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Evaluation of the Physiological State of Tilapia *Sarotherodon melanotheron* from the Lead and Cadmium-Polluted Lake Nokoué- Porto-Novo Lagoon of in Benin

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Abstract Water pollution remains an important concern for aquatic biodiversity, and threatens fish life. In Lake Nokoué - Porto-Novo lagoon complex, one of the most productive in West Africa, fish do undergo the harmful effects of water pollution, which affect fish physiology and therefore their survival. This study aimed to assess the physiological state of *Sarotherodon melanotheron*, one of the most abundant fish species in this complex. To this end, lead and cadmium concentrations in fish, obtained from the complex during periods of high and low water, were determined using atomic absorption spectrometry. Hematoxylin & Eosin staining was used to examine the histology of their gonads and livers. Hematological and biochemical analysis was conducted on fish blood samples. The analysis revealed that lead (12.65 ± 11.63 mg/Kg) and cadmium (0.14 ± 0.07 mg/Kg) were accumulated in fish. During low-water, fish had decreased hemoglobin, hematocrit, white blood cells and lymphocytes, while blood glucose and increased ALT transaminase activity. Histological analysis of the gonads and liver revealed numerous alterations, particularly during periods of high-water. Collectively, these data show that pollution of the complex promote bioaccumulation of these heavy metals resulting in altered fish physiology and health.

Keywords Hematology, Histology, *Sarotherodon melanotheron*, Lake Nokoué- Porto-Novo Lagoon

1. Introduction

Inland fishing plays an important role in Benin's economy, accounting for 18.8% of gross national agricultural product, and is the main source of fish products in Benin, accounting for 79% of fish production [1]. Inland fishing is of vital importance to people living along rivers and lakes, as it contributes to food and nutritional security and also provides cash income and other socio-cultural benefits [1]. The Lake Nokoué - Porto-Novo lagoon complex, despite its small size, contributes to inland production in West Africa. According to the [2], inland fisheries in West Africa produce around 600,000 to 700,000 tons per year, while the Lake Nokoué-Porto-Novo Lagoon complex contributes 34,000 tons per year [3], representing approximately 4 to 5% of West Africa's production. At the national level, its fish production alone accounted for 65% to 80% of Benin's continental production [4].

Unfortunately, fish production has fallen over the last five years, reaching 17.98% between 2019 and 2023 [5]. One of the major causes of this drop in production is the pollution of Benin's major ecosystems [6], notably Lake Nokoué Porto-Novo lagoon. This complex is under heavy pressure, receiving waste of all kinds from the surrounding towns [6]. Among the pollutants of greatest concern, there are toxic metals, which are non-biodegradable and accumulate in various ecosystem compartments: water, sediments, suspended matter [7] and living organisms [8]. Lead and cadmium are dangerous, extremely toxic metals that are widespread in the environment. They are among the three metals classified as priority water pollutants by the European Economic Community (EEC) and the World Health Organization (WHO) [9]. Many studies have highlighted the presence of these metals in the sediments, water and organisms of this complex, such as fish, crabs, etc. [8,10,11,12]. These pollutants disrupt ecosystems and cause great damage. One of the most devastating effects of pollutants, especially heavy metals, on ichthyological fauna is the disruption of the endocrine system [13], which hinders species survival by affecting their reproduction. Pollutants are also at the root of several other disturbances, such as the immune system, the neurological system and fish growth [14].

Most studies carried out on these ecosystems are based on assessing pollution levels, but there are few studies on ecotoxicological aspect. It is important to know the damage that pollutants have caused to fish physiology, so that appropriate measures can be taken to safeguard its long-term survival. This research focuses on the effects of these pollutants during the two major seasons on the physiology of *Sarotherodon melanotheron*, a euryhaline species and the second most abundant in Lake Nokoué - Porto-Novo lagoon [4]. Findings from this work will increase ecotoxicological data availability and awareness of decision-making authorities in charge of this water body.

2. Methodology

Lead and cadmium were measured in *Sarotherodon melanotheron* fish to assess the level of accumulation of these toxic metals in this species. A multi-biomarker approach was used to analyze the physiology of fish exposed to pollutants. This approach includes blood count, blood glucose, creatinine, transaminases (AST and ALT) and histological analysis.

2.1. Sampling Sites in the Complex

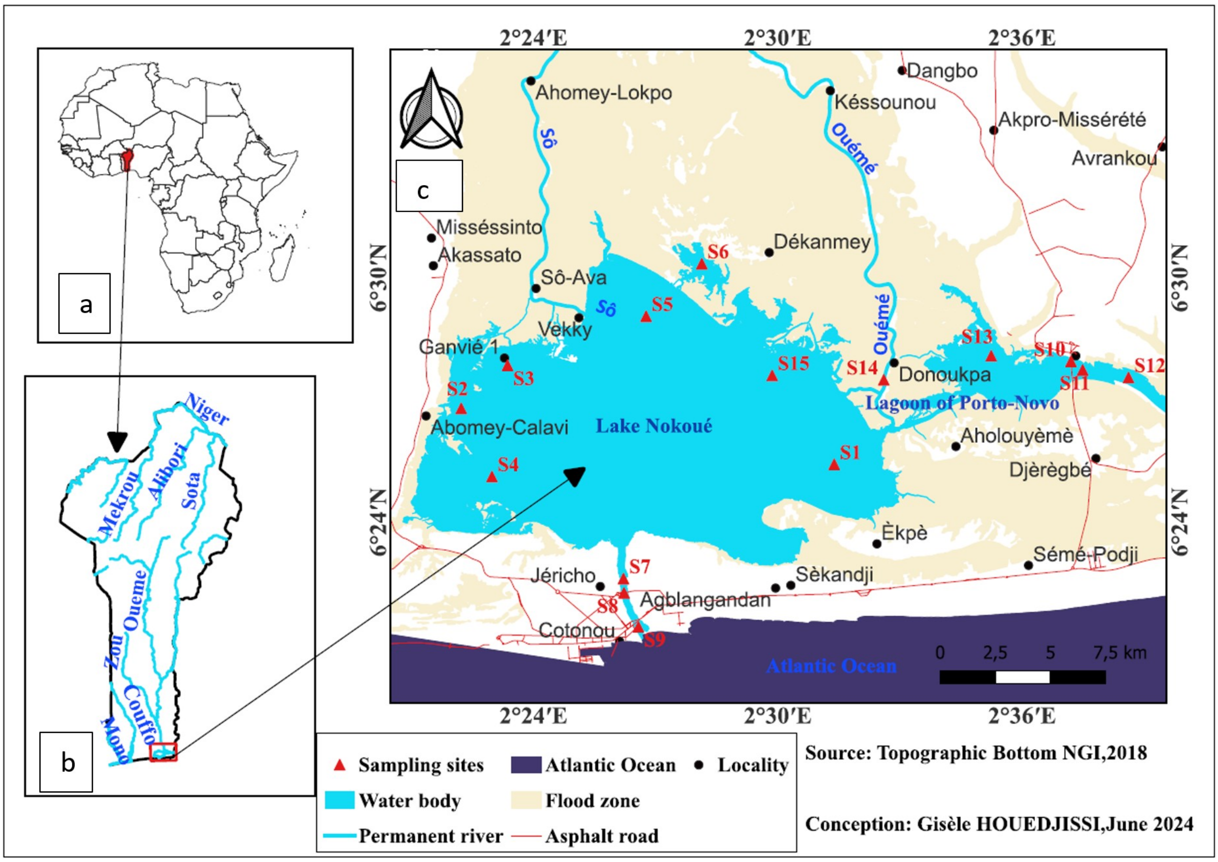
The samples of fish from their living environment were collected on the lagoon complex of Lac Nokoué - Porto-Novo lagoon complex, the location of which is shown in Figure 1. This brackish water complex is connected (to sea) by the Totché channel [15] and receives water from rivers such as the Sô and Ouémé, which discharge their contents, including chemical pollutants, into it. This irrigation defines two annual periods of water levels, high and low, directly dependent on rainwater, runoff and watercourses. Figure 1 shows the study environment and the sampling sites.

2.2. Sample Collection

Specimens of *Sarotherodon melanotheron* with an average weight of 114.98 ± 55.38 g were collected from various identified sites in the complex during the high water period of October 2023 and the low water period of March 2024. A total of 160 fish were studied, including 80 males and 80 females. For each fish, weight and length were measured to calculate biometric parameters. Blood was collected using 2 ml syringes and then transferred to dry tubes and tubes containing EDTA. The liver and gonads were removed after dissection for histological analysis. All fish used were approximately the same size. The rest of the fish was used for lead and cadmium analysis. The sample size is summarized in the Table 1.

