

RESEARCH ARTICLE

CONTRIBUTION TO THE ASSESSMENT OF PERSISTENT ORGANIC POLLUTANTS (POPS) IN MARINE ORGANISMS: THE CASE OF SAROTHERODON MELANOTHERON (RÜPPELL, 1852) AND MUGIL BANANENSIS (PELLEGRIN, 1928) IN THE SALOUM DELTA

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Abstract

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It is known that the consumption of seafood has several health benefits for humans. However, the accumulation of organic pollutants, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), in their flesh could pose public health problems. Thus, the concentrations of PCBs and OCPs in edible fish species (Mugil bananensis, Sarotherodon melanotheron) from the Sine Saloum Estuary located in central-western Senegal are investigated in order to assess the potential risks of these contaminants to human health. After analysis, PCB52 and chlordane show the highest concentrations in the edible flesh of these two species. The concentration of PCB52 is almost identical in these two species, while that of chlordane is higher in S. melanotheron. Regarding the distribution of PCBs and OCPs in M. bananensis, the highest concentration is found in Fimela for PCBs and in Sokone for OCPs. For S. melanotheron, the highest concentration is found in Toubacouta for PCBs and in Félir for OCPs. The cancer risk values obtained after calculation do not reveal any serious risk because they do not exceed the threshold value established by the U.S. EPA (2005) which is 1×10^{-4} . However, it is recommended to the populations a moderate consumption because of the phenomenon of bioaccumulation.

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Introduction:-

Estuarine ecosystems are quite important from a biological and ecological point of view (Costanza et al., 1998). They are highly productive and serve as nurseries for various marine species (Albaret, 1999; Beck et al., 2001; Peterson, 2003). They ensure the renewal of fisheries resources (Albaret, 1999) and constitute essential habitats for juveniles of various marine species (Rubec et al., 1999; Whitfield, 1999; Beck et al., 2001; Le Pape et al., 2003; Barletta et al., 2005). They also support an important fishing activity and provide part of the animal protein needs of local populations (Houde and Rutherford, 1993; Blaber, 1997). In spite of their importance, these ecosystems are subject to important anthropogenic disturbances such as pollution. Indeed, in recent decades, pollution has had a definite impact on aquatic ecosystems (Harley et al., 2006). Several chemicals such as trace metals and persistent organic pollutants (POPs) are introduced into aquatic ecosystems.

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Anthropogenic pollutants are ubiquitously present in marine and estuarine environments (Bowen and Depledge, 2006), which may present a risk to both biodiversity and productivity of marine ecosystems, as well as human marine resources (Jenssen, 2003). Persistent organic pollutants (POPs) tend to accumulate in the sediment and food web, thus reaching concentrations that potentially cause toxic effects (Moore et al., 2002; Magnusson et al., 2006; Fair et al., 2009). POPs are among the most dangerous pollutants released by humans into the environment each year. They are organic compound that are highly resistant to photolysis, biological and chemical degradation. They come almost entirely from anthropogenic sources associated with the manufacture, use, and disposal of certain chemicals. There are also natural sources of organochlorine compound. Because of their high degree of halogenation, they have very low solubility in water and are readily soluble in fat. Thus, persistent organic pollutants are able to pass through the lipid structure of biological membranes and accumulate in fat deposits. Because of their semi-volatility, POPs are able to travel great distances through the atmosphere to reach distant regions (Wania and Mackay, 1993). Their persistence, stability, long-range transport, bioaccumulation and biomagnification ability, as well as toxicity to aquatic life and potential carcinogenicity to higher organisms (Smith et al., 1999; Persky et al., 2001; Weintraub and Birnbaum, 2008; Su et al., 2014) arise from features such as halogenation, low vapor pressure (low volatility) and high hydrophobicity (Nicklisch et al., 2016).

