratios between 52 and 99% [17, 18].

Correlation between total mercury and methylmercury

It is interesting to make a correlation analysis between total mercury and methylmercury contents in all analysed samples in order to make predictions in biota of an aquatic ecosystem. In this case, it was found a significant positive correlation between both variables as it was shown in Figure 3. A good regression ($p<0.0001$) was obtained with a satisfactory correlation coefficient ($r = 1$). The intercept of the regression is practically negligible (1E-05) and the slope is lower than to unit (0.4254), indicating that total mercury always exceeds methylmercury. So, the increase of total mercury provides an increase of methylmercury bioaccumulation, which supposes a growth of health risks, due to its high toxicity [19].

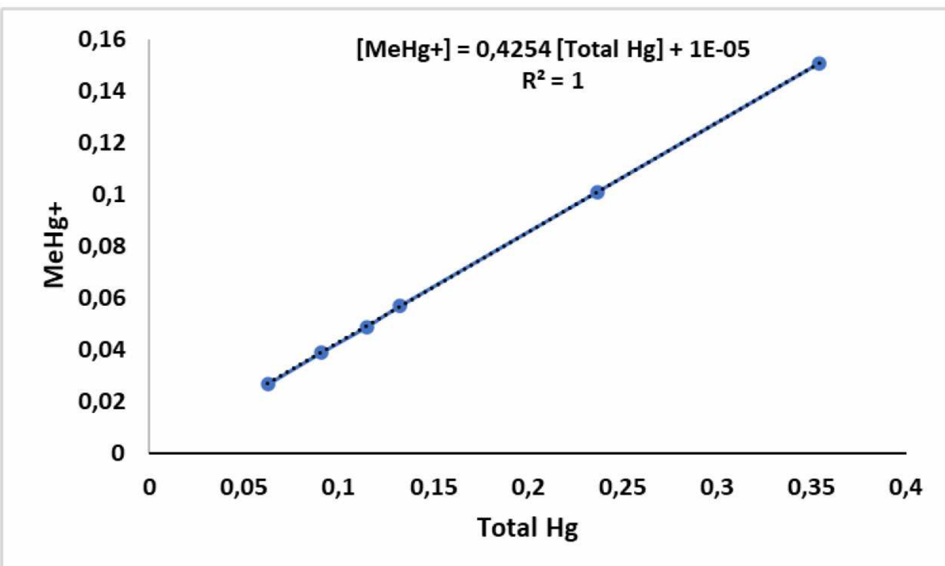

Figure 3. Correlation between total mercury and methylmercury content in fish.

Conclusions

The results obtained in fish analyzed samples indicate that, in fish present mercury contents. The total mercury content in fish varied between 0.0626-0.3542 µg/g, dry weight. In fish species, metal accumulation mainly depends on their size and different metal uptake. In addition, it is important to indicate that, in all analyzed samples, the values obtained were considerably lower than the European legislation limits for fish, which assure a safety exploitation of the marine resources in the studied area. In all fish samples studied, the content of methylmercury was considerably lower than total mercury but, in general, slightly greater in fish species. In fact, the percentages obtained of methylmercury respect total mercury varied between 42.3-42.8 % in fish tissues. The ANOVA analysis allows to conclude that significant differences ($p<0.05$) were not found between fish samples. Finally, it was also found a satisfactory correlation ($p<0.0001$; $r = 1$) between total and methylmercury in fish samples studied, which permits to estimate the bioaccumulation of methylmercury in muscle tissues when the total mercury is varied.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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