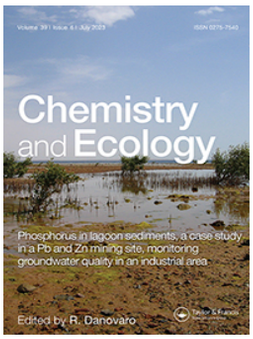

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Assessment of the health status of Lake Nokoué (Benin, West Africa) exposed to multiple pollutants during flood season

Agbohessi Prudencio[a], Onibon Chabi[a], Pelebe Edéya Orobiyi Rodrigue[a,b], Mattah Dzorgbe Agbeko Precious[b], Guedegba Nicresse Léa[a], Imorou Toko Ibrahim[a] and Chikou Antoine[c]

[a]Research Laboratory in Aquaculture and Aquatic Ecotoxicology (LaRAEAq), University of Parakou, Parakou, Benin; [b]Africa Centre of Excellence in Coastal Resilience (ACECoR), Centre for Coastal Management, University of Cape Coast, Cape Coast, Ghana; [c]Laboratory of Hydrobiology and Aquaculture, University of Abomey-Calavi, Abomey-Calavi, Benin

ABSTRACT

This study used the levels of pollutants and the histopathological analysis of the gonads and liver of *Sarotherodon melanotheron* sampled in Lake Nokoué and Lake Toho, both in Benin (West Africa) to assess the health status of the ecosystems. Sixty adults (30 males and 30 females) of this species were caught during the flooding seasons from both Lake Nokoué, and Lake Toho (as a reference). The physicochemical parameters of the water and the heavy metal contents (Pb, Cd, Hg) in water/sediment/fish of three sites (S1, S2, S3) in Lake Nokoué and two sites (S4, S5) in Lake Toho were analysed. Results confirm very strong organic status with organic pollution index ranging from 1.25 to 1.5. Pb, Cd, and Hg levels in the water/sediment/fish muscle tissue of Lake Nokoué were far exceeding international standards. The histopathology of gonads revealed testis-ova up to 40% in males and follicular atretic oocytes up to 33.33% in females, indicated oestrogenic effects from the pollutants with a delay of spermatogenesis and stimulation of oogenesis. The histopathology of the liver revealed the presence of regressive changes. The findings call for actions to protect the ecosystem from pollution and attention in terms of health of humans consuming fish products from Lake Nokoué.

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Fish; Lake Nokoué; pollutants; gonads and liver histopathology; oestrogenic effects

1. Introduction

Surface water bodies found in and around urban and metropolitan areas are prone to different forms of pollution [1]. The pollutants are either from domestic, industrial, and agricultural sources. The effect of the pollutants is not only enormous on the aesthetics of the environment of the water body but also on the riparian populations and communities. The most affected are the biodiversity including flora and fauna of the waterbody [1]. Certain types of fishes, macroinvertebrates, and other organisms which do not thrive in pollution die off, while others that are hardy species withstand different forms of pollution of the environment but live in poor state of health [2].

CONTACT Agbohessi Prudencio agbohessip@yahoo.fr Research Laboratory in Aquaculture and Aquatic Ecotoxicology (LaRAEAq), University of Parakou, Parakou, Benin

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According to Lalèyè et al. [3], Lake Nokoué (Figure 1) is the most productive lake in West Africa with a fish production of over two tonnes per hectare. Lake Nokoué is fed especially in the rainy season by rivers Ouémé and Sô, which flow from its northern side. Cotonou lagoon is a manmade reservoir, created in 1885 to connect Lake Nokoué to the Atlantic Ocean forming a complex of wetlands in the south of Benin [4]. The connection between the lake and the ocean therefore caused seasonal fluctuation in the salinity of the lake. Lake Nokoué therefore has brackish water in the dry season and fresh water in the rainy season [4]. The complex system has a very high biodiversity with a large diversity of fish from marine, freshwater, and lagoon sources. Other important species also include molluscs (oysters and tarets), shellfishes (crabs, shrimps, etc.), amphibians, etc. [5]. Unfortunately, these resources have declined rapidly as a result of several factors of which the most important is increased pollution. For example, in their inventory intended to identify species threatened with extinction, Lalèyè et al. [3] listed 51 species while Gras [6] listed 87. This difference of 36 species in 42 years clearly shows a huge decline in biodiversity. Pollutants in Lake Nokoué are from various sources but the most important are from organic and inorganic/chemical sources including metals.

Organic pollution of Lake Nokoué comes from the daily dumping of branches of trees to create an '*acadja*' system or '*fish pen*' fishing, a special refuge, feeding, and reproduction area for fishes as well as shrubs placed in the water to create passages for catching fishes. These organic matters overtime get rotten in the water. Also, the dumping of household refuse and animal wastes as well as the discharge of liquid waste made up of human and animal faeces in the surroundings of the water are sources of organic

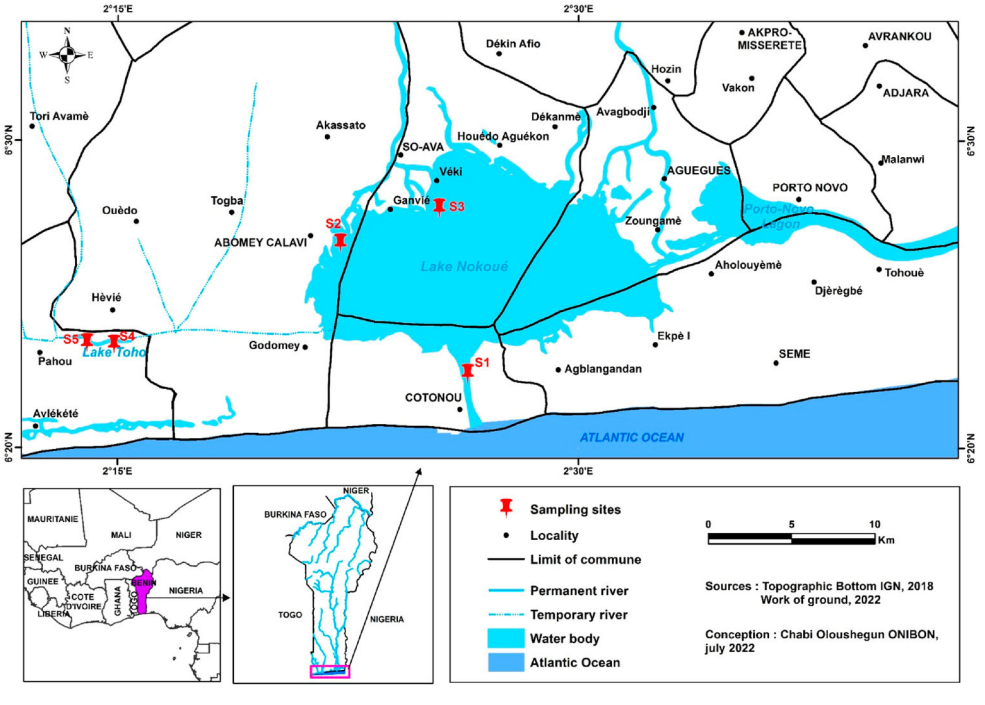

Figure 1. Map of Lake Nokoué and Lake Toho showing the study sites (S1-S3 sites on Lake Nokoué and S4-S5 on Lake Toho as reference sites)

pollution. Inorganic pollutants are mainly mineral fertilisers from the Ouémé River or from diffuse sources such as farms surrounding the lake [7]. The mineral fertilisers include ammonium, sulphates, phosphates, nitrates, and nitrites. In their extreme concentration in the water body, these pollutants cause eutrophication and hence increase the deficiency of oxygen from the water with very low primary productivity [8]. Increased concentration of these pollutants has been observed in Lake Nokoué by Capo-Chichi et al. [9] with concentrations of nitrates between 0.56 and 17.7 mg/L, nitrites from 0 to 2.64 mg/L, ammonium from 0.01 to 1.89 mg/L, phosphates from 0.01 to 1.89 mg/L and Biological Oxygen Demand from 7.96 to 31.20 mgO_2/L.

Chemical pollution of Lake Nokoué comes from spills of petroleum products from vehicular and water transport around and on the lake and depositions of barrels of petroleum products in the water [10]. Other sources of chemical pollution include effluents of biomedical waste into the lake [8], effluents from the pulp and paper industries [11], runoff and silage untreated wastewater from the neighbouring urban areas of Cotonou and Abomey-Calavi [7]. Regarding petroleum products, several studies showed that the products contain various kinds of pollutants such as petrogenic polycyclic aromatic hydrocarbons (PAH), the most dangerous of which are benzo(a)pyrene, benzo(k)fluoranthene and benzo(ghi)perylene [12,13] as well as Lead which is known for its toxicity especially to fish species [10]. Biomedical waste consists of solid and liquid hospital waste from radiology, laboratory analysis, dental waste, expired and left over drugs, broken apparatus like thermometers, etc. [7]. Dovonou et al. [13] discovered that certain fish species including Guinean tilapia *Coptodon guineensis*, Banded jewelfish *Hemichromis fasciatus* and Blackchin tilapia *Sarotherodon melanotheron,* all from Lake Nokoué are contaminated up to 32, 29, and 40% respectively with petrogenic PAH including naphthalenes, acenaphthenes, anthracenes, fluoranthenes, and acenaphthylenes. Toxic metals such as Lead, Cadmium, Mercury, Arsenic, Zinc, and Copper have been found in the sediments of Lake Nokoué [12]. Shellfish species such as Bigfisted swimcrab *Callinectes amnicola* and Rainbow crab *Cardisoma armatum* captured in and around the vicinity of the Lake Nokoué have all shown some level of toxicity with heavy metals [14]. Yèhouénou et al. [4] recorded varying levels of Cadmium (21.78 µg/L), Mercury (2.19 µg/L), Lead (25.42 µg/L), and Zinc (64.33 µg/L) in Lake Nokoué. In very high levels, organochlorine pesticides have also been observed in certain fish species such as West African ladyfish *Elops lacerta*, Delta goby *Gobbienellus occidentalis*, Bonga shad *Ethmalosa fimbriata*, and Atlantic spotted grunter *Podamasys jubelini* caught in the Lake Nokoué [4].

