RISK OF INTOXICATION BY HEAVY METALS (Pb, Cd, Cu, Hg) CONNECTED TO THE CONSUMPTION OF SOME HALIEUTIC SPECIES IN LAKE NOKOUE: CASE OF THE PENAEUS SHRIMPS AND THE *SAROTHERODON MELANOTHERON*

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Summary

Lake Nokoue is one supplying source of water species in Benin. Many populations, especially those South of Benin and part of Nigeria carry out a great deal of activities around that lake. Meanwhile, huge quantities of wastes are dumped into the receptacle.

The main objective of this work is to estimate the risk of intoxication by some heavy metals (Pb, Cd, Cu, Hg), following the consumption of two water species, the most numerous ones in the lake and in the markets.

Lead turned to be the toxic metal highly accumulated by both species studied with an average content of 0, 99µg/g of dry weight with the shrimps (that is virtually twice the level accepted which is 0,5 µg/g) and of 0,92µg/g for the fishes dry weight (virtually five times the standard which is of 0,2µg/g)

Although penaeus shrimps concentrate more the metals, what exposes to a greater risk of lead intoxication, is the consumption of seratherodon melanotheron when no account is take of the eating habits.

Mercury on the other side seems to be, according to its content, t5he metal which have less risk on health nowadays when we consume the water products.

However, we have noticed a negative correlation between its content and the organic carbon dissolved by lixiviation in both species studied. Thus, it is likely that the methyl form (highly poisonous at low dose) be present in lake Nokoue 's biocenosis.

Although copper is a metal intervener that contributes to the metabolism of various living organisms, its contents with the sarotherodon melanotheron, exceeds forty-five times the level recommended.

Key word: prawn or shrimps, sarotherodon melanotheron, heavy metals intoxication.

INTRODUCTION

For many years, full consideration has been given to the dispersion of polluting agents in the environment by the scientific community. Heavy metals are listed among the main environment polluting agents because of their toxicity, their potential bioaccumulation in many animal species and their wide dispersion in the environment. (Katemo *et al., 2010*). In an aquatic ecosystem, those metals are often soluble in water streams or connected with the sediment particles and may pile up in living organisms at concentration levels superior to the water ones (Ramade, 1992;).They may lead to destructive effect on the ecological balance of the aquatic environment (Katemo *et al.*, 2010; Atolaye and Aremu, 2007).and probably on human health.

In the city of Cotonou, and in the local neighboring areas where, facing the high population density, a policy of efficient waste management is paining to be emplemented. The bank of

Lake Nokoue are used as wild dumping ground of all kinds of liquid and solid wastes.(see pictures I to 3).Moreover, Oueme, the main watercourse in Benin, being used for irrigation and transport of various commodities among which hydrocarbons (see picture 4) flows into that Lake.

Picture1: water collector on the bank of Agbalilame

Picture 2: bank of lagoon at Yenawa overloaded by solid domestic wastes

Picture 3: battery on the bank at Cotonou

Picture 4: Canoe full of fuel cans at Zogbohoue

And yet, Lake Nokoue is supposed to be the most productive lagoon in West Africa with an output of more than 1 ton of fish per Hectare (Yehouenou, 2005).
Fishing is very developed and constitutes an important activity carried out by the riversides inhabitants.(Direction des Pêches du Bénin, 2006).
Lake Nokoue plays a key role in the feeding of the population by supplying it its main source of animal protein. (Lalèyè, 1995). A chemical or microbiologic pollution of Lake Nokoue waters might bring about serious public health problems.
Many studies have mentioned both chemical and microbiologic pollution of lake Nokoue. Niyonkuru, 2007 ; Mama *et al.*, 2011).Thus, it is worth estimating the poisoning risk connected to the consumption of some water speciesfrom the lake in order to help prevent possible health problems.
The choice of paneus shrimps and of the tilapia (carpus) sarotherodon melanotheron is links to their high level of consumption by the riversides population. In fact, a visit paid to our

different markets has shown that shrimps are the most sold shellfish. As for the tilapia sarotherodon melanotheron, they constitute the most fished fish in Benin lagoon.(Niyonkuru, 2007).

By this study, we aim at two objectives: on the one hand, estimate the heavy metals content (Pb, Cd, Cu, Hg) with aquatic animals scheduled, and on the other hand check whether the daily consumption of these species represent health risks referring to the levels set by international institutions.

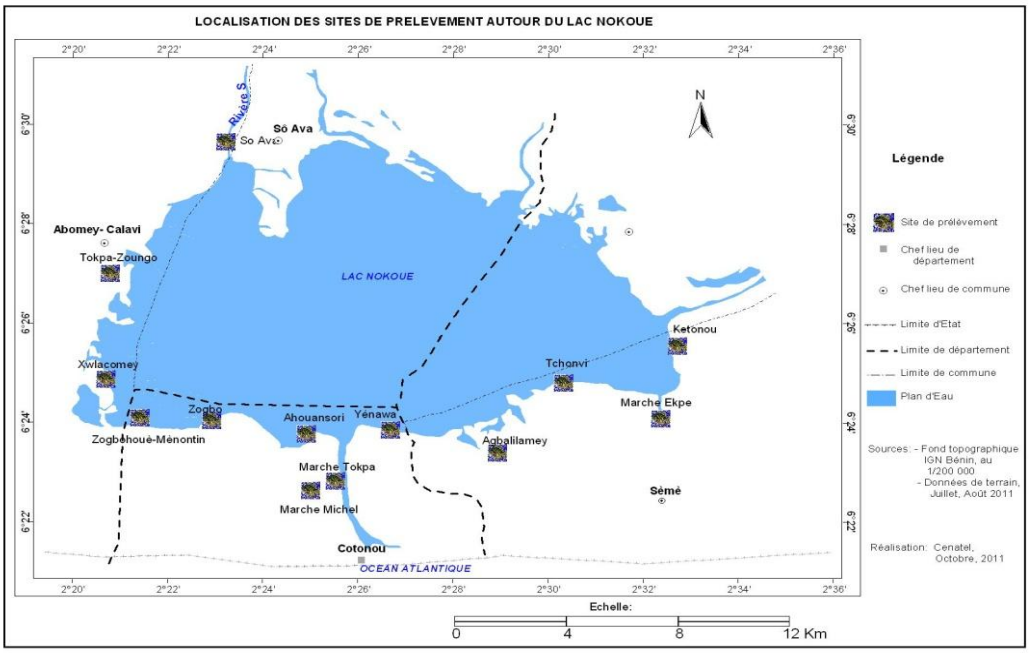
2- Methodology - Material

2-1 – Sampling

Choice of sites.

