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Histopathological evaluation of *Oreochromis mossambicus* gills and liver as biomarkers of earthen pond water pollution

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Abstract

Oreochromis mossambicus were sampled from a semi-intensively managed polyculture earthen pond in Bagauda, Nigeria for histopathological changes in the gills and liver as early warning signs of pond water pollution. Pond water was sourced from nearby Bagauda dam through a single 28 inches water pipe. The physicochemical parameters of the earthen pond water were within acceptable limits for the growth and survival of *O. mossambicus*. Although observed histopathological lesions were significantly (p<0.05) higher in the liver than in the gills of sampled fish, lesions were within the normal functioning of respective organs based on the degree of tissue change protocol. The observed lesions indicated low level pollution of the earthen pond water. The result of this preliminary work notwithstanding, there is a need for constant monitoring of the earthen pond water and its water source (Bagauda dam) for pollutants. This is to ensure the continuous well-being and increased productivity of stocked fish within the earthen pond because of the large catchment area being drained by the dam relative to increasing human anthropogenic activities within the area.

Keywords: Earthen pond, Histopathology, *Oreochromis mossambicus*, Polyculture, Semi-intensive management

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Introduction

Aquaculture is arguably the most vibrant sector of the global food system, being the fastest growing sector alongside terrestrial crop and livestock production (Troell *et al*., 2014). This is because of increasing human population and, by implication, increasing demand for cheap protein coupled with declining wild fish stock (Naylor *et al*., 2000). This is in addition to the fact that time of fish harvest can be synchronized to coincide with market demand (Ardill, 1982). Water quality is the important limiting factor in pond fish culture (Edun *et al*., 2014). Water pollution greatly affect aquaculture yield (Akanni & Akinwumi, 2007) hence aquatic pollution has become a problem of great concern worldwide (Dane & Sisman, 2015). Aquatic pollution causes severe morphologic and physiologic alterations in aquatic organisms (Mazon *et al*., 1999), especially in chronic and sub-lethal exposures where structural and functional changes are more frequent than mass mortality (Flores-Lopes & Thomaz, 2011). Morphological examination of fish organs have been used to evaluate effect of pollutants in fish (Poleksic & Mitrovic-Tutundzic, 1994). That is why histopathological assessment of fish allows for early warning signs of disease and detection of long term injury in cells, tissues and organs (Marchand *et al*., 2009) before obvious manifestations.

Tilapia is one of the most important food fish cultured worldwide (Sevilleja, 1985) although these warm water fishes are indigenous to Africa (Popma & Masser, 1999). However, *Oreochromis* spp is reportedly the most important in modern aquaculture (Yadar, 2006), especially *O. mossambicus (*Peters, 1852) that is known for fast growth rate and tolerance of wide range of environmental conditions (Canonico *et al*., 2005). Most times Tilapia is cultured with other fish species to take advantage of many natural foods available in ponds as well as to produce a secondary crop and/or control tilapia recruitment, in addition to improving water quality for enhanced productivity (Rakocy & McGinty, 1989). Although semi-intensive pond management of tilapia aquaculture requires low capital input, it is greatly influenced by environmental conditions (Yakubu *et al*., 2014) hence the need for this study. Reports of histopathological evaluation of toxicants effects in *O. mossambicus* exist (Midhila & Chitra, 2015; Patel *et al*., 2016) but there is dearth of information on histopathological assessment of *O. mossambicus* from semi-intensively managed polyculture earthen pond as early warning signs of pond water pollution. The work therefore, aimed to perform histopathological evaluation of the gills and liver of *O. mossambicus* from a semi-intensively managed polyculture earthen pond in Bagauda, Nigeria in order to ascertain the extent of cellular damage as early warning signs of the earthen pond water pollution.