Increasing pollution of the lake with a cocktail of pollutants is having untold effects on aquatic organisms, including fish [15]. Records show the disappearance of many fish species from the lake [3], but those that still remain suffer the effects of this widespread pollution. Despite the widespread pollution of Lake Nokoué, there is no study, to our knowledge, on the impact of these toxic substances on fish in the waterbody. In fact, there is no study at the moment that examines the impairment of endocrine regulation and liver status of wild fishes despite their key importance in the reproductive and growth processes of the organisms. The purpose of this study is to assess the effects of the pollutants mentioned above on the reproductive and liver systems of Blackchin tilapia. The Blackchin tilapia typically are hardy species by nature and withstand heavy pollution yet are highly consumed by populations of riparian communities of coastal

West Africa. The current study assessed the health status of Lake Nokoué relative to a reference lake, Lake Toho, using a paired-lake approach with the following objectives to:

1) assess the level of organic pollution using dissolved oxygen, lack of which is caused by excess nutrients in lake.
2) study the extent of heavy metals contamination using Pb, Cd, and Hg, measured in three media including water, sediment, and Blackchin tilapia muscle tissues.
3) examine the degree of histopathology of Blackchin tilapia using gonad and liver histological analysis and biometric measurements.

2. Materials and methods

2.1. Location of the study site, sampling site selection, and characterisation

The paired-lake study was conducted on Lake Nokoué and Lake Toho, located in the southeast of the Republic of Benin. Lake Nokoué (6° 25′N, 2° 26′E) has a surface area of 150 km^2 (Figure 1). The lake measured 20 km from east to west and 11 km from north to south direction. In the east, Lake Nokoué is linked to the Porto-Novo lagoon with which it forms a brackish water system with a surface area of about 180 km^2. Lake Nokoué is also connected to the Atlantic Ocean by the 4.5 km long channel known as Cotonou lagoon [16]. Lake Toho (latitudes 6°37′09″N and longitudes 1°46′36″E) is found in between the Agamé plateau and the Bopa plateau. The lake covers an area of 9.6 km^2 at low water and 15 km^2 when flooded. The lake is, on average, 7 km long and 2.5 km wide in the south and around 500 m wide in the north [17].

Five (5) sampling sites were selected based on their location and major economic activities that take place in the areas (Figure 1). The first three sites (S1, S2, and S3) were located along the Lake Nokoué in Dantokpa market (Cotonou), Onatho (Abomey-Calavi) and pier (Sô-Ava), respectively. Site 1 (S1) was chosen because of its location close to the Dantokpa market where both organic and chemical wastes are produced. Waste from the market including garbage and chemicals is dumped into the lake by local communities. Site 2 (S2), found near Onatho was selected because of its major road intersections with heavy vehicular traffic associated with huge emissions of petroleum products [13]. It is also a site commonly known for widespread practice of '*acadja*'. The third site (S3) is found near Sô-Ava pier, a place also known for its high population density and heavy fuel transportation.

The remaining two (2) sampling sites, S4 and S5, were located along Lake Toho, which was assigned to be the reference lake in this study because it is free of this type of contamination [17]. Site S4 is located close to the Pahou pier and S5 is located near the '*Tonon Foundation*'. Another reason for selecting Lake Toho was the fact that it is located in the same geographical area as Lake Nokoué. It was also selected because Blackchin tilapia is available throughout the year.

2.2. Physicochemical parameters of water

Three (3) physicochemical variables including temperature, dissolved oxygen, and pH of the water bodies were measured once a week from July 30 to October 30 2020 and 2021 *in situ* between 7 and 9 am (local time). Water temperature and dissolved oxygen were

measured at a depth of 50 cm using a calibrated Oxythermometer (WTW oxi 197-S). The pH was measured using an ATC/HANNA/pHep3 pHmeter with an accuracy of 0.1. One (1) litre of water samples were then collected in 1.5L containers at each site and kept in an ice chest at 4°C and transported to the Research Laboratory of Aquaculture and Aquatic Ecotoxicology in Parakou for the analysis of nutrients such as nitrites, nitrates, phosphate, and ammonium. The samples were subjected to spectrophotometric analysis using a HACH LANGE DR 2800 spectrophotometer. For the biological oxygen demand (BOD5), the water samples were taken using a BOD bottle and its measurement was made by the respirometric method OxiTop in a thermostatically controlled enclosure (or a BOD-meter) at 20°C after five (5) days of incubation at 20°C. The analyses of all these chemical parameters are carried out following the procedures described by Strickland and Parsons [18].

The organic pollution index (OPI) [19] was calculated in order to assess the degree of organic pollution in the lake. In fact, the OPI indicates the degree of alteration of water by chemical variables revealing organic pollution of the environment. It is calculated from four parameters divided into five (5) classes (Table 1). The value of the parameter obtained is compared with the values in the reference table to determine the class to which it belongs. The OPI for a sample is equal to the mean of the parameter classes. It is given by the following formula:

$$\mathrm{OPI} = \left(\sum_{K \neq 0} c_k, \cdots\cdots\cdots, c_i \right) / n \tag{1}$$

with Ci: the class number of the parameter and n: the number of parameters analysed.

2.3. Sampling and heavy metal analysis

2.3.1. Sampling

Sampling was carried out twice (July 30 and October 30 2020 and 2021), when the Nokoué water was still fresh. Water samples were collected in the five sites (S1, S2, S3, S4, and S5) directly with glass bottles previously sterilised and rinsed with 10% nitric acid and distilled water. Sediment was sampled from each sampling point using a grab sampler. The sediment samples were carefully placed in glass, which were previously rinsed in 10% nitric acid and distilled water.

One hundred and twenty sexually mature individuals of the Blackchin tilapia species including 60 (30 males and 30 females) from Lake Nokoué and 60 (30 males and 30 females) from Lake Toho were carefully selected from fishermen's catches and bought for the study during sampling periods. Samples were kept in an ice chest with temperature maintained at 4°C and transported to the laboratory at the University of Parakou. In

Table 1. Limits of classes for the calculation of the organic pollution index [19].

Classes	$NH4^+$ (mg/L)	$NO2^-$ (µg/L of N)	$PO4^{3-}$ (µg/L)	DBO5 (mg/L)	Class averages	Organic pollution levels
5	<0.1	≤5.0	≤15.0	<2.0	5.0–4.6	Null
4	0.1–0.9	6.0–10.0	16.0–75.0	2.0–5.0	4.5–4.0	Weak
3	1.0–2.4	11.0–50.0	76.0–250.0	5.1–10.0	3.9–3.0	Moderate
2	2.5–6.0	51.0–150.0	251.0–900.0	10.1–15.0	2.9–2.0	Strong
1	>6.0	>150.0	>900.0	>15.0	1.9–1.0	Very strong

the laboratory, the dorsal muscle of the Blackchin tilapias was removed and lyophilised. Heavy metals analysed in this study were Lead (Pb), Cadmium (Cd), and Mercury (Hg) and these toxic metals were selected because of the type of economic activities around the water bodies under investigation.

2.3.2. Heavy metal analyses from water, sediment, and fish dorsal muscle

Water samples were filtered. After filtration, 2 drops of nitric acid (HNO3, 62%) were added and then aspirated directly into the flame Atomic Absorption Spectrophotometer (Thermo Electron Corporation with Solaar correction) for metal determination [20]. To confirm the coherence and accuracy of the results, sample analyses were performed at least three times with the same protocol. The detection limit for the heavy metals analysed in lake water is 10 ng/L.

Sediment samples were oven-dried (5–7 g). Dry samples were grounded into a homogenous mixture using a ceramic mortar and pestle and sieved through a 2 mm mesh screen to remove coarse materials because particles >2 mm may consist of shells, rocks, wood, and other detrital materials and are usually not a source of bioavailable contaminants [21]. Besides, large debris, shells, and visible organisms were removed prior to grinding. For metal analysis, 2 g of dried and grounded sediment were weighed, and mineralised in 5 mL H_2O_2 (32%) for 24 h and then 5 mL HNO_3 (62%) for 30 min using Teflon container. The contents of the Teflon container were digested gently and slowly, by heating in a sand bath till the contents got to near dryness. They were then set aside for cooling. The digest was filtered into a 50 mL volumetric flask, specially made for using distilled water. Each digested sediment sample was analysed for trace metals using a flame Atomic Absorption Spectrophotometer (Thermo Electron Corporation with Solaar correction). The digestion and analytical procedures were checked by E. Merch Darmstadt, Germany and BDH Chemical Limited with known concentration for heavy metals. The detection limit for the heavy metals analysed in sediment is 1 ng/g.

The dorsal muscle of fish collected were dried and grounded in a ceramic mortar. Five (5) g of each sample were weighed in a quartz crucible. Five (5) g of distilled water in another crucible to serve as blank. The crucibles containing samples and water as blank were put in a programmable oven. The initial temperature was lower than 100°C. The temperature increased to 450°C at a rate of 50°C/h. After this calcinations step, crucibles were removed and allowed to cool down. One (1) to three (3) mL of water was added and evaporated in a water bath. Five (5) mL of hydrochloric acid were added to each crucible and were evaporated. Residues were dissolved in a volume of 10–30 mL of nitric acid 0.1 mol/L. Crucibles were stirred thoroughly to dissolve the ashes for 1–2 h then filtered in a 50 mL flask. The resulting solution was then analysed by atomic absorption spectrophotometer. The detection limit for the heavy metals analysed in fish muscle tissue is 1 ng/kg.

