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Research Article

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Cytotoxicity of Waters of a Tropical Lagoon and Contamination of Tilapia Guineensis by Lead, Zinc, Copper and Cadmium: Case of Agbokou and Porto-Novo bridge sites (South of Benin)

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Abstract The demographic explosion and the industrial revolution had consequences as the discharge of domestic and industrial waste in the lakes and lagoons which polluted them, involving serious environmental problems and of public health. The objective of this study is to know the level of total toxicity of two sites of the Porto-Novo lagoon and to determine the content of lead, zinc, copper and cadmium in the fish Tilapia guineensis. The aim of this study is to evaluate the toxicity of water of two sites by the test of cytotoxicity on the inhibition of the growth of the roots of onions and to determine the content of the Metal Elements Traces (MET) such as lead, zinc, copper and cadmium in the flesh, the head, the liver and the edges of the fish Tilapia guineensis fished in the Porto-Novo lagoon by the spectrophotometric method of atomic absorption. The test of cytotoxicity showed that waters of sampling of two sites do not have global toxicity because the average lengths of the roots of onions exposed to various concentrations of water of the two sites does not depend on those. Thus the inhibition of the growth of the roots of onions does not depend of the concentrations on water on the two sites. But the malformations observed such as the deformed or turned over roots constitute precursory signs of a genotoxicity. The mineralisation of various parts of the fishes Tilapia guineensis was made by protocol HACH and the content of lead, zinc, copper and cadmium was determined using the spectrophotometer of atomic absorption. The analyses revealed that the Metal Elements Traces such as lead, zinc, copper and cadmium are concentrated in the various parts of the fish tilapia guineensis to the variables degrees.

Keywords Aquatics species; Cytotoxicity; Genotoxicity; Inhibition of the growth; Onion; Tilapia guineensis

Introduction

The impoverishment of biological diversity, the environmental problems and of public health are caused by toxic pollutants which are poured in the rivers in general and in the littoral lagoons in particular.

This pollution can have irreversible effects on the aquatics ecosystems such as disappearance of animals and vegetables species, the contamination of the trophic chain with very significant economics consequences.

Toxic pollution of the aquatic environments is especially trained by hydrocarbons derived from petroleum, domestics and industrialists wastes which accumulated gradually to reach toxics thresholds which exceed those tolerable by the organism thus causing harmful consequences on health. The demographic growth, poverty and pollution are three major problems which undermine the development of the african countries [12]. As the industrial activities and urban intensify in all the areas of the african continent, the problems involved in toxic pollution become increasingly worrying.

But, the studies undertaken on the african continent are very rare and disseminated. Among the first which were interested in the studies on pollution in Africa, we can quote Dejoux in 1988 which made a study on the various ecological problems in a monograph devoted to the african interior water pollution [9] and Phillips in 1991 which made a study of the tropical marine ecosystems [23].

The need for managing the watery resources rationally and for controlling discharges of waste which destroys the environment gradually pushed some african researchers to be interested in the abatement of water surfaces pollution; we can quote the work of Abba and al. in 2008 on the contribution to the physicochemical study of the lake ecosystem Dayet in Morocco [1] and research of Kanangire in 2001 on the food of fish with azolla on the agropiscicole ecosystem in Rwanda [15].

In Benin, research on aquatic pollution is very small. We can quote the work carried out by Mama and al in 2009 on the evaluation of the incidence of the activities of dyeing on water and the organics with the accesses of Cotonou lagoon [17] then on the methodology and the results of the diagnosis of the eutrophisation of the lake Nokoué in 2010 [18]; Dèdjiho in 2011 evaluated the trophic chain of a marine surface protected in relation to its physicochemistry: Case of Gbézoumè in the town of Ouidah [8]. Chouti in 2011 studied the chemical pollution of a tropical lagoon (waters, sediments, fishes) of Porto-Novo and noted that the quality of water and sediments of the lagoon of Porto-Novo are not likely to protect the watery life [7]. The death of fish often noted on the level of the lagoon could be justified by the high concentrations of heavy metals (Pb, Cd and Hg) then of the impoverishment of the dissolved oxygen lagoon which results from the chemical degradation of the algae and waste finding in the lagoon without treatment.

