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Assessment of Some Heavy Metals in Fresh Fish (*Oreochromis aureus*): Case of Toho Lake in South Eastern Benin, West Africa

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Abstract

The protection of aquatic ecosystems is of great importance to maintain the biological balance necessary to secure healthy foods therein imbedded. Toho Lake, located to the south east of Benin, is threatened by pollution emanating from anthropogenic activities with the use of chemical fertilizers, effluent of domestic waste, particularly human and animal excrement with neighbourhood effluents. This study aimed at assessing some heavy metals in fresh fish (*Oreochromis aureus*) of Toho Lake in order to secure healthy food for the population and protect the ecosystem. Samples of water and fishes were collected and analyzed by HACH DR 3900 after treatment. Results revealed a mean oxygenation of water (4.95 mgo$_2$/l), Biochemical Oxygen Demand (21.5 mgo$_2$/l), Chemical oxygen Demand (149, 39 mg/l) with the nitrogen in Nitrate (NO_3: 0.18 mg/l), ammonium nitrate (N-NH_4^+: 0.47 mg/l), and phosphorous (2.06 mg/l) to be very high. The highest contents of heavy metals obtained in the fresh fish were cadmium (32.25 mg/kg), copper (115 mg/kg), lead (8.25 mg/kg), and zinc (90.75 mg/kg) and revealed that the fishes of Toho lake were polluted. The finding allowed us to conclude that the pollution of the lake and the fish carnage are due to anthropogenic pollution through chemicals spilling. Some protection methods of the lake and ecosystem are proposed in order to keep the fish safe and protect the well-being of the population.

Keywords

Fresh Fish, Heavy Metals, Pollution, Toho Lake, South Eastern Benin

1. Introduction

Pollution of surface and ground water is a major problem due to rapid urbanization and industrialization. The large scale urban growth due to increase in population and migration of people from rural areas to urban centers has increased domestic effluents while industrial development revives the issue due to setting up of new plants or expansion activities, generating large volumes of industrial effluents (Avnish & Saksena, 2010). Natural water contains different types of impurities as well as introduced impurities in different ways such as weathering of rocks and runoff of soils surface, atmospheric deposition of aerosol particles and from several human activities (Adeyeye, 1994).

The pollution affects dangerously available water resources and caused many issues to the drinking water in the world. These past years, many African countries saved a demographic growth which leads to the acceleration of the urbanization and the use of large surface by agricultural and industrial activities. All these side effects of development are not well managed, particularly the solid and liquid garbage, effect of various pollutants thrown in the watercourses, and disturbing the different aquatic environmental components and reduce the fishing productivity. Water quality monitoring has one of the highest priorities in environmental protection policy (Pesce & Wunderlin, 2000; Simeonov et al., 2002) to control and minimise the incidence of pollutant Problems (Mophin-Kania & Murugesan, 2010). Moreover, heavy metals pollution is a worldwide problem causing challenges to regions for the quality of their water resources inherited (Belhamra, 2001). In some ecosystems, the presence of the chemical products can cause the death of animals and plants but also can be the source of animal or plant extinction, leading to the dysfunction of the trophic chain (Gold et al., 2002).

By using water both for our health and environment, we contribute to pollute and deteriorate its quality by our activities. For this reason, available water, especially clean water in quality and aquatic ecosystem conservation becomes a challenge and many countries try to resolve it. Benin is also concerned.

Thus, Toho lake, located in the South-East Benin underwent an accidental pollution around mid-May 2018, resulting in the death of thousands of fish whose cost is estimated at millions of dollars. The results of water analysis by the Central Laboratory of Food Safety of the Ministry of Agriculture, Livestock and Fisheries allowed that the officials to conclude that the waters of the lake have not been contaminated by an external product, but rather human activities on the lake. These results could not satisfy the concern of the farmers located on the lake in the district of Kpinnou because of the conclusion that the fish carnage on the lake could be due to anthropogenic pollution due to a spill of chemicals. As for the traditional faith people, this drama is due to an endogenous phenomenon of the lake because certain practices and rituals that were customary are no longer implemented and this is what may have angered the gods of the lake. The panoply of hypotheses put forward following this tragedy has pushed the scientific community and various actors of the water sector to conduct research on the lake in order to verify these hypotheses.