2.3.3. Biometric indices measurements

Prior to the collection of dorsal muscle samples from fish for heavy metals analysis, the total length and weight of each sampled fish were recorded to calculate the Fulton

condition factor according to the following formula:

$$K = 100 \times W/L^3 \quad (2)$$

where W = body weight (g) and L = total length (cm).

Each fish was then macroscopically examined to identify external and internal abnormalities, parasites, or lesions. On the spot fish were immediately anesthetised by placing them for 10 min in benzocaine hydrochloride and killed by severing the spinal cord anterior to the dorsal fin and the back muscles were removed. The liver and gonad weights were recorded to calculate the Hepatosomatic index (HSI = liver weight/body weight × 100) and Gonadosomatic index (GSI = gonad weight/body weight × 100). The mid-sections of the left gonads (male and female) and of the liver were fixed in 4% acetic formaldehyde solution for histological studies.

2.4. Gonad histology

In the histology laboratory of the Institute of Biomedical and Applied Sciences (ISBA) of Cotonou, tissues were dehydrated through a graded series of methanol, cleared with toluene, and embedded in paraffin.

In males, the testes were cut into a series of 5 µm sections and a paraffin section was mounted on every 50 µm. For the histological analyses, slides were stained with haematoxylin, eosin, and safran (HES) and were examined using light microscopy at a range of magnifications (10–40×). The sections were observed to detect oocytes within the testicular tissue (testis-ova) as an indication of feminisation. In feminised testes, the oocyte stages were described. Stages of spermatogenesis were determined according to [2,22–24], and quantified by counting cells in a delimited surface under a light microscope. The counting was repeated three times randomly in each testis section. There are six (6) stages of spermatogenesis with well-defined characteristics: spermatogonia A, spermatogonia B, spermatocytes I, spermatocytes II, spermatids, and spermatozoa.

In females, 5 µm sections were also mounted on glass slides, stained with trichrome haematoxylin, phloxine B, and light green (Tri-green) and examined under light microscopy at a range of magnification (10–40×). Six oocytes maturation stages were estimated according to Agbohessi et al. [2] and [24] and OECD [23], with some modifications. These are: chromatin nuclear oocytes, perinuclear oocytes, cortical alveolar oocytes, early vitellogenic oocytes, late vitellogenic oocytes, and spawning oocytes stage. The percentage of each stage was determined for 100 cells per ovary.

For both sexes, a qualitative histopathological assessment was conducted using a multi-headed Olympus light microscope aimed at creating more objective results. The recorded pathologies were also semi quantitatively assessed using a scoring system, which was modified from a protocol of Bernet et al. [25]. For each histological alteration, a score value (indicative of the extent of the lesion throughout the tissue) was multiplied by an importance factor (indicative of the degree of reversibility of the lesion). Results for all the alterations identified were then summed to give a gonad index, namely testis index (I_t) and ovary index (I_o), for each male and female specimen, respectively. According to Bernet et al. [25], the formula used to calculate

these indices is:

$$I_{org} = \sum_{rp} \sum_{alt} (\alpha_{orgrpalt} \times w'_{orgrpalt}) \quad (3)$$

where org = organ (constant), rp = reaction pattern, alt = alteration, α = score value and ω = importance factor.

This index was used to compare the severity of occurrence of gonadal histological alterations between fish from each lake. In order to classify the I_t and I_o results according to the severity of the histological response, the results were evaluated according to a classification system of Van Dyk et al. [26] and [27] based on the scoring scheme proposed by Zimmerli et al. [28].

Class 1 (index <10): Normal tissue structure with slight histological alterations.

Class 2 (index 10–25): Normal tissue structures with moderate histological alterations.

Class 3 (index 26–35): Pronounced alterations of organ tissue.

Class 4 (index >35): Severe alterations of organ tissue.

Besides the indices calculated by extent (score value) and pathological importance (importance factor) of lesions, a further point of interest is the prevalence of histopathological features. The prevalence of every alteration was calculated as the percentage occurrence of an alteration within all fish of each lake. The formula was:

$$\textit{Prevalence of histological alteration} = \frac{(\text{Number of fish with the alteration}) * 100}{\text{Total number of fish}} \quad (4)$$

2.5. Liver histology and overall health status

Each liver was cut at 6 µm. The sections were mounted on glass slides and stained with HES. The procedure for evaluation of liver samples was similar to that used for the gonads.

To compare the overall health status based on the histological lesions, a Total Index (I_T) was calculated according to [25], by adding up all of the organ indices of individual fish. This index had the general formula:

$$I_T = \sum_{org} \sum_{alt} \sum_{rp} (\alpha_{orgrpalt} \times w'_{orgrpalt}) \quad (5)$$

For abbreviations, see the organ Index I_{org} formula. The I_T for each fish was calculated as follows:

$$I_T = Ig + I_L \quad (6)$$

where Ig = the gonad (testis or ovary) index and I_L = the liver index. The average Total Index per sex and lake were therefore determined.

2.6. Data analysis

Results were expressed as mean ± standard deviation. When the normality and homogeneity of the data were verified, the analysis of variance with two classification factors (Lakes and sites) for the physicochemical parameters (ANOVA 2) was performed. To minimise the effect of gender, the data were tested separately for males and females. The differences

between the means were evaluated with an analysis of variance with one classification factor (ANOVA 1) using the lake as factor. An ANOVA 1 was followed by Dunnett's comparison as the post hoc test to compare the means for Lake Nokoué with those of the reference lake for biometric indices (K, GSI, and HSI). For the concentrations of heavy metals, an ANOVA 1 test was followed by Duncan's multiple range tests because some heavy metals are absent in reference sites. Histological descriptions of tissues were qualitative and [28–31] were used as comparison methods. However, we used an ANOVA 1 and Dunnett's test for the Total Index. Calculations were made using Statistica for Windows, version 5.0 Software. The probability (p) value of 0.05 or less was considered significant. For means expressed in percentages, a statistical analysis data set was log transformed (log[x + 1]) for normalisation.

3. Results

3.1. Physicochemical parameters

The water temperature recorded from the two Lakes ranged between 27.8°C and 32.0°C (Table 2). Dissolved oxygen recorded from Lake Nokoué (4.2–4.6 mg/L) was lower than what was observed in Lake Toho (5.2–6.0 mg/L) but with no significant difference ($p > 0.05$). The pH recorded from Lake Toho was between 7.0 and 7.1 and that of Lake Nokoué was slightly lower (5.8–6.0) but not statistically different ($p > 0.05$). All the other parameters recorded including NO^{2-}, NO^{3-}, PO_4^{3-}, NH_4^+, and BOD_5 were higher on Lake Nokoué as compared to Lake Toho ($p < 0.05$). The OPI calculated for the various sites revealed that the sites of Lake Nokoué were lower (1.25–1.5) as compared to the sites located on Lake Toho (3.75–4). This shows that Lake Nokoué was organically more polluted than Lake Toho.

3.2. Heavy metal content

Table 3 shows the heavy metal (Pb, Cd, or Hg) compositions of the water and sediments from the various sampling sites and in Blackchin tilapia from Lake Nokoué and Lake Toho. The data show that heavy metal concentrations in both water and sediments were higher

Table 2. Physicochemical characteristics and OPI recorded at selected sites of Lake Nokoué and Lake Toho.

	Lake Nokoué			Lake Toho	
	S1	S2	S3	S4	S5
Water temperature (°C)	28.1 ± 1.0	27.8 ± 0.9	31.3 ± 1.1	31.9 ± 1.1	32.0 ± 0.8
Dissolved oxygen (mg/L)	4.3 ± 0.5	4.6 ± 0.6	4.2 ± 0.3	6.0 ± 0.4	5.2 ± 0.2
pH	5.8 ± 0.6	6.0 ± 0.5	5.8 ± 0.5	7.1 ± 0.9	7.0 ± 0.5
NO^{2-} (mg/L)	0.9 ± 0.7a	0.8 ± 0.8a	0.2 ± 0.1b	0.03 ± 0.01b	0.01 ± 0.01b
NO^{3-} (mg/L)	200.0 ± 13.7a	160.0 ± 16.8a	105.0 ± 1.8a	20.1 ± 3.5b	19.0 ± 2.9b
$PO4^{3-}$ (mg/L)	0.6 ± 0.2a	0.8 ± 0.2a	0.8 ± 0.1a	0.011 ± 0.01b	0.012 ± 0.01b
$NH4^+$ (mg/L)	30.0 ± 5.1a	24.0 ± 2.3a	5.2 ± 1.0b	0.5 ± 0.1b	0.4 ± 0.1b
Biological oxygen demand (BOD_5) (mg/L)	33.2 ± 5.6a	30.4 ± 5.3a	28.1 ± 3.4a	5.1 ± 1.1b	6.0 ± 1.5b
OPI	**1.25**	**1.25**	**1.5**	**3.75**	**4.0**
	1.33 ± 0.14			**3.875 ± 0.18**	

Table 3. Heavy metal (Pb, Cd, and Hg) contents in water, sediment, and Blackchin tilapia from Lake Nokoué and LakeToho.