In Benin, the industrials and domestics wastes constitute the true sources of pollution of our lakes and lagoons which are transformed into worn water dumps of the factories and the bordering populations. However pollution decreases in a drastic way the quality of life and affects health dangerously then the lifespan [12]. Pollution can involve very serious affections such as cancer, the atheroscleroses, the diseases cardiovascular and premature old age [12].

Several studies showed the existence of a cytogenotoxicity in worn water coming from the industrial activities or urban [2] [12] [13] [16]. Environmental pollution and the cytotoxicity can be evaluated by the test in vivo onions relating to the cells of the ends of the roots of onions [11]. The test of onions was used to evaluate the toxicity and the genotoxicity of the industrial effluents [2] [12] [13] [25]. Samuel et al. carried out into 2010 a test of cytogenotoxicity to evaluate the toxicity of the water samples of two effluents of the town of Lagos in Nigeria which respectively receive the discharges and worn water of the factories of painting and textile [26].

Two sites of taking away were selected for this study, the first with an aim of determining the contributions of the domestic activities and waste of the chemicals used for the manufacture of the soap and poured in the lagoon is located just behind factory IBCG of Porto-Novo; the second, located beside the bridge of Porto-Novo, to study the impact of domestic waste of the bordering fishermen then those of all the city poured in the lagoon by a worn water main sewer.

The various techniques used to determine the content of the metal elements traces can be gathered in four types of methods which are: chemical methods, physicochemical methods, nuclear methods and biochemical methods. These various types of analyses make it possible to measure the content of the micropolluants in water, the sediments, the plants and in the watery species such as fish, shellfish, algae etc. But the majority of these methods are very expensive and their employment is mentioned only for one very small number of studies carried out recently in Africa [3] [14] [22].

In Africa also, the method by far most usually used for proportioning of heavy metals is that of the spectrophotometry of atomic absorption (AAS). It has being fast, significant, simple advantage and of being able to analyze complex mixtures without preliminary separation. For the majority of heavy metals, the basic technique include atomization with the torch, while for some metals very slightly concentrated, they use the technique of the furnace with graphite which proceeds by thermoelectric atomization. The techniques of analyses generally used to measure the content of heavy metals in the flesh of fish are spectrophotometry and x-ray fluorescence

In the African under-area, several techniques of analyses were used to determine the lead content in Sarotherodon melanotheron and the tilapia guineensis such as mineralization normalizes (NF IN ISO 15587-1et-2.INERIS 2003) [27]; the spectrophotometry of atomic absorption [5] then method of kits MERCK to spectrophotometer NOVA 60 [6].

As water of the lagoon of Porto-Novo becomes increasingly hostile with the watery life, we studied the width of this hostility with respect to the living organisms in the lagoon by choosing two sites of taking away on the lagoon and by using the test of cytotoxicity on the inhibition of the growth of the roots of onions then sought to know the content of lead, zinc, copper and cadmium in the fish Tilapia guineensis.

Materiel and Methods

The onions have a size ranging between 5 cm and 6 cm diameter and their weights vary between 47.74g and 84.69g. They were bought at AWHANGBÔ market of Porto-Novo. The fishes were bought at the fishermen on Porto-Novo lagoon and were preserved in a refrigerator containing of the ice to the laboratory. The weight and the size of fish vary respectively between 10 g and 110 g then between 11 cm and 18.5 cm.

The water used for this test was taken on two sites of the lagoon of Porto-Novo. The first site (S1), just located behind the factory of the IBCG at AGBOKOU in the second district of the town of Porto-Novo has as geographical coordinates N: 06°27.768 ' and E: 002°38.575 ' then the second site (S 2), located beside the bridge of Porto-Novo in the first district of the town of Porto-Novo has as geographical coordinates N: 06°28.20 ' and E: 002°37.197 '.

