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A comparative evaluation of the effects of pollution on selected organs of *Oreochromis niloticus* (Linnaeus, 1758) from two subtropical lakes with different pollution levels in Zimbabwe: Histopathological comparison

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ABSTRACT

Zimbabwean aquatic systems are threatened by pollution. The objective of the study was to compare the impacts of pollution in Lake Kariba (Sanyati basin) and Lake Chivero. Water samples were collected in June, July and August 2018, while *Oreochromis niloticus* were sampled in August 2018. In Lake Kariba, the physicochemical parameters and micronutrients were within acceptable limits, while the organs of fish showed mild histological alterations. The mild histological alterations serve as an early warning sign of the water quality of the Sanyati basin, particularly with the pollutants continuously entering the lake. Poor water quality parameters, such as high phosphorous, ammonia and low dissolved oxygen recorded in Lake Chivero resulted in a higher prevalence of histological alterations in the fish, some of which are irreversible. These observations indicate that Lake Chivero is more impacted than Lake Kariba. In addition, it was noted that most of the histological alterations observed in Lake Chivero are linked to high organic loads. With pollutants continuously pouring into Lake Chivero, the water will soon be unsuitable for a large number of aquatic organisms. Therefore, regular monitoring and the assessment of the lake are strongly recommended to preserve the aquatic biodiversity.

INTRODUCTION

Water is becoming a scarce commodity in most parts of the world due to the increase in population growth **(UNDP, 2006)**. The development of human communities, industrialization, the use of fertilizers and increase in the irresponsible use of water resources have deteriorated river and lake water qualities **(Sanchez & Manuel, 2007)**. However, the availability of good quality water is an indispensable feature for preventing diseases and improving the quality of life **(Patil *et al.*, 2012)**. Therefore, monitoring and controlling surface water is critical to guarantee high quality water for various applications **(Bollinger *et al.*, 1999)**.

In aquatic ecosystems, fish are regarded as a valuable indicator of environmental pollution since they are at the top of the aquatic food chain that accumulate toxicants **(Authman *et al.*, 2012)**. A variety of approaches have been used to evaluate the effects of stress on the health of fish populations **(Adams *et al.*, 1993)**. Most are necropsy-based, although some of the more commonly used approaches are based on age and growth analysis, condition factor **(Busacker *et al.*, 1990)**, organosomatic indices **(Goede & Barton, 1990)**, numerous measures of biochemical, physiological and pathological conditions **(Niimi, 1990)**. Of these approaches, histopathological investigation of fish tissue provides early warning signs of disease and contributes to understand the nature of stress responses. **Ayas *et al.* (2007)** stated that, histopathological conditions are fast and efficient for the detection of acute and chronic adverse effects in fish and may express the health condition of exposed fish.

Environmental stress in fish triggers the hypothalamic-pituitary-inter-renal axis, which helps fish to adapt to environmental change **(Mommsen *et al.*, 1999)**. This mechanism causes an increase in adrenocorticotrophic hormone (ACTH) and cortisol, which alter fish metabolism and physiology, and if the stressor is chronic, changes in organ morphology occur thereby inducing a number of lesions in different organs **(Mallatt, 1985; Harper & Wolf, 2009)**. The gills, liver and kidneys are the primary markers for aquatic pollution **(Bernet *et al.*, 1999)**. Furthermore, physiological evaluation through somatic indexes such as condition factor (CF) is indicative of metabolic fish condition that increases the information about water quality **(Schibatta, 2005)**. In addition, by using a scoring system, it is possible to quantify histopathological changes and correlate them with the level of environmental stressors **(Bernet *et al.*, 1999)**.

The lakes of Kariba and Chivero are two important lakes in Zimbabwe since they form vital sources of fish, which provide cheap protein and a source of livelihoods for many families. *Oreochromis niloticus* is the common fish species in both lakes. A once off study by **Mabika & Barson (2013)** reported mild histological lesions on the gills of *O. niloticus* in the Sanyati basin, Lake Kariba. However, it is important to note that domestic effluents from municipal sewers, as well as crocodile and fish farm effluents are finding their way into the waters of the Sanyati basin of Lake Kariba. Furthermore, boating and tourism, hydropower generation, among others contribute heavy metal, persistent organochlorines and chemical pollution to the basin, and these are potential pollutants to the lake water. Lake Chivero, situated about 35 km downstream of Harare (capital city of Zimbabwe), is hypereutrophic **(Moyo, 1997)**. The major sources of pollution include urban runoff, sediments, sewage effluents, industrial effluents and leachate from land filled areas along the river banks within its catchment **(Mathuthu *et al.*, 1997; Zaranyika, 1997)**. The high levels of heavy metals in water, sediment and fish tissues were recorded in Lake Chivero and within its catchment **(Zaranyika, 1997; Nhiwatiwa *et al.*, 2011)**. **Utete *et al.* (2019)** compared the histological alterations of *O. niloticus* between Lake Chivero and Lake Manyame (which are 15 km apart and lie on the same river). The authors reported similar histological alterations since the two lakes are subjected to similar sources of pollution.

In the current study, Lake Kariba and Lake Chivero are located in two different agro climatic zones and are subjected to different sources of pollution and climate patterns. The objective of the study was therefore to carry out a physicochemical and semi-quantitative comparison of the histological alterations of selective organs of *O. niloticus* between Lake Kariba (Sanyati basin) and Lake Chivero.

