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Assessing the mercury content of fishes (*Sarotherodon melanotheron* and *Chrysichthys nigrodigitatus)*, shrimps (*Penaeus spp*), and oysters (*Crassostrea gasar*) of complex "lake Nokoué -lagoon of Porto-Novo" in the Republic of Benin (West Africa)

Hermione W. DEGILA[1,2]*, Julien G. ADOUNKPE[1,3], N.B. Nadia AZON[1,2], Vincentia QUENUM[2] and Martin P. AÏNA[1,2]
[1]Laboratoire des sciences et Techniques de l'Eau (LSTE) Institut National de l'Eau (INE), Université d'Abomey-Calavi, Benin
[2]Laboratoire de Surveillance Environnementale Ministère Cadre de Vie et du Développement Durable, Benin
[3]Laboratoire d'Ecologie Appliquée, Faculté des Sciences Agronomiques. Université d'Abomey Calavi, Benin
hermywolf@gmail.com

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Abstract

By ratifying the Minamata Convention related to mercury, the Republic of Benin committed itself to assess the metal pollution sites within the country. Aquatic mercury pollution represents a potential health threat to the population whose protein source is the fish from the water bodies across the country. Lake Nokoue-Lagoon of Porto-Novo complex represents the most important fish provider in West Africa. But this watershed is contaminated by mercury. The present study aims at investigating the mercury content of the most consumed fish, shrimp and oysters. Samples of sarotherodon melanotheron, Chrysichthys nigrodigitatus, shrimps (Penaeus spp), and oysters (Crassostrea gasar) were taken at different fishing sites of the complex and different period of the year. The mercury content of the samples was determined by the Direct Mercury Analyzer 80. Its turns out that 1.87 to 239 µg/kg wet weight with an average of 21.08 ± 21.62 µg/kg wet weight was found in C. nigrodigitatus, 0.48 to 49.81 µg/kg wet weight in S. melanotheron with an average of 6.84 ± 5.21 µg/kg wet weight; 0.84 to 17.17 µg/kg wet weight in penaeus spp shrimp, 4.46 to 8.98 µg/kg wet weight in C. gasar oyster. Those values even though well below the Benin Republic standards for non-predatory fish are above the Canadian standards and twofold the Check Republic ones. However, spatial distribution study of mercury in fishery products and health risk assessment needs to be undertaken to guide decision makers.

Keywords: Mercury pollution, fishery products, mercury content of fish, lake Nokoué , Porto-Novo lagoon.

Introduction

Sources of metallic trace elements in the aquatic ecosystem are both natural and anthropogenic. However, anthropogenic contamination is much more important. Indeed, the ratio of anthropogenic fluxes versus natural fluxes of toxic metals such as arsenic, cadmium, lead and mercury are respectively 1.7; 9.6; 20.4 and 19.8[1]. Mercury, the only one metal that is liquid at room temperature, has useful physicochemical characteristics in various fields. This explains its presence in various industrial processes and products, hence, its release into the environment. In addition, the mining sector with the production of gold is one of the largest users of mercury thus leading to significant releases into the environment[2,3]. Given the health and environmental risk posed by the strong presence of mercury in the environment, the Benin Republic ratified in 2016 the Minamata Convention on mercury which requires, among other things, the identification of contaminated sites and people at risk.

The present study aims at assessing the presence of mercury in the most consumed fish species in the "Lake Nokoué, Porto-Novo lagoon" complex, one of the most productive water bodies in West Africa. Previous studies have shown the contamination of this body of water with various toxic metals. This contamination is of natural origin with metallic inputs from telluric, atmospheric, marine, fluvial and soil leaching during significant rainfall events[4, 5]. but also anthropogenic[6-8].

Indeed, this water body is subject to: i. the discharge of runoff water collectors and domestic wastewater, ii. the discharge of various solid and liquid waste from household and industrial directly into the lake, on the bank or on the flood plains in order to fill the swamps; and iii. the anarchic occupation of the banks and the creation of lakeside villages which do not benefit from any sanitation system. Thus, the lake has become the receptacle of household waste and excreta. It should also be noted that small-scale artisanal gold production activities in the departments of Atacora and the use of various agricultural phytosanitary inputs in cotton growing areas induce mercury contamination of the Ouémé river, its tributaries, and by ricochet, the lagoon of Porto-Novo as well as Lake Nokoué; the two outlets of this river towards the ocean[9,10]. In addition, there is a source of groundwater with a high mercury content in the

Porto-Novo lagoon and mercury was found in the sediment of Lake Nokoué[5,11].