Water of the two sites is taken in plastic cans and is transported to the laboratory then preserved in a refrigerating apparatus at 4°C [21]. To the beginning of the test the temperature taken water is brought back to that of the laboratory (26 ± 2°C) then diluted with water distilled to obtain the various water concentrations to be tested.

Process of the test

The onions were laid in the pyrex bechers containing beforehand various taken dilutions of water. Distilled water was used as control (0%) and to obtain the dilutions used for the test of cytotoxicity.

Test of inhibition of the growth of the roots

The test of cytotoxicity was carried out with the Laboratory Agriculture and Health (AGRI AND HEALTH) of the IITA and rests on the inhibition of the growth lengths of the roots of onions exposed during 96 hours in various dilutions of the water taken on the two sites [24].

The concentrations of dilutions of water of the two sites of taking away are: 0 %, 25 %, 50 %, 75%, 100 %. Each concentration was retorted 5 times.

The various dilutions used for the test of cytotoxicity are replaced every 24 hours with new diluted solutions. At the 4 days end of exposure, the length of the roots of onions was measured.

Method of analysis of various parts of fish

The fresh fishes were weighed and left again according to their weight in tubes test hermetically closed with a stopper then transported to the laboratory in a refrigerator containing of the ice. Each sample of various parts of fresh fish was retorted 4 times that to say 20 samples.

The mineralization of various parts of fish was made according to HACH protocol and Metal Elements Traces was determined by a colorimetric proportioning with the spectrophotometer of atomic absorption while using Spectr AA 110 of the Laboratory of Sciences of the Ground, Water and Environment (LSEE) of the National Institute of the Agronomic Research of Benin (INRAB).

Analyze statistical

The statistical analyses of the test of cytotoxicity based on the inhibition of the growth of the roots of onions and the determination of the content of lead, zinc, copper and cadmium in the fish Tilapia guineensis were made with Microsoft Excel computer program.

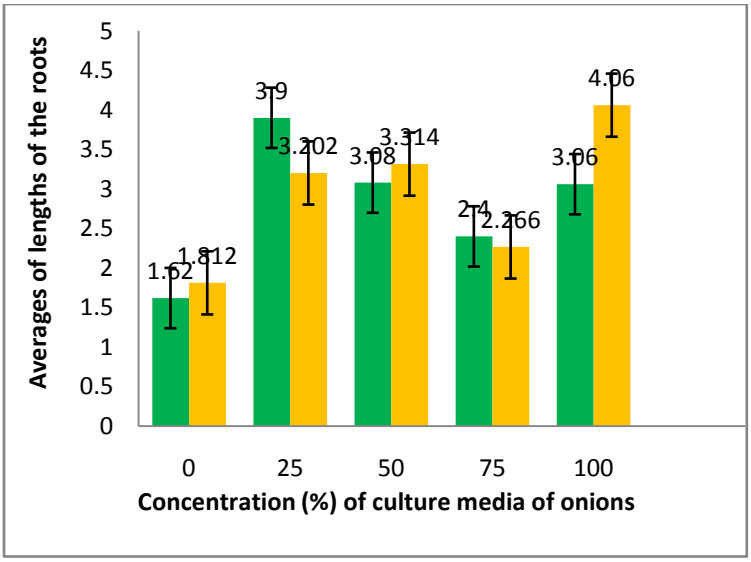
Results and Discussion

At the end of various carried out work, the investigations into the ground and the analyses in accordance with the methods described previously, the quality of water of the two sites of taking away having made the object of this study and the evolution of the content of lead, zinc, copper and cadmium in the fish Tilapia guineensis fished in the Porto-Novo lagoon were evaluated.