2.3. Chemical Analysis of Heavy Metals

After blood and organ sampling for histology, the fish were oven-dried at 99 °C. Mineralization by nitric acid attack was used for solubilization, which took place in a closed environment at high temperature (150 °C). Briefly, to 1g sample of dried and grounded fish, 4 mL of 65% nitric acid was used for mineralization. Beforehand, 10 mL of 9% hydrogen peroxide (H_2O_2) was added to the sample for 24 hours prior to acid etching. Gradual heating was carried out at a temperature of around 150 °C for 2h on a hot plate. The samples were then filtered to obtain preparation containing the chemical elements to be assayed. The method used for lead and cadmium determination was atomic absorption spectrometry using the flame technique (SAA Ice 3000 SERIES THERMO FISCHER).



Fifteen sampling sites (S1-S15) were identified on the complex. a: Map of Africa showing location of Benin, b: map of Benin showing the location of the complex and c: magnified map of the complex showing the sampling sites S1-S15.

Figure 1. Study environment and sampling sites

Table 1. Number of samples for each parameter analyzed

Type of analysis	Samples of male fish	Samples of female fish
Hematological parameters	80	80
Biochemical parameters	80	80
Histology of the gonads	40	40
Liver histology	40	40

2.4. Hematological Analysis

The blood cell count (CBC) was used to determine several hematological parameters that reflect the physiological state of the fish. These parameters include hemoglobinemia, hematocrit, red cell or erythrocyte volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin content (MCHC), white blood cell or leukocyte volume (NB or WBC), leukocyte forms, etc. The analysis was carried out on the SYSMEX KX 21N hematology analyzer. Blood previously collected in EDTA tubes and labelled was placed in the machine, and after reading, the results were printed.

To ensure the authenticity of the automation, blood smears were taken. The slides were stained in May-Gruwald diluted 50% with Giesma diluted 20% with neutral water. Slides were observed under a EUROMEX microscope. Neutrophils, eosinophils, basophils, lymphocytes, monocytes and white blood cells were counted.

2.5. Biochemical Analysis

Biochemical analyses were carried out using a DR 3900 spectrophotometer. Operating mode: transmission (%) - absorbance and concentration - scanning. The wavelength range of the device is 340 to 1100 nm, with a bandwidth of 5 nm. The resolution is 1 nm, which means it can distinguish length variations as small as 1 nm. A scan step of 1, 2 or 5 nm can be selected, which determines the interval between each wavelength measurement during a scan. The unit is equipped with a color TFT touch screen, offering a user-friendly interface. These include blood glucose, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Fish blood samples collected in dry tubes were centrifuged to separate the serum from the pellet. For each biochemical parameter, the serum was taken and mixed with its corresponding reagent (gly; creat; asalt-alate). Each mixture produced is filled into the spectrometer, which after absorption displays the result on the screen.

2.6. Histological Analysis

After blood sampling, gonads and liver were removed from fish and weighed to calculate the hepatosomatic index and the gonado-somatic index. Biometric indexes (condition factor "K", gonado-somatic index (GSI) and hepatosomatic index (HSI)) were used to assess the general physiological status of the fish.

$$\text{Condition factor } K = 100 * \left(\frac{P}{LT^b}\right)$$

P: body weight (g); LT: total length (cm) and b: allometric coefficient = 3

This factor is used to assess the biological condition and corpulence of the fish. A high K value means that the fish are overweight and live in an environment where conditions are favorable to them.

Hepato-Somatic and Gonado-Somatic Indexes (HSI and GSI):

$$HSI\ (\%) = \left(\frac{Wf}{We}\right) * 100$$

$$GSI\ (\%) = \left(\frac{Wg}{We}\right) * 100$$

With Wf: weight of liver, We: weight of eviscerated fish and Wg: weight of gonads.

For the histological technique, organs were preserved in 10% formalin at neutral pH for fixation. Histological sections were prepared following the standard protocol (fixation, dehydration and thinning, paraffin embedding, sectioning and staining). Sections were stained with hematoxylin and eosin (H&E). The organs were sectioned at thicknesses of 4 µm. The slides were observed using a Euromex optical microscope.

The alterations were classified into six types: (i) circulatory disturbances, (ii) regressive changes, (iii) progressive changes, (iv) inflammatory disorders, (v) neoplasia and (vi) intersex (applicable only to gonads) according to Van Dyk *et al.* [16]. A semi-quantitative analysis was carried out using occurrence scores according to Bernet *et al.*'s [17] protocol. The occurrence score indicates the extent of the alteration in the organ of each alteration. The scores used are: (0) absent (unchanged), (2) present in small quantities (low occurrence), (4) present in moderate quantities (moderate occurrence), (6) present in large quantities (severe occurrence). The importance factor (indicating the degree of reversibility of the alteration) of each alteration was also estimated: (1) minimal pathological importance (easily reversible), (2) moderate pathological importance (reversible after the pollutant has disappeared), (3) high pathological importance (irreversible leading to partial or total loss of organ function). For all alterations, the occurrence score was multiplied by the importance factor to give the organ index (I(testis) for the testis, I(ovary) for the ovary and I(liver) for the liver). According to Bernet *et al.* [17], the formula is

$$\mathbf{I_{org}} = \sum_{\mathbf{rp}} \sum_{\mathbf{alt}} (\boldsymbol{\alpha}_{\mathbf{org\ rp\ alt}}\ \mathbf{x}\ \boldsymbol{\omega}_{\mathbf{org\ rp\ alt}})$$

Where I_{org} = organ index, rp = reaction pattern or type of alteration, alt = alteration, org = organ (constant), α = occurrence score, ω = importance factor.

The calculated indexes were classified according to Van Dyk *et al.* [16] to assess the severity of damage to each organ.

Class 1 (organ index <10): organ with histological structure and slight alterations.

Class 2 (organ index 10 - 25): organ with moderate histological changes.

Class 3 (organ score 26 - 35): organ with histological structure showing pronounced alterations.

Class 4 (organ index >35): organ with histological structure showing severe alterations.

Next, the overall health of the fish was assessed by calculating the total index (I_T), which is the sum of the organ indexes:

$$\mathbf{IT} = [\mathrm{I(testis)}\ \mathbf{ou}\ \mathrm{I(ovary\ )} + \mathrm{Iliver}]\ [17]$$

The prevalence of each alteration considered as percentage of occurrence (%) was calculated according to the formula: [12]

$$\mathbf{P} = (\mathbf{N(alt)}\ \mathbf{x100})\ /\mathbf{NT}$$

P: prevalence, N(alt): number of individuals with the alteration, NT: total number of individuals examined.

The different stages of development of male and female gonads were counted for each sample. An average was calculated for each stage of development. The percentage of each stage of gonad development was estimated. This allowed us to examine the development of the reproductive organs [16].

2.7. Statistical Analysis

The data is processed using XLstat statistical software. The fish represents the experimental unit, and the factors considered are flood, low-water and the sex of the fish. Results are expressed as mean ± standard deviation of the mean. The analysis of variance (ANOVA) test was used to evaluate the difference between means. Means were compared between flood and ebb and male and female. To show the difference between the data, Duncan's test was used, and a difference was considered statistically significant if the p-value was less than 0.05. A Pearson correlation test was performed between blood parameters, biometric parameters and lead and cadmium concentrations in fish.

3. Results

3.1. Lead and Cadmium Concentrations in Fish

Lead and cadmium concentrations in *Sarotherodon melanotheron* fish sampled from this complex ranged from 0.2 ± 0.87 mg/Kg to 12.65 ± 11.63 mg/Kg and from 0.09 ± 0.01 mg/Kg to 0.14 ± 0.07 mg/Kg respectively (Figure 2). Lead concentration was significantly higher during low-water than during high-water (p= 0.014). However, no significant difference was observed for cadmium between seasons.

3.2. Effect of Water Quality on Hematological Parameters

Hemoglobin and hematocrit levels are shown in Figure 3 A and B. Hemoglobin and hematocrit in both seasons ranged from 7.32 ± 1.89 to 9.39 ± 2.74 g/dL; 21.64 ± 8.83 to 28.95 ± 9.10%, respectively. During the high-water season, hemoglobin (p = 0.048) and hematocrit (p = 0.009) were significantly lower than during the low-water season.