To date, mercury contamination of the fish species found in this body of water, unlike other toxic metals such as lead and cadmium. This study, which examines the mercury content of some of the most consumed fish species is meant to fill up this gap for decision makers actions.

Materials and methods

Study Area: The complex "Lake Nokoué- Porto-Novo lagoon " constitutes the outlet of the Ouémé river towards the ocean. Thus, this complex receives various chemical contaminants from the agricultural regions of the country[10]. In addition, the Ouémé river crosses several large cities in the country where poor waste management makes it the receptacle of various waste that it carries to the "Lake Nokoué, Porto-Novo lagoon" complex.

Choice of fish species: The identification of species was based on their abundance in the catches, their wide spatial distribution and finally, their potential to accumulate contaminants from the environment. Thus, referring to the report on fishing, fish farming and aquaculture in Benin[12], two species of fish (*Sarotherodon melanotheron* (Rüppell, 1852; and *Chrysichthys nigrodigitatus* (Lacepède, 1803); a crustacean (*Penaeusspp*) and a mollusc (the oyster *Crassostrea gasar* (Deshayes, 1830)) were selected for the present study.

Identification of sampling sites: The main selection criteria are the intensity of fishing activities, the presence of housing, solid waste discharge on the Lake Nokoue-Lagoon of Porto-Novo complex bank and other anthropogenic activities likely to promote the release of heavy metals into the water such as manual dredging of the river. Thus, following a preliminary site visit carried out in January 2018, nine fishing zones were selected on the "Lake Nokoué and the Porto-Novo lagoon" complex. Table-1 shows the sampling zones and their code while Figure-1 shows their geographical position in the lagoon complex.

Table-1: Catch sites for fishery products and their code.

Catch site	CODES
Agbalilame Tchonvi	S1
Aguegues	S2
Djassin Honvie	S3
Ekpe Ketonou	S4
Ganvie Sozounko	S5
Houedogbadji	S6
Pont_Porto_Novo	S7
Totche	S8
Zogbo Menontin Godomey	S9
Total	09