MATERIALS AND METHODS

Study areas

Lake Kariba and Lake Chivero are located about 400km apart. *Orechromis niloticus* is heavily fished from both lakes. Lake Chivero is Harare's main water supply and is located to the southwest of the city. It was constructed over two-and-a-half years and opened to the public in 1952 **(Zimbabwe National Water Authority, 2014)**. The lake is 16 km long and its shoreline is approximately 48 km. The lake area holds about 250 000 million litres of water and is approximately 26 km^2 (2632 ha). At its widest point the lake stretches for 8 km. Lake Kariba was built in 1958 to provide hydro-electrical power to Zimbabwe and Zambia. It covers 5100 km^2 and forms more than 200 km of the border between Zimbabwe and Zambia **(Chenje, 2000)**. The lake is 320km long, 40 km in maximum width and a surface area at average water level of 5 250 km^2. The Sanyati basin occupies about one fifth of the total lake area and drains a large proportion of Zimbabwe's farming areas **(Balon & Coche, 1974)**. Three sampling sites were chosen from each lake.

Water quality parameters

Temperature, pH, conductivity and dissolved oxygen (DO) and percentage oxygen saturation were measured in the field once a month for three months (June, July, August 2018) in both lakes. Water temperature, DO and oxygen saturation were measured using a HACH oxygen 330i meter, while pH and conductivity were measured using a HACH pH meter and WTW 330i conductivity meter, respectively. Transparency was measured by a sechhi disc. Subsurface water samples from the different points in the basins were collected in pre-cleaned glass bottles. They were analyzed for the following micronutrients in the laboratory; total phosphorous, total nitrogen, nitrate and ammonia. These micronutrients were measured with a HACH water analysis kit (HACH DR/2010portable data logging spectrophotometer) using filtered water samples filtered through 0.45µm Millipore sized Whatman GF 47 mm filters, except in the case of total nitrogen and phosphorus. Water samples were analyzed in triplicate for each nutrient in order to improve the accuracy of results **(Utete *et al.*, 2013)**.

Fish sampling

Sixty fish specimens (20 per site) of *O. niloticus* were collected by seine netting from Lake Chivero during the first week of August 2018. In the third week of August 2018, 60 fish specimens (20 per site) were collected by seine netting from the Sanyati basin of Lake Kariba. In both samplings, fish specimens were sampled from the same sites where water samples were collected. Fish samples were transported to the respective laboratories in aerated tanks full of lake water. In the laboratory, they were killed by dorsally severing them just behind the head through the spinal cord. They were weighed and their sizes were determined by measuring both total length and standard lengths. For each individual, about 1 cm piece of the gill, liver and kidney were excised.

Histological processing

The tissue specimens were immediately fixed in 10% buffered formalin for 48 hours after removal. The fixed tissues were dehydrated and infiltrated with molten wax on a processing machine. They were embedded in paraffin wax, sectioned into 5 µm slices by a rotary microtome and stained using haematoxylin and eosin **(Bancroft & Gamble, 2008)**. Specimens were coded before being processed for histological examination. Slide examination and scoring were done blindly. The sections were examined and photographed using a Leica Leitz Labor Lux microscope fitted with a camera (Leica DX 0.32X) and an automatic light exposure unit (Leica Orthomate E).

Histological analysis

The prevalence of each lesion was calculated following **Cuevas *et al.* (2016)** as; **[(number of cases) total cases analysis) * 100]**. A scoring system proposed by **Bernet *et al.* (1999)** was applied for the assessment of pathological changes in fish that were caused by different degrees of pollution. According to this method, pathological changes are classified into four reaction patterns, namely; circulatory, regressive, progressive and inflammatory. An importance factor ranging from 1 (minimal alteration) to 3 (marked importance) was assigned to each alteration, determining the relevance of a lesion and its pathological importance. 1) = minimal pathological importance, the lesion is easily reversible as toxicant exposure ends; (2) = moderate pathological importance, the lesion is reversible in most cases if the stressor is neutralized and (3) = marked pathological importance; the lesion is generally irreversible, leading to partial or total loss of the organ function. The importance factor reflects the ability of the alteration to be reversible following removal of the stressor. For instance, degenerative alterations (general necrosis) are given the highest importance factor (three). Most proliferative alterations are given an importance factor of two. The circulatory (altered blood flow) alterations were given an importance factor of one **(Agamy, 2012)**.

Depending on the degree and extent of lesions, a score value ranging from 0 (unchanged) to 6 (severe occurrence) was determined following **Bernet *et al.* (1999)**; (0) no histological alteration; (2) mild histological alteration; (4) moderate alteration and (6) severe alterations (diffuse). The final value for each alteration results from the multiplication of the score value by the importance factor. Summing up these final values for each reaction pattern (the index for circulatory disturbance (IC), the index for regressive changes (IR), the index for progressive changes (IP) and inflammatory index (II) gives the organ index (OI). The mean organ index was calculated for *O. niloticus* from each lake. Organ index values were used to classify the severity of histological response using classes based on the scoring scheme proposed by **Zimmerli *et al.* (2007)**; Class I (index ≤ 10)—normal tissue structure with slight histological alterations; Class II (index 11–20)—normal tissue structure with moderate histological alterations; Class III (index 21–30)—moderate modifications of normal tissue; Class IV (index 31–40)—pronounced histological alterations of the organ; Class V (index N>40)—severe histological alterations of the organ. The sum of the multiplied score values and importance factors of all diagnosed changes for each specimen resulting in different indices were statistically analyzed. The sum of the three organ indices per fish yielded a total fish index value indicating the combined histological response of the sampled organs for that individual fish. A mean fish index was then calculated for the total sample group from both lakes.