Today, the poor functions have many negative effects on the ecosystems, the aquatic species health and surface water too. For the best environmental functioning of the lake water, the following questions are formulated:

- What is the present quality of Toho Lake water in Benin?
- What are the fishes "flesh quality" of Toho Lake in Benin?

Surface water quality appreciation is based on physicochemical and chemical parameters evaluation and the detection of the aquatic microorganisms, best indicator of water quality. Some data came from the sediment analysis which are useful and constitute the memory of the river life. The presence of pollutants could have some effects on the naturel ecosystem (Dèdjiho, 2013). Despite the recovery of aquatic life after the huge death of Toho lake fish, this pollution will not be without repercussion for the ecosystem. Availability of quality data on the lake water will allow to design the best protection strategy which will lead to the ecosystem conservation. Furthermore, the assessment of the quality of Toho lake fish will allow us to guarantee safe food to the population. It is the aim of this paper titled: *Assessment of some heavymetals in fresh fish* (*Oreochromis aureus*): *case of Toho lake in South Eastern Benin, West Africa*

The richness of hydro-ecosystemic heritage and the accidental boom death of the fish in mid-May 2018 is the reason of Toho lake choice as the study area. Toho Lake is a large watershed with various ecosystems, rich in biodiversity such as a wide variety of fish species and other aquatic resources.

2. Materials and Methods

2.1. Area of Study

The study area is Toho Lake which is located between Agamé plateau and North-west plateau of Bopa (in Benin Republic). It extends from 6˚35 to 6˚40 north latitude and from 1˚45 to 1˚50 East Longitude. The area, in dry season, is 9.6 km^2 and 15 km^2 in a rainy season. It has 7 km of length and 2.5 km of meridional width and approximately 500 km northern width (Ahouansou, 2003). Toho Lake has a crescent form oriented south-north and covered by tree districts: Kpinnou in the municipality of Athiémé, Zoungbonou in the municipality of Houéyogbé and Houin in the municipality of Lokossa (**Figure 1**).

2.2. Station Choice

In order to have a panoramic view of the parameters variability of the study area, the lake was subdivided according to the longitudinal stratification proposed by Tachet et al. and Ahansou. Thus, from upstream to downstream of the lake, four sampling stations were selected.

The stations were selected according to the fish carnage taking place on the lake as a result of pollution from the livestock farm on the lake and the change in