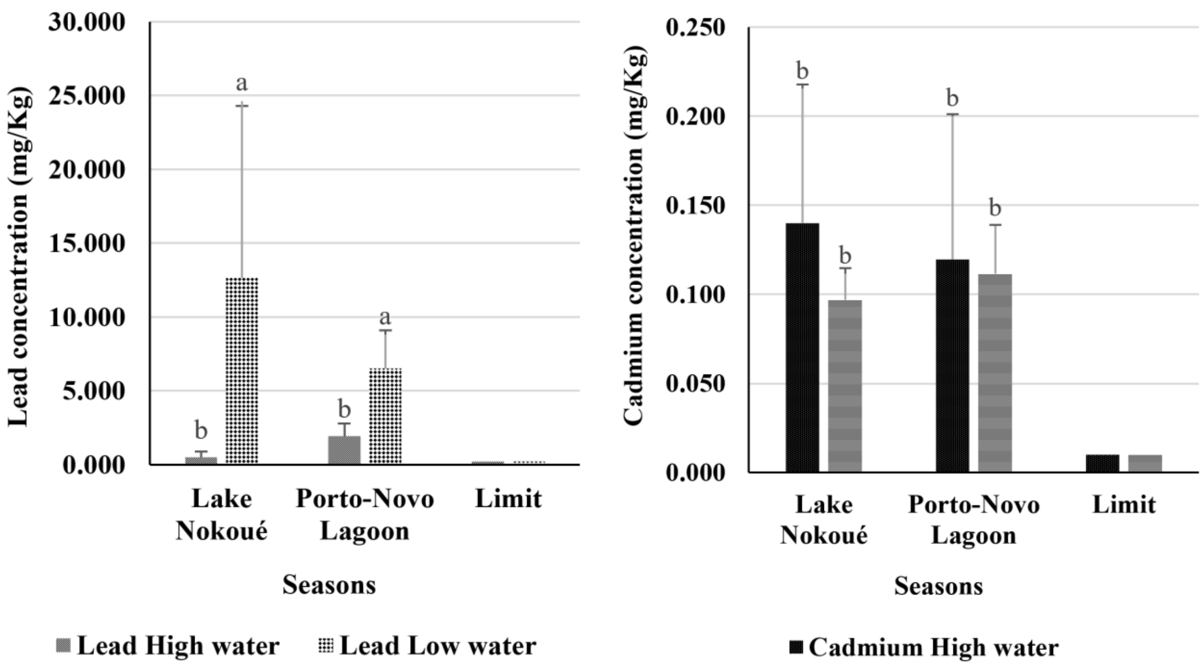


Figure 2. Concentration of lead (A) and cadmium (B) in fish (Lower-case letters indicate the difference between seasons)

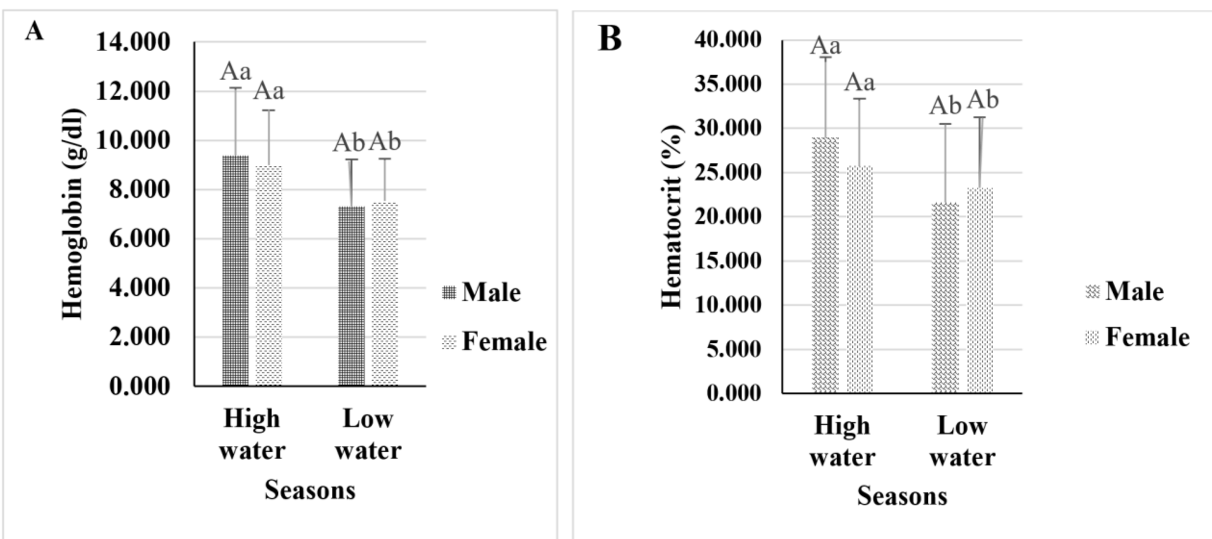


Figure 3. Hemoglobin (A) and hematocrit (B) levels

White blood cells and lymphocytes ranged from 107.56 ± 68.13 to 300.66 ± 484.06 $10^3/mm^3$ and 84.70 ± 13.79 to 93.15 ± 5.12 $10^3/\mu L$ respectively in both seasons (Figure 4 A and B). During low water, they were significantly lower ($p = 0.039$).

Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin content (MCH) and mean corpuscular volume (MCV) are presented in Table 2. No significant differences were observed between seasons or gender ($p > 0.005$).

Neutrophils, eosinophils and monocytes (Table 3) show no variation between the two seasons and gender ($p>0.005$). Monocytes, however, were significantly low during high-water ($p<0.05$).

The correlation between lead and cadmium concentrations in fish and hematological parameters (Table 4) shows that white blood cells, hemoglobin and lymphocytes are strongly correlated with lead. A negative correlation showing that the two evolve in opposite directions, i.e. increasing lead causes a decrease in these hematological parameters.

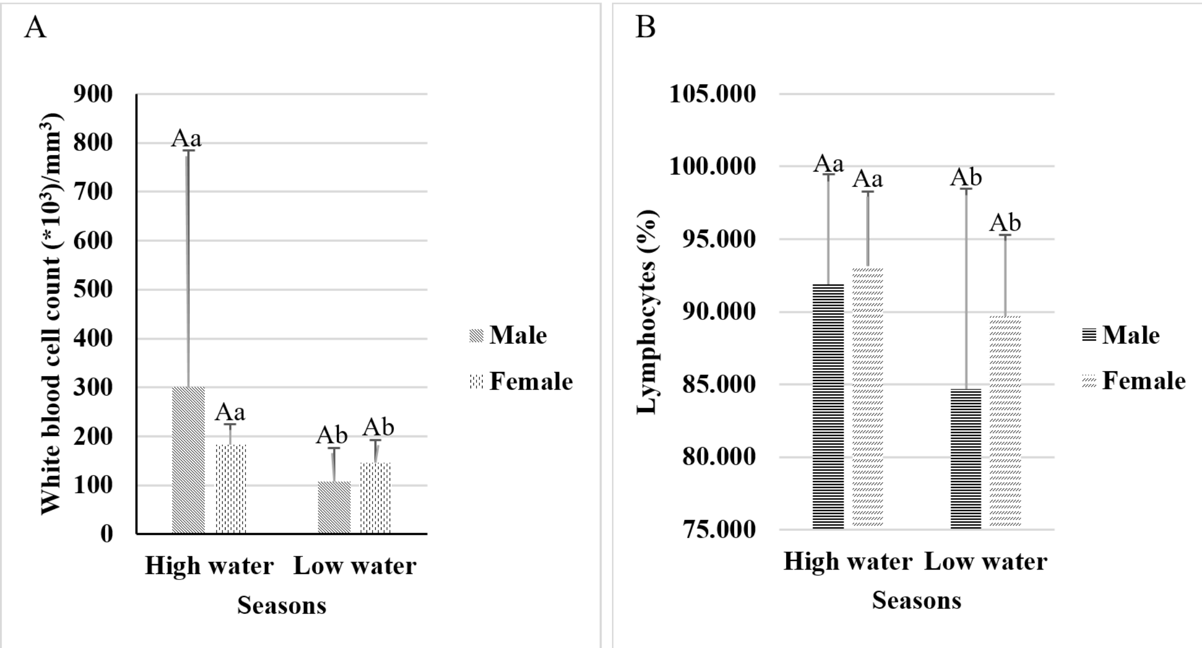


Figure 4. White blood cell (A) and lymphocyte (B) (Upper-case letters indicate a significant difference between sexes, lower-case letters a difference between seasons)

Table 2. MCHC, MCH and MCV levels

	High water		Low-water	
	Male	Female	Male	Female
MCHC (%)	33,18 ± 5,19 Aa	36,46 ± 8,02 Aa	37,9 ± 12,41 Aa	35,41 ± 11,21 Aa
MCH (%)	44,59 ± 6,79 Aa	46,77 ± 9,01 Aa	47,18 ± 9,94 Aa	44,33 ± 10,58 Aa
MCV (μm^3)	136,99 ± 14,56 Aa	129,41 ± 12,86 Aa	129,6 ± 22,46 Aa	128,25 ± 19,71 Aa

"The capital letters indicate the significant difference between the sexes, and the small letters the difference between the seasons".