Statistical analysis

The condition factor (CF) was estimated stepping the method of **Pauly (1983)** as follows:

$$CF = W * 100/L^3$$

Where, **CF** is the condition factor;

W is the total weight of the fish (g), and

L is the standard length of the fish (cm)

Comparisons of water quality parameters and the histological scheme of **Bernet *et al.* (1999)** between the two lakes were calculated using the non-parametric Mann-Whitney-U test. A level of probability p<0.05 was considered statistically significant.

RESULTS

1. Water quality parameters

The physicochemical variables for the two lakes are shown in Table (1). Conductivity was significantly higher (p<0.05) in Lake Chivero compared to Lake Kariba, while percentage oxygen saturation, dissolved oxygen, temperature and transparency were significantly higher (p<0.05) in Lake Kariba than in Lake Chivero. Both lakes recorded an alkaline pH which was statistically insignificant (p>0.05).

2. Micronutrients and condition factor

All micronutrients in Lake Chivero were significantly higher (p<0.05) than those in Lake Kariba (Table 2), with phosphorous levels higher by a factor of about 32, followed by ammonia with a factor of about 20. The mean CF of fish was higher in Lake Kariba than in Lake Chivero, and this difference was statistically significant (p<0.05).

Table 1. Water quality parameters for Lake Kariba and Lake Chivero

Parameter	Lake Kariba	Lake Chivero
pH	7.84±1.66	8.10±0.17*
Conductivity	88.76±18.89	517.48±25.98
% Oxygen	89.45±20.04	50.11±2.01
DO	6.84±1.61	4.55±0.27
Temp. (°C)	25.93±5.67	20.36±0.48
Transparency	1.93±0.64	0.82±.027

* Denotes statistically insignificant between lakes (Manny Whitney U test) P<0.05

Table 2. Micronutrients and the condition factor of *Oreochromis niloticus* for Lake Kariba and Lake Chivero

Micronutrient	Lake Kariba	Lake Chivero
Ammonia	55.70±47.14	1092.39±419.56
Total P	25.55±8.41	812.69±82.41
Nitrates	33.40±29.04	227.90±68.08
Total N	202.92±230	1320.50±385.74
Condition factor	2.093±0.616	1.868±0.171

3. Prevalence and severity of the histological alterations

The histological alterations were grouped into four reaction patterns; namely, circulatory disturbances, regressive changes, progressive changes and inflammation (Table 3).

Table 3. Prevalence and severity of histopathological lesions (%) of *Oreochromis niloticus* in Lake Kariba and Lake Chivero

Reaction pattern	Lesion	Lake Karana		Lake Chivero	
		No	Severity	No	Severity
Gills					
Circulatory disturbances	Hyperemia	7	Mild	30	Moderate
	Aneurysm	10	Mild	53	Severe
	Oedema	7	Mild	30	Mild
Regressive changes	Epithelial lifting	52	Mild	63	Moderate
	Curving of secondary lamellae	0	Nil	20	Mild
	Globate tips on secondary lamellae	0	Nil	13	Mild
	Eosinophilic fragments	0	Nil	7	Mild
Progressive changes	Hyperplasia	23	Moderate	73	Severe
	Secondary lamella fusion	17	Mild	60	Severe
Inflammation	Inflammatory cell infiltration	7	Mild	30	Moderate
Liver					
Circulatory disturbances	Hyperemia	10	Mild	47	Moderate
	Vascular congestion	0	Nil	40	Moderate
Regressive changes	Necrosis (karyolysis)	10	Mild	43	Moderate
	Clear hepatocytes	0	Nil	20	Mild
	Hemosiderin deposits	0	Nil	57	Severe
	Vacuolation	3	Mild	47	Moderate
	Melano macrophage aggregates	3	Mild	53	Moderate
	Disintegration of parenchyma	0	Nil	43	Moderate
Progressive changes	Nil	0	Nil	0	Nil
Inflammatory	Inflammatory cell infiltration	17	Mild	53	Moderate
Kidney					
Circulatory disturbances	Hyperemia	10	Mild	37	Mild
Regressive changes	Melano macrophage aggregates	3	Mild	27	Mild
Progressive changes	Nil	0	Nil	0	Nil
Inflammatory	Inflammatory cell infiltration	7	Mild	23	Moderate

4. Histological alterations in gills

Circulatory disturbances observed in fish specimens from both lakes were hyperemia (Fig. 1A), aneurysm (Fig. 1B) and edema (Fig. 1C). These alterations were mild in Lake Kariba, while in Lake Chivero they were moderate, severe and mild, respectively. The prevalence of these histological alterations was higher in fish from Lake Chivero than in Lake Kariba (Table 3). Regressive changes observed in Lake Chivero were epithelial lifting (moderate) (Fig.1E), curving of secondary lamellar (mild) (Fig. 1D), globate tips at the end of the secondary lamellae (mild) (Fig. 1C) and eosinophilic fragments (mild) (Fig. 1F). In Lake Kariba, mild epithelial lifting was the only regressive change observed. The prevalence of epithelial lifting in Lake Chivero was higher than in Lake Kariba. Progressive changes in both lakes were hyperplasia (Fig. 1B) and fusion of secondary lamellar (Fig. 1A). These changes were severe in Lake Chivero. Compared to Lake Kariba, the prevalence of these lesions was higher in Lake Chivero. Inflammatory cell infiltration (Fig. 1D) was mild in Lake Kariba and moderate in Lake Chivero. The prevalence of inflammatory cell infiltration was higher in Lake Chivero compared to that in Lake Kariba.