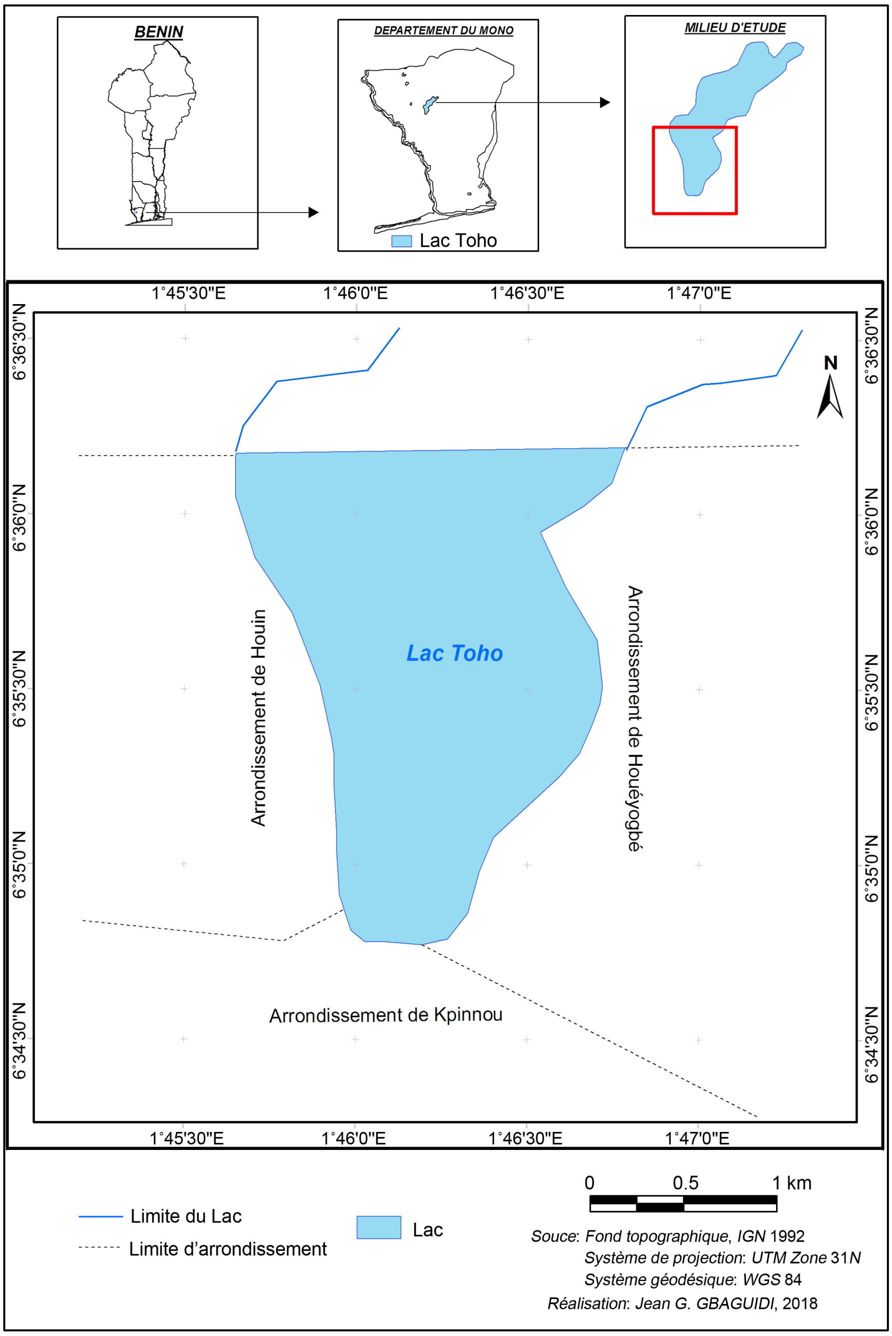

Figure 1. Map of the study area.

water colour. The three close stations are located in the Farmland of the fish where we observed the most death of the fish. The last point which is very far from the three stations is where the fish did not die but lost their balance (Figure 2 and Table 1).

2.3. Sampling

Water and fish specimen sampled were collected during the small rainy season. Sampling work has taken place in September, October and November 2018.