		Lake Nokoué			Lake Toho	
Matrice	Heavy metal	S1	S2	S3	S4	S5
Water (mg/L)	**Pb**	4.02 ± 1.2 a	2.14 ± 0.93 b	2.11 ± 0.91 b	0.16 ± 0.08c	0.11 ± 0.05 c
	Cd	1.25 ± 0.8 a	1.31 ± 0.31 a	0.98 ± 0.61 a	<dl	<dl
	Hg	0.82 ± 0.3 a	0.21 ± 0.1 ab	0.11 ± 0.06 ab	<dl	<dl
Sediment (µg/g)	**Pb**	18.21 ± 0.2 a	9.81 ± 0.3 b	2.78 ± 0.24 c	0.011 ± 0.0 d	0.001 ± 0.0 d
	Cd	11.06 ± 0.01a	4.09 ± 0.09 b	0.93 ± 0.06 c	0.001 ± 0.0 c	<dl
	Hg	4.19 ± 0.3 a	1.22 ± 0.011b	1.93 ± 0.11 b	0.023 ± 0.01 c	0.013 ± 0.01 c
Fish (mg/kg)	**Pb**	2.83 ± 0.04 a	0.10 ± 0.05 b			
	Cd	2.12 ± 0.12 a	0.10 ± 0.05 b			
	Hg	0.153 ± 0.05 a	0.05 ± 0.0 b			

Data are mean ± SD. For each metal, different letters indicate significant differences between sites or Lakes ($p < 0.05$). dl = detection limit.

for all sites in Lake Nokoué as compared to the sites in Lake Toho with a statistically significantly difference ($p < 0.05$). Similarly, the levels of the same heavy metals in the dorsal muscle of Blackchin tilapia from Lake Nokoué were higher ($p < 0.05$) than those from Lake Toho. Pb is the most prevalent metal among the three assayed in water, sediments, and fish followed by Cd.

3.3. Biometric indices

The condition factor (K) of Blackchin tilapia from the two Lakes were statistically comparable ($p > 0.05$). Figure 2A, however, shows an increasing tendency in K comparing male to female as well as Lake Nokoué to Lake Toho. The lowest K is obtained in male Blackchin tilapias from Lake Nokoué (1.36) and the highest K in females from Lake Toho (1.73). Moreover, the GSI (Figure 2B) and HSI (Figure 2C) of Blackchin tilapias from Nokoué were significantly higher ($p < 0.05$) compared to that of the reference lake. The lowest GSI was obtained in males from the reference lake (0.1%) and the highest (4.25%) in females from Nokoué. Regarding the HSI, the lowest was found in male fishes from Lake Toho (1.31%) and the highest in males from Nokoué (2.72%).

3.4. Histology of Blackchin tilapia gonads

3.4.1. Males

Sections of Blackchin tilapia testis exhibited all stages of spermatogenesis in fish from both lakes (Figure 3A). Spermatids and spermatozoa were poorly represented (12.1% and 9.99%, respectively) in the testes from the contaminated Lake Nokoué as compared to those from the reference Lake Toho (20.0% and 30.0%, respectively). Spermatogonia A and B are the most represented (29.99% and 20.01% respectively) in Lake Nokoué compared to Lake Toho (5.0% and 10.1% respectively).

Alterations of the testes are shown in Figure 4 and Table 4. The fish sampled in the reference lake (Lake Toho) showed the lowest percentages of gonad alterations (3 alterations), whereas the fish sampled in polluted Lake Nokoué displayed the highest (9 alterations). Regressive changes i.e. interstitial fibrosis, necrosis, vacuolation of the testicular parenchyma, presence of immature cells in the lobular lumen, detachment of lobe

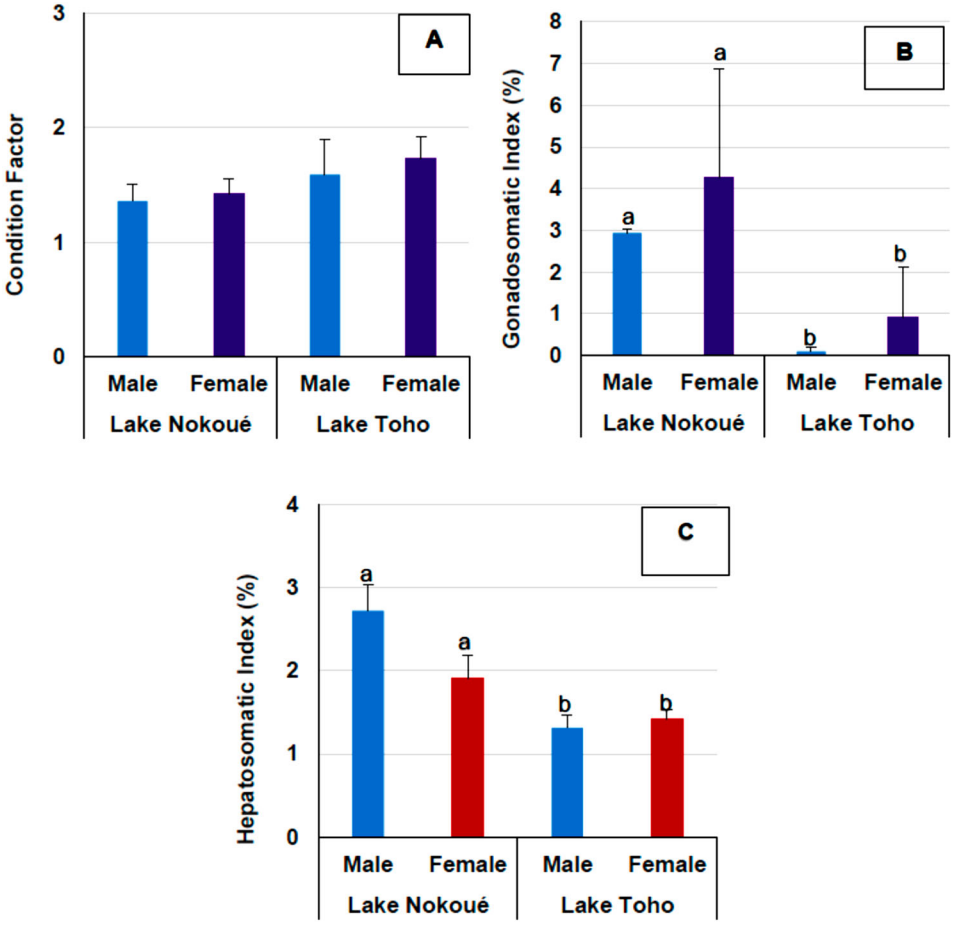

Figure 2. Condition Factor (A), Gonadosomatic Index (B) and Hepatosomatic Index (C) of Blackchin tilapia caught in Lake Nokoué and Lake Toho. Data are mean ± SD (with n = 30/sex/lake). For each sex, different letters indicate significant differences between Lakes ($p < 0.05$).

basal membrane, cysts disorganisation, germ cell syncytia and infiltration of adipocytes in the lobe lumen, were found to be more prevalent in Blackchin tilapia from Lake Nokoué compared to those of Lake Toho. The presence of testicular oocytes (previtellogenic oocytes) was detected in Blackchin tilapia sampled from Lake Nokoué, and in close to 40.0% of male Blackchin tilapia examined. No testis-ova was found in Blackchin tilapia from the reference lake.

3.4.2. Female

All stages were present in the ovaries of Blackchin tilapia sampled in both Lakes, with a marked preponderance of perinucleolar oocytes (24.2%–28.4%) and late vitellogenic oocytes (8.2%–25.5%). A lower proportion of chromatin nucleolar oocytes (19.6%), perinucleolar oocytes (24.2%), cortical alveolar oocytes (16.5%), early vitellogenic oocytes (12.5%) and spawning oocytes (1.7%), and a higher abundance of late vitellogenic

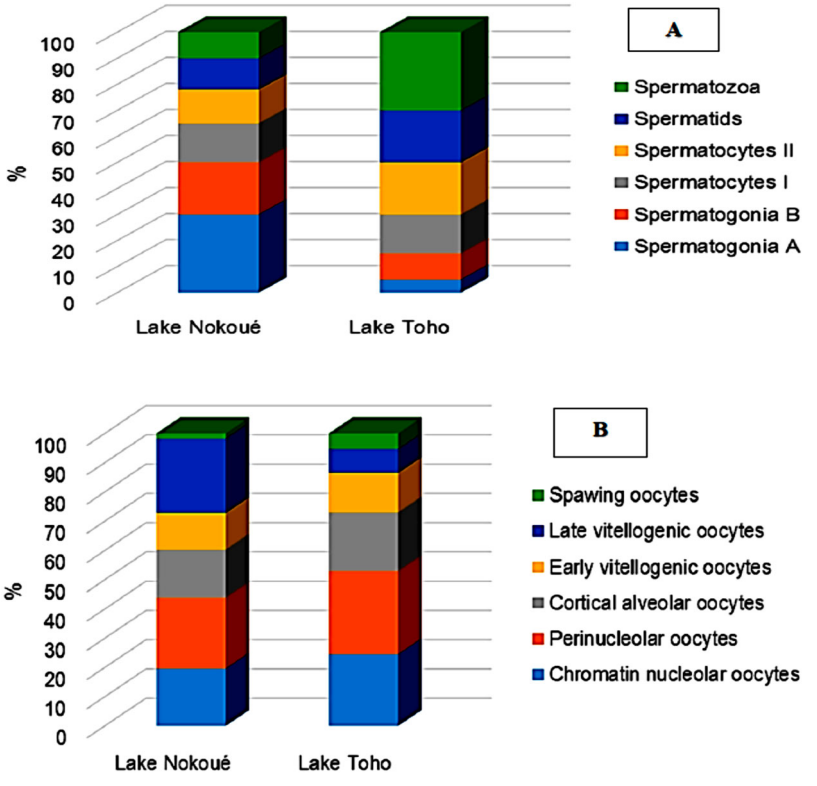

Figure 3. Percentages of different testicular stages (A) and different oocyte stages (B) in Blackchin tilapia caught in Lakes Nokoué and Toho. Data are given as mean values, n = 30.

oocytes (25.5%) were observed in Blackchin tilapia from Lake Nokoué compared to those sampled in the reference lake (Figure 3B).