5. Histological alterations in liver

Two circulatory disturbances hyperemia (moderate) (Fig. 2A) and vascular congestion (moderate) (Fig. 2B) were recorded in fish from Lake Chivero. Whereas in Lake Kariba, the only circulatory disturbance was hyperemia (mild), whose prevalence was lower than that from Lake Chivero. Regressive changes observed in Lake Chivero included necrosis (moderate) (Fig. 2D), clear hepatocytes (mild) (Fig. 2B), hemosiderin deposits (severe) (Fig. 2C), vacuolation (moderate) (Fig. 2D), melanomacrophage aggregates (moderate) (Fig. 2A) and disintegration of parenchyma tissue (moderate) (Fig. 2E). In Lake Kariba, three mild regressive changes; namely, necrosis, vacuolation and melanomacrophage aggregates were observed, whose prevalences were lower than those from Lake Chivero. No progressive changes were observed in fish from both lakes. Inflammatory cell infiltration (Fig. 2C) was mild in Lake Kariba while moderate in Lake Chivero. This histological alteration had a higher prevalence in Lake Chivero than in Lake Kariba.

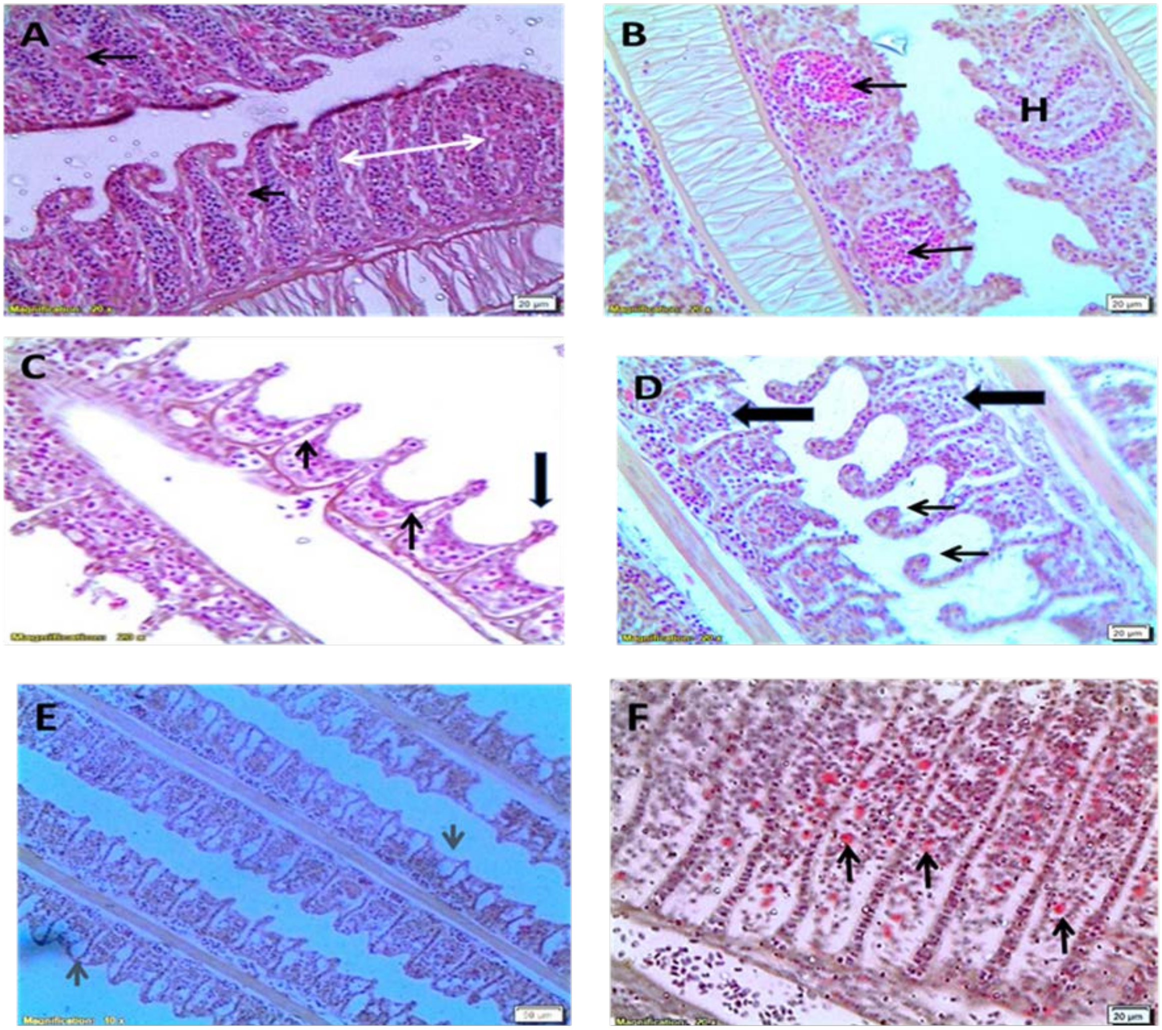

Fig 1. Micrographs of *O niloticus* gill from Lake Chivero stained with H&E. A) Hyperemia(black arrow) and lamellar fusion(double white arrow) 400X; (B) Aneurysm (arrow) and hyperplasia (**H**) 400X; (C) Edema (thin arrow) and globate tip of secondary lamellar (thick arrow) 400X; (D) Inflammatory cell infiltration(thick arrow) and curving of secondary lamellar (thin arrow) 400X; (E) Epithelial lifting (arrow) 200X; (F) Eosinophilic fragments (black arrows) 200X