Our objective being to estimate the level of contamination of water products from Lake nokoue and consumed, we chose to carry out our sample taking at river banks and in markets. Owing to the help of the President and some leading members of the Association of fishermen of lake Nokoue, we got from the data base of the fishing Department in Benin, we got a list of about forty mostly frequented places by fishermen and wholesale fish merchants alongside lake Nokoue. A reasoned choice of 25% of sites by Lake Nokoue riverside municipalities (Cotonou, Sèmè-kpodji, Abomey-Calavi, Sô-ava) has been implemented taking into account the rising number of attendance. In addiction to these places, we've selected three markets where fishes and shrimps from many landing stages can be found. Picture 5 shows where the various sites alongside Lake Nokoue could be found.

An average sample per site was made for each species studied.



Picture 5: location of taking sites around Lake Nokoue

Collection and analysis of the samples:

As for the shrimps, about ten are bought from all the fishermen and wholesale fish merchants who come to the selected sites between 7:00and 9:00 Am. As the shrimps were taken at night, their unloading ends after 9:00 in the morning. Thus, account the abundance of unloadings, a sample of 200g to 300g is set up per site. The samples are automatically put into an ice box containing accumulators and are transported cool to the laboratory of science and technique of water of Abomey Calavi Polytechnic School.

As far as the tilapas are concerned, a few fishermen unload their fish before 9:00 but the majority of them as well as wholesale fish merchants come after 9:30.Therefore, as soon as the first fisherman or the wholesale fish merchant arrives, one to two fish are bought from a wholesale fish merchant out of four for about half an hour. Those samples are identified and put in an icebox containing accumulators and are transported to the laboratory. At the level of Tokpa and St Michel markets, the taking of the fish and the shrimps are carried out only next to the sellers who notify that their commodities come from Lake Nokoue. The samples thus taken are rinsed with distilled water and dried up to 105oC until the weight becomes constant2-2 collection and analysis of data

Lead contents, cadmium and copper have been determined by an atomic absorption spectrophotometer with oven ((Varian, Spectra AA 800 having a Zeeman correction system of non atomic absorption due to the residual matrix) the limit of detection is about 0, 1 µg/L in the laboratory of soil, water and environment sciences (LSSEE) of the Agricultural Research Center (CRA).The dosage was preceded by hot mineralization. Mercury dose was measured out by molecular absorption spectrometry following HACH method. The spectrophotometer used is HACH-LANGE DR 2800 after mineralization and extraction by cold vapor.

To make the Health risk evaluation starting from metal average content and the average private weight of a shrimp or a fish, we calculated the daily contributions taking into account cooking and eating habits. Thus we figured out that shrimps consumption fall between the intervals of 5 to 10 units and the fish one to 2 units a day. These are the values we compared to the daily dose required per age group.

Excel software 2007 and SPSSS (statistical package for social sciences) version 12.1 have been used to process data statistically. Student tests and ANOVA (Analysis of variance) have been used according to the needs.

3- RESULTS

3-1 Heavy metals content per site

After dosage of heavy metals in the ground and dried up samples, we have accessed the content of µg par gram of shrimp and fish dry weight (see pictures 6; 7; 8; 9).

The standards used in this study are metal values accepted with shrimps and fish for their edibility. Those standards are the ones authorized by the Benin Ministry of Agriculture of Reaning and Fishing in (2003). Except the one of copper in the fish which is recommended by France Highest Council of Public Hygiene of (Katemo et *al.*, 2010). As for the case of lead among the sampling of both species in our study, the contents vary from one site to the other. Lead contents in shrimps are higher than the standard accepted almost at the level of all sites except Houalacomey. The highest value obtained at Tokpa is little less than four times the threshold. As for the fish, the contents are higher than the standard. The highest values reach 8 to 10 times the standards and have been obtained respectively in the markets of Tokpa and Ekpe (picture 6).

Concerning the cadmium, the values are all under the threshold of edibility defined by the Ministry of Agriculture for shrimps. Besides, for the fish, those values are on top in eight sites.(Picture 7). It is with the copper that we note the most important excesses. The standards show that the copper values in the shrimps are above the standard in 8 of the 12 sites checked. On the contrary, as far as the fish are concerned, that copper content is higher than the threshold at the level of all the sites. The highest values have been seen at Zogbohoue as well for the shrimps (1.5 times the standard) and for the fish (more than 100 times the values recommended which is 0, 1µg/g. Picture 9 shows us that with the shrimps and the fish as well, the mercury contents are considerably low according to the threshold values.