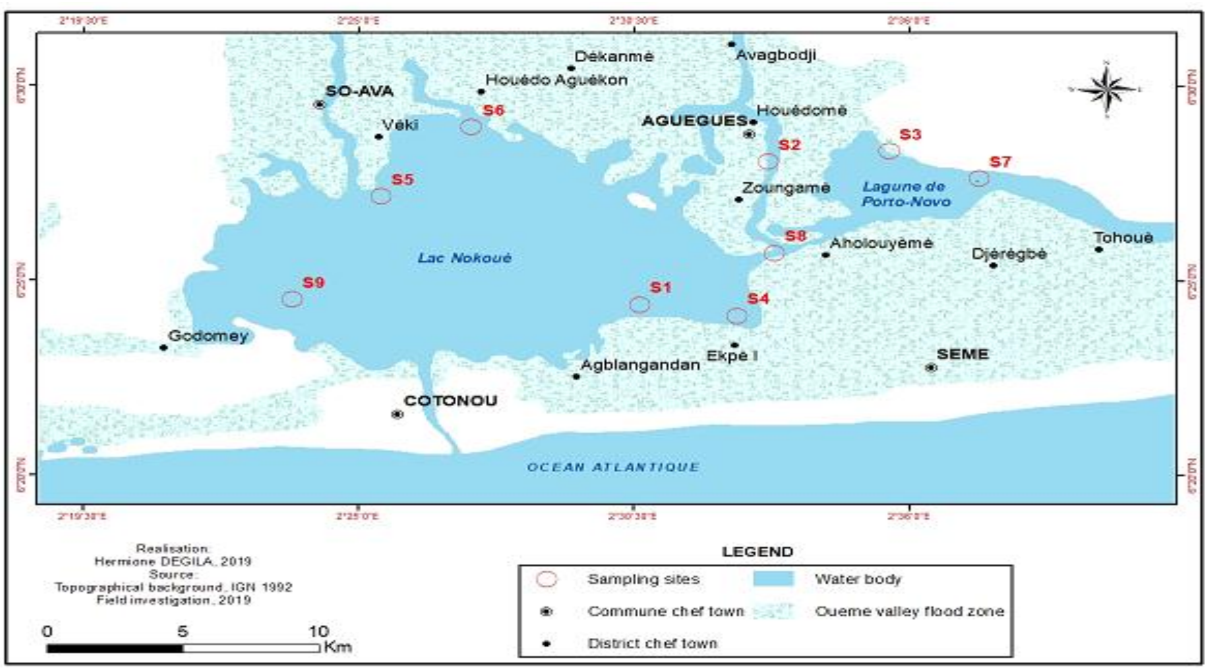

Figure-1: Map of the study area and sampling sites.

Collection, transport and storage of fish samples: A prior agreement with a fisherman identified by the head of the fishermen's association in each study area made it possible to collect samples of fish, shrimp and oysters on a monthly basis, for one year (April 2018 to March 2019). The high-water period experienced some fluctuations. There were twelve (12) months of campaign for *S. melanotheron* and *C. nigrodigitatus* fish, ten (10) months for *penaeus spp* shrimp, and five (05) months for

***Crassostrea Gasar* oysters: Collection and transport of samples:** For each sample, three individuals of each species of fish are taken from the fisherman per size class (a maximum of three classes depending on the catch of the day). For oysters and shrimps, a sample of thirty individuals is made up per site and per campaign is successful. These samples are placed in coolers containing accumulators and transported to the laboratory.

Samples preparation and storage: For fish, the sizes of the individuals are measured in centimeters. The fish are then cleaned if necessary and then thoroughly washed under running water. The dorsal flesh is then removed with forceps and scalpel, crushed and frozen at $-40^{o}C$ for the mercury content assessment. Concerning the shrimps, the samples are crushed and frozen until the determination of total mercury. For oysters, the shells are cut open with a knife and the flesh is crushed and frozen until the determination of total mercury.

Determination of total mercury content: The mercury determination was made by direct analysis of mercury using DMA 80. This device is fully described elsewhere[11,13]. Succinctly it operates by atomic absorption spectrophotometer with thermal decomposition and gold sensor. The light source is a low-pressure mercury lamp with a wavelength of 253.65nm. It is coupled to a UV photodiode detector. Its detection limit for solids, liquids and gases is 0.003ng (double cell). 0.001ng (tri-cell).

Results and discussion

Description of the samples: A total of six hundred and six (606) fish samples were collected for this study at a rate of three individuals of fish per sample. The work thus focused on approximately 1800 individuals of fish including 897 *C. nigrodigitatus* and 921 *S. melanotheron*, all sizes combined. Table-2 shows their numbers by size class. For Penaeus spp prawns, there were 32 samples (960 individuals). As for the *C. Gasar* oysters, 15 samples corresponding to 450 individuals were collected.

Results: Mercury content of different species of fishery products: It appears, for the 12 months of campaign, that the highest concentration of HgT in the fresh state of fish flesh is found in *C nigrodigitatus* with an average of 21.08 ± 21.62µg / kg. A minimum of 1.87µg/kg wet weight and a maximum of 239µg/kg wet weight were obtained (Table-3). It is followed in descending order by *S. melanotheron* which have an average of 6.8 ± 5.21µg/kg wet weight, *C. gasar* oysters (6.0 ± 1.6µg/kg wet weight) and finally *penaeusspp* shrimp (4.7 ± 3, 5µg/kg wet weight) (Table-3).

These mercury content values are well below the Benin standard which sets the mercury concentration in the fresh flesh of non-predatory fishery products at 500µg/kg wet weight. It should be noted that apart from the *C. Gasar* oysters which is only found at Lake Nokué, the samples with the highest levels were fish samples at site 7 located on the Porto-Novo lagoon.

Table-2: Number fish samples by size class.

Definition of size categories with *S. melanotheron* Gt : T > 16cm ; Mt : 16 < T ≤13cm; Pt : T< 13cm				
Numbers per size class	size Gt NE : 102 ; NI : 306	Size Mt NE : 105 ; NI : 315	Size Pt NE :100 ; NI : 300	All class sizes combined NE :307 ; NI :921
Average size value	16,20 ± 2,11cm	12,76 ± 1,64cm	10,76 ± 1,19cm	14,43 ± 2,90cm
Definition of size categories with *C. nigrodigitatus* Gt : T > 30cm ; Mt : 30cm < T ≤ 20cm ; Pt : T < 20cm				
Numbers per size class	Big size Gt NE : 53 ; NI :159	Average size Mt NE : 121 ; NI :363	Small size Pt NE= 125 ; NI :375	All class sizes combined NE :299 ; Ni :897
Average size value	30,77 6,35	19,97 3,35	14,60 1,82	22,76 7,70

T = size without the caudal swimmer; NE: number of samples; NI: Number of individuals.

