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Light Microscopy Observation of the Gills of Post Juvenile Africa Cat Fish (*C. gariepinus)* Exposed to Sub-lethal Concentrations of Glyphosate Herbicide (IPA 360g/L)

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Abstract
The histopathological studies of the sectioned gills of post Juvenile Africa catfish C. gariepinus exposed to sub-lethal concentrations of glyphosate based formulation (Isopropylamine salt 360 mg/L) were observed for 28 days. Epithelial lifting, hyperplasia and hypertrophy of the epithelial cells with partial fusion of lamellae were associated with various concentrations of glyphosate. Severity of alteration was both time and concentration dependent. All the fishes held in the control tanks showed no histological alterations. The observed changes in the gills under light microscopy showed that the use of histopathological techniques will allow investigators to examine specific target organs and cells as they are affected by exposure to environmental chemicals like herbicide. Investigating histopathological alteration offers a means of detecting acute and chronic harmful effects of exposure in the tissues and organs of individual animals.

Keywords: Histopathology, Gills, Assessment tool, Glyphosate toxicity, Biomarker, fish.

Introduction
Histopathological alteration can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem [1]. The gills of Fish have been described as efficient tools for biomonitoring potential impacts [2] because of their large area in contact with the water and high permeability [3, 4, 5]. The gills are particularly sensitive to changes in environmental conditions and perform such vital functions as ion osmoregulation, gas exchange and nitrogen excretion. Gill morphology has been described as a good indicator of the water quality and the general health condition of cultured fish [6]. Szakolczai [7] studied the histopathological changes induced by environmental stress in common carp (*Cyprinus carpio*), the Japanese colored Carp-Cyprinus), and the African cat fish (*Clarias gariepinus*). In all the species treated, it was observed that the goblets cells of the gills and the skin increased in number and there was slight detachment of the epithelium of the secondary gill lamellae.
The severity of damage to the gills depends on the concentration of the toxicants and the period of exposure [8, 9]. Haaparanta *et al.,* [10] reported chloride cell proliferation as a significant pathological change in roach *Rutilus rutilus,* collected from polluted lakes in Finland [11]. Epithelial lifting, hyperplasia and hypertrophy of the epithelial cells with partial fusion of lamellae are defense mechanisms which result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants [12, 13]. Lamellar aneurysms and blood congestion with dilation of marginal channels together with leukocyte infiltration could be considered part of an inflammatory response and occur when fishes suffer a more severe type of stress [14]. Aneurysm is related to the pillar cells rupturing [15], as a result of a bigger flow of blood or direct effects of chemicals on these cells.
Gill lesions in response to a wide range of contaminants, including Organochlorines, petroleum compounds, Organophosphates, Carbamates, herbicides and heavy metals have been reported [16, 17, 18]. Changes have also been reported in the gills of the fishes exposed to organic toxicants [14]. Different fish species can exhibit markedly different sensitivities to toxic contaminants in the environment based on such factors as habitat, prey type, life span, migratory behavior, and genetic constitution (USEPA, 1987). (19)
Histopathology alteration in gills of fish due to pesticides and other contaminant have been reported by several authors [21, 22]. Since the gills are the primary route for the entry of pesticides. Gills in fish are critical organs for

respiratory and osmoregulatory functions. Respiratory distress is one of early symptoms of pesticides poisoning [23]. According to [24], in the gills the toxicants appear to breakdown the adhesion between epithelial brachial cells and the underlying pillar cells and the pillar cells: this is accompanied by a collapse of the structural integrity of the secondary lamellae and subsequent failure of the respiratory functioning of the gills. Gill epithelium is a major site of gaseous exchange [25] and is an important site for disease production, because they are a rich source of blood, an important media for the infectious agents. Since gaseous exchange takes place through the gills, they may easily become contaminated from external sources [26]. Sublethal levels of detergents have been reported to induce gill damage and impaired active oxygen uptake [27] and gill damage in form of haemorrhage has also been observed in *Gambusia affinis* exposed to Diquat [28]. Coutinho and Gokhale [29] found epithelial lifting in the gills of carps *Cyprinus carpio* and tilapias *Oreochromis mossambicus* exposed to the effluents of a waste water treatment plant. Engelhardt [30] observed epithelial lifting and lamellar fusion in rainbow trouts *Oncorhynchus mykiss* exposed to petroleum residues. Similar alterations in the gills have also been reported in the fishes exposed to metals [2, 31, 15]. The objective of this research is to establish the importance of histological biomarkers as good endpoint for assessing the health condition of exposed aquatic organism to toxins.