Table 3. Neutrophils, eosinophils and monocytes

	High water		Low-water	
	Male	Female	Male	Female
Neurophils (%)	4,25 ± 6,01 Aa	2,75 ± 1,55 Aa	6,25 ± 10,23 Aa	4,4 ± 3,06 Aa
Eosinophils (%)	0,7 ± 1,26 Aa	0,65 ± 1,38 Aa	1,25 ± 1,20 Aa	1 ± 0,72 Aa
Monocytes (%)	3,3 ± 3,57 Aa	3,45 ± 3,06 Aa	7,8 ± 5,78 Ab	4,9 ± 3,65 Ab

"The capital letters indicate the significant difference between the sexes, and the small letters the difference between the seasons".

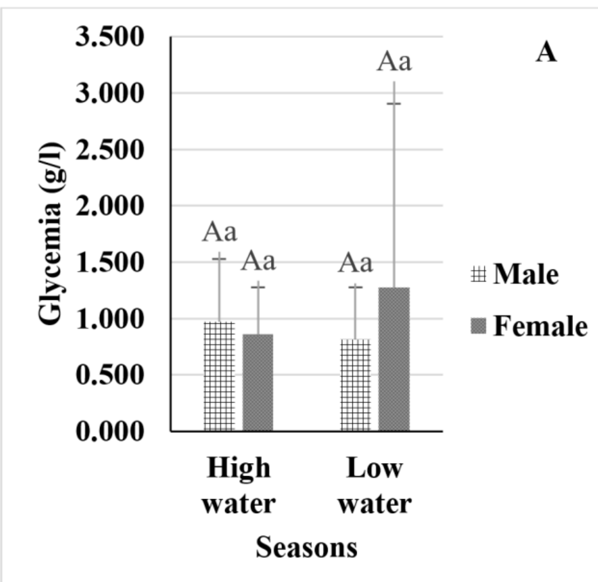
Table 4. Correlation between lead, cadmium and hematological parameters

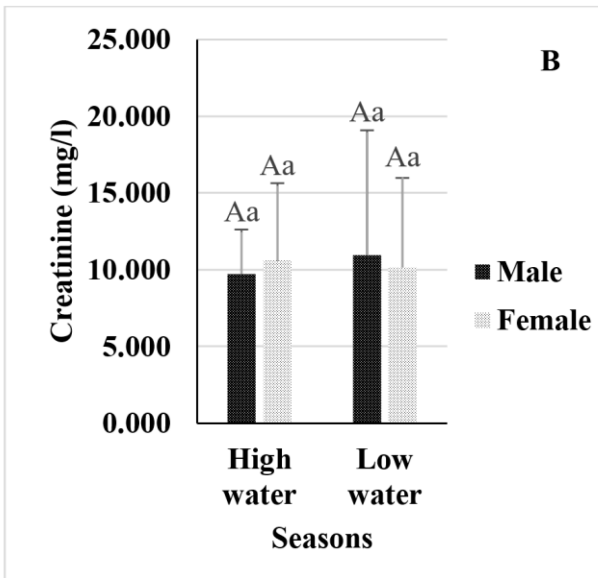
Variables	r and p	Cadmium	Lead	Red blood cells	Hemoglobin	Hematocrit	Lymphocytes
Cadmium	r	1					
	p	0					
Lead	r	0,41	1				
	p	0,72	0				
White blood cells	r	-0,45	**-0,99**	-0,97			
	p	0,70	**0,02**	0,15			
Hemoglobin	r	-0,40	**-1,00**	-0,95	1		
	p	0,73	**0,01**	0,18	0		
Hematocrit	r	-0,60	-0,97	-0,99	0,97	1	
	p	0,58	0,14	0,03	0,15	0	
Lymphocytes	r	0,44	**-1,00**	0,96	-0,99	-0,98	1
	p	0,71	**0,015**	0,16	0,02	0,12	0

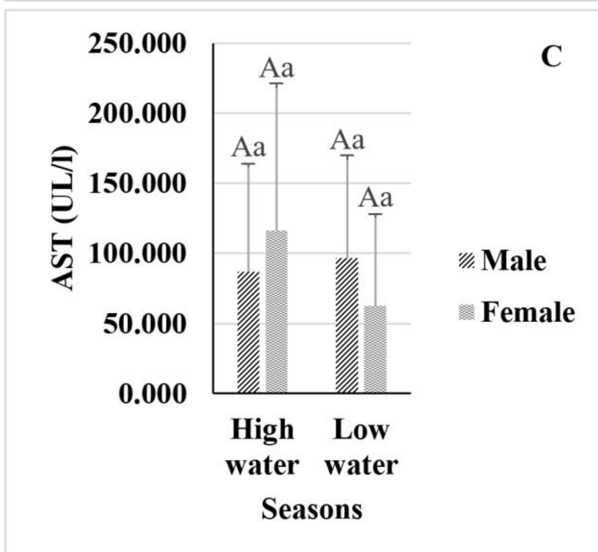
"r" represents the coefficients of determination and 'p' represents p-values

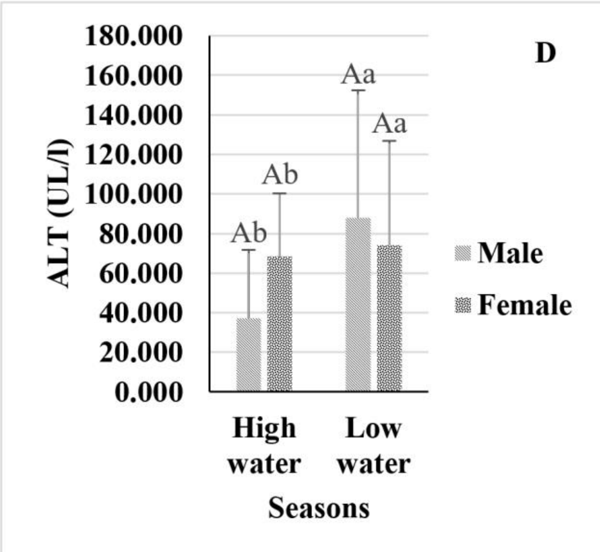
3.3. Effect of Water Quality on Fish Liver and Kidney Function

Blood glucose, creatinine and transaminase (ALT and AST) levels are expressed as mean plus or minus standard deviation (Figure 5). In both seasons, blood glucose, creatinine and transaminases (AST, ALT) ranged from 0.85 ±0.41 to 10.95 g/L±8.10; 9.71 ±2.88 to 10.95 ±8.10 mg/L; 62.66 ±65.39 to 116.5 ±105.01 IU/L; 37.29 ±34.51 to 88.08 ± 64.42 IU/L, respectively. For glycemia, creatinine and ASAT transaminase, there was no significant difference between seasons or sexes ($p > 0.05$). However, ALAT transaminase was significantly higher during the low-water period than during the high-water period for both sexes ($p = 0.008$).









Upper-case letters indicate a significant difference between sexes, lower-case letters a difference between seasons.

Figure 5. Glycemia (A), creatinine (B) and transaminase (C et D) levels

3.4. Liver and Gonad Histology

3.4.1. Biometric Parameters (K, HSI and GSI)

Values for condition factor K, Hepatosomatic Index (HSI) and Gonado-Somatic Index (GSI) are expressed as mean ± standard deviation (Table 5). Condition factor significantly lowers during low water than during high water (p = 0.002). As for the GSI, it is lower during high-water than during low-water. Also, the GSI of males is significantly lower than that of females (p = 0.0001). There were no significant differences in HSI between seasons or gender. However, the results show a tendency for the HSI to decrease during high-water.

The correlation matrix between heavy metals and biometric parameters shows a negative correlation between lead and cadmium and these parameters (Table 6). This shows that increasing the concentration of these metals in fish causes a decrease in K, GSI and HSI. This correlation is significant between Cadmium and HSI (p = 0.04).

3.4.2. Liver Histology

Prevalence of liver alterations

Seven alterations were identified in the fish livers, classified as regressive changes and circulatory disorders (Table 7, Figures 6 and 7). In fact, fish presented more liver damage during high-water than during low-water. But the dominant alterations were melano-macrophagic centers and hepatocyte hypertrophy.

Table 5. Biometric parameters

	High-water		Low-water	
	Male	Femelle	Male	Femelle
IGS (%)	0,26 ±0,25 Bb	1,91 ±3,32 Aa	0,29 ±0,24 Ba	7,60 ±6,15 Ab
HSI (%)	1,56 ±2,38 Aa	1,23 ±2,02 Aa	0,98 ±0,65 Aa	2,21 ±1,89 Aa
K	2,39 ±1,62 Aa	1,90 ±0,22 Aa	1,59 ±0,42 Ab	1,48 ±0,32 Ab

"The capital letters indicate the significant difference between the sexes, and the small letters the difference between the seasons".