Table-3: Average mercury content of the different species studied.

Fishery products	Avg HgT in µg/kg wet weight	Standard deviation	Minimum in µg/kg wet weight	Maximum in µg/kg wet weight
Fish *S. melanotheron* all sizes	6,84	5,21	0,48	49,81
fish, *C nigrodigitatus* All sizes	21,08	21,62	1,87	238,92
Shrimps *Penaeus spp*	4,70	3,48	0,84	17,13
Oysters *C. gasar*	6,03	1,56	4,46	8,98

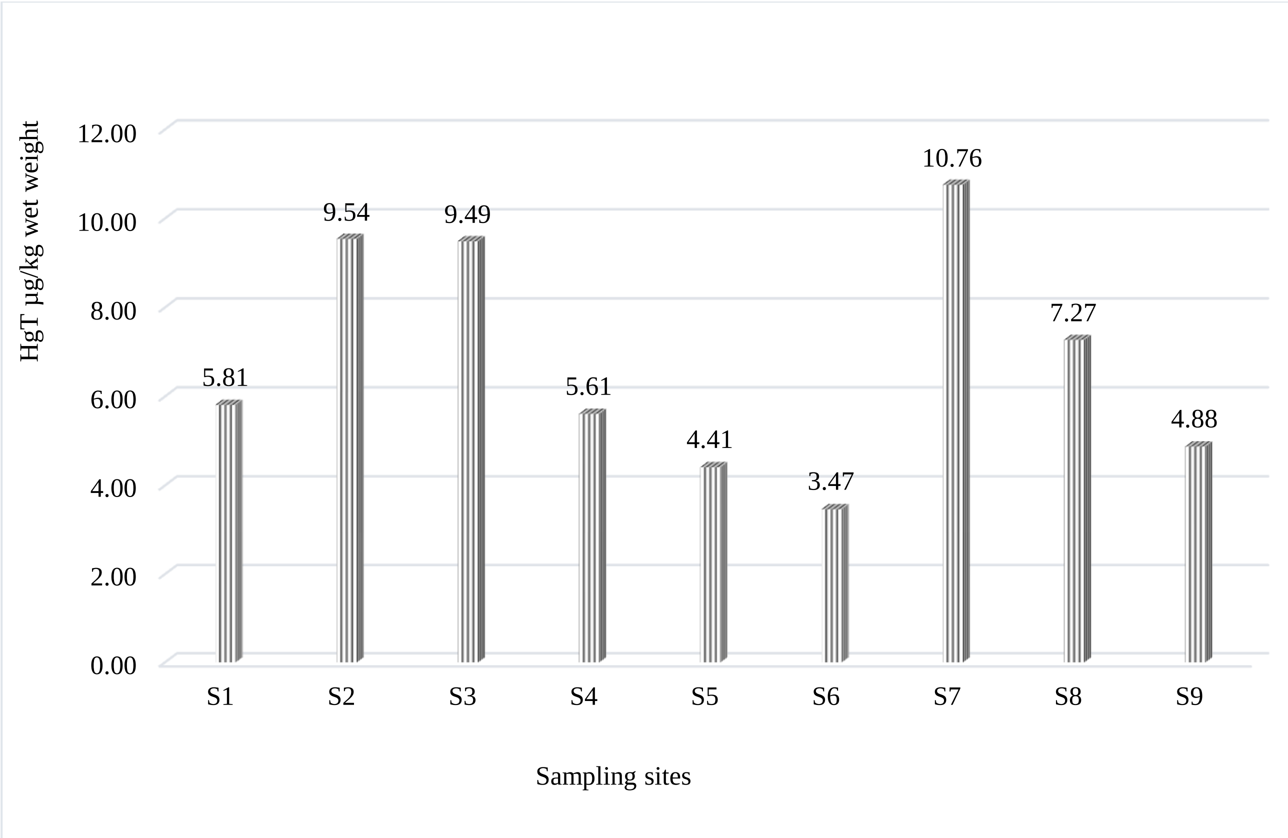

Figure-2: Sarotherodon melanotheron: mercury content by site.