Materials and Methods

The temperature, pH and dissolved oxygen (DO) content of water samples were determined with conventional clinical mercury-in-glass thermometer submerged in an inclined position about elbow deep, Hanna portable pH meter (Hanna Instruments) and the modified Winkler-Azide method (Lind, 1979; APHA, 1985), respectively within the first four (4) hours of sampling after stabilizing each sample with Manganous sulphate. Biological oxygen demand and ammonia contents of the pond water were measured with HI83200 multiparameters photometer for laboratories (Hanna Instruments). The study earthen pond, with a surface area of 1.83 acres, belonged to Kano state Ministry of Agriculture and Natural Resources fish seed multiplication centre, Bagauda, Kano state, Nigeria. The farm is situated between latitude $11^{o}21'$ and $11^{o}45'$ N and longitude $8^{o}15'$ and $8^{o}31'$ E, and about 57 km from Kano town along Kano - Tiga - Jos road in Wak District of Bebeji Local Government Area, Kano state, Nigeria (Bichi & Dawaki, 2010). The pond had both thin and broad leaf plants growing within it and was stocked with about 3000 *Clarias gariepinus*, *Heterotis niloticus*, *Oreochromis niloticus*, *Oreochromis mossambicus* and *Tilapia zillii* juveniles each 10 months prior to sampling. The pond was initially filled with water from Baguada dam through a 28 inches water pipe via gravity (Figure 1) into three stilling basins with filters supplying five concrete ponds, 19 earthen ponds and four *Lates* spp raceway ponds through water channels. Bagauda dam has a water storage capacity of about 22,000,000 m^3 liters (UNDRO, 1988). The dam is bordered on all sides by arable agricultural land outside its southeastern border occupied by Nigerian Law School, Kano study centre, Baguada, Nigeria. Fish pond water was subsequently topped once on monthly basis during the rainy season but twice weekly during the dry season from the same Bagauda dam. Fish were occasionally fed with certain amounts of groundnut cake mixed with wheat bran. The engaged local fishermen were able to catch 16 adult *O. mossambicus* (152.30 ± 25.16 g mean weight and 20.24 ± 0.64 cm mean total length) with drag nets during sampling. Sampling was done just once in the early hours of the morning so as to eliminate seasonal variations (Kutović *et al*., 2008).

The gills and liver of sampled fish were harvested for histopathological evaluation after fish were euthanized with 40 % ethyl alcohol (Fafioye *et al*., 2005). Tissues were immediately fixed in Bouin solution prior to histopathological processing using standard method of paraffin embedding, sectioning at 5 µm and staining with haematoxylin and eosin (Bancroft & Cook, 1994). Prepared slides were viewed under various magnifications for lesions or histopathological changes. The degree of tissue change (DTC) were determined by modifying the semi-quantitative method described by Poleksic & Mitrovic-Tutundzic (1994) using the formula: $DTC = (1 \times \sum I) + (10 \times \sum II) + (100 \times \sum III)$ after screening the number of tissue lesions in stages *I*, *II* and *III* for the particular organ, respectively. Stage *I* alterations were lesions, which did not impair the normal functioning of the tissue while stage *II* alterations were more severe lesions that impaired the normal functioning of the tissue. Finally, stage *III* alterations were very severe lesions, which induced irreparable tissue damage. Calculated DTC value for each organ was interpreted as follows: 0 -10 for normal organ, 11 – 20 for slightly damaged organ, 21 – 50 for moderately damaged organ, 51-100 for severely damaged organ and above 100 for irreversibly damaged organ, respectively.

Data were expressed as mean ± SD and analyzed for statistical significance ($P<0.05$) with student *t*-test using GraphPad software programme (GraphPad Prism version 4.0, San Diego, USA).