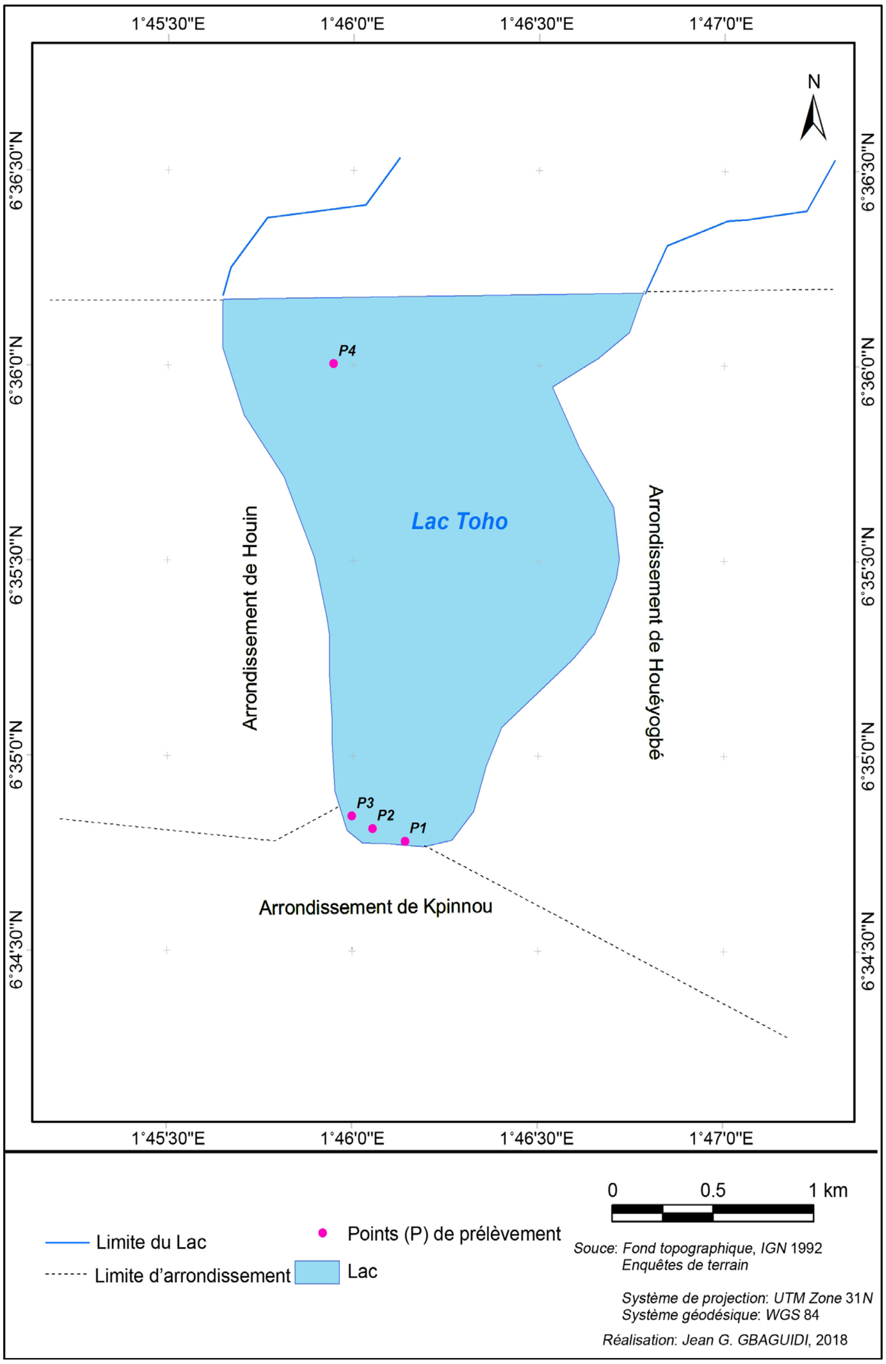

Figure 2. Location of the sampling sites of water and fish.

Table 1. Sampling stations of Toho Lake.

Sites	Names	Reason of the Choice of the Site	Geographic Coordinates
P 1	Kpinnou 1	In front of the farmhouse of the fish farmed	6˚33'N; 1˚46'10"E
P 2	Kpinnou 2	Medium of the farmhouse of the fish farmed	6˚33'5"N; 1˚46'5"E
P 3	Kpinnou 3	Behind of the farmhouse fish farmed	6˚34'N; 1˚46'E
P 4	Houin	Out of the farmhouse fish farmed	6˚36'N; 1˚45'90"E

Some parameters as temperature, pH, turbidity, salinity, Electrical Conductivity (EC) and Total Dissolved Solid (TDS) were measured with a multi-parameter on the site. Dissolved oxygen was measured at the laboratory by chemical method. The depth of the lake was measured with the graduated cord and riband meter.

Water for the physicochemical analysis was sampled in 500 mL polyethylene bottles with double plugs and sterilized. It was conserved in the icebox at 4˚C and carried to the laboratory.

2.4. Methods of Analysis

2.4.1. Physicochemical and Chemical Parameters

To reach the aim of this work, many parameters as temperature, pH, turbidity, salinity, Electrical Conductivity (EC) and Total Dissolved Solid (TDS) were measured with multipara meter PCSTest+TM35. The different parameters of sampled water were measured in-situ with multipara meter PCSTest+TM35. The DBO was measured by a DBO meter OxiTop label. The respirometric method was used. The DCO was determined by colorimetric method.

2.4.2. Eutrophisant Substances

Nitrate, nitrite, total phosphorus, phosphate and ammonia are nutrients and were evaluated with HACH DR 3900 spectrophotometer in the laboratory. Nitrate was measured by method 8039 called cadmium reduction method, nitrite by method 8507 named Diazotation method, NTK by Nessler and Rochel salt, phosphate and Total Phosphate by Phosphaver 3 method (method8048).

2.4.3. Heavy Metals

The zinc determination was carried out by the Zincon method (Metod 8009) allowing measurements in the concentration range of 0.01 to 2.00 mg/l. The metals form complexes with cyanide. The addition of cyclohexanone causes a selective release of zinc. The zinc then reacts with the indicator, 2-carboxy-2-hydroxy-5-sulfoformazyl benzene (zincon) to form blue-coloured species. The blue coloration is masked by the brown coloration of the excess indicator. The intensity of the blue coloration is proportional to the zinc concentration. The reading is obtained at 620 nm.