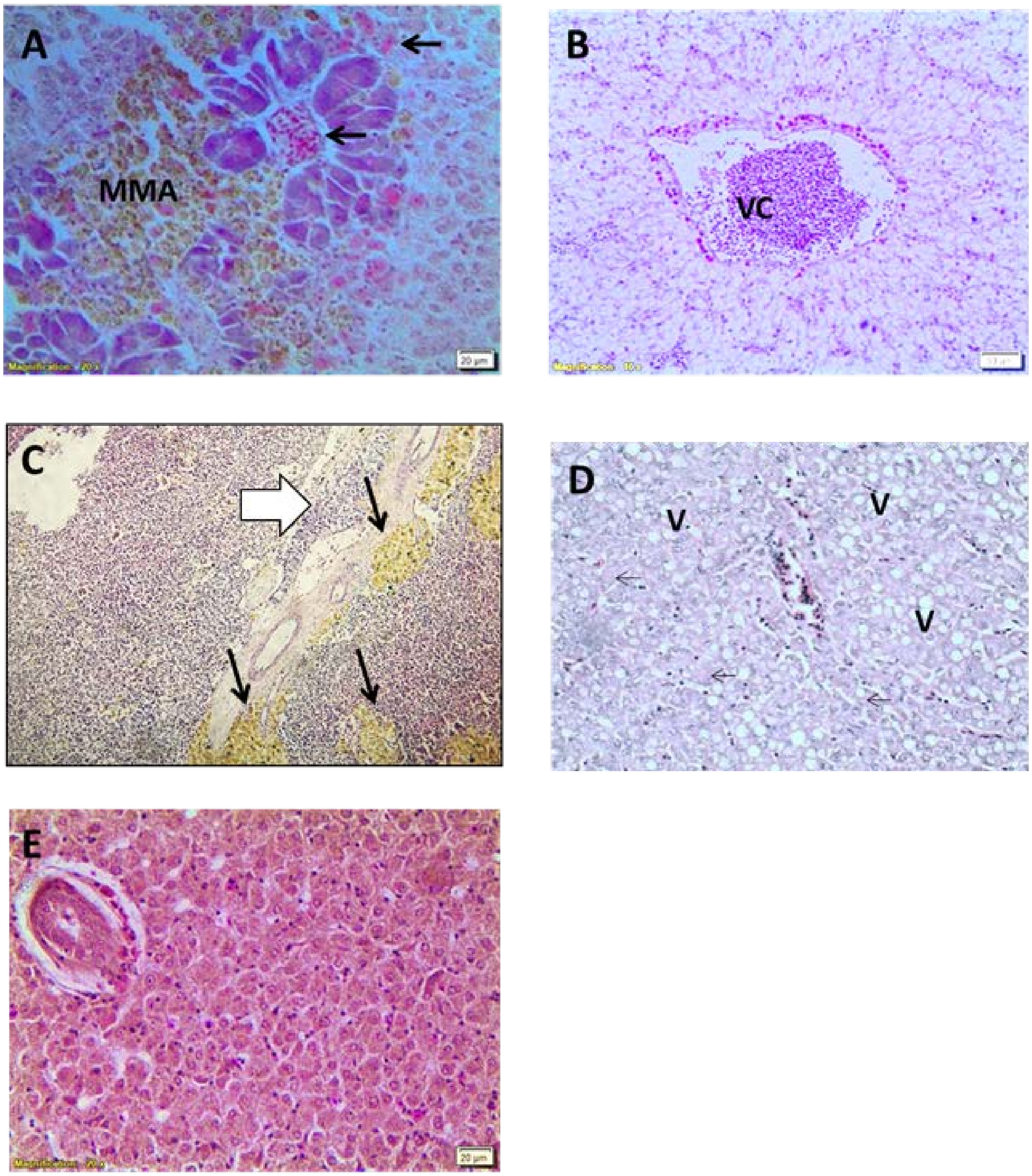

Fig 2. Micrographs of *O niloticus* liver from Lake Chivero stained with H&E. (A) Hyperemia (arrow) and melanomacrophage aggregates (**MMA**) 400X; (B) Vascular congestion (**VC**) and clear hepatocytes 400X; (C) Inflammatory cell infiltration(thick white arrow) and hemosiderin deposits (black arrows) 400X; (D) Vacuolation (**V**) and necrosis (arrows) 200X; (E) Parenchyma disintegration 400X

6. Histological alterations in kidney

Fish from both lakes had a single circulatory disturbance; hyperemia (Fig. 3A) which was mild in both lakes. The prevalence of hyperemia was higher in Lake Chivero than in Lake Kariba. Melanomacrophage aggregates (Fig. 3B) were the only regressive change observed in both lakes. This lesion was also mild in both lakes. The prevalence of melanomacrophage aggregates was higher in Lake Chivero than in Lake Kariba. No progressive changes were observed from both lakes. Inflammatory cell infiltration (Fig. 3B) was mild in Lake Kariba and moderate in Lake Chivero. The prevalence of inflammatory cell infiltration was higher in Lake Chivero than in Lake Kariba.

7. Organ indices

Results in Table (4) present the organ indices and their classification according to **Zimmerli *et al*. (2007)**. The gill index for Lake Chivero fish was higher (41.41±13.94) than that of Lake Kariba (18.20±4.65). This difference was statistically significant (p<0.05). Gills from Lake Chivero fell within Class V, while those from Lake Kariba fell within Class II. The liver index for Lake Chivero (35.67±14.37), which fell within Class IV was significantly higher (p<0.05) than that of Lake Kariba (7.83±2.63), which fell within Class I. The kidney index for Lake Chivero (7.73±1.44) was slightly higher than that of Lake Chivero (7.43±1.91). The difference was statistically insignificant (p>0.05). Kidneys from both lakes fell within Class I. The fish mean index for Lake Chivero (84.13±24.12) was significantly higher (p<0.05) than that of Lake Kariba (33.73±11.40).