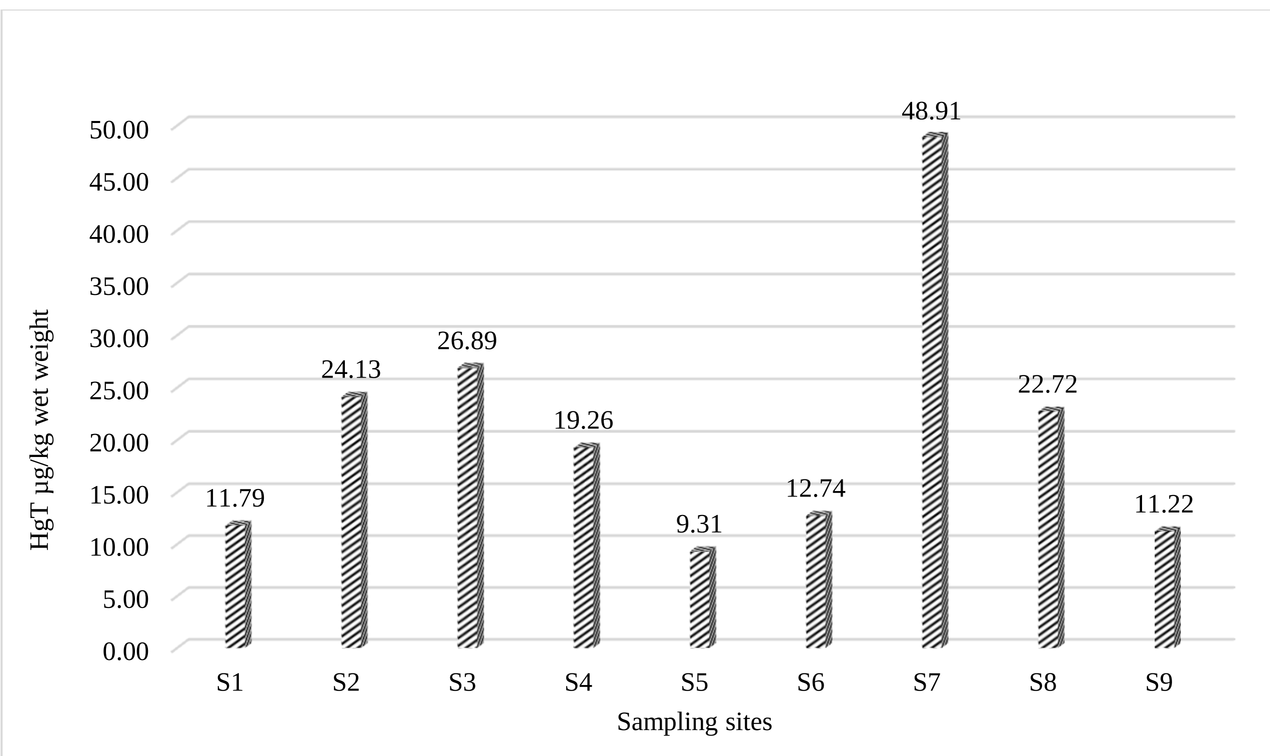

Figure-3: Chrysichthys nigrodigitatus mercury content by site.