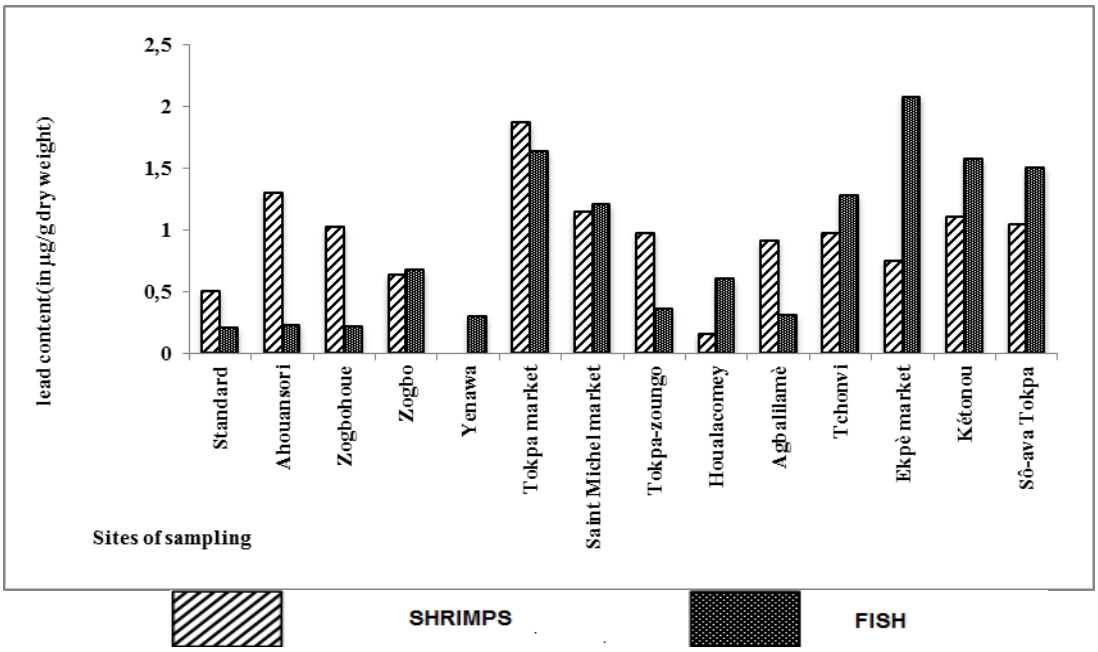
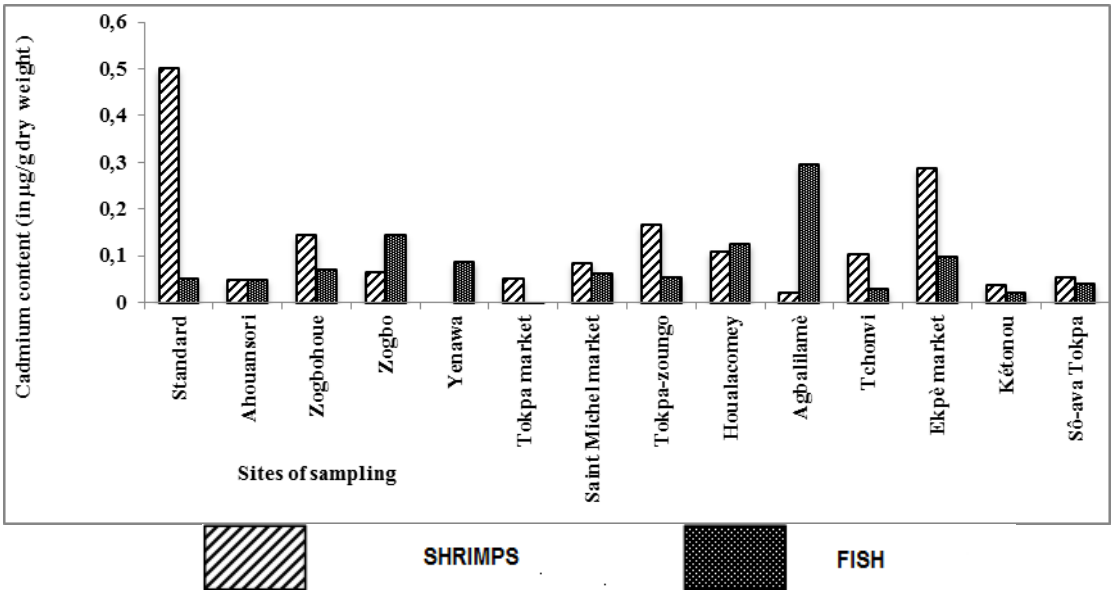
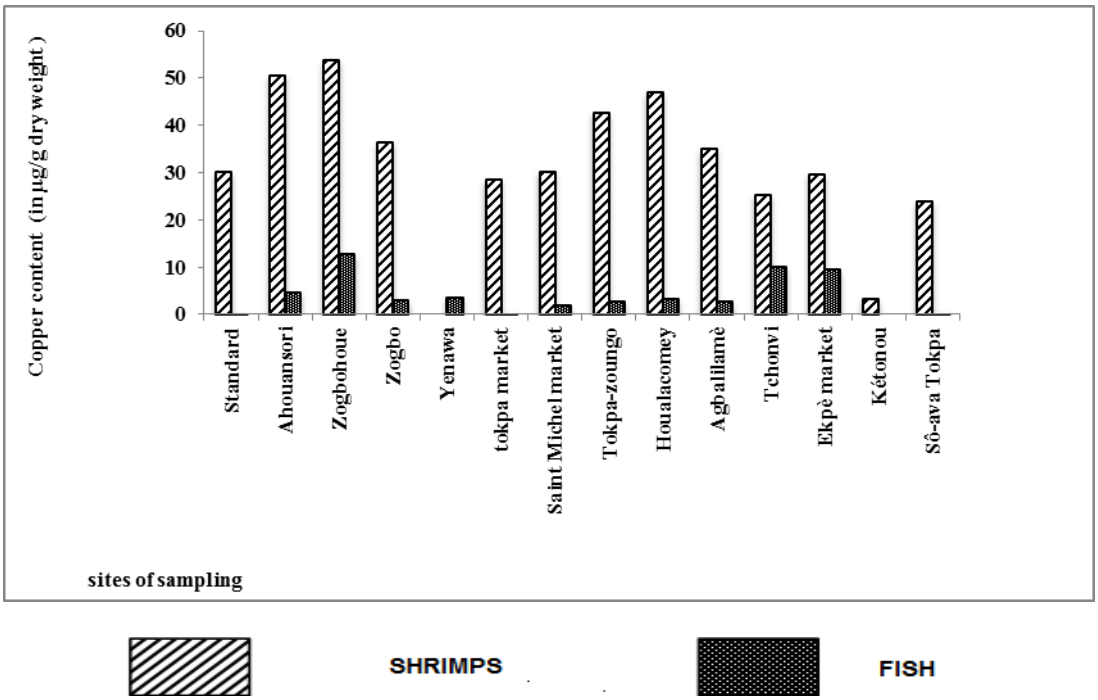