Seven types of alteration, all classified as regressive changes (RC) were identified in Blackchin tilapia females (Table 4 and Figure 5). These are melano-macrophage centres (MMC), necrosis, fibrosis, preovulatory atretic follicles, vacuolation, proteinaceous fluid in the interstitium of ovaries, and detachment of the cytoplasmic membrane in perinucleolar oocytes. Blackchin tilapia from Lake Toho showed the lowest number of alterations (4 damages). The most prevalent alteration in fish sampled in the reference lake (Lake Toho) was the presence of MMC (30.0%). Vacuolation and detachment of cytoplasm membrane of oocyte were also respectively identified in 20.0% and 13.33% of female Blackchin tilapias from Lake Toho. Among the high-importance alterations (factor 3), atretic preovulatory follicles were the most prevalent with 33.33% of females in the more contaminated lake, Lake Nokoué.

3.5. Liver histology

In male Blackchin tilapias sampled from Lake Toho (Table 5 and Figure 6), the data showed the lowest number of liver alterations (7 alterations) as compared to the male Blackchin tilapias from Lake Nokoué, in which 11 alterations were found. Regressive

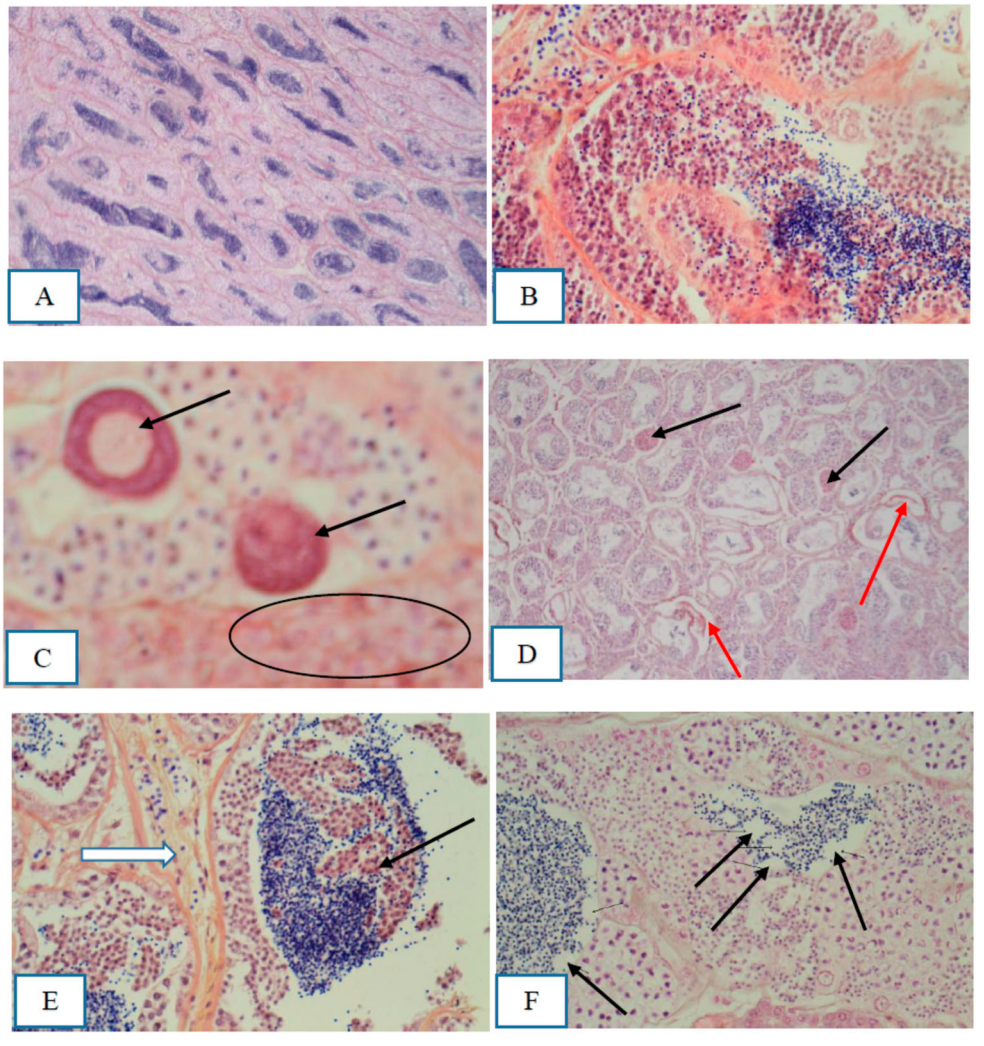

Figure 4. Light micrographs of testis sections (stained with HES) of Blackchin tilapia (**A**) from Lake Toho showing normal testicular organisation. (**B**) From Lake Nokoué showing interstitial fibrosis (orange colour) and cyst disorganisation. (**C**) From Lake Nokoué showing 2 testis-ova (black arrows) and vacuoles (black circle). (**D**) From Lake Nokoué showing large germ cell syncytia (black arrows) and basal membrane detachments (red arrows). (**E**) From Lake Nokoué showing interstitial fibrosis (white arrow) and immature cells in the lobular lumen (black arrow). (**F**) From Lake Nokoué showing infiltration of adipocytes (arrows). Magnifications: 10× (**A, D, F**), 20× (**B, C, E**).

changes were found to be more prevalent compared to any other of the reaction patterns. In the male Blackchin tilapias from Lake Nokoué, the most prevalent lesions were steatosis (80.0%), nuclear degeneration (80.0%), and hepatocellular degeneration (80.0%), which were all regressive changes. In male Blackchin tilapias from the reference lake, the most prevalent is hypertrophy cells (33.33%), a progressive change.

Females from Lake Nokoué showed 11 lesions while those from Lake Toho showed six alterations. Regressive changes were more prevalent than progressive ones. MMC, necrosis, steatosis, dilatation of sinusoids, hepatocellular degeneration, and hypertrophy cells,

Table 4. Prevalence (%) of histopathological alterations identified in the gonad of Blackchin tilapia from Lake Nokoué and Lake Toho.

Organs	Reaction pattern	Alterations	Lake Nokoué	Lake Toho
Testis	RC	Interstitial fibrosis (3)	**33.33**	–
		Necrosis (3)	**30.0**	**23.33**
	IS	Testis-ova (3)	**40.0**	–
	RC	Vacuolation (1)	**33.33**	**26.67**
		Immature cells in lobular lumen (2)	**23.33**	–
		Detachment of membrane (2)	**20.0**	–
		Cyst disorganisation (2)	**10.0**	–
		Germ cell syncytia (2)	**20.0**	–
		Infiltration of adipocytes (2)	**30.0**	**10.0**
Ovaries	RC	MMC (2)	**23.33**	**30.0**
		Necrosis (3)	**23.33**	**10.0**
		Fibrosis (3)	**26.67**	–
		Vacuolation (1)	**30.0**	**20.0**
		Detachment of cytoplasm membrane of oocyte (2)	**70.0**	**13.33**
		Proteinaceous fluid in the interstitium of ovaries (2)	**23.33**	–
		Pre-ovulatory atretic follicles (3)	**33.33**	–

Data are given as mean values, $n = 30$. The importance factor is indicated in parentheses for each alteration. RC = regressive changes, IS = intersex, MMC = Melano-macrophagic centres.

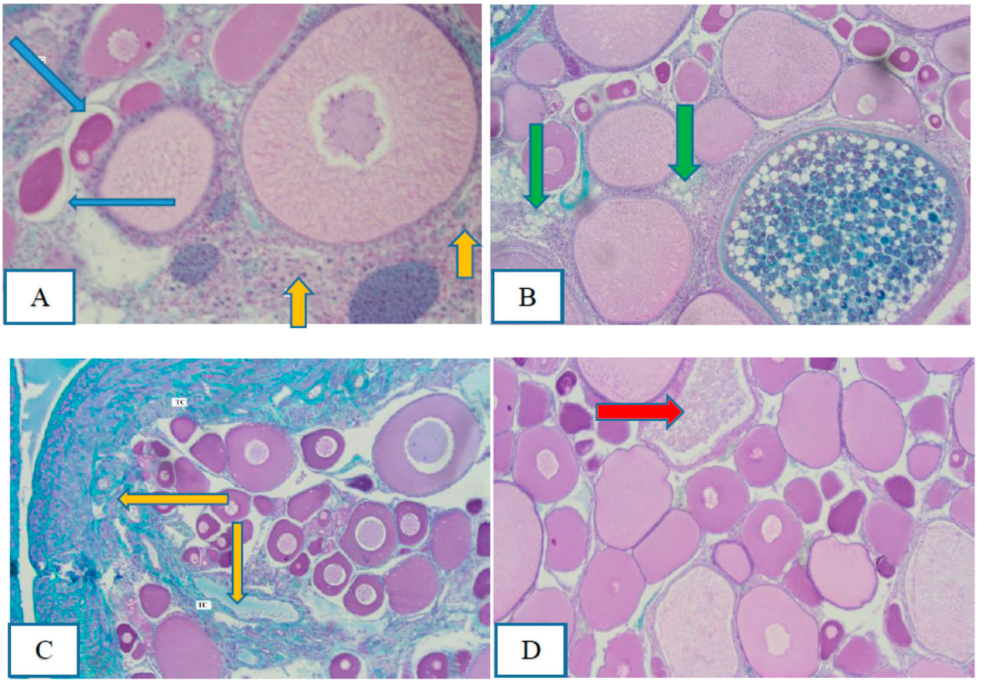

Figure 5. Light micrographs of representative ovary sections (stained with tri-green) of Blackchin tilapia (**A**) from Lake Nokoué showing detachment of the cytoplasmic membrane of perinucleolar oocytes (blue arrows) and proteinaceous fluid in the interstitium of ovaries (yellow arrows). (**B**) From Lake Nokoué showing vacuoles in the interstitium of ovaries (arrows). (**C**) From Lake Nokoué showing fibrosis (arrows). (**D**) From Lake Nokoué showing pre-ovulatory atretic follicle (arrow). Magnifications: 10× (**C, D**), 20× (**A, B**).