More precisely, on the one hand the two sites of the lagoon were analyzed for the evaluation of the toxicity of water which constitutes the medium of life of fish and on the other hand the proportioning of the content of lead, zinc, copper and cadmium in the fish Tilapia guineensis. We have the various results which are then followed analysis and interpretation of the data.

Level of water toxicity of the two sites of Porto-Novo lagoon

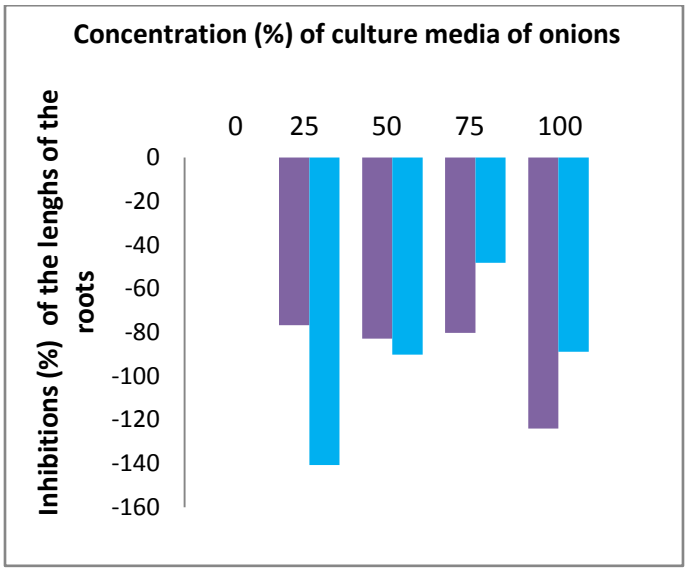
Figures 1 and 2 respectively present the averages of lengths of the roots and inhibition of the growth of the roots of onions put in culture in the water samples taken on the two sites.



Captions **Average of lengths of the roots of onions exposed in water of the site S2**

Average of lengths of the roots of onions exposed in water of the site S1

Figure 1: Averages of lengths of the roots of onions exposed in water of the two sites S1 and S2



Captions

Inhibitions of the roots of onions exposed in water of the BRIDGE site (S2)

Inhibitions of the roots of onions exposed in water of the IBCG site (S1)

Figure 2: Inhibition of the growth of the roots of onions exposed in water of the two sites S1 and S2

The results obtained starting from this test of cytotoxicity carried out on the roots of onions show that there exists on the level water samples taken on the lagoon of Porto-Novo a selective inhibition of the growth of the roots which appears by the fact that some roots of onion do not push whereas others push very well.

Inhibitions of the growth of the roots of the two sites of taking away show that the roots develop less quickly with the level of the site S2 than on the level of the site S1 thus we deduce that the water of the site S2 is polluted than those of the site S1.

It is to be observed that selective toxicity can not be the only cause of the inhibition of the growth of some roots; this phenomenon can be also explained is by the fact why there is a nutritional competition which prevents some roots from developing or is by the fact that some onions are can be attacked interior by the pathogenic micro-organisms which prevent the development of the roots of onions.

The absence of a total toxicity on the two sites can be also justified by the fact that the onions developed a defense (production of antibody) to protect the roots against the toxic pollutants being in water from the two sites or that there was a phenomenon of detoxification caused by the production of enzymes which metabolize the toxic organic molecules and eliminate them.

It is noted that for water of the two sites, inhibitions of the growth of the roots are all negative in other words there is rather a growth of the roots. Therefore there is not a total toxicity on the two sites.

This report is corroborated by the averages of lengths of the roots and inhibitions of the growth of the roots (figures 1 and 2) which show that the roots of onions placed in the water taken on the two sites S1 and S2 of the Porto-Novo lagoon of concentration 25%, 50%, 75%, 100% pushed more than those of onions placed in distilled water (0%).

