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HISTOLOGICAL EXAMINATION OF HEPATIC CELLS OF *CLARIAS GARIEPINUS*: AN ECOTOXICOLOGICAL EVALUATION OF MGBUOBA FISH POND

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ABSTRACT
Background: Ecotoxicological assessment the aquatic life has become an urgent need and an integral part of human survival. As such this study was undertaken to assay the status of the fishes bred and sold for consumption in commercial fish farms. **Materials and Methods:** The sampling involved harvesting of table-sized fish: twenty fishes from MGBUOBA and ten fishes from ARAC. The histological assessment involved the determination of the qualitative and semi-quantitative analysis of the liver cells of the harvested fishes. **Results:** The following results were obtained for Circulatory Disturbance (CD) [Haemorrhage 22.0% for ARAC, 24.3% for Mgbuoba; Vacuolation 40.0% ARAC, 34.0% Mgbuoba]; Regressive Change (RC) [Necrosis 16.0% ARAC, Mgbuoba 3.9%]; Foci of Cellular Alteration (FCA) [Vacuolated Foci 4.0 ARAC, Mgbuoba 27.2%, Necrotic Foci 18.0% ARAC, Mgbuoba 10.7%]. Average percentage prevalence 20.0% ARAC and 20.02% Mgbuoba. **Conclusion:** Assessment of the depth of alterations in the Liver of the fishes indicates that the fishes were exposed to pollutants and it is speculative that the pollution could have ensued from poor management or inadequate facility.

KEYWORDS: Liver, ARAC, Haemorrhage, Mgbuoba.

INTRODUCTION
Ecotoxicological assessment the aquatic life has become an urgent need and an integral part of human survival. The rate of fish consumption and fish farms have hit the sky in most nations especially Nigeria. The proliferation of fish farms and the standard practice have become a concern not all to Nigeria but the African nations at large.

Poor management practices which include impure water constituting the fish habitat, dirty surrounding, incomplete draining of pond due to lack of proper drainage, inadequate water supply, infrequent inspection and repairs are major ways of unconsciously exposing commercial fishes to contaminants. With time, pollution sets in, leading to changes in the gross anatomy of the fish, histological changes specifically. These changes include: hypertrophy, hyperplasia and even degeneration of the whole cell. This ultimately leads to growth and sales of unhealthy fishes which pose a subtle but great threat to consumers.[1-3]

STUDY AREA
Experimental Site (Commercial Pond in Mgbuoba Community, Port Harcourt, Rivers State, Nigeria). Mgbuoba is a community located in Obio/Akpor local government area of Rivers state. Its geographical coordinates are 4o 50’ 51” North, 60 58’ 47” East. The surrounding communities are Ozuoba and Rumuokwuta.[3]

The commercial fish pond located in Mgbuoba community is made of concrete. There are two sections of the pond, one for fingerlings and the other for mature fishes of 600grams to 1kilogram. Each pond section has demarcations made of concrete which divide each column into two compartments each. Approximately 800 fishes inhabit both sections of the pond per time. [3]

Fishes cultivated in the pond are fed with a product of Livestock Feeds Plc-Aquamax. The nutrient composition of this feed includes 40% crude protein, 12% fat, 2.6% fibre, 1.0% ash, 2.0% calcium, 2.4% lysine, 1.1% methionine, 12.0% moisture. The antibiotic-Fish Cure is administered to the fishes suffering from ill health. Signs like pale white patches on the head or body of the fishes and shortened barbells indicate ill health.[3]

REFERENCE AREA (ARAC)
The chosen reference centre, African Regional Aquaculture Centre is situated in Omuihuechi Aluu, Ikwerre Local Government Area, Rivers State. It covers

an area of 81hectares. The activities done in this centre comprise research, training and development of sustainable aquaculture options in sub-Saharan Africa.[3]

The Aquaculture was established in 1980 as a result of recommendations of the Aquaculture Planning Regional Workshop that was held in Accra, Ghana in 1975. ARAC develops scientific databank, builds partnerships and linkages across local regional and international boundaries, monitor research outcomes regularly with the view of studying impacts and providing quality of technologies developed.[3]

STUDY SPECIES

Kingdom: Animalia, Phylum: Chordata, Class: Actinopterygii, Order: Siluriformes, Family: Clariidae, Genus: clarias, Species: Clarias gariepinus

Fig. 1: African Sharptooth Catfish, Clarias gariepinus (Source: Food and Agricultural Organization of the United Nations).