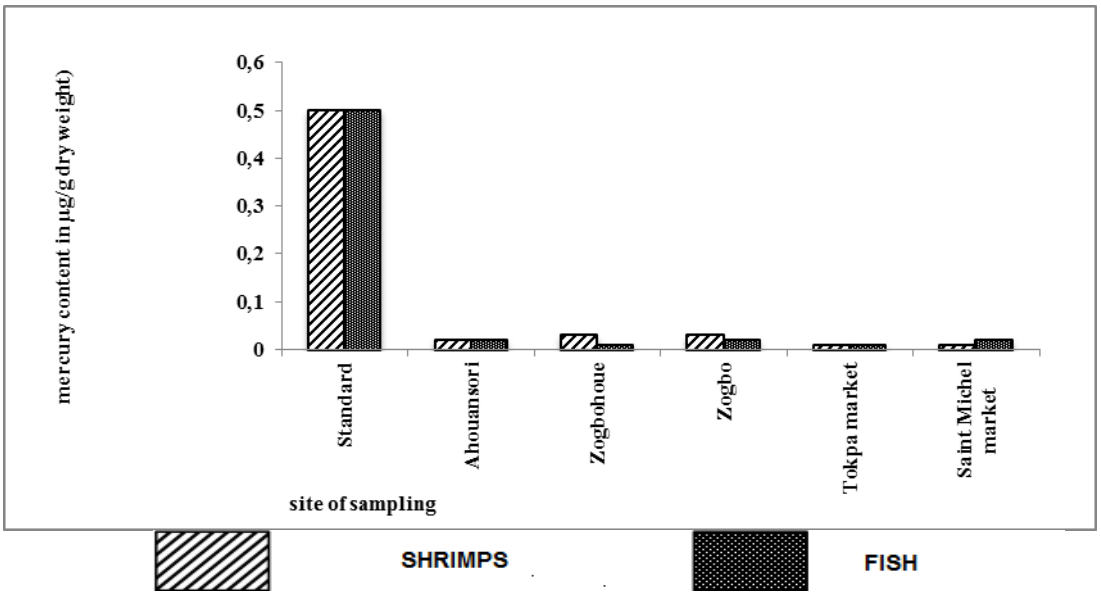
Figure 6: shrimps and fish lead contents per site



Picture 7: Shrimps and fish cadmium content per site



Picture 8: Shrimps and fish copper content per sites.



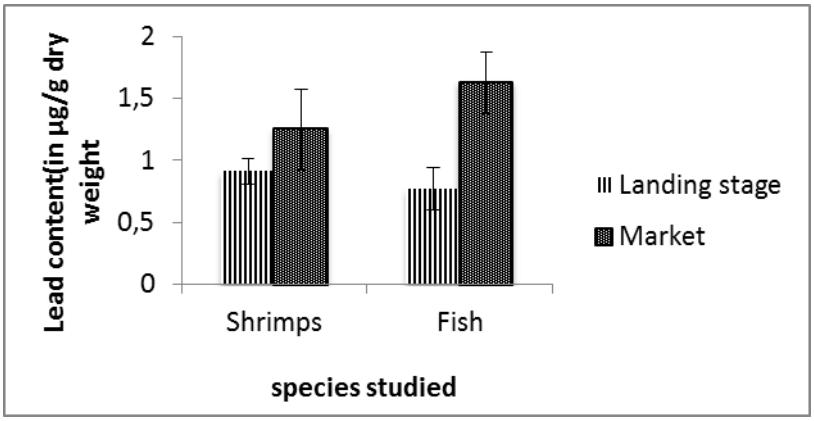
Picture 9 : Mercury content in shrimps and fish per sites.

3.2-Metal contents according to the methods of the place of taking

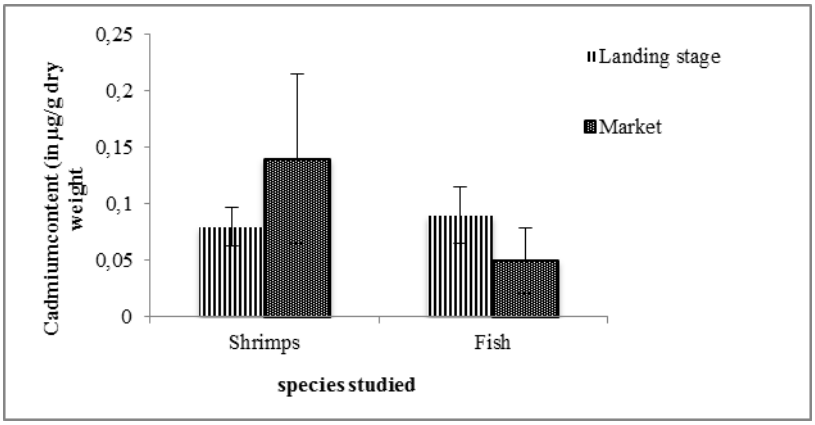
What is generally noted about the population as far as the place of supplies is concerned is that people buy directly fresh fishing products for the cooking or at the level of the markets or directly at the landing stages. That's why we found it worth gathering the ''taking sites'' following two methods:

Landing stage and market. Pictures **(10; 11; 12; 13)** show us the average contents with both species according to the taking places (market or landing stage). As far as lead is concerned, **(Picture 10),** we've noticed that the average contents are higher at the level of the markets, in comparison with those of the landing stages. However, that difference is only significant with the fish **(p=0.03).**

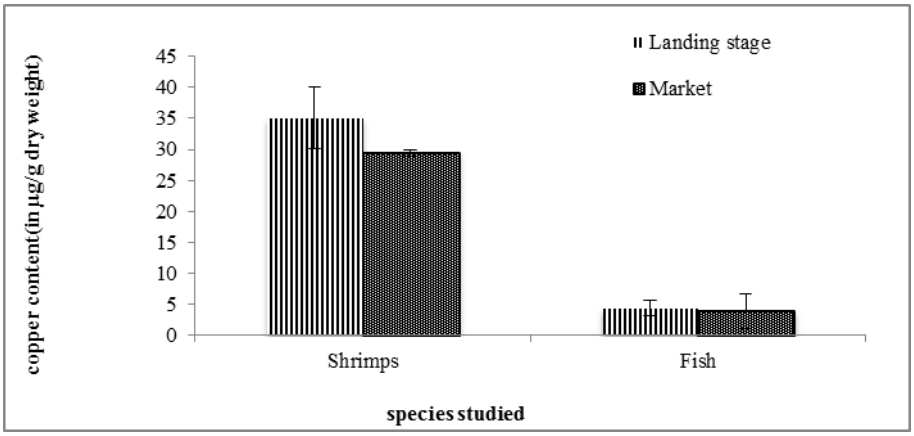
Picture 11 about cadmium shows that the average contents are higher at the levels of the market places in comparison with landing stages for shrimps whereas it is the reverse for fishes. Nevertheless, the difference is not very significant.
As for copper **(Picture 12),** the average contents in shrimps are high in the landing stages but the difference is not significant. **(P-value of the ANOVA test = 0,53)** whereas they are almost equal with fish. Moreover, the picture shows us that shrimps of short height (short if compared to fish) have a content significantly higher than the ones of the fish at the level of the two types of ‘’taking”. As for mercury (Picture 13), it is noticed that the average contents are higher at the level of landing stages, comparison made with those of market places and that difference is significant with the shrimps. **(p-value = 0.03).**