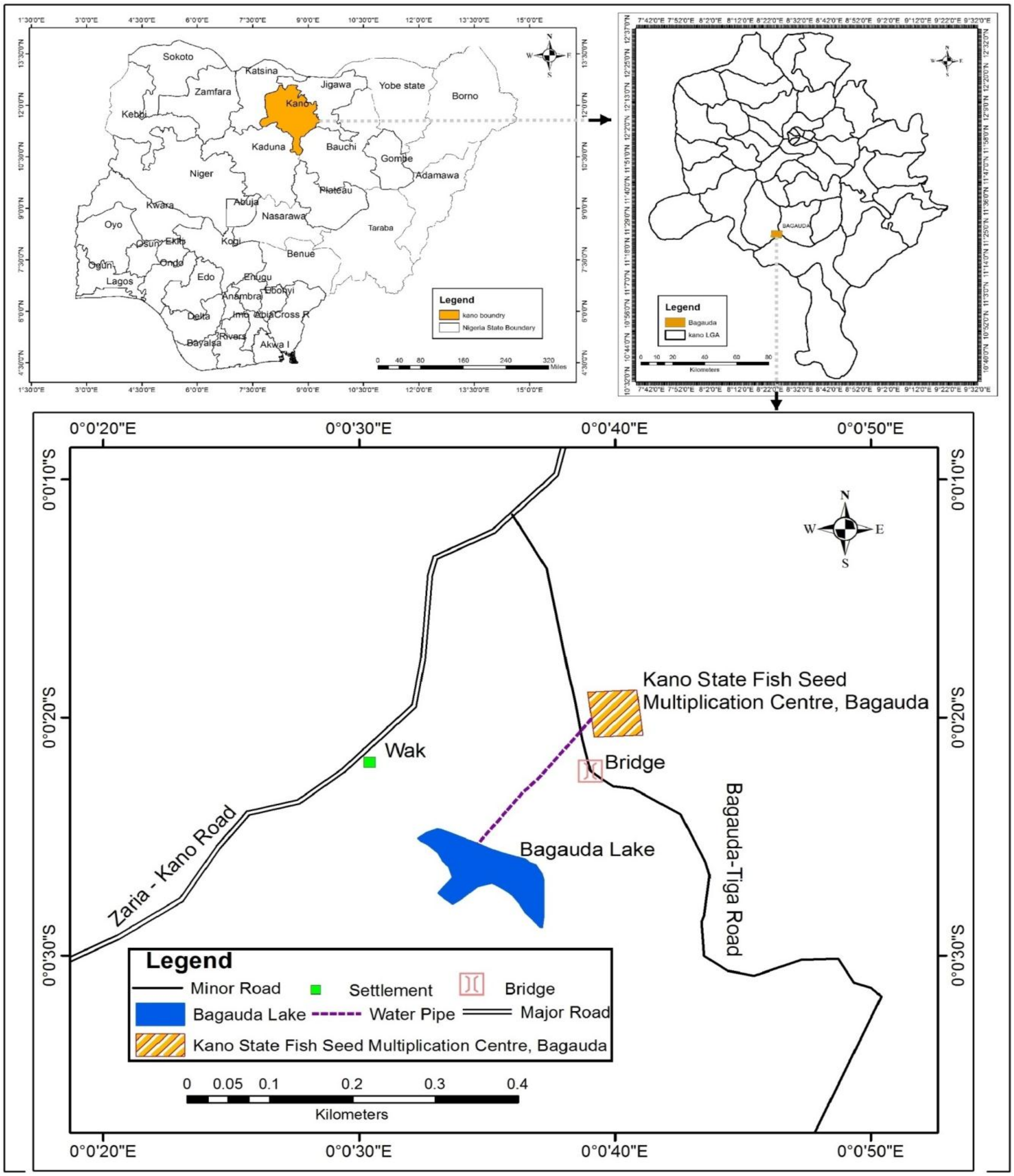

Figure 1: Map of Bagauda, Kano Sate, Nigeria.
Source: Modified from Administrative Map of Kano 2016

Results

The physicochemical parameters of the sampled semi-intensively managed polyculture earthen pond water were as shown in Table 1. Histopathological changes, incidence and the DTC in the gills and liver of *O. mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria were as presented in Tables 2 & 3 as well as in Plates 1 – 7, respectively. The degree of tissue change was significantly (p<0.05) higher in the liver compared to the gills of sampled fish.