STUDY SPECIES DESCRIPTION

Clarias gariepinus is a large, eel-like catfish of African origin. It is a sharptooth catfish with dark gray or black colouration on the back which seemingly becomes faded towards the belly, giving a white belly.[4-6]

This specie of catfish reaches a maximum length of 1.7m and can weigh up to 60kg (130lb).[4-6] They possess slender bodies, flat bony heads and broad terminal mouths with four pairs of barbels. They also possess large accessory breathing organs which comprise modified gill arches and only the pectoral fins have spines.[4-6]

Researchers have to a large extent done works on ecotoxicology and commercial fish ponds which have been reported by different authors.[3-26]

AIM

The aim of this study was to carry out a qualitative histological analysis of the Liver of *clarias gariepinus* (catfish), the semi-quantitative histological analysis and the pollution status of the fish pond.

MATERIALS AND METHODS

PHASES OF STUDY

PHASE 1 (Preliminary study): The experimental site was visited and enquiries were made on the quantity of fish in the pond, type and frequency of fish feed used, treatment administered to fish in poor health condition, mode and frequency of changing the water content of the pond. A sample fish was harvested and taken to the African Regional Aquaculture Center for identification by a taxonomist.

PHASE 2 (Sampling of Fish)

CONTROL

Control fishes were harvested. This was done by first collecting some water content of the pond into a plastic container which would contain the fishes from the control site to the laboratory. The essence is to maintain the original aquatic habitat of the fishes. Afterwards, the remaining water content of the pond was drained and with the aid of a seine; ten table-sized cat fishes were harvested, put into the plastic container in which had exactly the same water content of the pond.

EXPERIMENTAL

Experimental fishes were harvested following the standard procedure. Twenty table-sized cat fishes were harvested from the pond.

HISTOLOGICAL ANALYSIS

This analysis involved the microscopic study of the liver tissues gotten from the harvested fishes. The analysis is thus divided into two: a qualitative and semi-quantitative assessment.

QUALITATIVE HISTOLOGICAL ASSESSMENT

This involved tissue processing and microscopy. The steps were as follows:

STEP 1 (RESECTION)

This is the surgical excision of an organ or tissue, either partially or wholly. Using a dissecting kit, the fishes were sacrificed using pithing method.

STEP 2 (FIXATION)

This was done by immersing the organs in 10% formal saline (10mls formaldehyde in 90mls of water) after excision. The formalin solution slowly penetrated the tissues, caused them to harden and preserved the tissues. They were left in the fixative for about 24hours to allow the fixative penetrate into every part of the tissue.

STEP 3 (DEHYDRATION)

The tissue samples were dehydrated to remove their water content. Alcohol was used as the dehydrating agent (50%, 70%, 90%, 95% and absolute alcohol) of increasing concentrations.

STEP 4 (CLEARING)

The alcohol used for dehydration of the tissue had to be cleared off the tissues; therefore xylene was used for the clearing process. The solvent (xylene) displaced the alcohol content in the tissue.

STEP 5 (IMPREGNATION)

After clearing, the tissues were transferred into molten paraffin wax for about 30 minutes. Paraffin wax is the most common infiltration and embedding medium. A typical wax is liquid at 60°C and can be infiltrated into tissues at this temperature then allowed to cool to 20°C where it solidifies to a consistency that allows sections to have a uniform cut.

STEP 6 (EMBEDDING)

The tissue samples which had been thoroughly infiltrated with wax were formed into "tissue blocks" which could be clamped into a microtome for sectioning. This step was carried out using an embedding mould which was filled with molten wax and the specimen placed into it.

STEP 7 (SECTIONING)

This was done to the already embedded tissues thereafter the tissues were sectioned at 3-5 microns and were picked up on a glass microscopic slide. The glass slides were placed in a warm oven for about 15 minutes to help the section adhere to the slide.

STEP 8 (STAINING)

The process was reversed in order to get the paraffin wax out of the tissue and allow water soluble dyes to penetrate the sections. Therefore, Routine H&E (haematoxylin and eosin) was done using the following procedures:

a. The tissues were dewaxed in xylene 1 and 2.
b. Then hydrated in descending grades of alcohol and brought to water.
c. They were stained in haematoxylin for 25-30minutes, brought to water.
d. Differentiation in 1% alcohol was done thereafter, rinsed in water immediately.
e. The slides were rinsed in 1% ammonia water, rinsed in water & stained in eosin for 2mins.
f. The slides were placed in the oven to dry.