For copper, the Hach method used is the porphyrin method ($C_{20}H_{14}N_4$). It is a method that allows measurements in the concentration range from 2 to 210 µg/l. The method is free of most interferences and does not require extraction or preconcentration. Interference from other metals is eliminated by the copper masking reagents.

Lead and cadmium are determined by the dithizone method. Lead is more soluble in chloroform than in water. Mixing the sample with chloroform causes the metal to migrate into the chloroform phase. In this phase, the dithiver combines with the lead to give a specific coloration whose intensity is proportional to the lead concentration.

Cadmium is more soluble in chloroform than in water. Mixing the sample with chloroform causes the metal to migrate into the chloroform phase. In this phase the dithiver combines with Cadmium to give a specific coloration whose intensity is proportional to the concentration of Cadmium.

2.5. Data Processing Methods

To test the variability of median heavy metal concentrations in fish flesh, the non-parametric Kruskall-wallis test was used. This was preceded by Shapiro-wilk and Ryau Joyner tests to check the normality and homogeneity of the data, respectively. Box plots were constructed for illustrative purposes. The physico-chemical parameters of the lake were characterized by the Student's test with one sample (test of conformity of a mean) was used. The limit values considered are those set by the WHO, the French standard and the relevant regulations. All analyses were performed in R software version 3.5.1 (R Core Team, 2018).

3. Results and Discussion

3.1. Physicochemical Parameters

Table 2 presents the conformity test which gives the water state through physicochemical parameters limit values. It suggested a significant statistic difference ($P < 0.005$) for the Electric conductivity, the temperature, (32.33˚C), the salinity (0.18 g/l), the Biochemical Oxygen Demand (21.5 mgO_2/l), the Chemical Oxygen demand (149.39 mgO_2/l, the Total Dissolved Solids (180.25 mg/l) and the turbidity (33.78 NTU) (Table 3).

3.2. Eutrophisant Substances

Table 4 shows the conformity test which gives the water state through chemical parameters limit values. It displays a significant statistic difference ($P < 0.005$) for the following parameters: Nitrate ($N\text{-}NO_3$: 0.18 mg/l), total phosphorus (2.06 mg/l), phosphate (0.05 mg/l), and ammonia ($N\text{-}NH_4^+$: 0.47 mg/l), NTK (0.80 mg/l) amount (Table 5).

Table 2. Conformity test results for the water state through physicochemical.

Components	Statistic	
	Value of t	Probability
pH (Potentiel d'Hydrogène)	0.14	0.897
TDS	−89.04	0.0003
Oxygen Dissolved	−0.2	0.854
Turbidity	−16.44	0.00005
Electric Conductivity (EC)	−71.82	0.00005
Salinity	−37.77	0.00004
Temperature	15.08	0.0006
Biochemical Oygen Demand	5.69	0.011
Chemical Oxygen Demand	9.65	0.002

Table 3. Descriptive statistic (means and error types, of physicochemical parameters).

Components	Mean	Error Type	Minimum	Maximum
pH	7.94	0.46	7.02	8.96
Electric Conductivity (EC)	355.25	2.02	352.00	361.00
Temperature	32.33	0.29	31.50	32.80
Turbidity	33.78	2.21	28.30	37.40
TDS	180.25	3.59	176.00	191.00
Oxygen Dissolved	4.95	0.25	4.40	5.60
Salinity	0.18	0.01	0.16	0.20
Biochemical Oxygen Demand	21.50	2.02	18.00	27.00
Chemical Oxygen Demand	149.39	11.34	128.13	176.61

Table 4. Conformity test result for the water state through lake pollutants.

Components	Statistic	
	Value of t	Probability
N-NH_3	8.67	0.003
N-NO_2	2.42	0.094
NTK	−7.87	0.004
N-NO_3	6.6	0.007
Total Phosphorus	3.46	0.040
Orthophosphate	−6.33	0.008

Table 5. Descriptive statistic (means and error types, of lake pollutants).