Table 5. Prevalence (%) of histopathological alterations identified in the liver of Blackchin tilapia from Lake Nokoué and Lake Toho.

		Lake Nokoué		Lake Toho	
Reaction pattern	Alterations	Males	Females	Males	Females
RC	MMC (1)	50.0	40.0	10.0	23.33
	Necrosis (2)	50.0	66.67	30.0	20.0
	Fibrosis (3)	40.0	33.33	–	–
	Steaotosis (1)	80.0	80.0	6.67	10.0
	Dilatation of sinusoids (1)	26.67	10.0	6.67	6.67
	Nuclear pleomorphism (2)	20.0	33.33	–	–
	Hepatocellular pleomorphism (2)	23.33	30.0	–	–
	Pycknosis (2)	10.0	6.67	–	–
	Nuclear degeneration (2)	80.0	33.33	6.67	–
	Hepatocellular degeneration (2)	80.0	26.67	3.33	6.67
PC	Hypertrophy cells (1)	40.0	26.67	33.33	20.0

Data are given as mean values, $n = 30$. The importance factor is indicated in parentheses for each alteration. RC = regressive changes, PC = progressive changes, MMC = Melano-macrophagic centres

were present in both Lakes. Liver alterations in males were more pronounced than those in females.

3.6. Overall health status of Blackchin tilapia

Table 6 shows that the alterations observed in testis could be grouped in class 4 ($It > 35$) in Blackchin tilapia sampled in the contaminated Lake Nokoué compared to the reference lake which were grouped in class 2 ($10 \leq It \leq 25$). Also, the alterations of ovaries from Blackchin tilapias sampled from Lake Toho were grouped in class 2 while those from the more polluted Lake Nokoué were grouped in class 3. A comparison between the testis and ovary alterations indicated that the testis structures were more impacted than the ovarian structures.

Liver index (I_L) of male Blackchin tilapia was higher in the contaminated lake, reaching class 3 ($26 < I_L < 35$) while I_L of fish from Toho Lake remained in the class 2 ($10 \leq I_L \leq 25$). In female, the same classes of liver alterations (class 2) were determined in both Lakes. In fish sampled in the reference lake, I_L was similar in males and females. In fish from Lake Nokoué, I_L of males was higher than that of females.

The total index was calculated for Blackchin tilapia per sex for both Lakes as shown in Table 5 to determine and compare the general health status of fish. In both sexes, I_T from the contaminated lake was higher than that of the reference with a statistically significant difference ($p < 0.05$ or $p < 0.01$). In fish from Lake Nokoué, I_T of males was also higher than that of females ($p < 0.05$).

Table 6. Gonad index (I_t and I_O), Liver index and Total index in Blackchin tilapia from Lake Nokoué and Lake Toho. Data are given as mean ± standard deviation, $n = 30$.

			I_L		I_T	
	I_t	I_o	Males	Females	Males	Females
Lake Nokoué	38.78 ± 4.76	26.04 ± 3.14	32.68 ± 8.12	22.71 ± 2.36	71.46 ± 6.18^{aA}	48.75 ± 8.43^{aB}
Lake Toho	12.55 ± 2.18	11.31 ± 6.01	14.62 ± 4.11	13.51 ± 2.26	27.17 ± 3.81^{b}	24.82 ± 7.44^{b}

In I_T, for each sex, different letters indicate significant differences between Lakes ($p < 0.01$), for Lake Nokoué, different capital letters in sex indicate significant differences between Lakes ($p < 0.05$).

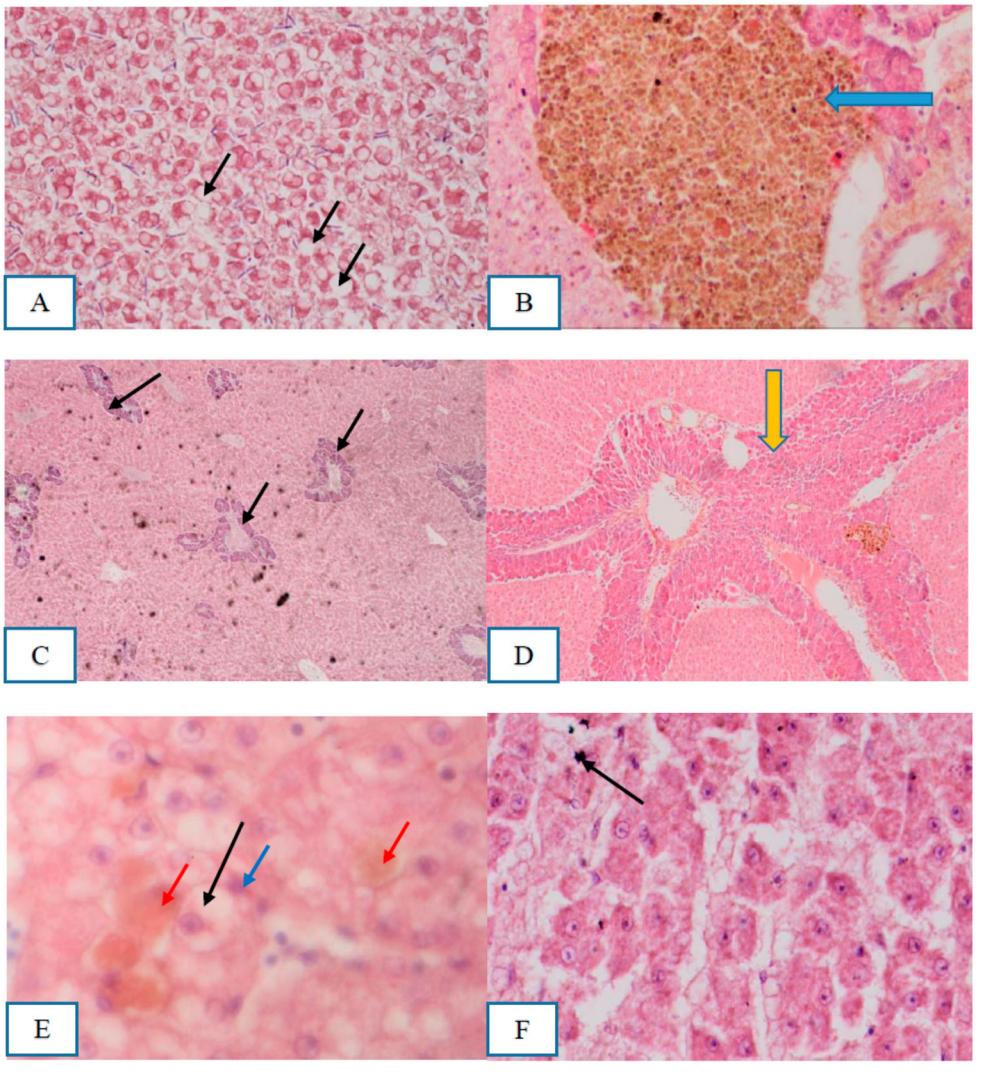

Figure 6. Light micrographs of representative liver sections (stained with HES) showing histopathological alterations identified in Blackchin tilapia from Lake Nokoué (**A**) hepatocytes with micro-vesicular steatosis. (**B**) MMC. (**C**) Highly vascularised areas. (**D**) Dilatation of sinusoids. (**E**) Hepatocyte degeneration (red arrows), cytoplasm vacuolation (black arrow) and nuclear degeneration (blue arrow). (**F**) pycknosis. Magnification: 10×.

4. Discussion

4.1. Organic and heavy metal (Pb, Cd, and Hg) pollutions in Lake Nokoué

The very low values of IPO in Lake Nokoué can be attributed to the high concentrations of ammonium and phosphate ions earlier recorded in the water [29]. Being close to the large Dantokpa market (Cotonou), S1 is an area of uncontrolled dumping of household wastes, dead animals, and all other waste from the market. The S2 is also close to the municipality of Abomey-Calavi where the practice of '*acadja*' fishing is rampant and waste from the large market of Abomey-Calavi municipality pollutes the aquatic environment. The S3

is in municipality of Sô-Ava, a densely populated area with residents whose mode of fishing is also mainly the '*acadja*' system. These different sites are therefore heavily polluted as a result of intense human activities. The decomposition of wastes generated by economic activities in these areas contributes to the decrease of oxygen in the water particularly at the water/sediment interface. This explains the relatively low levels of dissolved oxygen recorded on these sites of Lake Nokoué. Chemical reactions by autotrophic nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) constantly released nitrites and nitrates from ammonium ions [30]. This could justify the higher concentrations of these ions in Lake Nokoué. The IPO obtained in the present study for Lake Nokoué is lower than those found by Capo-chichi et al. [9] for the same lake (2.0–3.25). This difference can be explained by the reduction in the number of sites (3) in our study compared to these authors who worked on 11 sites. The high organic pollution of Lake Nokoué found in this study is comparable to what was found in the EL Tarf region of Algeria, with an IPO of 1–2.33 [31]. The findings are however slightly lower than what was found by Buhungu et al. [32] in the Kinyankange River (1.91 and 2.38), which is a tributary of Lake Tanganyika in Burundi.

The pollution in Lake Nokoué is not only organic but chemical in nature as well [4]. Lake Nokoué is polluted by several chemical substances including polycyclic aromatic hydrocarbons, dioxins, alkylphenol polyethoxylates, trace metal elements, etc [13]. Being impossible to analyse all the pollutants present, the determination of the level of pollution in toxic metals was made as an indication.