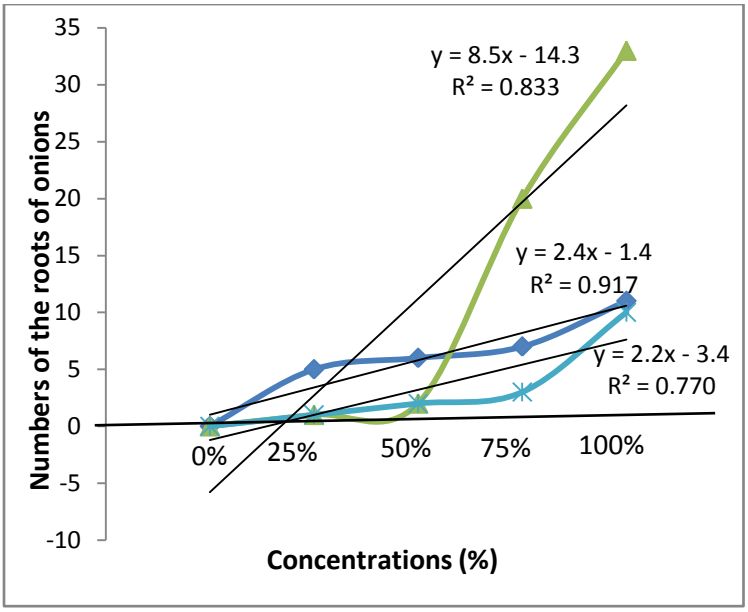
This absence of total toxicity can be explained by abundance in the water taken on these two sites, of the nutritive organic substances which made it possible the roots to develop and to grow without too many difficulties this is plausibly because worn water is poured in the lagoon of Porto Novo by the means of a drain which emerges in the lagoon right at the entry of the town of Porto-Novo. This result is contrary with that obtained by Samuel et al. in 2010 [26] and confirms that of Chouti in 2011 had noted that water of the lagoon of Porto-Novo was not toxic with the test of cytotoxicity carried out on the shrimp larvae [7].

The waste of the substances and chemicals products used by the industry of the greasy substances (IBCG) to prepare their products such as the cosmetic soaps, oils and other products then domestic waste of the residents are poured in the lagoon. This waste contains fertilizing organics substances which could be used by the roots for their growth.
The results of the calculation of the number of the deformed, turned over or round roots of onions are consigned in table 1 for the two sites.

Table 1: Numbers of the roots of onions deformed, turned over or round cultivated in water of the two sites

Site of sampling	Concentrations (%)	Number of deformed roots	Number of roots having a round end	Number of turned over roots
Site 1	0%	0	0	0
	25%	1	1	5
	50%	2	2	6
	75%	3	20	7
	100%	10	33	11
Site 2	0%	0	0	0
	25%	1	8	0
	50%	2	13	1
	75%	4	17	2
	100%	8	20	3

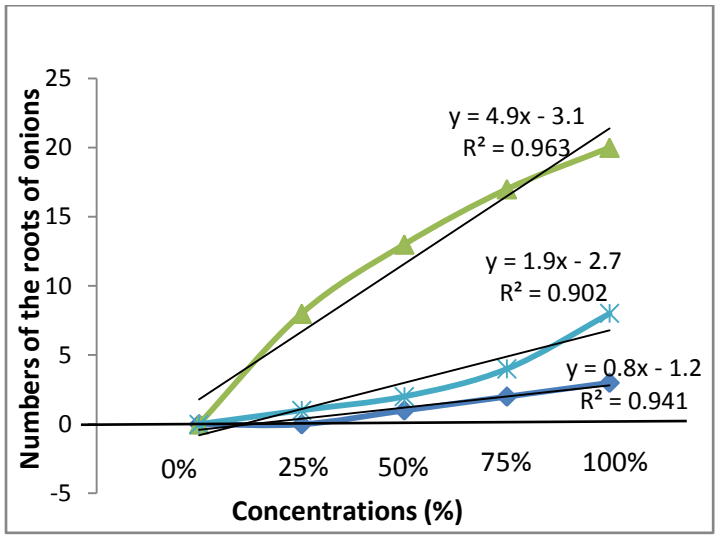
This absence of total toxicity on the level of the two sites does not exclude a chronic toxicity which is also foreseeable. Indeed, sixteen (16) deformed roots, fifty six (56) roots having a round end and twenty-nine (29) roots turned over upwards on the site S1 then fifteen (15) deformed roots, fifty eight (58) roots having a round end and six (6) roots turned over upwards on the site S2 were obtained (Table 1).