STEP 9 (MOUNTING/COVER SLIPPING)

The stained section on the slide were covered with a thin piece transparent plastic or glass to protect the tissue from being scratched, to provide better optical quality for viewing under the microscope, and to preserve the processed tissues. Thereafter, the stained tissues on the slides were covered using a plastic coverslip.

SEMI-QUANTITATIVEHISTOLOGICAL ASSESSMENT

A qualitative assessment protocol was used to qualify histopathological alterations observed in the sections of each of the organs. A qualitative histopathological assessment was done using CX31 Olympus light microscope. Tissue sections were scanned on 400x magnification. Tissue sections were semi-quantitatively assessed using part of a scoring system[18] modified from the protocol.[19] In brief, the tissue samples were assessed by identifying histopathological alteration in terms of reaction patterns including: circulatory disturbance, regressive changes, inflammatory responses, neoplasia.

Neoplasia, if identified, the alteration was given an importance factor which represents the potential of the alteration to affect fish health: 1 (alteration is reversible); 2 (alteration is reversible if the stressor is neutralized); 3 (alteration is irreversible). A score value representing the occurrence of the alteration throughout the tissue was also assigned: 0 (absent), 2 (mild), 4 (moderate), and 6 (severe).[18-20] The score value and the importance factor for the each alteration were multiplied. The liver index was calculated for each sample group (experimental and control group) and was compared between the groups. This index indicates the combined histological response of the liver for the individual fish.

RESULTS

LIVER HISTOPATHOLOGY

A variety of histological alterations were identified in the liver tissue. These alterations included vacuolated hepatocytes, necrosis, haemorrhage, foci of cellular alteration (FCA) which includes vacuolated and necrotic foci. Vacuolated hepatocytes and necrosis were more prominent in fish specimen from Mgbuoba.