Numerous studies have shown that the human populations most contaminated with POPs were those that consumed the most fish (Schwartz et al., 1983; Grimvall et al., 2000; Hanrahan et al., 1999; Weintraub and Birnbaum, 2008; Gobas and Arnot, 2010; Chovancová et al., 2011; Fromberg et al., 2011). In coastal West African countries, notably Senegal, fish has been central to the local economy for decades. The share of animal protein it provides is very high: 75% of the nutritional requirements in animal protein (FAO/WHO, 2006). In the Sine-Saloum estuary, one of the most densely populated areas in Senegal, fishery resources are of paramount importance as they constitute the main source of animal protein. Among the species most consumed in the Sine-Saloum estuary are the tilapia Sarotherodon melanotheron and the mullet Mugil bananensis.

Information with sufficient spatial coverage to identify hotspots of contamination is lacking despite the high number of hazardous compounds contaminating the marine environment (Hedge et al., 2017).

Thus, this study proposes to evaluate the levels of POPs in the fish *Sarotherodon melanotheron* and *Mugil bananensis* in the Sine-Saloum estuary. The aim is to evaluate the variations of these contaminants in the fish, as well as the possible risk to human health associated with their consumption.

Materials and Methods:-

Study area

The Sine-Saloum Estuary (SSE) is a natural region located north of the Gambia and south of the Petite Côte of Senegal (Fig 1). It has an area of 546 km² and is a deltaic system characterized by flat valleys with variable water levels depending on seasonal flooding (September to December) and maritime inflows. The outflow of continental waters to the sea is normally rare because the hydrological regime is characterized by a deficit of freshwater inflow from January to September (Albaret, 2005). The Sudanian climate is characterized by two seasons: a dry season from November to May and a rainy season, also called "wintering", from June to October. The Sine-Saloum estuary is a rather unique ecosystem with a permanently reversed haline gradient (increasing from the mouth upstream). The extreme upstream parts of the system are even in a hypersaline situation (>70 per thousand upstream of Foundiougne) and in some sectors, upstream of Kaolack, salinity can reach 130 per thousand, i.e. nearly 4 times the average salinity of sea water (Simier et al., 2004). It consists of three main branches: the Saloum to the north, the Bandiala to the south and the Diomboss in between.

Persistent organic pollutants (POPs)

POPs are organic compound that are highly resistant to photolysis, biological and chemical degradation. They originate primarily from anthropogenic sources associated with the manufacture, use and disposal of certain chemicals. There are also natural sources. POPs are halogenated and most often chlorinated compound. The carbon-chlorine bonds in these compounds are very stable to hydrolysis (Stimman et al., 1985). Because of their high degree of halogenation, POPs have very low water solubility and are readily soluble in fats. Being lipid soluble, they are able to pass through the lipid structure of biological membranes and accumulate in fat deposits. They are semi-volatile, a property that subjects them to transport. These compounds are able to travel great distances through the atmosphere to reach regions far from their place of origin. They have been measured on all continents, at sites

representing all climatic zones around the world (Wania and Mackay, 1993). This includes remote regions such as open oceans, deserts, the Arctic and Antarctic where no significant local sources exist.

Sampling protocol

Site selected

Five sites located in the Saloum Delta were targeted: Félir, Fimela, Sokone, Toubacouta and Missirah. These sites were selected based on their geographic distribution and accessibility.

Sampling

Sarotherodon melanotheron and *Mugil bananensis* samples were collected from fishermen from September to October 2017. At each site a weight (2 kg) was sampled for each species. The collected individuals were kept in a thermostat containing ice before being transported to the laboratory where they are kept in a refrigerator.

Sample pretreatment and analysis

Fish grinding: the samples were ground with a grinding machine after evisceration and the resulting leg was dried in an oven.

Extraction:

After drying, 5 g of the sample was weighed and 15 g of sodium sulfate and an inert drying agent were added. The mixture was then dried overnight before being transferred to cartridges into which 100 μ l of PCB112 was added to dope it. The extraction solvent (200 ml hexane/acetone (3:1; v/v) for each sample was prepared. This solution is introduced into 250 ml flasks where pumice stones have been added for gentle boiling. Thus the extraction device (soxhlet) is mounted and the analyte is extracted after 16 hours.