Table 1: Physicochemical parameters of water sampled (n=6) from a semi-intensively managed polyculture earthen pond in Bagauda, Nigeria

S/no.	Physicochemical parameters	Values (mean ± SD)	Acceptable range
1.	Temperature (^oC)	28.35 ± 0.12	15 – 35*
2.	pH	7.24 ± 0.16	7.0 – 9.5*
3.	Dissolved oxygen (mg/L)	5.39 ± 0.69	> 3 – 5*
4.	Biological oxygen demand (mg/L)	5.60 ± 1.26	3 – 6*
5.	Ammonia (mg/L)	0.50 ± 0.52	0.05 – 0.1**

(*) Bhatnagar & Devi (2013)
(**) Francis-Flyod *et al*. (2009)

Table 2: Histopathological changes, incidence and the degree of tissue change (DTC) in the gills and liver of *Oreochromis mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria

DTC	Histopathological lesions (incidence)	
	Gills	Liver
I	- Congestion (18.75%) - E. hyperplasia (56.25%) - E. lifting (6.25%) - Clubbing (12.50%) - Oedema (50.00%)	- H. cytoplasmic vacuolation (56.25%) - HP. cytoplasmic vacuolation (68.75%)
II	- Aneurysm (31.25%)	- Congestion (81.25%) - Bile stagnation (87.50%)
III	-	-

E: Epithelial; H: Hepatic; HP: Hepatopancreatic

Table 3: The degree of tissue change (DTC) between the gills and liver of *Oreochromis mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria

Fish specie	*Gills*	*Liver*	t-value	P-value
Oreochromis mossambicus	0.11 ± 0.06	0.19 ± 0.08	3.15	0.0037*

Value with asterisk (*) is statistically significant (P<0.05)

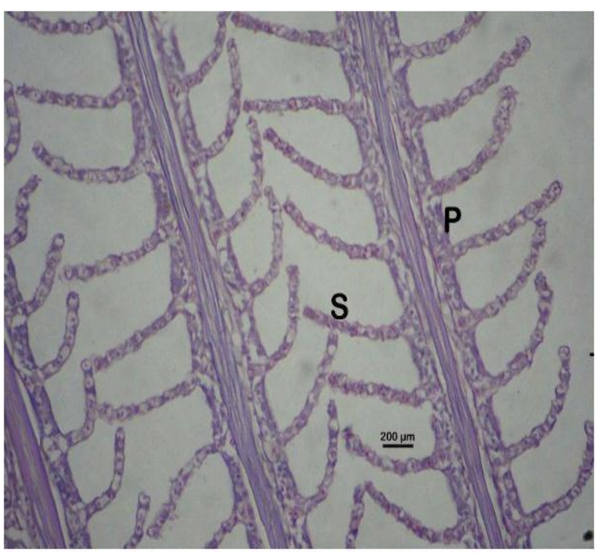

Plate 1: Photomicrograph of a section of the gill of *Oreochromis mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria: Note primary lamellae (P) and secondary lamellae (S). H & E

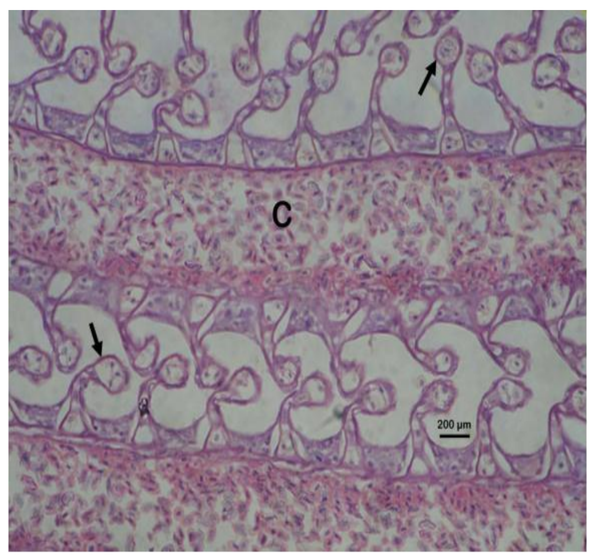

Plate 2: Photomicrograph of a section of the gill of *Oreochromis mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria: Note congestion (C) and lamellar clubbing (arrows). H & E