Component	Mean	Error Type	Minimum	Maximum
N-NO_3	0.18	0.03	0.10	0.20
N-NO_2	0.17	0.07	0.08	0.36
N-NH_4	0.47	0.05	0.33	0.58
Azote Total-NTK	0.80	0.41	0.12	1.93
Orthophosphate	0.05	0.01	0.03	0.06
Phosphorus	2.06	0.45	1.31	3.37

3.3. Principal Component Analysis (PCA)

Principal Components Analysis indicated that the two axes explain 84.91% of information, which is good enough to have a precision in the interpretation (Table 6).

Figure 3 shows the correlation coefficient between physicochemical parameters and the principals component axis that indicates a high positive correlation between the first axis (axis 1), the PH, EC, TDS, N-NO_2 and the DBO in the first part. In the second part, it indicates a high negative correlation between the first axis, temperature, turbidity, the ammonia (N-NH_3), and the NTK amount. The positive side of the first axis shows that when water presents a high pH we notice that the DBO, Electric Conductivity, TDS and nitrite ($N\text{-}NO_2^-$) are also high. Furthermore, the negative side indicates that when water presented a high temperature, the value of ammonia (NH_3^+) and NTK were also high.

Table 6. Correlation coefficient between physicochemical parameters and principal axis components.

Physicochemical Parameters	Axis 1	Axis 2
PH	0.89	−0.40
Electric Conductivity (EC)	0.98	0.06
Temperature	−0.86	−0.49
Turbidity	−0.94	0.23
Total Dissolved Solids (TDS)	0.97	0.24
Oxygen dissolved	−0.43	0.89
Salinity	0.73	0.66
Azote in Nitrate ($N\text{-}NO_3$)	0.49	−0.78
Azote in Nitrite ($N\text{-}NO_2$)	0.94	0.33
Azote in Ammonia ($N\text{-}NH_3$)	−0.93	−0.20
Azote NTK	−0.63	0.51
Orthophosphate	0.09	0.78
Phosphorus	0.03	−0.90
Biochemical Oxygen Demand	0.98	−0.16
Chemical Oxygen Demand	0.13	0.90
Depth	0.12	−0.36

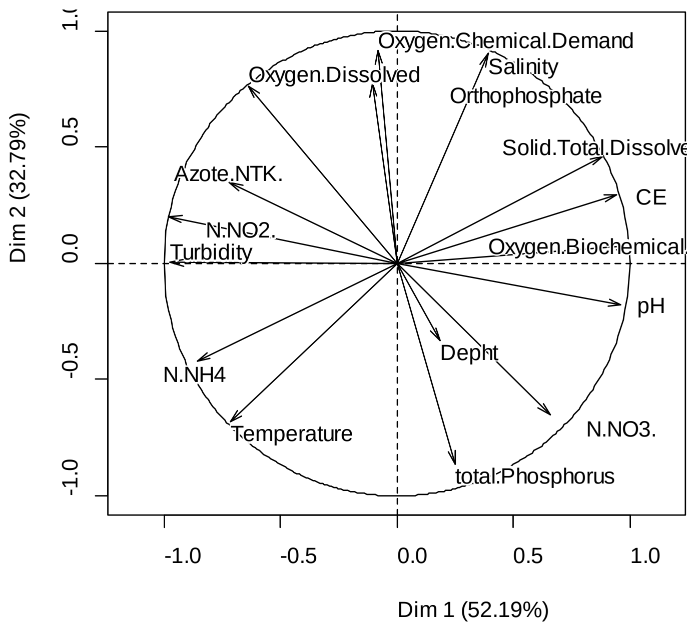

Figure 3. Correlation between physicochemical parameters and principal axes.

However, Figure 3 indicates high positive correlation between the axis 2 and the Dissolved oxygen, salinity, phosphorus concentration, and the DBO. Furthermore, there is a high negative correlation between the same axis 2, the nitrate (N-NO_3) and the phosphorus. The positive side of the axis 2 shows that when BDO is high other parameters like Dissolved oxygen, salinity, and orthophosphorus are also high. The negative side of the axis 2 shows that when nitrate concentration is high in the water, phosphorus concentration is also high.

Taking in account obtained results of the present study one can say, when water presents a high pH value, the values of TDS, nitrite, Dissolved oxygen, salinity, orthophosphorus, DBO, and the Conductivity Electricity are high whereas when water presented a high temperature then high amounts of ammonia (N-NH_3), NTK, nitrate (N-NO_3) and phosphorus were also observed.