Evaporation with the rota-vapor:

After extraction 1 ml of isooctane is added to each flask to evaporate the POPs extract to about 3 ml at a temperature of 40°C.

Cleaning with alumina oxide (Al₂O₃):

The cleaning of the extract had started first with alumina. After rinsing the glass columns with hexane, 15 g of alumina followed by 1cm of Na_2SO_4 was added. Each column was conditioned with 15 ml of hexane. After column conditioning, a rinsed 250 ml flask was placed under the column as soon as the sample extract was eluted. The rinse was repeated with 1 ml of pentane two more times to then elute the column with 170 ml of hexane.

Second evaporation with the rota-vapor:

Once the elution was completed, 2 ml of isooctane was added to each flask containing extract which was then evaporated to about 3 ml. After that, the extract was transferred to a clean tube and the flask was rinsed 3 times with 1 ml of hexane.

Fractionation with silicate gel:

The fractionation had allowed to recover the polychlorinated biphenyls (PCBs) on the one hand and the Organochlorine pesticides (OCPs) on the other hand. To do so, a small amount of hexane and a small plug of silanized glass wool had been deposited respectively in the appropriate column for SiO₂. After that; 1.8 g of 1.5% SiO₂ was poured and 1 cm of Na₂SO₄ is put on top. It was important to tick the side of the column to allow the SIO₂ to settle in the column. Next, the column had been conditioned with 4 ml of hexane; both tubes (fraction 1 and 2) had been sprayed with some hexane.

After this conditioning, the extract was transferred to another column until it was eluted. Then the sample tube was rinsed with 1 ml of hexane. The extract was then collected in two tubes.

Fraction 1: Polychlorinated biphenyls (PCBs) recovery.

Tube 1 was placed under the column and rinsed with 1ml of hexane. The operation was repeated three times and 10 ml of hexane was added to the column after the elution of the last rinse.

Fraction 2: Organochlorine pesticides (OCPs) recovery

Then, the second tube was placed under the column and the column was eluted with 10 ml (solution (hexane (85): diethyl ether (15)).

Evaporation with nitrogen gas: after fractionation 1 ml of isooctane was added to both tube and the 2 fractions were evaporated to 0.5 ml each. The extracts were homogenized and transferred to autosampler vials with the correct codes after weighing their empty weight and their weight with the extract.

Sample reading: a gas chromatograph (GC) was used to assess the contamination of POPs in fish.

The GC chain consisted of an oven heated to variable temperatures (initial temperature: 75 °C for 0.5 mn, final temperature: 300°C with a step of 10 °C/mn and the rest at 300°C for 7 min) in which was a capillary column of type DB-XLB of diameter 0.25 mm and 30 m length, helium carrier gas of a micro electron capture detector (micro ECD) heated to 350 °C and a system of injection without division (injected volume: 1 μ L). These data were programmed into a computer allowing the processing of the results.

Results:-

PCBs concentration in fish

The concentration of the different types of PCBs (PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180) found in the meat consumed from *M. bananensis* and *S. melanotheron* at the five sites were recorded. On average, PCB52 obtained in the products collected at all sites (except Missira) had the highest concentration for all species (Figure 2). PCB138, only noted in Fimela, was the compound with the lowest concentration in *M. bananensis*, while it was absent in *S. melanotheron*. The average concentration of PCBs was relatively higher in *S. melanotheron* with 0.07 \pm 0.04 µg/kg in *M. bananensis*.

OCPs concentration in fish

Different OCPs recorded in the flesh of *M. bananensis* and *S. melanotheron* collected at the five sites were represented in the Figure 3. The analyze of the figure related that all the OCPs were found in *S. melanotheron*. For *M. bananensis*, only mirex was not detected. Chlordane was the most important OCPs detected in the flesh of both species with a mean concentration of $0.71\pm0.85 \,\mu$ g/kg in *M. bananensis* and $1.03\pm1.62 \,\mu$ g/kg in *S. melanotheron*.