Captions

Curve of deformed roots
Curve of turned over roots
Curve of roots having a round end

Figure 3: Evolution of the deformations of the roots of onions according to the concentrations of site S1 sampling



Captions

Curve of deformed roots
Curve of turned over roots
Curve of roots having a round end

Figure 4: Evolution of the deformations of the roots of onions according to the concentrations of site S2 sampling

In addition, it is noted that the number of deformed, turned over roots or having a round end believes when the concentration of water of the sites increases. It varies from zero (0) to ten (10) on the site S1 and from zero (0) to eight (8) on the site S2 for the deformed roots; from zero (0) to thirty three (33) on the site S1 and of zero (0) to twenty (20) on the site S2 for the roots having a round end then of zero (0) to eleven (11) on the site S1 and of zero (0) to three (3) on the site S2 for the turned over roots. Thus, the water samples taken on the two sites are hostile for their development and which it is possible that the two sites of taking away can present a genotoxicity (figure 3 and 4).

Toxicity of fish

Determination of the Metal Elements Traces (Pb, Zn, Cu, Cd)

The content of the Metal Elements Traces (ETM) such as lead, zinc, copper and cadmium was given in the flesh, the head, the liver and the edges of the fish *Tilapia guineensis.* The results obtained made it possible to represent the graphs of figure 5.

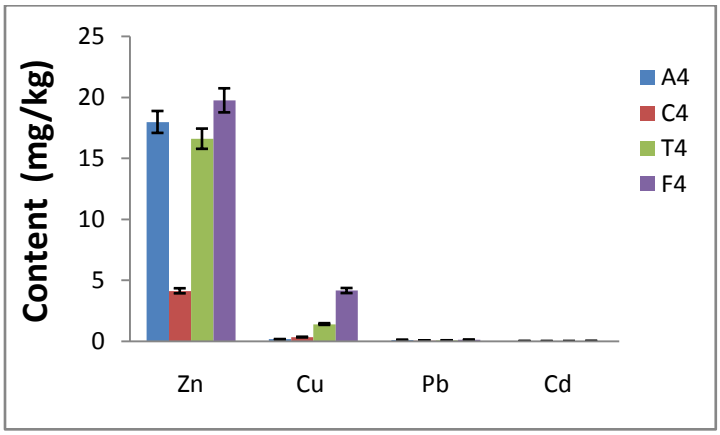


Figure 5: Evolution of the content of heavy metals in the various parts of Tilapia guineensis

In the fish Tilapia guineensis, zinc is concentrated more in the edges A4 than in the flesh C4. The zinc contents in the edges A4 exceed of 13,84 mg/kg those of C4. The zinc contents are maximum in the livers F4 of the fish Tilapia

guineensis. They exceed of 3,14 mg/kg those found in the head T4. Copper concentrated preferentially in the livers of studied fish. The contents of copper are weak in the other parts of fish. Lead is slightly concentrated in all the parts of fish. It has a minimal content equal to 0,068 mg/kg in the flesh C4. The cadmium contents are extremely weak in all the parts of studied fish.

Several teams of research of the African western under-area were interested in proportioning of the metal elements traces in general and in particular lead in the species halieutics of great consumption like *Sarotherodon melanotheron* and *the tilapia guineensis.* The two methods generally used by these teams of research are the method of kits MERCK to the spectrophotometer and the spectrophotometry of atomic absorption.