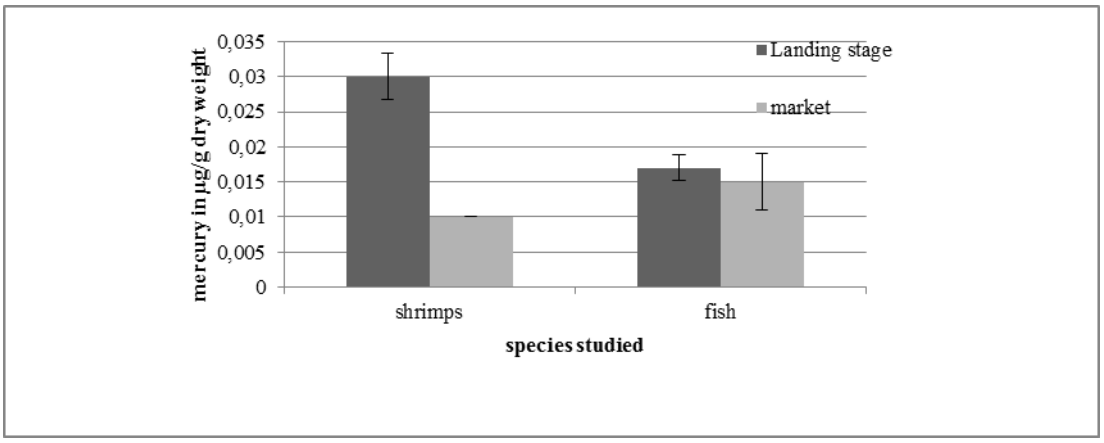
Picture 10: Lead average content in shrimps and fish according to the place of supplies.



Picture 11 : Cadmium average content with shrimps and fish according to the place of supplies.



Picture 12: Copper average content with shrimps and fish according to the place of supplies.



Picture 13 : Mercury average with shrimps and fish according to the place of supplies..

3.3- POISONING RISK

The toxicity of metals on living organisms especially on man depends both on the quantity input and the chemical form under which they are presented. We didn't make the specification of chemical species, but we tried to find out the possible correlation between those metals and the organic carbon which likely may imply the presence of metals in our samples.

Account taken of our observations, and from synthesis of previous works, we can assume that the consumption of shrimps might vary between 5 and 10 daily and fish consumption might be an average of 2 per person and per day; whish shows the real danger in consuming products from the lake if nothing is done to limit the dumping of wastes in it.

3.3.1.-Corrélation between metals and organic carbon

Content in organic substances

We determined the content of organic substances in dried samples in order to report the possible presence of metal in organic form. Table 1 shows that shrimps contain more of it than fish.

Table 1 : Organic substance, total organic carbon dissolved in shrimps and fish.

SITES	Shrimps			Fish		
	MO en g par g de crevette	COT en g par g de crevettes	COD en g/mL	MO en g par g de poisson	COT en g par g de poisson	COD en g de C /L
Zogbohouè	0,87	0,50	17,50	0,82	0,48	15,62
Zogbo	0,86	0,50	14,90	0,80	0,46	18,16
Ahouansori	0,84	0,48	27,70	0,81	0,47	17,56
yénawa				0,77	0,45	23,56
Tokpa market	0,89	0,50	27,40	0,79	0,46	16,76

Michel market	0,87	0,50	29,40	0,82	0,47	15,44
tokpa-zoungo	0,87	0,51	23,00	0,84	0,49	16,86
houalacomey	0,87	0,50	17,60	0,84	0,49	19,08
Agbalilamè	0,86	0,50	28,80	0,77	0,45	12,82
Tchonvi	0,87	0,50	23,40	0,79	0,46	16,20
Ekpè market	0,87	0,50	24,20	0,83	0,48	20,40
ketonou	0,87	0,50	28,80	0,82	0,47	22,40
Sô-ava tokpa	0,86	0,50	28,00	0,79	0,46	16,20

-Correlation with metals

According to the correlation matrixes presented in the tables 10 and 11, no correlation was noticed between metal and the total organic carbon with the shrimps. None was noticed with the fish either.

Let us mention that there is a good positive correlation **(0, 68)** between mercury and copper with shrimps. This leads us to say that they might be the same evolution between both elements.
As for the dissolved organic carbon, it presents a negative correlation with mercury in both species studied. A negative correlation also exists between copper and dissolved organic carbon with shrimps. As the content of carbonic gas is higher with shrimps, we can validly assert that copper is well mixed up with organic substances. This matches the results of literature (Azita *et al*,2008 ; Aina *et al*., 2009)

Table 2 : Correlation between metal and organic carbon with shrimps

	Pb	**Cd**	**Cu**	**Hg**	**COT**	**COD**
Pd	1					
Cd	-0,32	1				
Cu	-0,24	0,21	1			
Hg	-0,76	0,47	0,68	1		
COT	0,18	-0,49	-0,15	-0,35	1	
COD	0,26	-0,33	-0,76	-0,54	0,14	1

Table 3: Correlation between metal and organic carbon with fish

	Pb	**Cd**	**Cu**	**Hg**	**COT**	**COD**
Pb	1					
Cd	-0,41	1				
Cu	-0,02	-0,08	1			
Hg	-0,20	0,53	-0,39	1		
COT	-0,12	0,33	-0,26	-0,23	1	
COD	0,17	-0,32	0,23	-0,58	-0,34	1