3.4. Heavy Metal in the Flesh of the Fishes

Table 7 presents the results of the Kruskal Wallis test giving the average concentration of heavy metals in the fish's flesh. According to the different sampling stations, it indicates a significant statistic difference ($p < 0.05$).

In Figure 4, the graph (a) presents the lead average concentration at each station. This graph shows that lead average concentration is high at all stations. The lowest concentration is obtained at Kpinnou 2 (2.25 mg/kg) and the highest concentration was obtained at Houin (8.25 mg/kg), and at Kpinnou 3 (6.5 mg/kg). These high concentrations indicate that Toho lake fishes were polluted.

The graph (b) of Figure 4 presents cadmium median concentration. It shows that the highest values (3.25 mg/kg) and (30 mg/kg) are obtained respectively at Kpinnou 2 and at kpinnou 3. The lowest median concentration (1.25 mg/kg) is obtained at Houin.

According to Figure 4, the graph (c) relative to the median zinc concentration in each station, shows that the highest value (90.75 mg/kg) is obtained at Kpinnou 1and the lowest concentration (40.25 mg/kg) is obtained at Houin.

The graph (d) of Figure 4 shows copper median concentration in the fish's flesh. The highest concentration (115 mg/kg) and (95 mg/kg) are obtained respectively at Kpinnou 1 and at Houin. The lowest concentration (30 mg/kg) is obtained at Kpinnou 2.

Table 7. Results of the Kruskal Wallis test for the median concentration of heavy metal in the fish's fresh breadth stations.

Parameters	Statistic		
	Ddl	x-Square	Probability
Lead	3	40	0.000
Copper	3	40	0.000
Zinc	3	40	0.000
Cadmium	3	40	0.000

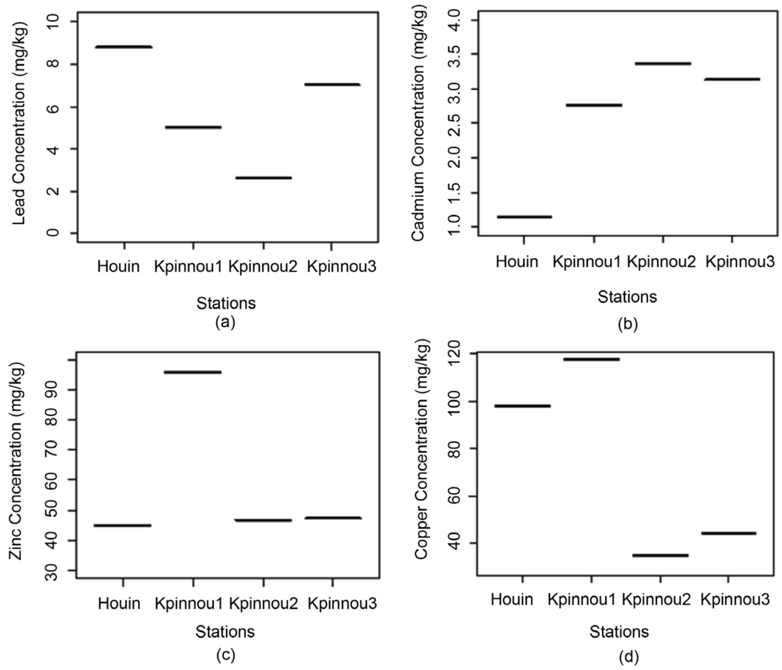

Figure 4. Moustache box presenting average concentration of the heavy metal in the fish's flesh.