The Pb levels in the water of Lake Nokoué are higher than those found (0.01–0.05 mg/L) in the waters of Pool Malebo on the Congo River [33]. The Lead levels in Lake Nokoué water are also higher than those found (0.006–0.020 mg/L) on aquaculture farms in Côte d'Ivoire [34] and those found (0.001–0.630 mg/L) on the Konkouré River in Guinée [35]. These Pb levels in the water of Lake Nokoué are well above the standards for drinking water set at 0.01 mg/L [36,37]. The Cd levels found in the water of Lake Nokoué are higher than those found by Coulibaly et al. [34] in water from farming in Côte d'Ivoire (0.019–0.074 mg/L), and also than those found by Jeff et al. [33] in water from Pool Malebo in the Congo River (0.01–0.02 mg/L). These Cd concentrations found are above the recommendations for drinking water according to WHO [36] and UE [37], 0.003 and 0.005 mg/L, respectively. The values obtained (0.11–0.82 mg/L) for Hg in Lake Nokoué water are well above the Hg concentrations in the Konkouré River water in Guinée (0.001 mg/L) as reported by Onivogui et al. [35] and from aquaculture farms in Côte d'Ivoire (0.008–0.013 mg/L) according to Coulibaly et al. [34]. These Hg contents of Lake Nokoué water are higher than the standards of 0.001 mg/L of WHO [36] and UE [37] for drinking water. The levels of trace metal elements in the water depend on the concentrations of these heavy metals trapped in the sediments which are released into the water each time [34].

The sediment contents in toxic metals of Lake Nokoué reordered in this study are clearly above the values found by Onivogui et al. [35] in sediments from the Konkouré River in Guinée (0.004–0.712; 0.002–0.004; 0.0001 mg/kg respectively for Pb, Cd and Hg) and by Coulibaly et al. [34] in sediments from aquaculture farms in Côte d'Ivoire (2.086 −12.270; 0.135–0.194; 0.169–0.321 mg/kg respectively for Pb, Cd and Hg). The Cd and Hg contents of the Nokoué sediments are higher than the Canadian guidelines for the quality of freshwater sediments of 0.6 and 0.17 mg/kg respectively for Cd and Hg [38,39].

Blackchin tilapia is primarily a pelagic fish spending more time in the water column with only temporary periods in or near water body sediments. It is therefore a species likely to be contaminated by trace metal elements. The average levels of Pb, Cd, and Hg found in the dorsal muscle of Blackchin tilapia from Lake Nokoué in this study are higher than those from Lake Toho. These values found for Pb and Cd are also higher than those present in the muscles of the Nile tilapia *Oreochromis niloticus* from farming systems in Côte d'Ivoire (0.052–0.105 and 0.018–0.027 mg/kg respectively) found by Coulibaly et al. [34] and fish from rivers in the Adour Garonne basin in France (0.007–0.235 and 0.01–0.465 mg/kg respectively) as observed by Ricoux and Gasztowtt [40]; but lower than the levels in fish from Lake Nokoué (26.85 and 2.01 mg/kg respectively) found by Hounkpatin et al. [41]. The Hg concentrations in Blackchin tilapia from this study are comparable to that revealed in fish from the Adour Garonne basin in France (0.04–0.755 mg/kg) by Ricoux and Gasztowtt [40] as well as those reported in fish from farms in Côte d'Ivoire (0.053–0.105 mg/kg) by Coulibaly et al. [34]. These Pb and Cd contents of Blackchin tilapia in the present study are well above the maximum limits set by WHO [36] of 0.2 and 0.05 mg/L respectively for Pb and Cd. These higher levels of these different trace metal elements found in these different matrices (water, sediments, fish) of Lake Nokoué would be linked to contamination from human activities such as the uncontrolled dumping of household and biomedical waste containing Pb, Hg, and Cd, urban effluents and industrial liquids, in particular those from dyeworks rich in heavy metals. Other sources are agricultural effluents from the cotton-growing areas of northern Benin and marshy sites, petroleum products (gasoline, engine oil, and diesel) deliberately or accidentally spilled into the lake during transport, marketing, and occasional engine oil changes that are also rich in heavy metals [7,41].

4.2. Effects of pollutants on gonads

The increase in GSI observed in fish from Lake Nokoué, was also noted by Hosseini Alhashemi et al. [42] in Shabout *Barbus grypus* and Bennifish *Barbus sharpeyi* captured in the largest wetland ecosystem in Iran contaminated with trace metal elements with a positive correlation between Pb and GSI. This result is also in agreement with those of Mdegela et al. [43] in wild African catfish *Clarias gariepinus* from sewage ponds in Tanzania, and those of Dorado *Salminus franciscanus* from the Abaeté River, a tributary of the upper Sao Francisco river basin (Brazil) exposed to toxic metals [44]. In the present study, despite the regressive alterations identified in the gonads of males and females from Lake Nokoué, the GSI remain higher than those of fish from the reference lake. Being adults, these fish from Nokoué captured during the floods would have already been adapted to the stressful conditions induced by the presence of pollutants and would have benefited from the food availability of the organic loads of these flood periods or from the niches unoccupied by species less tolerant, which favoured reproduction and increased GSI [45]. Another no less plausible hypothesis indicates that this species had to adapt to unfavourable environmental conditions and changed its reproductive strategy in order to ensure its survival [46].

Histological abnormalities similar to those found in the testes of male fish from Lake Nokoué in the present study have already been described in the literature as caused by several types of pollutants including trace metals, pesticides, xenoestrogens, etc.

[2,47,48]. In Grass goby *Zosterisessor ophiocephalus* males from lagoon Bizerta in Tunisia, these alterations have been attributed to toxic metals (Pb, Cd, and Hg) [47]. Abd Aziz et al. [48] revealed in Javanese ricefish *Oryzias javanicus* subjected to Hg for 14 days for a complete disorganisation of the cysts and a delay in spermatogenesis. These lesions noted in the male gonads in this study being all of the regressive type, mark a degeneration of these testis induced by the cocktail of pollutants [45,47]. According to OECD [23], the presence of germ cell syncytia, vacuolated germ cells, and individual or clustered apoptotic germ cells in gonads of males indicates testicular degeneration. Interstitial fibrosis found in this study indicates increased fibrous connective tissue (collagenous fibres and fibrocytes or fibroblasts) within the testis and is in line with previous studies reporting testicular fibrosis after exposure to oestrogens in male *C. carpio* [49]. Necrosis in the testes indicates the death of sex cells such as spermatogonia, spermatids, spermatozoa, etc., as found in male *Z. ophiocephalus* from lagoon Bizerta in Tunisia exposed to several pollutants including toxic metals (Pb, Cd, and Hg) [47]. Flammarion et al. [50] also observed necrotic cells in the testicular tissues of European chub *Squalus cephalus* exposed to oestrogens. Necrosis, vacuolation, and infiltration of adipocytes were, however, found to be very low in males from Lake Toho. The low alterations in male tilapias of Toho were probably due to the normal slight regression of testis. It is when these changes have a strong occurrence that they become pathological. However, the most significant alteration in males of Blackchin tilapia analysed in this study was the high frequency of testicular oocytes (40% of male samples) in fish from Lake Nokoué, clearly indicating the feminisation process of those males. Testis-ova were also found in Thinlip grey mullet *Liza ramada* sampled from a polluted estuary in central Italy [51]. Also in Spottail task *Notropis hudsonius* exposed to xenoestrogens [52]. More so, male flounder *Platichhys flesus* caught in the estuary of the rivers Tyne and Mersey in the United Kingdom had testis-ova in 7% and 9% of individuals respectively [53]. All testis-ova observed in this study contained previtellogenic oocytes. Agbohessi et al. [2] also revealed testis-ova at the previtellogenic oocyte stage in males of *C. guineensis* and *C. gariepinus* captured in the Alibori River receiving effluents from the cotton-growing areas of northern Benin. The molecular mechanisms underlying the appearance of testis-ova in fish are not fully understood; however, several authors have argued that the excess of oestrogens is capable of inhibiting spermatogenesis in males and causing the formation of oocytes from spermatogonia [54]. Nadzialek and Kestemont [54] further demonstrated that xenoestrogens (17α-ethinylestradiol) induced an in-vitro imbalance between apoptosis and cell proliferation in the testes of gudgeon, *Gobio gobio*, through their effects on some target genes such as Bcl-2 and caspase 3 [55]. Apart from the toxic metals analysed in this study, it is possible that other pollutants not measured here such as polycyclic aromatic hydrocarbons, dioxins, alkylphenol polyethoxylates, etc., are responsible for the oestrogenic effects observed.

The regression of spermatogenesis observed in fish from contaminated Lake Nokoué was also observed in summer flounder, *Paralichthys dentatus*, exposed to xenoestrogens, corroborating earlier findings on various fish species [56]. The delay of spermatogenesis is also viewed as an indicator of exposure to xenoestrogens [56].

These alterations observed in the ovaries of the reference lake's fish were probably due to the normal slight regression of ovaries. Martinez et al. [57] also revealed in female Threadfin shad *Dorosoma petenense* ovary from la Polvora urban lagoon in the Grijalva

basin, Mexico, a preovulatory atretic follicle, vacuolation, and proteinaceous fluid in the interstitium of ovaries, as found in the present study in Blackchin tilapia ovary of Lake Nokoué. The increased oocyte atresia was reported in *C. gariepinus* sampled from an urban nature reserve in South Africa by Pieterse et al. [58]. The increased pre-ovulatory atretic follicles have been identified as key histological features indicative of endocrine disruption induced by chemicals [2,24,59]. The vacuoles of the interstitial tissue identified in exposed fish most likely contained lipids, as had the characteristic spherical shape. This alteration has previously been identified in fish exposed to endocrine disruptors [59]. An increase in the prevalence of MMC identified in the ovaries however may indicate excessive oocyte degeneration [59]. The interstitial proteinaceous fluid is presumably vitellogenin within the ovarian interstitium. The presence of this fluid may cause a thickening of interstitial areas that might be misinterpreted as stromal proliferation [23]. The interstitial fibrosis indicates the presence of increased fibrous connective tissue (collagenous fibres and fibrocytes or fibroblasts) within othe varian interstitium (stroma).