3.3.2- Le plomb

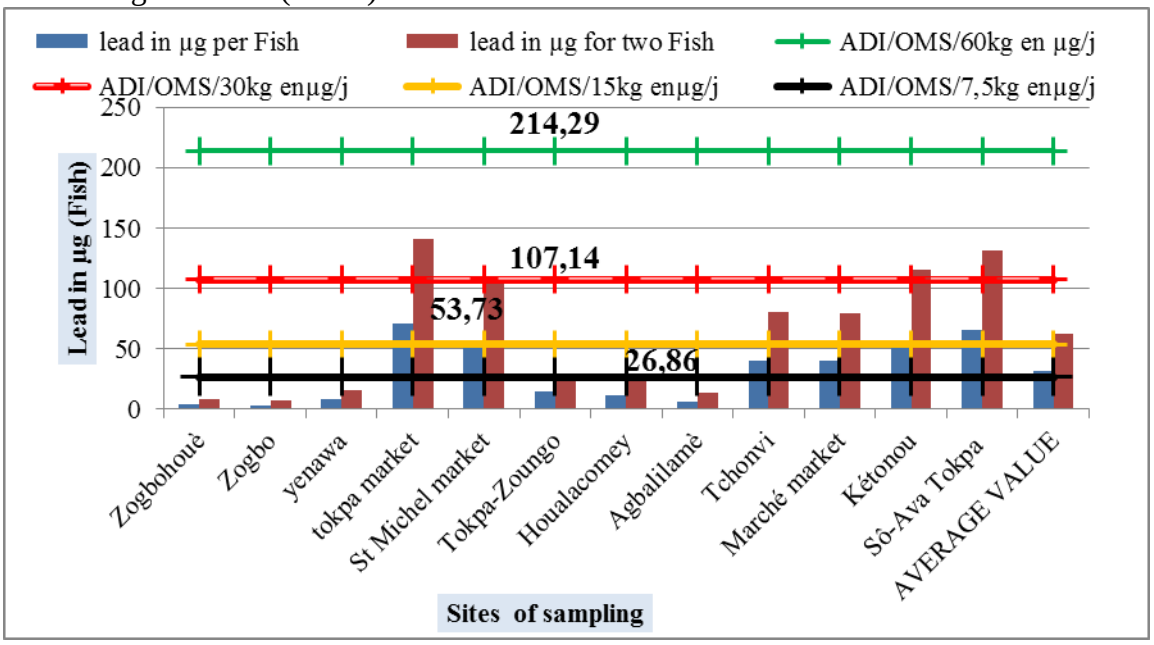
The daily supply of lead for about ten shrimps of which the average value is of 14,24 ± 6,23µg remains inferior to the Acceptable daily intake (ADI) recommended by the World Health Organization (WHO), whatever the weight of people old enough to consume some. Mention must however be made of the fact that the value obtained for the samples taken at Tokpa exceed slightly 7.5 kg children's ADI. (Table 4)

Tableau 4 : Shrimps lead supplies

sites	Pb in µg/shrimps	Pb in µg/5shrimps	Pb en µg/10 shrimps	Acceptable daily intake by WHO in µg according to body weight			
				60Kg	30Kg	15Kg	7,5Kg
ahouansori	0,97	4,83	9,66	214	107	54	27
Tokpa market	2,80	14,01	28,02	214	107	54	27
St michèl market	1,82	9,08	18,16	214	107	54	27
Agbalilamè	1,32	6,58	13,17	214	107	54	27
Tchonvi	1,22	6,11	12,22	214	107	54	27
Ekpè market	0,98	4,91	9,83	214	107	54	27
Kétonou	0,91	4,55	9,11	214	107	54	27
Sô-Ava Tokpa	1,38	6,89	13,78	214	107	54	27

As for fish, no excess of ADI was noticed with individuals of 60kg consuming 2 fish a day. But the excesses have already been noticed for consumption of 2 fish with a child of 30 kg. (Picture 14). The average lead content of 2 fish exceeds the DAI of 15 kg children. In addition, at the level of 4 sites of among which the two markets in Cotonou, the consumption of a single fish provides a 15kg child his daily dose of acceptable lead.

Remark that only one fish can provide a 7.5kg child with lead ADI according to the World Health Organization. (WHO)



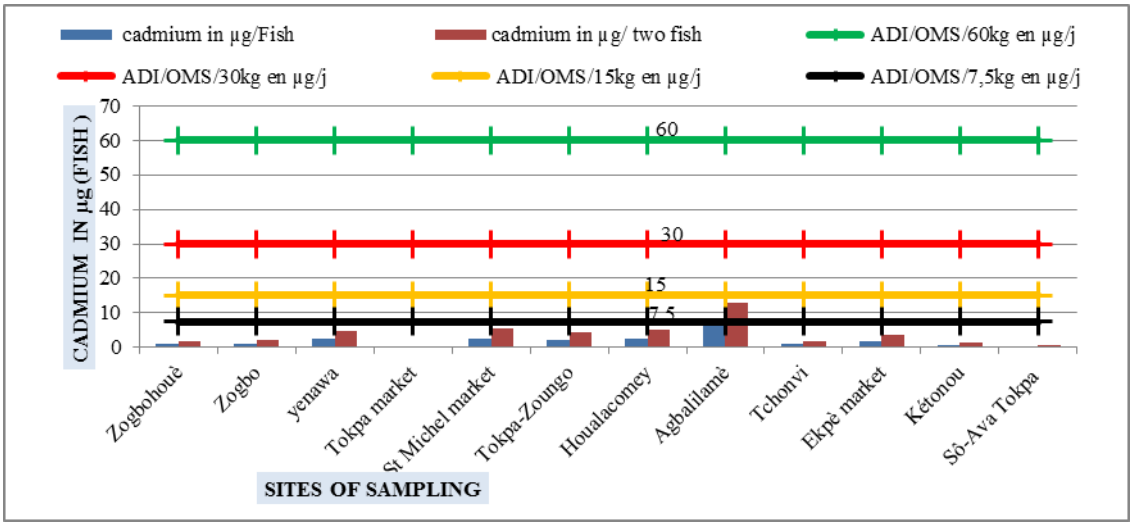
Picture 14 : Lead content in fish per site compared to the WHO ADI

3.3.3- Cadmium

The shrimps of Lake Nokoue supplies a very small quantity of cadmium (table 5) and the consumption of about 10 shrimps everyday doesn't expose to any health risk. The values obtained and of which the average is 1, 02 ± 0, 66µg for 10 shrimps is far inferior to the ADI (acceptable daily intake), even for a 7.5 kg child according to W.H.O. (7, 56µg).
As for the fish studied, it is only with 7.5kg children that eating 2 fish a day might bear some intoxication risk.