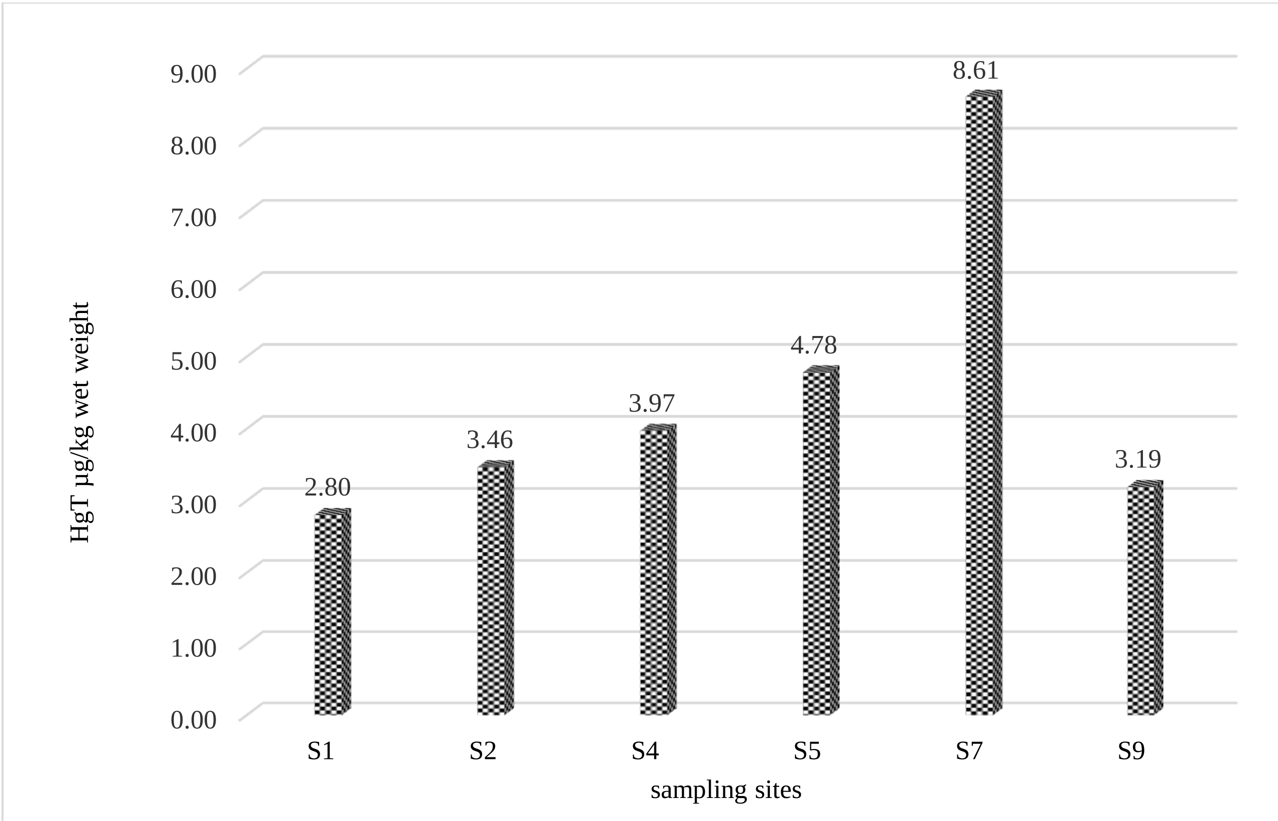

Figure-4: Shrimp penaeus spp: mercury content by site.

Table-4: Total mercury content in oyster *Crassostrea gasar* from one site to the other (April 2018 to march 2019).

Oyster; HgT(µg/Kg wet weight)					
Sampling site	Sampling site code	Average	Standard deviation	Min	Max
Agbalilame Tchonvi	S1	4,60	0,14	4,46	4,74
Ganvie sozounko	S5	7,28	1,56	5,26	8,98
Houedogbadji	S4	5,80	1,40	4,96	7,42
Zogbo menontin godomey	S9	5,72	1,38	4,91	7,78

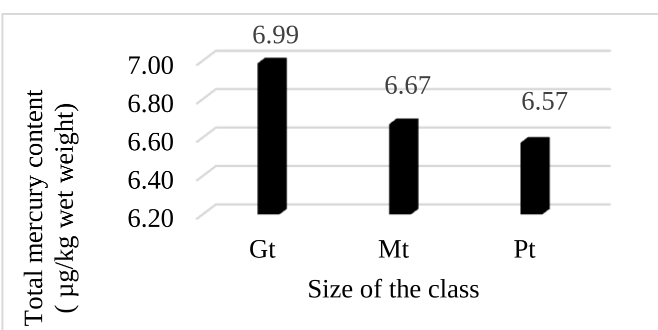

Figure-5: Variation of mercury content in relation with size (S. melanotheron Gt = large size, Mt =medium size, Pt =small size).