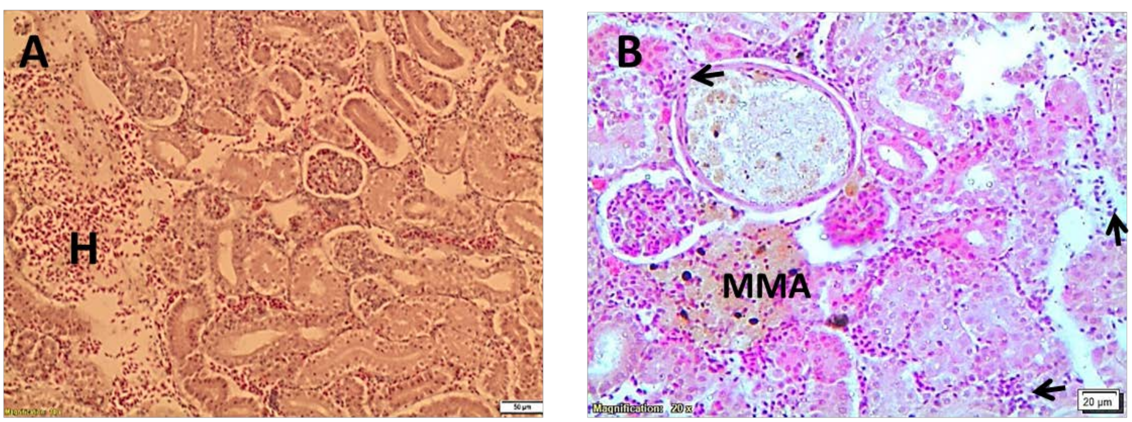

Fig 3. Micrographs of *O niloticus* kidney from Lake Chivero stained with H&E. (A) Hyperemia(**H**) 200X; (B) Melanomacrophage aggregates(**MMA**) and inflammatory cell infiltration (arrows) 400X

Table 4. Mean organ indices of *Oreochromis niloticus* and their classification according to **Zimmerli *et al*. (2007)**

Variable	Lake Kariba	Classification	Lake Chivero	Classification	*P*-value
Gill index	18.20±4.65	Class II	41.41±13.94	Class V	0.00001
Liver index	7.83±2.63	Class 1	35.67±14.37	Class IV	0.00001
Kidney index	7.43±1.91	Class I	7.73±1.44	Class 1	0.187*
Mean fish index	33.73±11.40		84.13±24.12		0.00001

*Not significant

DISCUSSION

It was observed that the water quality assessment values of micronutrients that increase with the increase in pollutants were higher in Lake Chivero than those observed from Lake Kariba water. For example, the levels of phosphorous (812.69±82.41 mg/L) in Lake Chivero were about 32 times higher than those from Lake Kariba. This high level of nutrients, especially phosphorus, supports growth of different phytoplankton and macrophyte species, including water hyacinth which depletes the water body of DO as was observed in Lake Chivero. The concentration of DO regulates the distribution of flora and fauna in the waterbody. In the present study, the mean concentrations of DO were 6.84±1.61 mg/L and 4.55±0.27 mg/L in Lake Kariba and Lake Chivero, respectively. **USEPA (1998)** defined the healthy water value of DO within the range of 5−14.6 mg/L, and less than 5 or greater than 14.6 indicates the impairment of the water body. Based on this view, Lake Kariba water had normal oxygen levels, while very low DO in Lake Chivero indicates the impairment of the water body. The low levels of DO may be a source of physiological disturbances in Lake Chivero that could be associated with some morphological damages observed for fish in the lake **(Alberto *et al.*, 2003)**. Ammonia concentration exceeding 2.0 mg/L can cause pathological changes in gills, liver and kidney of fish **(Ip *et al.*, 2001; Bakar *et al.*, 2016)**. Extremely high levels of ammonia were recorded in Lake Chivero, and these levels could probably contribute to the severe histological alterations observed in Lake Chivero fish.

Pollutants such as sewage, pesticides and agricultural run-off have been reported to find their way into the Sanyati basin **(Berg, 1995)**. Consequently, we expected high levels of the water quality parameters assessed. However, results observed in the study suggest that the levels of these pollutants seem to be overwhelmed by the dilution effect of the lake. On the other hand, we expected higher temperatures in Lake Chivero than in Lake Kariba because of the high organic load in the former, but this was not the case. The higher temperature in Lake Kariba could be attributed to the differences in agro-ecological zones as Lake Kariba is in climatic Region V, while Lake Chivero is in region II. Region II is cooler and receives more rainfall than Region V, which is hot and experiences high temperatures. However, the water temperatures recorded in both lakes were normal for metabolic activities of organisms such as fish **(Boyd & Lichtkoppler, 1979)**. The pH value for both lakes was in the alkaline region. We expected this to be lower in Lake Chivero due to high levels organic matter entering the lake. The effect of the organic load could probably be neutralized by some alkaline pollutants in the lake. This observation could probably indicate that there are more alkaline than acidic pollutants in both lakes. Further investigations are recommended to confirm this. However, pH below 4.8 and above 9.2 is deleterious for aquatic organisms, especially for fish **(Sharifinia *et al.*, 2019)**. Therefore, based on the observed results from both lakes, it can be confirmed that the pH conditions were suitable for fish growth during that period.