Spatial distribution of OCPs

The mean concentration of OCPs recorded in the flesh of *M. bananensis* and *S. melanotheron* at the different sampling sites were shown in Figure 4. The ANOVA test revealed that the spatial distribution of mean OCPs concentrations in the flesh of these two species was not significant (P>0.05). The Sokone site had the highest mean concentration of OCPs for *M. bananensis* (2.48±6.59 μ g/kg) while for *S. melanotheron* the highest mean concentration (0.57±1.34 μ g/kg) was observed at Félir. As for PCBs, the lowest mean concentrations of OCPs were noted at Missira for both species.

Spatial distribution of PCBs

The spatial distribution of mean PCBs concentration in the flesh of *M. bananensis* and *S. melanotheronwas* showed in Figure 5. It showed a rather uneven distribution of mean PCBs concentration among the localities. However, there was no statistically significant difference (P>0.05) between these different sampled sites. The highest concentration was recorded at Fimela for *M. bananensis* (0.16 \pm 0.14 µg/kg), while for *S. melanotheron* the site of Toubacouta recorded the highest concentration of PCBs (0.07 \pm 0.09 µg/kg). However, the Missira station recorded the lowest PCBs concentration for all species.

Estimated cancer risks (ECR) for PCBs

Table 1 showed the obtained ECR values related to the consumption of *M. bananensis* and *S. melanotheron*. The estimated cancer risk from the two species was about 0 to 5.92×10^{-18} for *M. bananensis*; 2.74×10^{-19} to 2.27×10^{-18} for *S. melanotheron*. For *M. bananensis* the highest ECR was obtained in Fimela, while for *S. melanotheron*, the maximum ECR was obtained in Félir. The lowest values were observed in Sokone and Fimela for *M. bananensis* and *S. melanotheron* respectively. For both species, all calculated ECR were below the standards established by U.S.EPA (United States Environmental Protection Agency).

Cancer risk assessment (CR) for OCPs

The ECR values obtained afterwards for *S. melanotheron* and *M. bananensis* regarding OCPs were recorded in table 2. Values ranged from 4.85×10^{-13} to 1.42×10^{-11} for *M. bananensis* to 2.82×10^{-14} to 2.94×10^{-11} for *S. melanotheron*. The lowest value is obtained at Missira for *M. bananensis* and at Toubacouta for *S. melanotheron*. The highest ECRs were recorded at Félir for both species. As for the PCBs, all the RCs calculated for the OCPs were lower than the U.S. EPA standards.

Discussion:-

PCBs and OCPs concentration in fish

This study revealed the presence of persistent organic pollutants (POPs) such as PCBs and OCPs in the edible flesh of *M. bananensis* and *S. melanotheron* at the different sites sampled. In other words, the waters of the Sine Saloum Delta are contaminated by these two pollutants. Indeed, POPs in the aquatic environment can accumulate in living organisms either by direct absorption of compounds dissolved in the water or by consumption of organisms belonging to the lowest trophic levels (Meador et al., 1995). Mean concentration of PCBs are higher in *S. melanotheron*, while those of OCPs are higher in *M. bananensis*. These differences seem to highlight the important role of ecological and physiological factors in the bioconcentration of these pollutants for each species (Diop et al., 2017). The other reason that could be mentioned concerns the trophic position of each species, which seems to be a determining factor in the importance of this contamination. Indeed, *S. melanotheron* belongs to a higher trophic level (2.1) (Diouf, 1996), whereas *S. melanotheron* is an omnivorous species capable of adapting its feeding mode according to environmental conditions. Its diet is much diversified but essentially composed of phytoplankton, zooplankton, organic detritus and algae (Kone and Teugels, 2003).