Table 5 : Shrimps cadmium supplies

Sites	Cd in µg/shrimps	Cd en µg/5 shrimps	Cd en µg/10 shrimps	Acceptable daily intake WHO in µg according to corporel weight			
				60Kg	30Kg	15Kg	7,5Kg
Ahouansori	0,10	0,48	0,97	60	30	15	7,5
Tokpa market	0,08	0,39	0,79	60	30	15	7,5
St Michèl market	0,12	0,61	1,22	60	30	15	7,5
Agbalilamè	0,02	0,12	0,25	60	30	15	7,5
Tchonvi	0,14	0,69	1,38	60	30	15	7,5
Ekpè market	0,24	1,18	2,37	60	30	155	7,5
Kétonou	0,05	0,24	0,49	60	30	15	7,5
Sô-Ava Tokpa	0,07	0,36	0,73	60	30	15	7,5

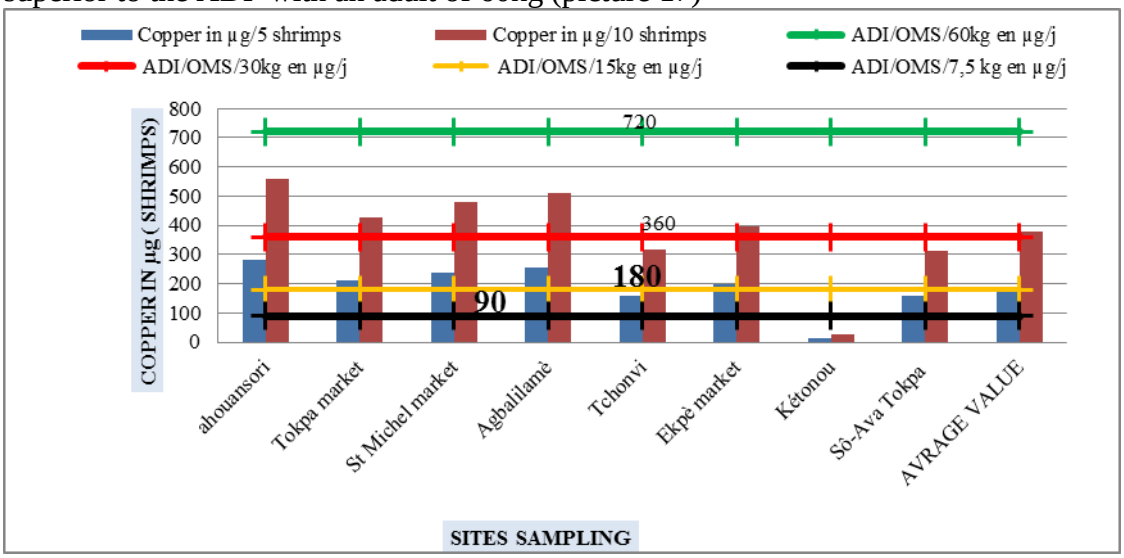


Picture 15: cadmium content in fish and per site compared to W.H.O. ADI

3. .3.4-Copper

If the consumption of 10 shrimps a day doesn't bring about any health problems for grown-ups of 60 years according to the W.H.O. , the consumption of 10 shrimps is risky for 30kg individuals. The average value which is of 378,42±166,66µg shrimps exceeds the ADI of 30kg children on six sites out of eight taken into account. In addition, the consumption of 5 shrimps everyday gives a 15kg child less copper than accepted dose set up by W.H.O.

(Picture 16). About fish, the average cooper supplies of 104µg/ exceeds the ADI set up by WHO. (90µg/day for 7,5kg children).Moreover, the daily consumption of 2 fish by children of 15kg and less show intoxication risks .The average value which is of 208µg for two fish is superior to the ADI with an adult of 60kg (picture 17)