High nitrate content (>1 mg/L) is not conducive for aquatic life **(Murdoch *et al.*, 2001)**. Nonetheless in unpolluted waters, the level of nitrate-nitrogen is usually less than 0.1 mg/L **(Chapman, 1996)**. The nitrate values recorded from both lakes, particularly in Lake Chivero indicates poor biotic conditions for aquatic life. However, nitrate is generally considered nontoxic to fishes **(Bromage *et al.*, 1988)**. The conductivity of water is a measure of capacity of a solution to conduct electrical current through it, depending on the concentration of ions and load of nutrients **(Srivastava *et al.*, 2012)**. Conductivity was 88.76±18.89 mg/L and 517.48±25.98 mg/L in Lake Kariba and Lake Chivero, respectively. According to **Dumont (1999)**, species number decreases in water with high conductivity. The high conductivity observed in Lake Chivero had the potential to decimate species diversity and also damage the fish's organs particularly the external gills **(Patil *et al.*, 2012)**.

The CF is an estimation of the general well-being of fish and is based on the hypothesis that heavier individuals of a given length are in better condition than less weight fish **(Bagenal & Tesch, 1978; Oni *et al.*, 1983)**. Consequently, CF decreases with the increase in length **(Fagade, 1979)** and is influenced by age of the fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development **(Pauly, 1983)**. Alterations in growth rates are induced by contaminants, and the explanation is the reallocation of energy to detoxification mechanisms, depleting reserves that were originally destined for growth **(Nicholson & Lan, 2005)**. The CF for fish from Lake Kariba and Lake Chivero were 2.093±0.616 and 1.868±0.171, respectively. **Ogamba & Abowei (2012)** reported that, the CF values of 1.60 and above indicates excellent condition. Therefore, based on the CFs observed from both lakes, *O. niloticus* is physiologically adapted to both lakes. This observation indicates that some parameters were not conducive for aquatic animal health. On the other hand, some parameters such as temperature and pH were conducive for aquatic life. This confirms the ability of *O. niloticus* to tolerate a broad ecological spectrum of extreme environmental conditions.

The gills are among the most sensitive vulnerable organs of the teleost fish because of their external location and intimate contact of their large surface area with the external environment **(Perry & Laurent, 1993)**. Subsequently, the presence of toxic substances in an aquatic environment causes alterations in the vital functions carried out by the gills and alterations in the morphologic structure of these organs. Three circulatory disturbances, including hyperemia, aneurysm and edema were observed in both lakes. However, these histological alterations were more prevalent and severe in Lake Chivero than in Lake Kariba. Because of the low levels of DO observed in Lake Chivero, blood flow in fish gills was much higher, and this might explain the higher value of gill circulatory changes in fish from Lake Chivero than Lake Kariba **(Booth, 1979)**. Epithelial lifting represents one of the most common alterations in the studies of fish gill histopathology **(Pandey *et al.*, 2008)**. This was confirmed in this study, as epithelial lifting was the common regressive change in both lakes though more prevalent and severe in Lake Chivero. This alteration may serve as a defence mechanism by increasing the pollutant blood diffusion pathway, across which contaminants must diffuse to reach the blood stream, thus interfering with gill respiration and thereby impairing oxygen uptake **(Fernandes & Mazon, 2003)**. Besides epithelial lifting, other regressive changes observed in Lake Chivero included curving of secondary lamella, globate tips on secondary lamella and eosinophilic fragments. These extra regressive changes in Lake Chivero may suggest higher level of pollutants or a diversity of pollutants entering into the Lake Chivero compared to Lake Kariba.

Hyperplasia is a common alteration in gill histopathology studies and represents the fastest and easiest adaptations to low water quality, with the purpose of decreasing the respiratory surface and increasing diffusion distance **(Mallat, 1985; Sollid & Nilsson, 2006)**. In extreme cases, hyperplasia leads to lamellar fusion, and thus the increased thickness of epithelium prevents further chemical absorption. Hyperplasia of gills was more prevalent and severe in Lake Chivero than in Lake Kariba. This progressive alteration is usually associated with a number of factors, including parasitism and exposure to urban sewage **(Huggett *et al.*, 1992)**. In Lake Chivero, this condition may be attributed to sewage exposure; whereas in Lake Kariba, this might have been caused by parasite infection. Additionally, the infiltration of inflammatory cells observed in both lakes was more prevalent and severe in Lake Chivero than in Lake Kariba. Fish leucocytes are part of its non-specific cellular defense, performing functions, such as phagocytosis and phagocyte killing, and the presence of leucocytes clearly indicates an inflammatory reaction and reflects an immunological response to environmental conditions of both lakes **(Yancheva *et al.*, 2019)**. Consequently, due to the severity of pollutants in Lake Chivero, inflammatory cells were more prevalent in fish from Lake Chivero compared to Lake Kariba. Based on the scoring scheme proposed by **Zimmerli *et al.* (2007)**, gills in Lake Kariba fell within Class II (index 11-20), indicating that they are normal with moderate histological alterations. Gills in Lake Kariba fell within Class V, suggesting that the organ had severe histological alterations.