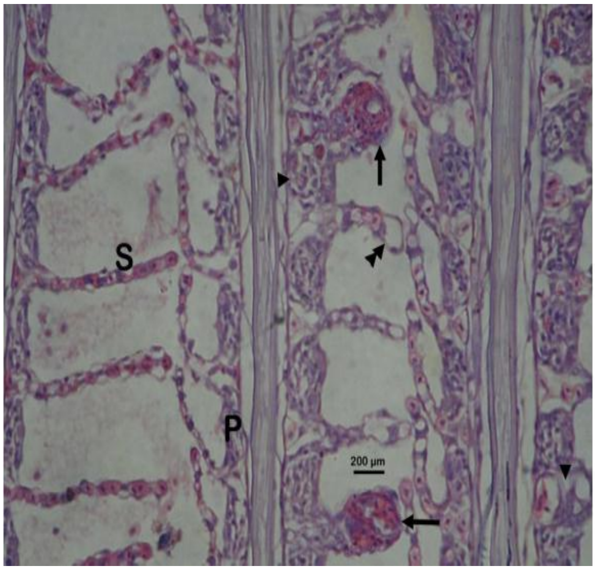

Plate 3: Photomicrograph of a section of the gill of *Oreochromis mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria: Note primary lamellae (P), secondary lamellae (S), oedema (single arrow heads), epithelial lifting (double arrow heads) and aneurysm (arrows). H & E

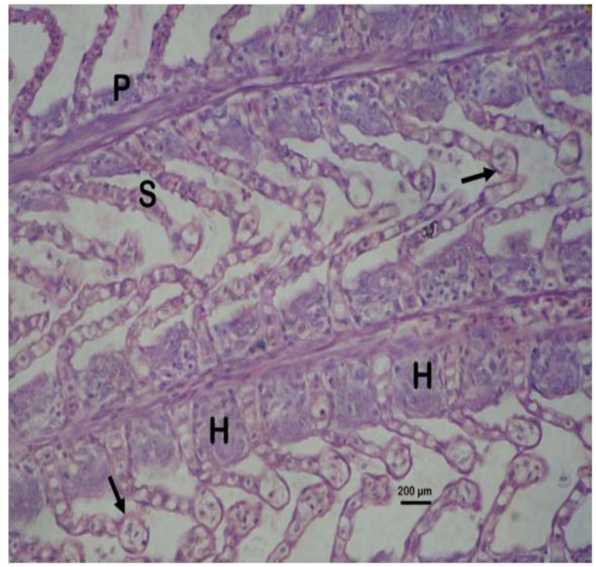

Plate 4: Photomicrograph of a section of the gill of *Oreochromis mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria: Note primary lamellae (P), secondary lamellae (S), lamellar hyperplasia (H) and lamellar clubbing (arrows). H & E

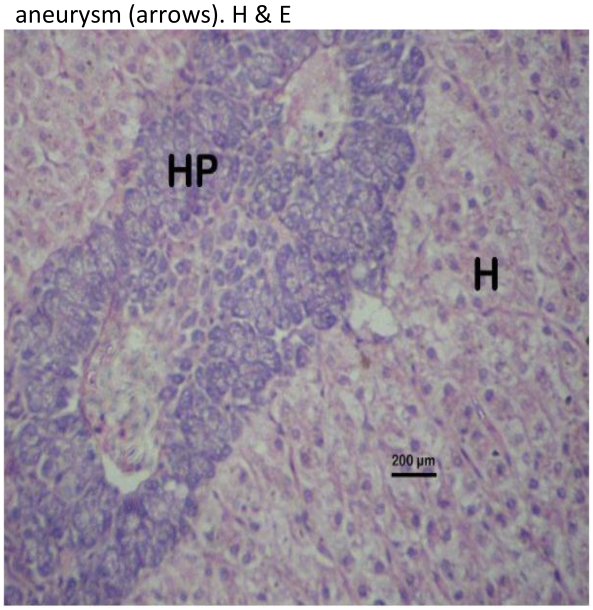

Plate 5: Photomicrograph of a section of the liver of *Oreochromis mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria: Note hepatocytes (H) and the hepatopancreas (HP). H & E