Table 6. Correlation matrix between lead, cadmium and biometric parameters

Paramètres	r et p	Cd	Lead	K	HSI	GSI
Cadmium	r	1				
	p	0				
Lead	r	0,418	1			
	p	0,725	0			
K	r	-0,916	-0,019	1		
	p	0,262	0,988	0		
HSI	r	**-0,998**	-0,356	0,941	1	
	p	**0,043**	0,768	0,220	0	
GSI	r	- 0,959	-0,659	0,765	0,938	1
	p	0,183	0,768	0,446	0,226	0

"r" represents the coefficients of determination and 'p' represents p-values

Table 7. Prevalence of liver damage

Alterations	Prevalence (%)			
	High-water		Low-water	
	Male	Female	Male	Female
Congestion	70	50	80	70
Sinusoidal dilatation	30	10	50	20
Vacuolization	30	40	20	40
Melano Macrophagic Center	100	40	100	80
Necrosis	30	30	70	60
Loss of configuration	50	30	50	40
Hypertrophy of nuclei	100	80	70	90

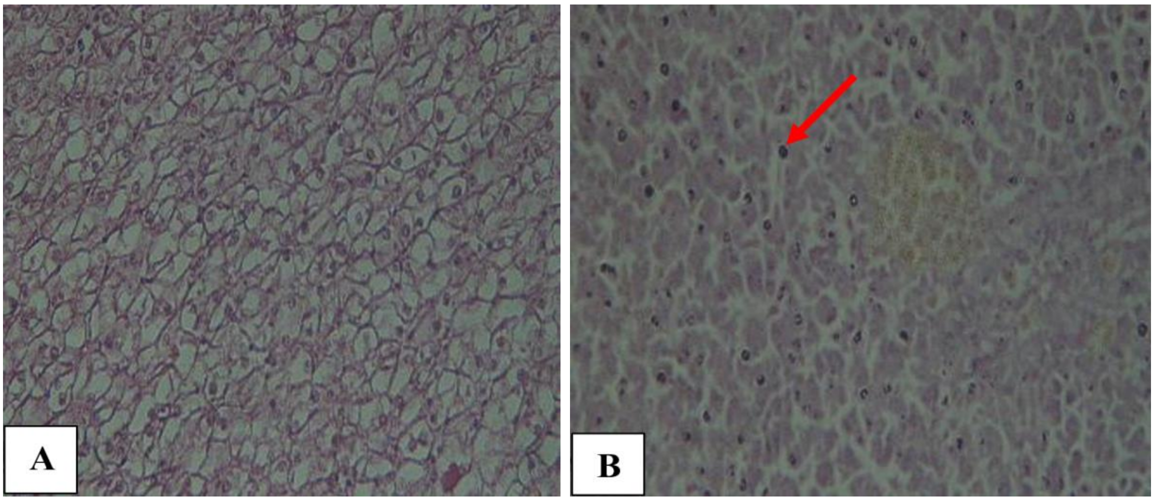
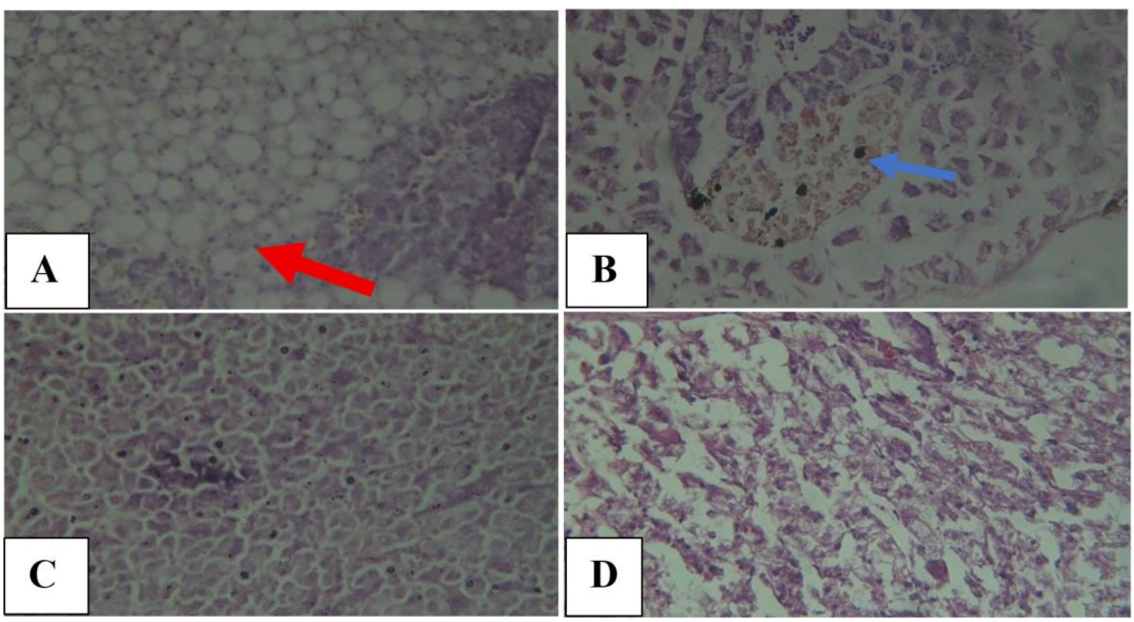


Figure 6. Normal liver structure in a fish (A) and nuclear hypertrophy (B); magnification 40X



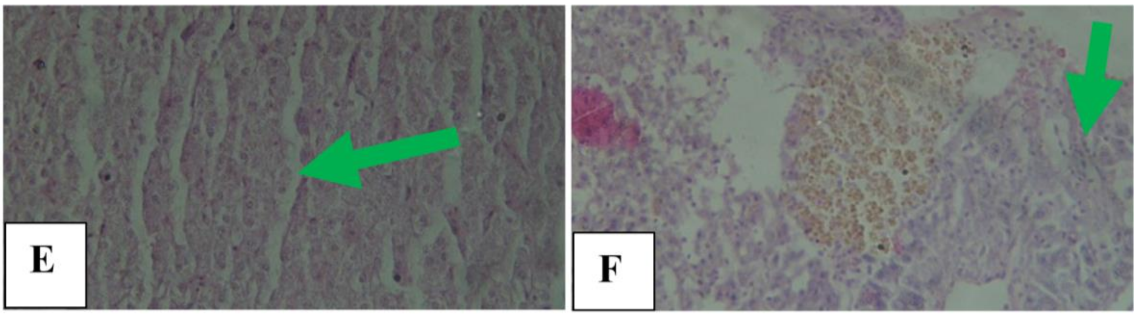
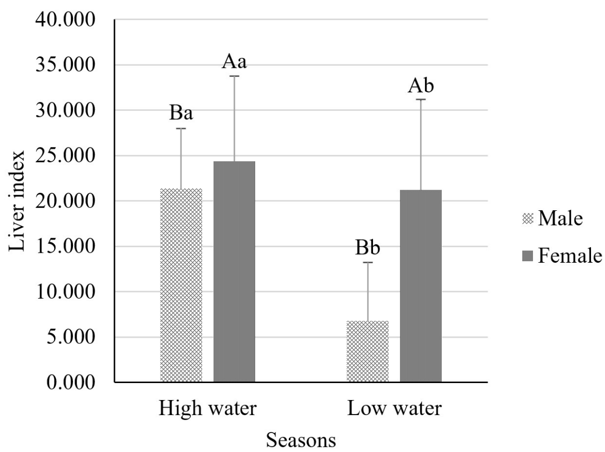


Figure 7. Histopathological alterations in the liver; A: vacuoles (Red arrow); B: melano-macrophagic centers (blue arrow); C: congestion; D: loss of lamellar configuration; E: Hepatic sinusoidal dilatation (green arrow); F: Necrosis (green arrow); 40X magnification

Histopathological indexes of the liver

Average histopathological indexes are classified according to the severity of alterations (Figure 8). Liver histopathological indexes were higher during high water than during low water (P = 0.002). Similarly, the livers of females were more affected than those of males. Also, the histopathological indexes of the liver of the high water are all class 2 (organ with histological structure with moderate alterations).



Upper-case letters indicate a significant difference between sexes, lower-case letters a difference between seasons.