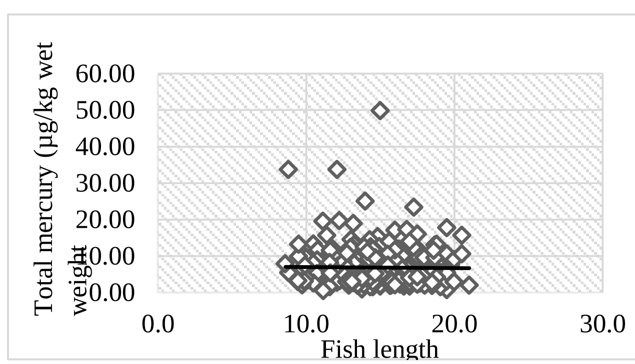

Figure-6: Relationship size- Total mercury content (S. melanotheron).

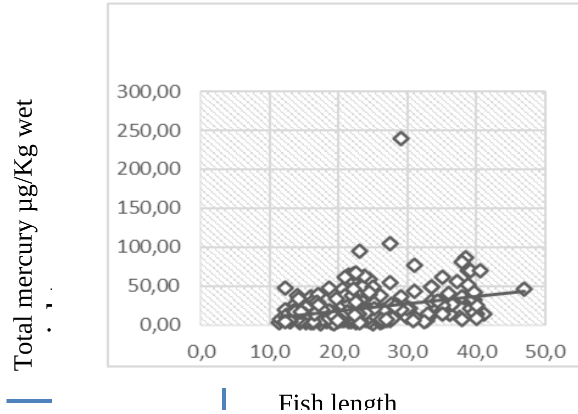

Figure-7: Relationship size- Total mercury content (C. nigrodigitatus)

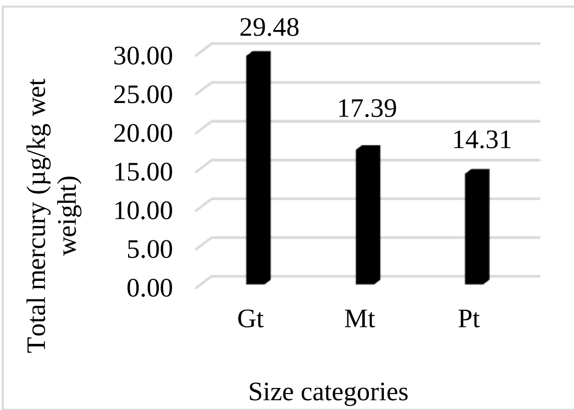

Figure-8: Variation of total mercury content in relationship of the size (C. nigrodigitatus Gt = large size, Mt = medium size, Pt = small size)

Hence of fish size on their mercury content: In *S. melanotheron* there is a slight decrease when moving from large to small categories. These values are respectively 6.99 ± 5.44µg /kg wet weight, 6.67µg/kg ± 4.97µg/kg wet weight, 6.57 ± 4.80 µg/kg wet weight for large, medium, and small size (Figure-5). Thus, at the *S. melanotheron* level, size has virtually no influence on the total mercury content of fish. This is confirmed by the insignificant very weakly negative correlation coefficient of -0.004 with a p-value of 0.9184 (Figure-6).

For *C. nigrodigitatus*, mercury levels vary with fish size (Figure-7). This is confirmed by a positive correlation coefficient of 0.21 significant with a P value of 1.12.10-7 between size and mercury concentration in fresh fish flesh. Thus, the samples of the Gt class, which are about 1.69 and 2.1 times the size of the Pt class, have an average mercury content of 1.54 and 2.02 times that of the Mt and Pt classes respectively. Also between the Mt class which is 1.36 times the size of Pt, its content is 1.21 times that of the last size class (Figure-8).

However, the Kruskal-Wallis comparison tests give a P-value of 3.10-4 and 4.10-4 respectively between the HgT contents of the Gt and Mt size classes and then between Gt and Pt. Whereas, the P-value between Mt and Pt is 0.4649. Thus, it is the content of the large size that differs significantly from that of the others.

Discussion: For non-predatory fish such as those covered by this study, and other fishery products, the Benin Republic has fixed by bill No. 0362 (2007) the mercury concentration limit at 0.5mg/kg of fresh flesh (wet weight), i.e. 500µg/kg wet weight. The extreme value obtained at the Porto-Novo Bridge (S7) for large *C. nigrodigitatus* (238.92µg/kg wet weight) is lower than this Benin standard which is identical to that of the European Union (regulation EU No 1881/06, 2006) and JEFCA (2006) for non-predatory fish. The same is observed for *S. melanotheron*, where the maximum content for the lagoon complex is 49.82µg /kg wet weight obtained at Djassin (S3) on the Porto-Novo lagoon. As for *Penaeus spp* shrimp and *C. gasar* oysters, their maximum content is respectively 15.26µg/kg wet weight 8.98µg/kg fresh body weight.