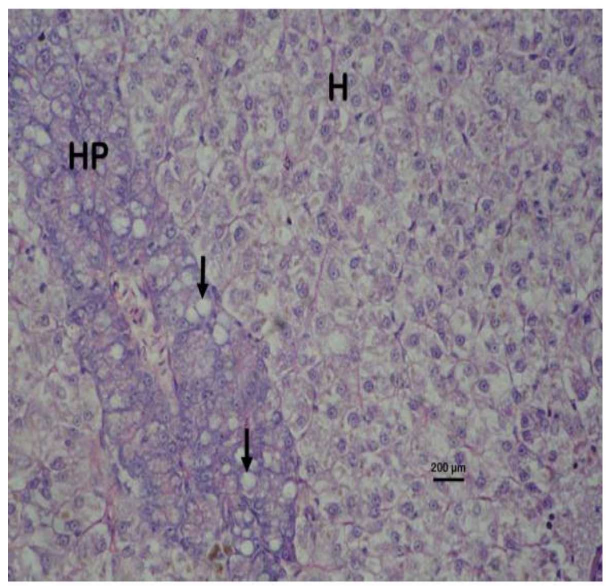

Plate 6: Photomicrograph of a section of the liver of *Oreochromis mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria: Note hepatocyte (H), hepatopancreas (HP) and hepatopancreatic cytoplasmic vacuoles (arrows). H & E

Discussion

The physicochemical parameters of sampled polyculture earthen pond water were within acceptable limits for the growth and survival of tilapia fish according to Bhatnagar & Devi (2013). This was responsible for the observed low level gills and liver alterations that did not affect the normal functioning of respective organs in sampled fish based on the DTC protocol. This is because water quality parameters outside these ranges stresses fish, reduces growth and predisposes fish to diseases (Estim & Mustafa, 2014) and/or causes structural tissue damage in exposed fish, especially ammonia whose water level is closely related to water pH and, to a lesser extent, lower fish water temperature and DO contents (Popma & Lovshin, 1996). Although the study earthen pond water ammonia level was above the recommended value, *Tilapia* spp are known to tolerate salinity, high water temperature, low DO and high ammonia concentration than most commonly cultured freshwater fish (Popman & Lovshin, 1996). They may tolerate ammonia level up to 2.4 mg/L in unacclimatized fish or 3.4 mg/L in those acclimatized to sub-lethal concentration (Redner & Stickney, 1979). However, ammonia concentration exceeding 2.0 mg/L is reported to cause gill and tissue damage, extreme lethargy and death in exposed fish (Bakar *et al*., 2016). The slightly elevated ammonia level within the study earthen pond might be due to the presence of carnivorous fish within it, which are known to excrete more ammonia than herbivorous fish (Ibrahim & El-Naggar, 2010). It might also be due to sewage and animal waste pollution of the pond water and/or its Baguada dam water source from

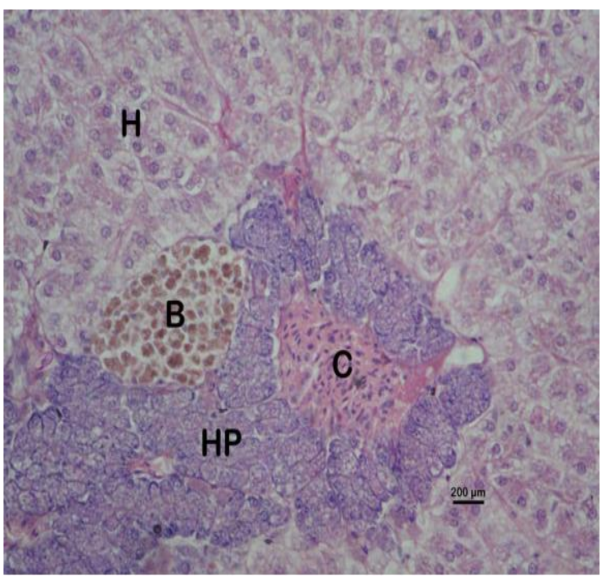

Plate 7: Photomicrograph of a section of the liver of *Oreochromis mossambicus* sampled from a semi-intensively managed polyculture earthen pond in Baguada, Nigeria: Note hepatocyte (H), hepatopancreas (HP), congestion (C) and bile pigment (B). H & E