Results of various proportionings of lead in these two fish species fished in the complex lagunaire Nokoué - channel of Cotonou, in the lagoon of Porto-Novo and the system of Large Lahou lagoon (Côte.d'ivoire) and in the lagoon of Burkina-Faso show that the content of lead in the fresh fish flesh varies from an area with another and exceeds sometimes that maximum tolerable by the WHO (2006) which is of 0.2 mg/kg to 0.4 mg/kg.

The lead contents in *Sarotherodon melanotheron* and *Tilapia guineensis* are respectively of 0.012 mg/kg and 0.075 mg/kg for the system of Large Lahou lagoon; 0.55 mg/kg and 0.4 mg/kg for the lagoon of Porto-Novo then of 1.51 mg/kg and 1.46 mg/kg for the complex of Nokoué lagoon – channel of Cotonou.

Although some of these contents are lower than the tolerable threshold of toxicity in the organism which is 0.3 mg/kg (EC, 1881/2006), it is noted that there is an accumulation of lead in the aquatic environment.

Moreover lead contents obtained in our study has average of 0.0878 mg/kg which exceed those obtained on the level of fish of the system of Large Lahou lagoon on the one hand but inferior to those obtained into 2011 by Chouti on the level of fish of Porto-Novo lagoon [6] and the complex of Nokoué lagoon – channel of Cotonou on the other hand.

Contents of various heavy metals obtained at the time of our study are surely bring about by the anthropic activities which enrich the environment of heavy metals [4]. In the same way, the importance of maregraphic dynamics supports the handing-over in suspension of the metal elements which adhere to surface organisms and gills among the fish [10]; that could justify the increase in their contents in fishes.

This situation could be also allotted to the food mode which differs at the two species. *Tilapia guineensis* have an omnivorous mode, nourishing organic refuses, phytoplanktons, shrimps, bivalves and larvae colonizing in particular, the calm mediums [5].

Thus, the permanent consumption of the bivalves and the detritus contained in the sediments could increase the content of metals at this species [19] [20]. On the other hand, *Sarotherodon melanotheron* are in general, phytoplanctonophages [5]. The content of lead and other heavy metals at this species, could be justified by the fact that the phytoplankton of the lagoon of Porto-Novo contains a strong quantity of Metal Elements Traces (MET), and by the pollution of toxic waste (lead necks of the fishermen, industrial and domestic waste).

This phenomenon of pollution of the rivers by the Metal Elements Traces is accentuated by the use of the rivers by conveyors smugglers and clandestine of petroleum products which dumped their cans of engine oils, gasoline, gas oil at the bottom of the lagoons to escape the pursuit from the customs officers. It is thus necessary to seek means effective and suitable to prevent the pollution of our rivers because, it generates harmful repercussions on the trophic chain in general and the human being in particular.

Conclusion

Works of research enabled us to study the cytotoxicity of the water samples taken on two sites with knowing the site S1 located behind industry of the greasy substances (IBCG) of Gbokou and that of the site S2 just located beside the Cotonou-Porto-Novo bridge. They also enabled us to study the content of lead, zinc, copper and cadmium in the fish Tilapia guineensis fished in the Porto-Novo lagoon. It comes out from this work that the two sites objects of our study do not reveal a gbobal toxicity but the indices such as the roots deformed and turned over to the top carry to believe that there is a specific toxicity which could with long causing a general toxicity of water of the lagoon.

It is probable that the deformations such as the swelling of the roots, the unfolding of the ends, malformations and the reversals of the roots to the top are caused by a genotoxicity on which we will investigate.

The research tasks also revealed that the various contents of the Metal Elements Traces such as lead, zinc, copper and cadmium obtained in the fish tilapia guineensis fished in the Porto-Novo lagoon vary according to studied heavy metals. This pollution is due certainly with the lead necks which are detached from the nets of the fishermen and are papered at the bottom of our rivers and lagoons; with various domestic, artisanal, agricultural and industrialists' wastes who are dumped in our rivers.

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