Unlike gills, the liver is protected from physical exposure to the external environment **(Harper & Wolf, 2009)**. The observed circulatory disturbances in Lake Chivero were hyperemia and vascular congestion, and both were moderate. In Lake Kariba, hyperemia (mild) was the only circulatory alteration observed. **Schmidt *et al.* (1999)** postulated that, vascular changes are frequently reported in fish exposed to organic contaminants. In the current study, the high levels of organic loads in Lake Chivero could be attributed to the frequent vascular congestion observed. This observation agrees with that of **Raskovic *et al.* (2013)** who considered that, circulatory disturbances are in general frequently observed lesions in fish exposed to organic contaminants. The observed congestion was probably an attempt to increase blood flow to the stressed hepatocytes. However, the observed circulatory alterations such as altered blood flow are easily reversible and do not alter the normal functioning of the tissue **(Agamy, 2012)**. Regressive changes were more common and severe in Lake Chivero than in Lake Kariba. In Lake Kariba mild alterations in the form of necrosis, vacuolation and melanomacrophage aggregates were observed. In Lake Chivero, all the regressive changes (necrosis, clear hepatocytes, vacuolation, melanomacrophage aggregates and disintegration of liver prarenchyma) were moderate with the exception of hemosiderin deposits that were severe. Regressive alterations such necrosis and disintegration of parenchyma observed in the study are given the highest importance factor since they form a direct effect of toxicants; they are generally irreversible, and their persistence or progression may lead to a partial or total loss of organ function **(Agamy, 2012)**.

Vacuolation recorded in the current study corroborates with the findings of **Marchand *et al.* (2009)** who reported vacuolation in liver of fish from sewage effluent polluted systems. According to **Pacheco & Santos (2002),** increased vacuolization of the hepatocytes is a signal of degenerative process indicating the interference of vacuoles, which acts as a cellular defence mechanism against substances harmful to hepatocytes leading to metabolic damage. Furthermore, **Panepucci *et al.* (2001)** noted that, glycogen acts as the storage of glucose to supply the higher energetic demand occurring in animals under stress. Thus during stress condition, glycogen in the hepatocytes is depleted and consequently leads to vacuolation **(Wilhelm Filho *et al.*, 2001)**. The occurrence of melanomacrophages centers in liver cells is normal **(Agius & Roberts, 2003)**. However, an increase in their size may be a result of environmental stressors as observed in Lake Chivero. In this context, **Marchand *et al.* (2009)** confirmed increased melanomacrophages centers in fish inhabiting systems polluted with sewage effluent. Severe hemosiderin deposits in Lake Chivero suggest high levels of iron in the lake, which may possibly lead to hemochromatosis thereby damaging the liver. Progressive changes in fish liver studies have generally been observed to be low **(Marchand *et al.*, 2012; Van Dyk *et al.*, 2012; Yancheva *et al.*, 2019)**. Moreover, this was confirmed in the current study as no progressive changes where observed in the liver from both lakes. The liver indices for Lake Kariba (7.83±2.63) and Lake Chivero (35.67±14.37) were lower than the gill indices from the same lakes, thus indicating that gills in both lakes were more affected than the liver. This observation contradicts with that of **Yancheva *et al.* (2019)** who considered the liver being more affected than the gills. The high liver index observed in Lake Chivero may indicate that this organ is overloaded, and at the same time it can be an alert for a possible failure of the liver, which would impair the detoxification capacity of the fish. According to the classification of **Zimmerli *et al.* (2007)**, the liver in Lake Kariba fell within Class I (index < 10), indicating that the organ is normal with slight histological alterations. The liver in Lake Chivero fish fell within Class IV (index 31-40), indicating pronounced histological alterations of the organ.

The kidney was the least affected organ in both lakes since relatively few mild alterations (hyperemia, melanomacrophage aggregates and inflammatory cell infiltration) were observed in both lakes. The kidney plays an important part in maintaining a constant internal environment by helping to regulate pH, water and sodium ion concentrations in the blood and tissues **(Thophon *et al.*, 2003)**. However in fish, the majority of nitrogenous wastes are excreted by the gills, and this could probably be the reason why the histopathology of the kidneys did not show sensitivity to the water quality parameters in the current study. However, this finding contradicts with that of **Takashima and Hibiya (1995)** who reported tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman´s space. These contradictions could be possible due to differences in the types and levels of pollutants in the current study compared to those of **Takashima and Hibiya (1995)**. Based on the scoring scheme proposed by **Zimmerli *et al.* (2007**), kidneys in both lakes fell within Class 1(I (index ≤ 10), indicating that the organ is normal with slight histological alterations.

The findings from the study therefore indicate that the gills were the most affected organ in both lakes, indicated by the multi-organ histopathological analysis (gills> liver> kidney). Similar results have been reported in the study of **Rašković *et al.* (2013)** on *Cyprinus carpio*. Nevertheless, our findings do not concur with those of **Costa *et al.* (2013)**, **Cuevas *et al.* (2016)** and **Yancheva *et al.* (2019)** who considered the liver being the most affected organ.

CONCLUSION

In Lake Kariba, the physicochemical parameters were within acceptable limits, while the organs had mild histological alterations. The mild alterations observed in Lake Kariba indicate low level of pollution, and this serves as an early warning sign of the water quality of the Sanyati basin, particularly with the pollutants continuously entering the lake. This calls for routine monitoring of the Sanyati basin. Poor water quality parameters, such as high phosphorous, ammonia and low dissolved oxygen have created a suboptimal environment that is stressful for the fish in Lake Chivero. This has resulted in a higher prevalence of histological alterations, some of which are irreversible in the lake. The fish mean index of Lake Chivero (84.13±24.12) was higher than that of Lake Kariba (33.73± 11.40). These observations indicate that Lake Chivero is more impacted than Lake Kariba. The gills and the liver of fish were the most affected organs in both lakes, and this confirms their reliability as biomarkers for aquatic pollution. It is also important to note that most of the histological alterations observed in Lake Chivero are linked to high organic loads. With pollutants continuously pouring into Lake Chivero, the water will soon become unsuitable for a large number of aquatic organisms. Therefore, a regular monitoring and an assessment of the lake are strongly recommended to preserve the aquatic biodiversity.

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