Figure 8. Liver histopathology index

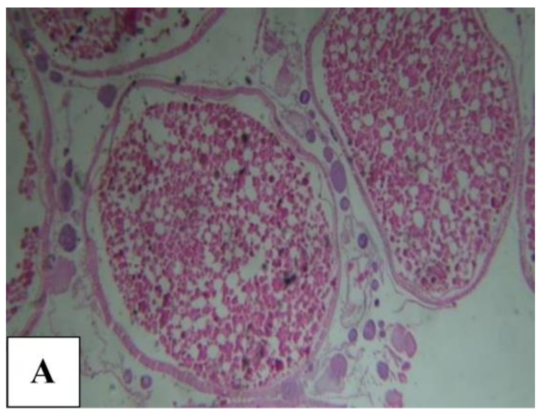
3.4.3. Histology of the Gonads

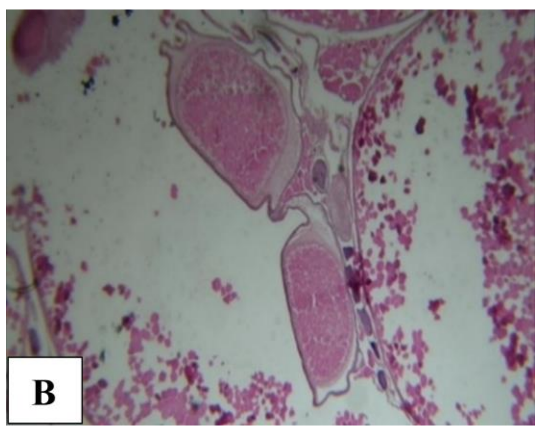
Prevalence of gonadal alterations

Six alterations were identified in the ovaries and five in the testes during both high water and low-water (Table 8 and Figures 9 and 10). These alterations are classified as regressive changes. In fact, 90% of high-water and 70% of low-water females have pre-ovulatory atretic follicles, and 90% of ovaries in all seasons are degenerating. The results always show that there is more alteration during high-water than during low-water.

Table 8. Prevalence of gonadal alterations

Alterations	**Prevalence (%)**			
	High-water		**High-water**	
	Male	**Female**	**Male**	**Female**
Necrosis	70	40	70	70
Cytoplasmic membrane detachment	-	20	-	10
CMM	70	90	90	60
Lobular disorganization	30	-	30	-
Vacuolization	10	50	30	10
Pre-ovulatory atretic follicle	-	90	-	70





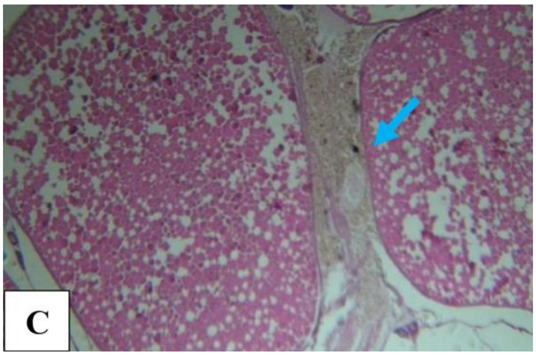


Figure 9. Histopathological changes in the ovaries. A: normal ovarian structure; B: preovulatory atretic follicles; C: melano-macrophagic centers. 40X magnification

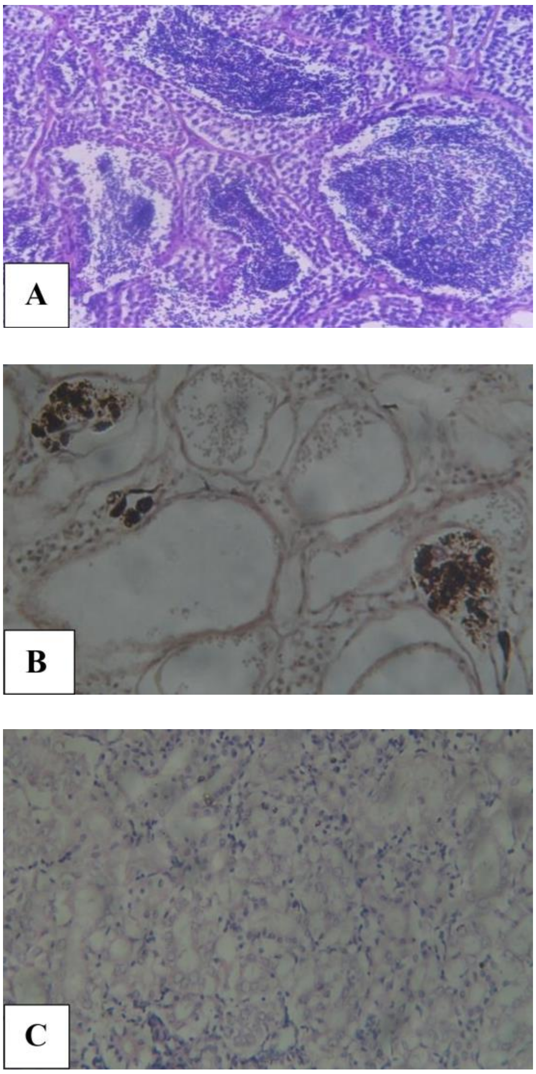


Figure 10. Histopathological changes in the testicular. A: normal testicular structure; B: melano-macrophagic centers and cellular necrosis; C: lobular disorganization. 40X magnification

Histopathological indexes of the gonads

Average histopathological indexes were classified according to the severity of alterations (Figure 11). Ovarian indexes were higher than testicular indexes, but this difference was not significant. The gonad indexes for the flood and low-water periods are class 2 (organ histologically with moderate alterations).

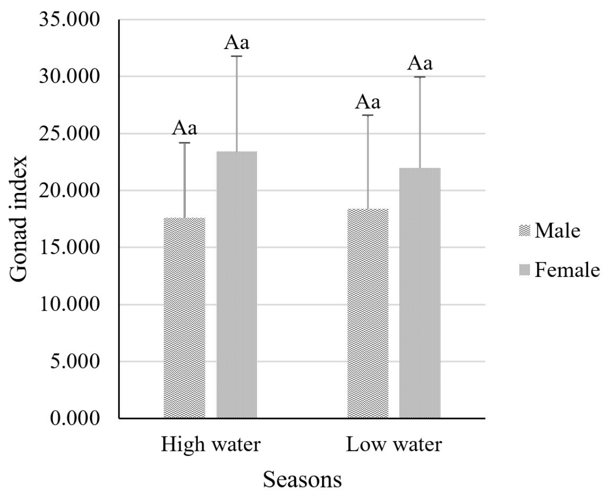


Figure 11. Histopathological index of the gonads. (uppercase letters indicate the significant difference between sexes, lowercase letters the difference between seasons)

Gonadal development stages

For male gonads, all stages of testicular development are present (Figure 12). Stage 6 (sperm) rates are highest during high-water. From the first to the fifth instar, no significant differences were observed between seasons. But the last stage (spermatozoa) is significantly higher during high water than during low-water (p = 0.0001).

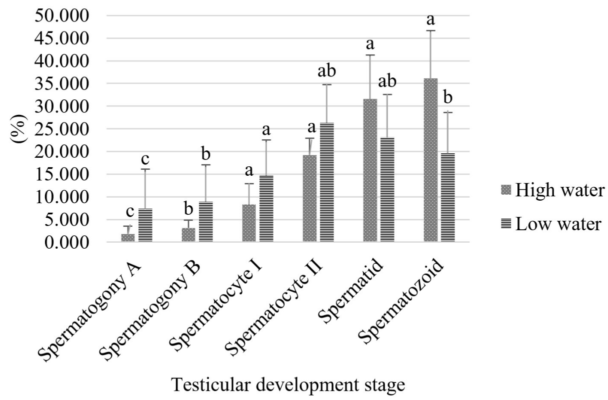


Figure 12. Stages of Testis development

In the ovaries, all stages of oocyte development were present (Figure 13). The rate for stage 1 (protoplasmic oocyte) was the highest. From the second to the sixth stage, no significant differences were observed between seasons. But the first stage (protoplasmic oocyte) was significantly higher during high-water than during low-water (p = 0.025). The low proportion of stage 6 oocytes confirms the high prevalence of degenerating follicles observed. In fact, very few cells reach normal maturity.