However, the Canadian standard for populations that consume large amounts of fish is 0.2µg HgT/g wet weight or 200µg/kg wet weight[14], whilst that of the Slovak Republic is 0.1µg of HgT /g or 100µg/kg wet weight[15]. Thus, the maximum mercury content obtained at the site S7 (238.92µg/kg wet weight) slightly exceeds the tolerated limit per the Canadian standard whilst it is twice higher than the Slovak's one for non-predatory fish.

Nevertheless, the average values obtained after the 12 months of sampling are not so alarming for the fish. Thus, the annual average for large *C. nigrodigitatus* at lagoon complex (29.48 ± 30.58µg/kg) represents 5.89% of the limit value of the joint FAO / WHO / JEFCA commission and the limit fixed by the Beninese standard. It is 14.74% of the limit set by Health Canada for heavy consumers (Health Canada, 2008). This average corresponds to 29.48% of the lowest limit value set by the Czech Republic for mercury in fish[15]. The average total mercury content of *S. melanotheron* in the present study represents respectively for JEFCA, the Republic of Benin, Health Canada and the Czech Republic about 1.4%; 3.4% and 6.8% of the limits set for the flesh of non-predatory fish.

As for *Penaeus spp* shrimps, *C. gasar* oysters, their average HgT concentration represents 0.84%, 1.21% of the limit retained by the Benin standard and JECFA, respectively. They are therefore insignificant compared to the lowest limit which is 100µg/kg wet weight set by Czechoslovakia.

The mean values of the HgT content of fish at the lagoon complex "Lac Nokoué lagune de Porto-Novo" are within the range of those obtained in Côte d'Ivoire in sardines (0.25µg/kg at 231±32µg wet weight)[16] as well as those obtained in tilapia *Oreochromis niloticus* in the same country[17]. Studies of fish from the Mali River show an average content of 0.047mg/kg wet weight with a maximum value reaching 0.270mg/kg wet weight[18] which are much higher than the average levels obtained in the flesh of *S. melanotheron* 6.84±5.21µg/kg wet weight with contents ranging from 0.48µg/kg to 49.81µg/kg wet weight. The mercury contents of *C. nigrodigitatus* which vary from 1.87 and 238.92µg/kg wet weight with an average of 21.08 ±21.62µg/kg wet weight are of the same order of magnitude as those obtained in Mali[18]. *C. nigrodigitatus* also has a total mercury content of the same order of magnitude as the levels observed in carp flesh in the Czech Republic (0.020 to 0.104 mg /kg wet weight) at uncontaminated sites[15] seem to be higher than the ones obtained at the level of the lagoon of Porto-Novo all sizes of fish combined (9.24±6.22µg/kg wet weight for *S. melanotheron* and 30.97±27.46µg/kg fresh body weight). Those values are within the range of the sub-region's values for mercury in fishery products.

Conclusion

The mercury content of fishery products in the complex lake Nokoué-lagoon of the Porto-Novo varies from: 1.87 to 239 µg/kg of wet weight with an average of 21.08±21.62µg/kg wetweight in *C. nigrodigitatus,* 0.48 to 49.81µg/kg wet weight in *S. melanotheron* with an average of 6.84±5.21µg/kg wet weight; 0.84 to 17.17µg/kg wet weight in *penaeusspp* shrimp, 4.46 to 8.98µg/kg wet weight in C. gasar oyster. It was noted that the samples taken at site S7 (Porto-Novo bridge) located on the Porto-Novo lagoon show the highest total mercury contents. Finally, with *S. melanotheron*, size does not seem to have a noticeable effect on the mercury content. However, for *C. nigrotigitatus*, the opposite phenomenon is observed.

In the light of these results, it is important that more in-depth studies be undertaken along the Ouémé river which flows into the studied complex, taking into account the spatial variation of the mercury content of the most consumed fish species. This will allow to identify with more precision the risks incurred by the consumers in function with the fishing sites in order to guide for remediation measures more effectively if necessary.

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