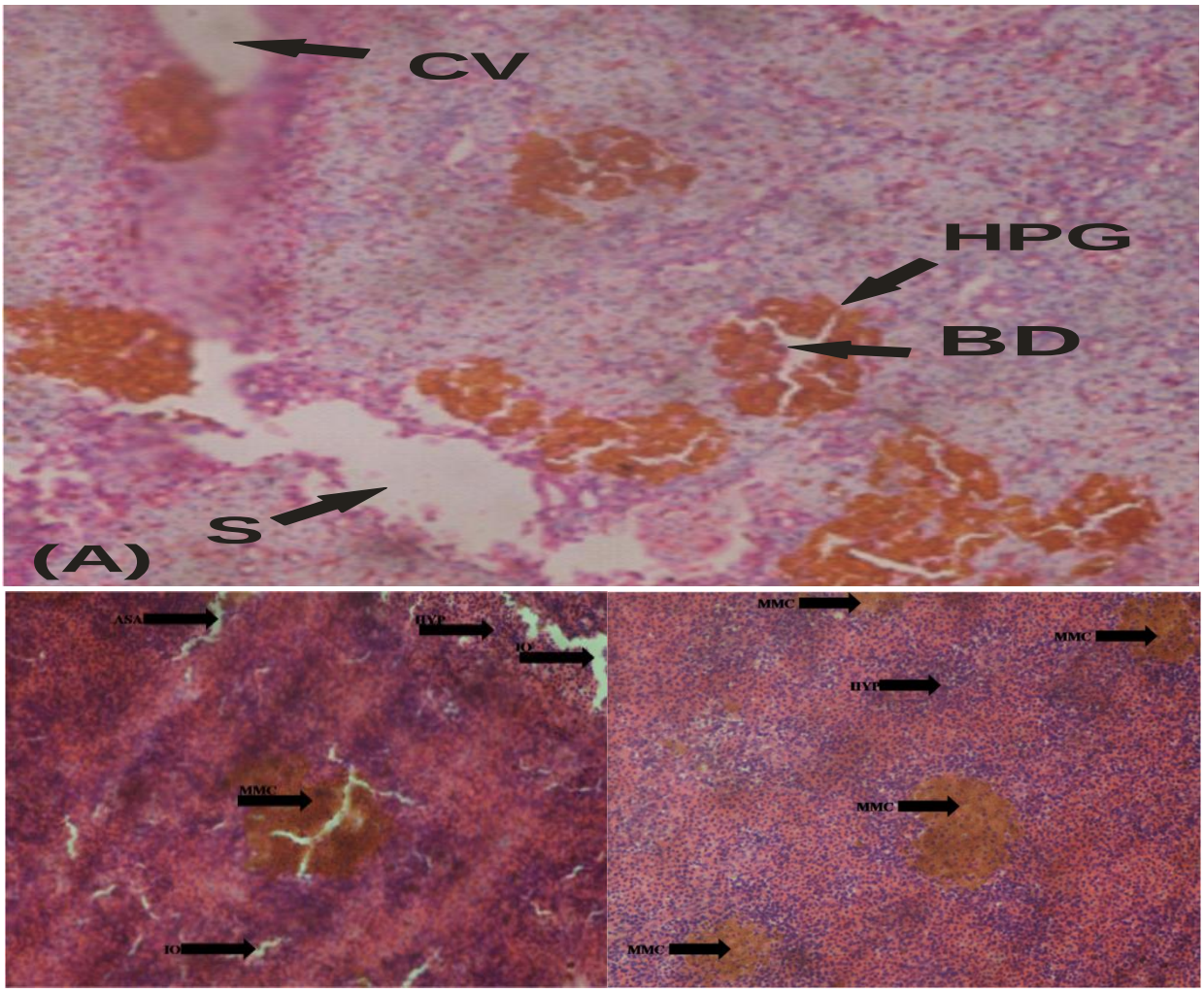

Fig. 2: Showing normal and histopathologic tissue micrograph of the liver. A.) Normal tissue at x 400 magnification, showing Sinusoid (S), Hepatopancreatic Gland (HPG), Bill ducts (BD) and Central Vein (CV). B) Histopathologic tissues at x 400 magnification showing, Vacuolation (VAC), Intercellular Oedema (IO), Hyperplasia (HYP), Architectural and Structural Alteration (ASA) and Melano Macrophage Centre (MMC). C) Histopathologic tissue at 400 X magnification showing Hypertrophy (H), and Melano Macrophage Centres (MMC).

Table 1: The percentage prevalence of Liver Histopathology.

Alteration	Prevalence (%)	
	ARAC (n=10)	MGBUOBA (n=20)
Circulatory Disturbance (CD)		
Haemorrhage	22.0	24.3
Vacuolation	40.0	34.0
Regressive Change (RC)		
Necrosis	16.0	3.9
Foci of Cellular Alteration (FCA)		
Vacuolated Foci	4.0	27.2
Necrotic Foci	18.0	10.7
AVERAGE % PREVALENCE	**20.0**	**20.02**

DISCUSSION

LIVER HISTOPATHOLOGY

The liver is a detoxification organ and is essential for both the metabolism and the excretion of toxic substances in the body. In the current study circulatory disturbances (vacuolation, necrosis of hepatic tissue and hemorrhage), regressive changes, and focal cellular alterations (FCA) were identified. The histological responses in the liver were vacoulation, necrosis and increase in FCA. These circulatory disturbances have also been mentioned in previous works.[2-12]

Comparison of the liver in both experimental and control groups showed that there is a level of distortion or alteration of the livers cells in the experimental group that is slightly higher than in the control. It therefore means that the habitat of the experimental group is polluted to a certain level which is seen by the amount of alteration recorded, though this magnitude of cellular alteration is not severe or lethal as to constitute nuisance to health at the moment. But it is worth noting that if nothing is done about this level of pollution, it might proliferate and become hazardous to health on consumption of the fish. Therefore, it is imperative that adequate and prompt attention be given to this ponds and aquatic habitats to ensure a health environment for growing fishes for commercial consumption.

Focal cellular alteration was seen in both experimental and control fish categories in the study. Vacuolated foci were more prominent in the experimental group than the control group (ARAC) which is a clear indication of a pathological condition in the experimental group which is a direct result contamination and pollution of the habitat. Considering necrotic foci, there was a high occurrence in the ARAC fishes compared to the experimental which is suggestive of a normal cellular morphology in an ideal situation. These results are consistent with the reports of other authors who have investigated the liver and reported similar findings.[10-12]

CONCLUSION

Assessment of the depth of alterations in the Liver of the fishes indicates that the fishes were exposed to pollutants and it is speculative that the pollution could have ensued from poor management or inadequate facility. However, it is important to note that these alterations were not toxicant specific but could be associated with pathogens, and metal pollution in the water.

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CONFLICT OF INTEREST

We write to declare that there is no conflict of interest.

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