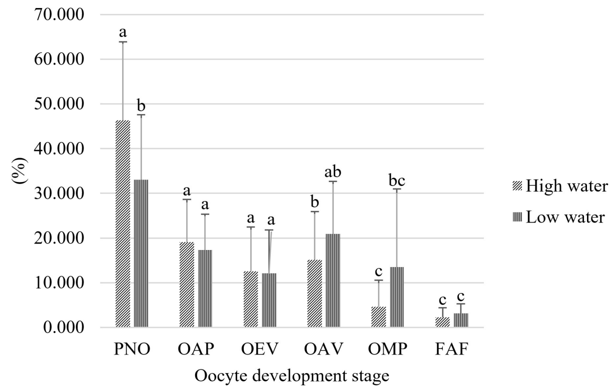


Figure 13. Developmental stages of the ovaries. PNO: Peri-nuclear oocyte, OAP: Oocyte in alveolar phase, OEV: Oocyte in early vitellogenesis, OAV: Oocyte in advanced vitellogenesis, OMP: Oocyte in maturation phase, PAF: Postovulatory atretic follicle

Total fish histopathological index

The total histopathological indexes of the fish (Figure 14) are class 3 and class 4; organs with histological structures show pronounced and severe alterations. Fish indexes for high water are significantly higher than those for low water (P = 0.007). Similarly, female fish indexes are significantly higher than male fish indexes during high water than during low water (P = 0.0001). The histological state of the fish is therefore more affected during high water than low water, particularly in female fish.

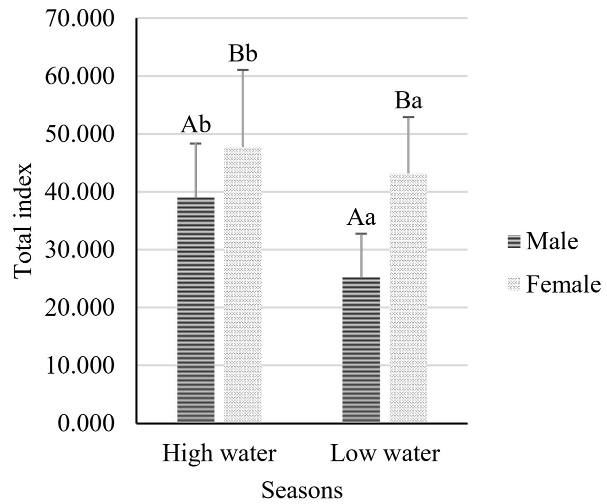


Figure 14. Total histopathological index of fish

4. Discussion

4.1. Pollution of the Complex with Toxic Metals and the Vital Functions of Fish

4.1.1. Lead and Cadmium Concentrations in Fish

The high concentration of lead (12.65 ± 11.63 mg/Kg) and cadmium (0.14 ± 0.07 mg/Kg) in *Sarotherodon melanotheron* fish from this complex during the low-water period (Figure 2) exceeds the WHO consumption limit (Cd: 0.05 mg/kg; Pb: 0.2 mg/kg). These concentrations found in this species of fish in the complex exceed those found by several authors who evaluated the same species in the complex [11,12,18,19,20,21]. These values are also higher than those found by Nakweti *et al.* [22] in the Congo River in *Oreochromis niloticus* and *Clarias gariepinus*. The high concentration of these metals in fish during flooding suggests that they are present in the water during high water, but their accumulation in the bodies of fish manifested the water during low water. Houédjissi et al. [23] showed that the concentration of lead and cadmium assessed at the same sites of the complex during the same period during high water and low water in water and sediment is higher in water (0.106 ± 0.116 mg/l and 0.010 ± 0.001 mg/l for lead and cadmium, respectively) during high water. These results indicate significant pollution of the complex, representing a serious threat to the survival and renewal of this species. Biodiversity is at risk, as the bioaccumulation of these heavy metals has toxic effects on aquatic life [14].

4.1.2. Effect of Water Quality on Fish Vital Functions

Hematological Parameters

Hemoglobin and hematocrit levels for both seasons were 7.32 ± 1.89 to 9.39 ± 2.74 g/dL and 21.64 ± 8.83 to 28.95 ± 9.10% respectively. These values are lower than those found by Adakole [24] in a river polluted by heavy metals. Low levels of these parameters were recorded during the low-water period. For fish of the cichlid family reared in good condition, hemoglobin and hematocrit levels are between 8.5 and 9.7 g/dL and 24 to 27% respectively [25]. This shows that during this period, fish are more exposed to environmental stresses that can affect their health. The values obtained in the present study during low-water are lower than those obtained by Kouassi et al. [25]. Hemoglobin is a protein that transports oxygen from the gills to the cells in the fish's organism, and hematocrit corresponds to the volume of red blood cells in the blood relative to the total volume of blood in the organism. Hemoglobin depletion is due to the destruction or reduced synthesis of hemoglobin in the blood. This directly indicates anemia in the fish. These alterations may be the result of microbial or chemical pollution, as microbes and chemical pollutants are capable of causing anaemia in fish [26]. It has been proven that Lake Nokoué - Porto-Novo lagoon - is heavily polluted with organic, chemical and microbial pollutants [6]. During the low-water, fish accumulated a high concentration of lead (Figure 2). The negative correlation between lead concentration in fish and hemoglobin (Table 4) shows that in this ecosystem, these pollutants are among those that affect the blood system of fish. Abdel-warith *et al.* [26] demonstrated that lead accumulation by *Clarias gariepinus* fish led to a significant reduction in hemoglobin and hematocrit. Exposure to lead causes structural damage to red blood cell membranes, leading to hemolysis, destruction of hemoglobin structures, and stimulation of hypoxia [27].

As for the fishes' immune systems, the low levels of white blood cells and lymphocytes (Figure 4 C and D) were recorded during the low-water period, the same period when the fishes accumulate more lead. These values are lower than those found by Fafioye et al. [28] in an ecosystem polluted with heavy metals, particularly lead, cadmium, and zinc. The negative correlation between lead concentration in fish, white blood cell counts and lymphocytes could explain a negative influence of lead on the fish's defense system. Al-Asgah *et al.* [29] have shown that chronic exposure of fish to lead causes destruction of white blood cells and lymphocytes. Indeed, several authors have shown that lead induces hematological dysfunction in fish [14]. These fish face numerous aggressors due to the high level of pollution in the ecosystem [6]. Monocytes, on the other hand, are low during high-water (Table 3). Whatever the season, the fish in this ecosystem are exposed to numerous stresses. This low monocyte count during high water could be caused by an aggression that particularly influences these cells during high-water, as rain drains all kinds of microbes and pollutants into the complex.

Hematology, used as a biomarker in this study, highlighted the effects of pollutants, particularly heavy metals, in this ecosystem on fish physiology. The destruction of erythrocytes or inhibition of erythropoiesis and decreased leukocytosis therefore indicate systemic stress.

Biochemical Parameters

The hematological results obtained in this study show systemic stress in fish under the effects of pollutants. These results were confirmed by biochemical results, particularly blood sugar, creatinine, and transaminases (ALT and AST), as the organs highlighted (kidneys and liver) are hematopoietic organs. Blood glucose, creatinine and the enzymes aspartate aminotransferase (AST) transaminase (Figure 5 A, B, C and D) show no significant difference between seasons and sex. However, the values observed show that the blood sugar levels of fish from Lake Nokoué-Porto-Novo lagune are higher than those recorded in the same species of fish from Lake Toho, which has a low level of toxic metal pollution [30]. These observed values are close to those obtained by Authman et al. [31] on fish sampled from a heavy metal-polluted watercourse in Egypt. Fish are exposed to environmental stress secrete glucocorticoids and catecholamines from adrenal tissues, which raise blood sugar levels [30]. This could explain the high blood glucose levels observed in the fish. The same observations were done by Agbohessi et al. [30] in the same ecosystem with the same species of fish. Creatinine is a chemical waste produced by the muscle in the blood during these activities and is eliminated by the kidneys [32]. Kidney function is assessed by creatinemia. A comparison of creatinine levels in *S. melanotheron* from the Lake Nokoué - Porto-Novo lagoon complex with those from Lake Toho found by Agbohessi et al. [30], shows that fish from this ecosystem have very high creatinine levels. This means that the kidneys of these contaminated fish are not functioning properly. Elarabany & Bahnasawy [33] have shown that cadmium induces kidney dysfunction in *Clarias gariepinus*.

The enzymes ALT and AST values recorded in fish from this ecosystem (Figure 4 C and D) exceed those observed in the same species from Lake Toho [30]. These values exceed those obtained by several authors in waterways polluted by heavy metals [31]. The enzymes ALT and AST are biomarkers used to assess the effects of toxic products on the liver [33]. Toxic metals affect the liver, causing hyperproduction of the AST and ALT enzymes in the blood [29]. Whatever the season, these AST and ALT values show that the livers of fish from Lake Nokoué Porto-Novo lagoon are being damaged.

These biochemical alterations indicate that the liver, kidneys, and muscles are impaired. In fish, the anterior kidney is the main hematopoietic organ, producing red blood cells (erythropoiesis), white blood cells (granulopoiesis, lymphopoiesis), and platelets, followed by the liver, which also participates in embryonic hematopoiesis [34].

4.2. Effects of Water Quality on Liver and Gonad Histology

The histology used as a biomarker in this study is the structural analysis of an affected hematopoietic organ (the liver) that has been demonstrated by biochemistry and reproductive organs that highlight the reproductive functions of fish.