Picture 16 : Shrimps copper content per site compared to WHO ADI

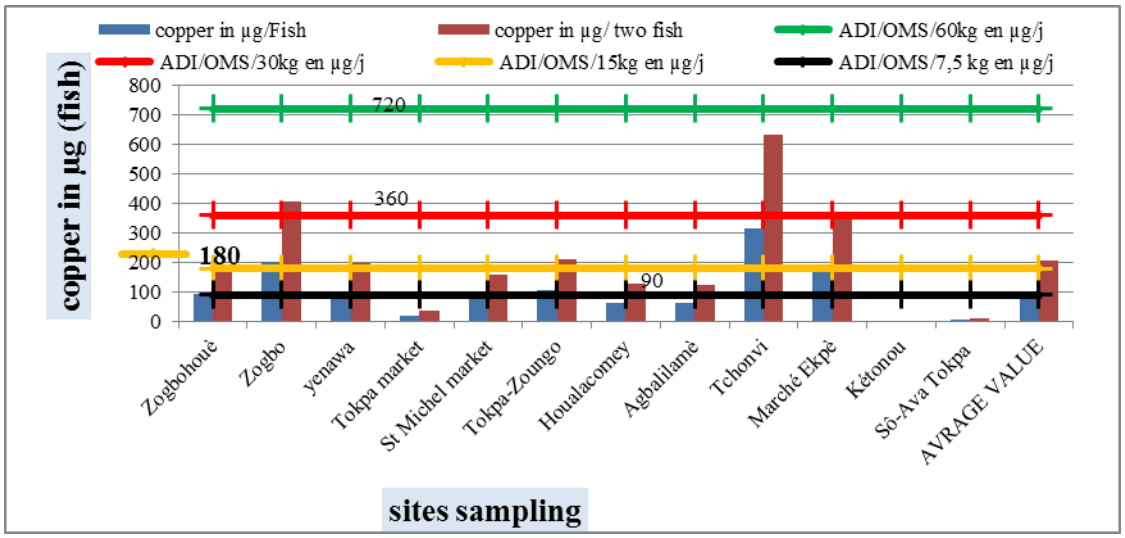


Figure 17: Fish copper content per site compared to WHO DJA

4-DISCUSSION

4.1 Accumulation of metals by both species.

Shrimps seem to accumulate metal more than sarotherodon melanotheron. In fact,the highest lead content is about four times the standard with shrimps whereas it reaches up to times the standard with fish. The same remark is made with the cadmium for which the contents at the level of shrimps, although high are all inferior to the standard whereas the ones of fish have reached almost six times the threshold value at Agbalililame. For the copper, no site presented copper content exceeding twice the standard level of shrimps whereas with the fish the values obtained vary between six and more than 100 time the threshold set up by the Public Hygiene Council in France.

This is probably linked to biological specificities of species. In fact, if we refer to Ministerial order N°425 (2003) of Benin Ministry of Rearing, Agriculture and Fishing setting up lead values cadmium ones and mercury ones in fishing in Benin. The contents authorized for lead and cadmium with shrimps (0,5mg/kg) are receptivity 2,5 and ten times the ones authorized for fish (0,2 et 0,05 mg/kg). And yet, both species are submitted to the same water pressure, that's what explains the excesses of standards with sarotherodon melanotheron. However, it is worth précising that having chosen to take into account eating and cooking habits in Benin, our research is not made on fresh flesh. Moreover, drying has made samples loose two thirds of their weights. (The weight after the drying up is 31,78 %±2, 33% of the weight of the fresh product for the shrimps and 31,50%±4,52% of the fresh fish). But the concentration provoked by the drying up cannot explain alone the contents that reach 100 times the standard.

Of the most poisonous metals studied, lead seems to be highly accumulated by both studied species. The highest values are noticed in Cotonou in Cotonou and Seme –Kpodji districts. That may be explained by population increase in both localities the endless traffic of hydrocarbons on reads and waters as well as economic activities. In fact, the bad management of a huge quantity of various wastes might bring about the contamination of the water ecosystem. Lead, being mainly used as additive to petrol (Audry, 2003). Petrol is supposed to be without lead since 2005, but is our petrol really without lead?

We failed to see studies devoted to the idea.

Copper is more abundant in shrimps than in fish (P-value = 0,00). Its average content with shrimps is seven times the one with fish. In addition, it is in the same metal that the highest excesses have been noticed. The

contents so high in comparison with the standards may be due to the presence of dumping places and houses stretched a long side the river or on piles.The higher concentration of copper in shrimps is understandable because that metal is highly mixed up with the organic substance which is more abundant in shrimps (86,4%) than in fish (80,8%). A brief comparison of our findings to those obtained with fishes in some rivers of the region have shown that the average contents of mercury are (0,02µg/g dry weight) inferior to those obtained by Biney (1991) in Ghana basin of Kpong and Wiwi river which are respectively of 0,053 and 037 µg/g fresh weight.. The same is true for the cadmium for which the values at the level of the river are respectively 0,10 et 0,19µg/g weight but our average is about 0,08µg/g but for lead the average we have with our fish (0,92µg/g)is almost the double of fish of both rivers. For copper, we have twelve times the average of fish from Kpon basin and 25 times of Wiwi river.

Compared to a study carried out lately in the Democrats Republic of Congo (Katemo *et al,* 2010) our averages are close to minimum values obtained for the same family of fish as far as the cadmium (0,064µg/g) end the copper (3,6µg/g). As for lead, our average is closest to the highest (1,23µg)

4.2 impact of the place of ''taking'' on the metals contents

It is mainly about lead that a difference is noticed between the contents of samples taken at landing stages and the ones taken in our markets with a difference of behavior between both species studied. In fact, the lead contents in the sample increase with shrimps and tilapias when we move from the landing stage to the market. However it is with the fish that the difference is significant (p-value = 0,03). The average obtained at the market is the double of the one of landing stages (0,77µg/g and 1,63µg/g dry weight). Conditions of transport and of conservation of both samples as well as the geographical location of our markets might explain the difference of behavior noticed between the two species (shrimps and fish). In fact, from our personal observations, on the field, the shrimps were convoyed to the market in waterproof cans in which they introduce some ice whereas the fish are put in baskets and are in permanent contact with the air. In addition, the traffic is heavy around our market with important volumes of exhaust gas.

3-2-3 health risk

The major health risk seen to from lead in the fish. In fact, even if the daily consumption of two fish doesn't bring the ADI planned for 60 kg adults; it supplies an average that exceeds the required dose for children of 15 kg. Consumption of a single fish is enough to provide a child of 7.5kg with his ADI. That lead accumulation in fish is very dangerous because children who need enough animal proteins for their growing are at the same time very sensitive to chronic lead intoxication (saturnism) with anemia, drop of intelligence quotient, congenital abnormalities, neuro – behavioral deficit etc. (Bisson *et al., 2003*).Moreover, fish remain one of the main sources of animal protein in south of Benin and the *Sarotherodon melanotheron* is one the most abundant species of Lake Nokoue (Niyonkuru, 2007).The main origins of contamination of water products might be the transport on the lake of hydrocarbon containing lead and which are accidentally or willingly thrown into the river. Exhaust gas, the second metal of which the content in the species studied might provoke metallic intoxication by copper in the shrimps. The excess of the ADI particularly with children less than 15kg for consumption of 5 shrimps is serious when one knows copper toxicity which acts by enzymatic inhibition, production of free radicals responsible for cell lesion at the level of the DNA, of mitochondria and lysosomes. (WHO IPCS, 1998)

Among the metals studied, mercury seems to be the one that shows less health risk to man. The contents obtained are low. The quantity of mercury likely to get into the shrimps is largely below the ADI set up by WHO even with children of 7.5kg. Nevertheless, the consumption of two fish may constitute to this weight group a real risk, knowing the danger of chronic metal intoxication and the fact that biomedical wastes and pharmaceutical wastes are daily thrown on the banks of our rivers. It is worth carrying out deeper studies with a larger sample.

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