4. Discussion

Chemical products released in environment by natural phenomena and anthropic activities can be dissolved in aquatic ecosystems and integrate the substance in the suspension. That can represent a danger for more aquatic organisms (Noumon et al. 2015). The average value of temperature: 32.33˚C (**Table 8**) is similar with Dèdjiho (2011) and Pouomogne (1998) data, these authors agree that temperature between 24˚C and 35˚C is favorable for the fish growth. Egan et Boyd (1997) reported by Abou (2001) thought that temperatures between 28˚C and 32˚C are optimal for the tilapia growth. Furthermore, the average amount of electric conductivity was 355, 25 µS/cm. That value is lower than data reported by Dimon et al. (2014) and Koudenoukpo (2017). The lake was moderately mineralized and the Electric Conductivity is near to the WHO standard. The average of Total Dissolved Solids value (180.25 mg/l) measured correlates with the Electric Conductivity value. The TDS informs about the water salinity. That value was different from the ones by Dovonou (2008). The amount of the salinity was lower than the WHO standard for the aquatic life. The salinity was low because sampling was done in the small rainy season. Additionally, The DBO value (21.5 mgO_2/l) was under the International standard limit. Toho lake was not polluted by biochemical oxidizing substances. That was confirmed by the available oxygen dissolved in the lake. The Chemical Oxygen demand value (149.39 mg/l) was high regarding the limit of French standard (40 mg/L). It reflects that Toho lake was polluted by organic substances. A lot load of organic substances rise to the tree branchs. The average nitrate value (0.18 mg/l) was superior regarding the limit of French standard (0.01 mg/l). It shows the pollution

Table 8. The comparison of the present study with some others authors' findings.

Heavy metals	Present study (2018) (Toho Lake in Benin)	Dimon et al. study (2014) (Ahémé Lake in Benin)	Chouti et al. study (2011) Porto-Novo lagoon in Benin)	Lafendi study (2017) (Pawns' fresh in Tlemcen
Cadmium	7 mg/Kg	-		0.012 mg/kg
Lead	60.75 mg/Kg	26 mg/kg	5.65 mg/kg	0.47 mg/kg
Copper	2100 mg/kg	-	0.16 mg/kg	0.019 mg/kg
Zinc	340 mg/Kg	170 mg/kg	7 mg/kg	0.38 mg/kg

of the lake come from human and animals dejection in the Toho lake. Elsewhere, the average ammonia (N-NH_3) value was high regarding limit French standard (0.02 mg/l) for the soft aquatic water. That value was different from the Koudenoukpo (2017) data in the Sô river and Dimon et al. (2014) value (3 mg/l of nitrate) respectively 2.06 mg/l and 0.05 mg/l which were high as compared to limit of French standard (1 mg/l: total phosphorus (1 mg/l) and orthophosphate (0.1 mg/l). So Toho Lake was polluted by phosphorus, effect of eutrophisation and complication of aquatic respiration when phosphorus was combined to the nitrate which leads to the lack of oxygen.

The dosage results of heavy metals in the Toho lake fish was: lead (8.25 mg/kg); zinc (90.75 mg/kg); cadmium (32.25 mg/kg) and coppery (115 mg/kg). Regarding the limits, the European standard of International Agency of Atomic Energy which recommends 0.18 mg/kg of cadmium, 3.28 mg/kg of copper; 0.12 mg/kg of lead and 67.1 mg/kg of zinc. We conclude that the fish's flesh of Toho Lake was polluted by lead, copper, cadmium and copper. According to Table VIII our results were different from the ones obtained by Lafendi in pawns flesh imported from China and sold at Tlemcen (Lafendi, 2017). Compared to the Dimon et al., 2014 results in the Ahémé lake and Chouti et al. (2011) results in the Porto-Novo lagoon, those results were different (**Table 8**). The highest concentration in the flesh of the fish of Toho Lake by bioaccumulation phenomenon could be found in human organism which has harmful effects on his health. Some authors showed that lead and cadmium are toxic trace elements for human (Testud, 2005). Lead has a harmful effect on the kidney and nervous system (saturnism). Lead could cause anemia for people. Cadmium doesn't have any importance for human metabolism because it didn't have any function for human organism (Miguel, 2001). Toxic cadmium effects for most exposition are a lot but the main effect for human and animal health was renal malfunction. Cadmium caused a disease called "Itaï-Itaï" described in Japan which was characterized by renal deficiency associated to osteoporose and osteomalacie (Payen, 2007).

5. Conclusion

In light of the findings of this work, some chemical parameters had high values above different standard limits. The concentration of heavy metals was very high in the fish's flesh. Toho Lake was polluted by azote component (nitrate, ammonia) and phosphorus. The highest value of Chemical Oxygen Demand confirms that Toho Lake was polluted by organic substance. Regarding different standard limits, the fish of Toho Lake were polluted by lead, cadmium, copper and zinc.

Conflicts of Interest

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

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