activities of inhabitants of bordering Nigerian Law School, Kano study centre as well as those of both arable and livestock farmers around the dam. This is because the presence of ammonia in any surface water indicates possible sewage and animal waste pollution of that aquatic environment (Obigacion, 2015). Similar physicochemical parameters have been reported in polyculture earthen ponds (Hassan *et al*., 2008; Saeed & Batran, 2014; Shoko *et al*., 2014). Ammonia level within the study fish pond water, although within tolerable level, might be responsible for the observed low level cellular damage in the gills and liver of exposed fish. This is because pathological changes are usually observed in the gills, liver and kidney of fish exposed to sub-lethal ammonia concentration (Ip *et al*., 2001) with fish mortality only occurring at toxic level in fish pond water (Hargreaves & Tucker, 2004).

The observed epithelial hyperplasia is a non-specific response induced by many gill irritants (Mallat, 1985) in exposed fish, including ammonia. It is considered a protective response of exposed fish to reduce absorption rate by increasing diffusion distance across gill surfaces (Mallat, 1985; Albassam *et al*., 1987). Although gill hyperplasia was mild in the study, these reactions might reduce oxygen diffusion across gill epithelium leading to hypoxia in extreme cases (Heath, 1987). This is even as fish has the capacity to compensate for low oxygen uptake by increasing ventilation rate (Fernandes & Mazon, 2003). The observed congestion was attempted to increase blood flow to damaged gills in order to improve oxygen uptake and supply to internal organs (Doherty *et al*. 2013).

The aneurysm was caused by damage to pillar cells and therefore, the structural integrity of lamellar vascular system (Martinez *et al*., 2004). Excessive aneurysm can impair respiratory efficiency, especially at higher temperature, when dissolved oxygen levels are low and metabolic oxygen demand is high (Roberts & Rodger, 2012). Although the observed gill lesions were not lethal, they might retard growth and affects reproduction (Das & Mukherjee, 2000). Fish liver suffers morphological alterations in toxic exposures (Camargo & Martinez, 2007) due to its position; function and blood supply (Van der Oost *et al*., 2003). Hepatic fatty degeneration evidenced by the observed cytoplasmic vacuolations within hepatocytes and hepatopancreatic cells were common responses of fish to toxicant exposures (Agamy, 2012). Such alterations are usually associated with protein synthesis inhibition, energy depletion or a shift in substrate utilization (Hinton & Lauren, 1990). The observed bile stagnation was indicative of possible hepatic metabolic damage (Fanta *et al*., 2003). However, the obtained DTC values of 0.11 ± 0.06 and 0.19 ± 0.08 in the gills and liver of sampled fish differed from 9.75 ± 3.00 and 14.06 ± 9.46 reported for *Auchenoglanis occidentalis* from Tiga dam, Nigeria by Abalaka (2015). Srivastav *et al*. (1997) and Evans *et al*. (2005) reported that although *Tilapia* spp have kidneys, most of the functions controlled by pulmonary and renal processes in mammals are actually performed by fish gills. Therefore, fish gills and liver represent important target organs suitable for histopathological evaluation of cellular damage (Rabitto *et al*., 2005; Oliveira Ribeiro *et al*. 2006) in polluted fish water.

In conclusion, the physicochemical parameters of the earthen pond and the observed histopathological changes in the gills and liver of the sampled fish were within acceptable limits and the normal functioning of respective organs base on the DTC protocol. However, these lesions indicated low level pollution of the study earthen pond water as early warning signs of the state of the earthen pond water quality. This calls for routine monitoring of the earthen pond water and its water source (Bagauda dam) for toxicants, especially heavy metals, in order to ensure the continuous well-being and survival of stocked fish for enhanced productivity. This is because of the large drainage catchment area of Bagauda dam, Nigeria in relation to the increasing human anthropogenic activities within the area.

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