Spatial distribution of PCBs and OCPs

Several studies conducted in Senegal and other countries have shown the presence of these POPs in aquatic organisms. The work of Diop et al. (2017) showed the presence of PCBs in *Mugil cephalus* and *Tilapia guineensis*, which belong to the family Mugilidae and Cichlidae respectively. However, the concentrations of PCBs encountered are much higher than those in our study. In Lake Ziway (Ethiopia), lower concentrations of OCPs than those obtained in this study are observed in the Cichlidae *Oreochromis niloticus* and *Tilapia zillii* (Yohannes et al., 2014). Regarding the spatial distribution of PCBs and OCPs concentrations, differences are observed between sites. However, these inter-site differences are not significant. The highest concentration in *M. bananensis* was found in Fimela for PCBs and at Félir for OCPs. For *S. melanotheron*, the highest concentration was found at Toubacouta for PCBs and at Félir for OCPs. For the moment, we do not have the necessary arguments to explain these differences between sites. However, the presence of these two pollutants could be due to the agricultural activities practiced in these areas, to the presence of numerous tourist sites, to market gardening and to growing urbanization. The lack of sewage disposal and treatment systems could also be partly responsible for the presence of these POPs in these sites. The presence of these compound could also be explained by the marine waters that penetrate these environments during high tide.

Estimated cancer risks

Concerning the health risks, the calculation of the ECR for PCBs and OCPs indicates that the consumption of these species does not present a risk for the consuming populations at the sampling sites for the moment. Indeed, the ECR values obtained in this study are largely lower than the acceptable limit value established by U.S. EPA (2005) which is 1×10^{-4} . However, even if the ECR values obtained seem insignificant, there may be a risk of developing cancer related to permanent consumption of these species. PCBs and OCPs, because of their high lipid solubility, which makes them highly susceptible to bioaccumulation and biomagnification (Baker and Walker, 1990; Kimbrough, 1987). Furthermore, ECR values (PCBs and OCPs) above established standards have been calculated on species of the family Mugilidae and Cichlidae (Yohannes et al., 2014; Diop et al., 2017).

Conclusion:-

Results presented here provide information on the presence of PCBs and OCPs in the edible flesh of two species (*Mugil bananensis* and *Sarotherodon melanotheron*) among the most consumed in the Sine Saloum Delta, as well as the health risks associated with their consumption. The data can also be used as a baseline for future monitoring programs. Among the compound analyzed, PCB52 and oxychlordane are the most present in the flesh of these two species. The differences in concentrations of PCBs and OCPs from one species to another and from one site to

another, thus underline the important role of anthropogenic sources and ecological and physiological factors in the concentration of pollutants. In addition, the concentrations of PCBs and OCPs in edible tissues of these two species are relatively low and generally below U.S. EPA limits. The cancer risk calculation reveals that, for PCBs and OCPs, respectively, ECR values do not pose an undue carcinogenic risk. Indeed, all ECR values are well below the recommended value. In terms of food safety, although both species have contaminant levels below the U.S. EPA recommended legal limits, this study suggests that they should be consumed in moderation due to the potential carcinogenic risks derived from PCBs and OCPs.

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 Table 1:- Estimated cancer risks for PCBs.

Table 2:- Estimated	l cancer	risks	for	OCPs.
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Sites	M. bananensis	S. melanotheron	Acceptable values defined (US EPA, 2005)				
Félir	1.42×10^{-11}	2.94×10 ⁻¹¹	$1 \times 10^{-6} - 1 \times 10^{-4}$				
Fimela	4.65×10^{-12}	1,63×10 ⁻¹³					
Sokone	1.22×10^{-11}	1.72×10^{-14}					
Toubacouta	1.34×10^{-13}	2.82×10 ⁻¹⁴					
Missira	4.85×10^{-13}	2.92×10^{-12}					



Fig 1:- Map of the study area (Sine-Saloum Estuary) in Senegal.





Fig 3:- Mean concentration of OCPs in *Mugil Bananensis* and *Sarotherodon melonotheron*. The vertical horizontal bars at the right end of each band represent standard deviations.



Fig 4:- Spatial distribution of mean OCPs concentrations in *Mugil bananensis* and *Sarotherodon melanotheron*. The vertical bars on each band represent standard deviations.







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