4.2.1. The Condition Factor K

High K values during high-water indicate overweight fish. Freshet is a period of abundant food for fish, when they are well nourished by organic matter that decomposes to increase seedling production. The low value of K during low-water indicates that the condition of the fish is affected. During low-water, the K is significantly low, which is justified by the fact that the concentration of toxic metals in fish is very high during this period, as toxic metals affect fish health [14]. The same observation was made by Okito *et al.* [35] in *O. niloticus* caught in the Ulindi and Elila rivers in DR Congo, polluted with toxic metals. The negative correlation observed between metals and K (Table 6) already shows that the condition of fish is affected by these pollutants. Several studies have shown that pollutants in this complex have affected the health of the fish living there [12].

4.2.2. Liver Alterations

The HSI obtained shows no significant difference between seasons and sex (Table 5), but a downward trend was observed during the high-water. However, these values are lower than those obtained by Agbohessi et al. [12] in Lake Toho, which is less polluted by heavy metals. They are also lower than those found by Okito *et al.* [35] Ulindi and Elila rivers in Congo. These same authors showed that the decrease in the HSI index is linked to severe liver alteration. This suggests that during high water, the concentration of pollutants is higher and could cause histopathological alterations in the livers of exposed fish. Houédjissi et al. [23] showed that the concentration of lead and cadmium in Lake Nokoué Porto-Novo lagoon is higher in the water during high water. This potential contamination did not result in a statistically significant difference in HSI between seasons. It is possible that pollutant concentrations during high water levels are not sufficient to induce alterations that could significantly reduce liver weight. Nevertheless, there is a downward trend in HSI during high water. This could be related to the histological sensitivity of fish to pollutants in the water. The negative correlation between cadmium and HSI also shows that fish have a histopathological sensitivity to the presence of these metals in the ecosystem. During low water periods, the HSI index is not as low despite high accumulation, which may indicate that fish have developed resistance to metal accumulation. The prevalence of liver alterations shows that there are more alterations during high water periods than during low water periods (Table 7). This confirms the low HSI index value obtained during periods of high water. Cadmium also correlates significantly negatively with the HSI index (Table 6), while cadmium concentrations in fish are high during periods of high water.

Highly prevalent alterations found were hepatocyte hypertrophy, Melano Macrophagic Center (MMC), congestion and necrosis. The same observations were made by Agbohessi et al. [12] in the same ecosystem. The liver is the main organ involved in the metabolism, detoxification and excretion of toxic substances, and hepatocyte hypertrophy is associated with increased liver detoxification activity [36]. Melano-macrophage centers are distinct aggregates of cells containing a variety of intracellular pigments (melanin, lipofuscin, hemosiderin) making them easily recognizable by light microscopy and widely used to assess the exposure of organisms to pollutants according to their prevalence [37]. They are present in all the tissues assessed, showing that the fish are highly contaminated. The fish's inability to regenerate a new liver after cell degeneration may also have resulted in necrosis [38]. This explains the large number of necrosis observed in the fish. The congestion found, with such a high prevalence rate in fish liver (whatever the season) indicates a disturbance in blood circulation that may result from environmental stresses of exposure to pollutants: trace metals, pesticides, hydrocarbons etc., and from infection or heart disease. All these alterations were observed by Okito *et al.* [35] in the Ulindi and Elila rivers in the Democratic Republic of Congo, also polluted with heavy metals during high-water. The histopathology index of the livers (Figure 8) shows that this organ is more affected during high-water, as the values are higher during high water and all organs are class 2, meaning organs with moderate alterations, whereas organs during low-water are class 1 and some class 2.

4.2.3. Alterations in the Gonads

As with the HSI observations, the mean GSI values of the high-water females are apparently low compared with those of the low-water females. This decrease in the gonado-somatic index during high-water can be explained by the presence of histopathological alterations in the gonads [40]. The same observation has been made by several authors in Oreochromis niloticus collected from the Ulindi and Elila rivers in eastern DR Congo [35]. In contrast, the GSI of males is significantly higher during high water than during low water. An increase in GSI in males has been reported in *Clarias gariepinus* in a heavy metal-polluted treatment basin in Tranzani [39]. The GSI values observed during high water are lower than those found by Agbohessi et al. [12] in Lake Toho in Benin and those found by Elarabany & Bahnasawy [33] in the Tranzani purifier basin. These low indices observed during the high water period could therefore be explained by much more serious histopathological abnormalities during the high water period, when pollutants are more prevalent in the water. The high prevalence of alterations during high-water (Table 8) confirms the low GSI values. Alterations with a high prevalence are CMM, necrosis and preovulatory atretic follicles. Preovulatory atretic follicles (Figure 9 B), identified in female gonads, are alterations linked to endocrine disruptors [38]. They were present in 90% of the ovaries studied during the high-water. The effects of toxic metals present in the environment could be one of the causes of the alterations identified. Several authors have shown that toxic metals are endocrine disruptors [31]. The necrosis identified in the testicles is the destruction of sex cells (spermatogonia, spermatids and spermatozoa) and these same observations have been made by other authors in this ecosystem [11]. The inability of the gonads to regenerate after the death of sex cells under the effect of pollutants leaves this necrosis observed in the gonads.

The developmental stages of the testes and ovaries (Figures 12 and 13) suggest that the testes and ovaries are undergoing normal development, as all stages are present. However, it should be noted that the sperm count was significantly higher during high-water than during low-water (Figure 12), and in the ovaries, the protoplasmic oocyte count was higher during high-water than during low-water in fish of the same size. This shows that gonadal cell development is disrupted during high-water, as all the alterations in the gonads that show the effect of endocrine disruptors are in high proportion during high-water. Also, the total histopathological index (Figure 14) of male and female fish is significantly high (classes 3 and 4) during high-water, with organs with pronounced and severe alteration. All this confirms that gonadal cells development is disrupted during the high-water season. This season is a period when the spillage of petroleum products, particularly leaded gasoline, is greatest, as boats are often capsized. All this could lead to physiological disruption of organisms. High sperm production is a sign of excessive production of Testosterone (T) and 11-Ketotestosterone (11-KT), hormones that control testicular cell development [40,41]. Toxic metals are capable of producing androgenic effects resulting in excessive male hormone production, or anti-androgenic effects inhibiting the production of T and 11-KT hormones, or estrogenic effects causing elevated estrogen levels in the blood, or antiestrogenic effects inducing a decrease in 17β-estradiol (E2) [38]. So, the high protoplasmic oocyte count could be an anti-oestrogenic effect of pollutants that could slow development during this season, or an oestrogenic effect that could underlie the high proportion of pre-ovulatory atretic follicles. If oocytes do not follow a normal development pattern, then it is obvious that not all stages are identifiable, which could explain the high proportion of the first stage (protoplasmic oocytes). A sex hormone assay could better explain and confirm these results.

The various biomarkers used in this study highlighted the effects of pollutants, particularly heavy metals, at different levels of biological organization: functional, metabolic, and structural. The degradation or inhibition of erythrocytes and low leukocytosis rates indicate general stress, which was confirmed by biochemical and histological analyses. Hyperglycemia and increased AST, ALT, and creatinine levels reflect the metabolic state of the fish and the functionality of their internal organs. These biochemical alterations indicate that the liver, kidneys, and muscles are affected. The histological alterations identified in the liver reveal one of the origins of the biochemical and blood disorders. The histology of the gonads also shows that the reproduction of these fish is disrupted. Measuring sex hormones would be important to better explain the effect of endocrine disruptors. These physiological impacts could compromise the renewal of local fish stocks. Regulating catches and fishing effort is not sufficient for sustainable fisheries management; it is essential to integrate measures to protect the quality of aquatic habitats and reduce pollutants.

5. Conclusions

Pollution of Lake Nokoué - Porto-Novo lagoon poses a serious threat to biodiversity, particularly fish populations. Reproductive organs, essential to the survival and renewal of species, are severely affected. The various alterations identified are mostly regressive, so if a plan to limit and clean up the complex is implemented, may be reversed over time.

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Conflict of Interest

We have no conflicts of interest.

Authors' Contributions

- Gisèle HOUEDJISSI: Conceptualization and conservation of data and Writing - original draft;
- Maxime Machioud SANGARE-OUMAR: Validation, Writing – revision, Supervision;
- Prudencio AGBOHESSI: Writing - editing;
- Mahugnon Alexis Bienvenu HOUNDJI: Writing - editing;
- Eric GLODJO: Visualization;
- Zoubérou Aboudou ABOUDOU : Data collection ;
- Jean-Michel AKPO: Monitoring and data collection;
- Condé-Abalou Roland BAMAÏ: Monitoring and data collection;

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