The lower proportion of the first four and the last stages in fish exposed to pollutants in Lake Nokoué compared to those in reference lake fish suggests a stimulatory maturation of oogenesis induced by this cocktail of pollutants [2]. However, this acceleration of oogenesis was not confirmed by a higher proportion of late vitellogenic oocytes in the ovaries of Blackchin tilapia from Lake Nokoué. In fact, it is the ovaries of fish from Lake Nokoué that contain the pre-ovulatory atretic follicles. These oocytes arrived at the stage of late vitellogenic oocytes degenerate due to the effect of the contaminants present. This is what explains the low proportion of spawning oocytes at the end of the process. Indeed, these toxics having an oestrogenic effect, the high 17β-oestradiol level acted on the maturation of the oocytes [2,23].

The effects on male individuals are higher than those on females. According to Van Dyk et al. [27] and Zimmerli et al. [28], the severity degree of histopathological changes (organ index) observed in the gonads (It = 38.78) indicates a severe alteration of testis; and that in ovaries (Io = 26.04) reveals a pronounced alteration of ovary compared to male and female fish from Lake Toho whose gonadal indices indicate normal tissue structures with moderate histological alterations. Van Dyk et al. [60] also found a similar testis index in *C. gariepinus* sampled in the polluted river of Roodeplast in South Africa.

4.3. Effects of pollutants on the liver

The histopathological lesions of the liver obtained in the present studies have already been observed in the liver of several species of fish exposed to several types of pollutants [2,24]. Several alterations observed in contaminated individuals were also found in male and female individuals from the reference lake at low occurrence. These are MMC, necrosis, steatosis, dilation of sinusoids, hepatocellular degeneration, nuclear degeneration and hypertrophy cells. Aware that there is no ecosystem with zero pollutants, these lesions are surely induced by toxicants or parasites from the reference lake not detected in the present study. MMC are distinctive groupings of pigment-containing cells normally located in the stroma of the haemopoietic tissue of the kidney [61]. In some fishes, they are also found in the liver [62] as in the case of Blackchin tilapia. In superior teleosts, they often exist as complex discrete centres, containing lymphocytes and macrophages, and may be primitive analogues of the germinal centres of lymph nodes. The MMC or

macrophage aggregates enclosed the absorbing region (artery/vein) and excretion (bile region) of the liver, and it is associated with metabolism of toxic compounds [61]. According to Agius and Roberts [63], MMC play an important role in the sequestration of the products of cellular degradation and in potentially toxic tissue materials, such as free radicals and catabolic breakdown products. High occurrence of MMC in the liver of Blackchin tilapia is related to the toxicants in Lake Nokoué [64]. Furthermore, large liver areas exhibiting disorganisation of the tubular cord-like structure, large melanomacrophage aggregates close to the blood vessels, and sinusoid dilatation are considered as indicators of severe alteration in the environment [62]. Necrosis of some portions of the liver tissue is characteristic of the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification. The inability of the fish to regenerate new liver cells may also have led to necrosis. During cell necrosis, the cytoplasm within the hepatocytes becomes eosinophilic and homogeneous; the cell nucleus also shrinks in size and undergoes pycknosis, karyolysis, and karyorrhea [65]. Nuclear degeneration and hepatocyte degeneration, observed in our study, lead to necrotic liver cells. Cytoplasmic vacuolisation was the most frequent hepatocyte change and it can be a signal of degenerative processes involving metabolic damage [66]. Blackchin tilapia in this study showed steatosis and vacuolisation, indicating a mobilisation of fat. This type of lipid deposition is a pathological process that can be used as an indicator of hepatic disturbances in fat metabolism [67]. Spisni et al. [68] described steatosis as a liver alteration due to an excessive dietary intake of lipid, which saturates the physiological capability of the liver. These histopathological hepatic observations suggest that Blackchin tilapia are suffering from exposure to sublethal concentrations of many pollutants discharged into the Lake Nokoué reservoir. This explains the hepatic indices $I_L = 32.68$ and $I_L = 22.71$ respectively for males and females from Lake Nokoué indicating for males 'pronounce alteration of liver' and for females 'normal tissue structures with moderate histological alterations' as found in males and females from Lake Toho. This result revealed by the liver indices (I_L) is contrary to that indicated by the hepatosomatic indices (HSI). Indeed, in the present study, the HSI of contaminated individuals is statistically higher than the one of individuals from the reference lake. Referring only to these HSI means that the livers of contaminated individuals are doing better than those of uncontaminated individuals. But in reality, the high HSI level in fish from Lake Nokoué can be linked, on one hand, to macroscopic abnormalities such as stones and nodules of fat [60] on the other hand, they may be attributed to the oestrogenic effects of the cocktail of pollutants highlighted in the present study. In fact, when there are oestrogenic effects of pollutants on fish, this means that there are strong syntheses of vitellogenin in the liver. This super-activity of the liver leads to the development of this organ [69]. There is therefore no contradiction between I_L and HSI in this study. Similar increase in HSI was also observed by Van Dyk et al. [60] in *C. gariepinus* caught in rivers impacted by mining industrial and urban effluents (South Africa), and by Corsi et al. [70] in *Z. ophiocephalus* caught in the Orbetello lagoon (Italy) and exposed to chlorinated and aromatic hydrocarbons.

4.4. Health status of Blackchin tilapia in Lake Nokoué

In this study, the condition factor K and the total index (I_T) [25] calculated from the histopathological study of the liver and gonads of fish were used to determine the health

status of the fish from Lake Nokoué and Lake Toho. The values of K of Blackchin tilapia from Lake Nokoué are statistically identical to those of the reference lake but with a downward trend. A decrease in the K of fish exposed to pollutants was also reported by Louiz et al. [47] in *Z. ophiocephalus* caught in lagoon Bizerta (Tunisia) contaminated with industrial and urban effluents containing several types of pollutants including the toxic metals Pb, Cd and Hg; by Hanson et al. [71] in *O. niloticus*, brackishwater catfish *Chrysichtys nigrodigitatus* and by Cook et al. [72] in Zebrafish *Danio rerio* reared in an environment contaminated with Malathion. Several hypotheses could explain the decrease in the condition factor, but the most convincing is the bioenergetic one. In fact, the presence of environmental pollutants can affect nutrient absorption by fish [73]. Even if fish are fed properly, a significant portion of nutrients from the catabolism of food could be converted into energy to cope with the energy demand caused by the chemical stress induced by the pollutants [2,74]. In reality, Blackchin tilapia is a filter-feeding species, and with the richness of the streams in plankton and suspended solids during the flood, there was therefore plenty of food for the fish during the sampling period. But it is the use of energy from this food that differs from one lake to another. As fish from Lake Nokoué have to deal with pollutants, most of the energy will be used to cope with the energy demand induced by the presence of toxicants. Gonadal indices (I_t and I_o) and hepatic indices (I_L) according to Bernet et al. [25] indicate that the gonads and livers of fish from Lake Nokoué are generally in poor condition due to pollutants. The I_T calculated according to Bernet et al. [25], indicated that the general health status of fish sampled in Lake Nokoué was much poorer than that of fish sampled in Lake Toho, particularly for males who are more affected due to the oestrogenic effects of pollutants. This feminising effect of male fish is a real threat to the survival of this species in this lake. The strong impact of xenoestrogens on the state of health of fish had also been proven by Agbohessi et al. [2] in Alibori River of Benin.

5. Conclusion

The results of this study confirm that Lake Nokoué is heavily polluted by several types of pollutants including organic and chemical pollutants such as trace metal elements (Pb, Cd, and Hg). This mixture of toxic substances, specifically Pb, Cd, and Hg, have been found to accumulate in the muscle tissues of Blackchin tilapia. Furthermore, these toxic substance mixtures impair the endocrine regulation of Blackchin tilapia inhabiting the lake, induce severe damages to the liver and gonads, and impair the health of the populations of Blackchin tilapia. This cocktail of pollutants has an oestrogenic effect on *Sarotherodon melanotheron* and constitutes a threat to the survival of the species. The consumption of fish especially Blackchin tilapia from Lake Nokoué may pose a serious health risk to human populations due to the negative effects associated with the bioaccumulation of such pollutants in the human body.

These findings suggest that long-term (multiple seasons and years) research efforts need to be done in Lake Nokoué and other brackish water ecosystems in Benin in terms of monitoring the quality of water, sediment, and biota. In addition, there will be great to further assess other biomarkers of reproduction such as sex steroids, vitellogenin in males, and enzymes namely 17β aromatase and 11β hydroxylase.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Notes on contributors

Agbohessi Prudencio is a Senior Lecturer and Specialist in Aquatic Ecotoxicology.

Onibon Chabi is a PhD student in Aquatic Ecotoxicology.

Pelebe Edéya Orobiyi Rodrigue is a Postdoctoral Fellow, Specialist in Hydrobiology.

Mattah Dzorgbe Agbeko Precious is a Senior Research Fellow, Specialist in Hydrobiology.

Guedegba Nicresse Léa is a Postdoctoral Fellow, Specialist in Aquatic Ecotoxicology.

Imorou Toko Ibrahim is a Professor, Specialist in Hydrobiology.

Chikou Antoine is a Professor, Specialist in Hydrobiology.

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