Materials and Method

Experimental fish specimen and chemicals

One hundred and twenty normal post juvenile *Clarias gariepinus* of both sexes, with a mean weight of 135.44±1.99 g and mean length of 28.32±0.844 cm were purchased from Osayi farms in Benin City, Edo state. They were kept in 60 l aquaria at 27.5±0.4 ◦C, pH 7.3, with 12:12 h photoperiod. They were left unfed in the first 2 days to adapt to a change in environment before feeding them with the fish diet. Laboratory aquaria were well aerated and provided with external filtration and a layer of gravel on the bottom. Fish were normally fed once a day with pelleted commercial food (Durante Aquaculture fish concentration-2mm). They were allowed to acclimate to captivity conditions for two months prior to taking the blood samples. Careful netting and handling was implemented to minimize stress. The commercial formulation of glyphosate (360 g/l-41 w.wt IPA) at five nominal concentrations 72, 54, 32 and 18 mg/L were used. These concentrations were defined taking into account, the result of the range finding test.

The sub-lethal test

The sub-lethal concentrations were used to perform the experiment according to the OECD procedure [19] for the static renewal technique. The tests consisted of a control and four concentration groups, three replicates per group, with ten fish in each replicate. The gills of the test animals (fish) were excised, keeping the filaments and rakers intact and rinsed in normal saline. Thereafter, they were fixed in 10 % formalin for about 24 h at 4°C and dehydrated through series of graded alcohol. Specimens were cleared in xylene, infiltrated with paraffin at 56°C and then embedded in paraffin wax. Thin sections of the selected gill tissues of about 6 – 7 Nμm) were cut by means of a rotatory microtome. They were dehydrated and stained with haematoxylin and eosin [20]. The sections were examined and photomicrographs taken, using an Olympus BH2 microscope fitted with photographic attachment. The prepared slides were used to describe the histological structure observed under light microscopy.

Result and Discussion

Gill epithelium of *C. gariepinus* exposed to 72 mg/L glyphosate showed necrosis and haemorrhage after the 7 day of exposure. Fish exposed to same concentration after the 14 day showed hyperplasia of epithelial cells at the base of the secondary lamellae. After the 21 day, it showed elastic cartilage of the filament, raised lamellar epithelium and interstitial oedema. As the time increased, there were severe alterations observed in the gills. Haemorrhage at primary lamellae, intraepithelial oedema, hypertrophy and lifting up of epithelial cells of secondary lamellae characterized the fish gills exposed to 72 mg/L of glyphosate after the 28 day(Fig1a). Fish gills of post juvenile Catfish exposed to 54 mg/L glyphosate showed hyperamia in the filamentous gill rays after the 7 day. While hyperplasia of epithelial cells at the base of the secondary lamellae and different degrees of lamellar fusion characterised the gills after the 14 day of exposure to 54 mg/L. Fish exposed to same concentration after the 21 day showed severe hyperplasia in the epithelial lining the secondary lamellae, degenerative changes,

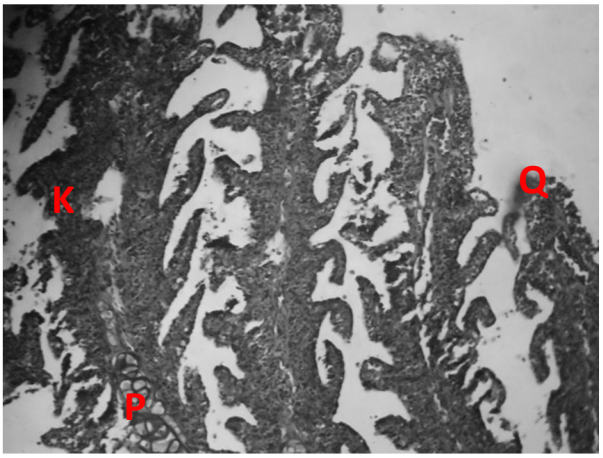

Fig.1A: Photomicrograph of gill epithelium of *C. gariepinus* exposed to 72 mg/L glyphosate. Necrosis (P), fusion of secondary lamellae(K) and oedema (Q) (H & E stain x100)

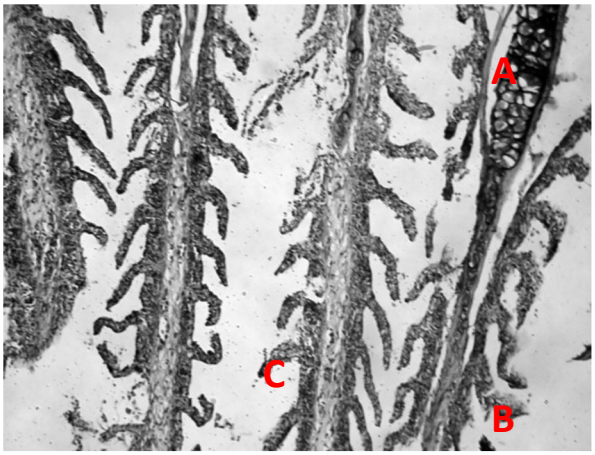

Fig.1B: Photomicrograph of gill epithelium of *C. gariepinus* exposed to 54 mg/L glyphosate. Necrosis (A), degenerated secondary lamellae and telangictasis in some of secondary lamellae(C)(H & E stain x100)

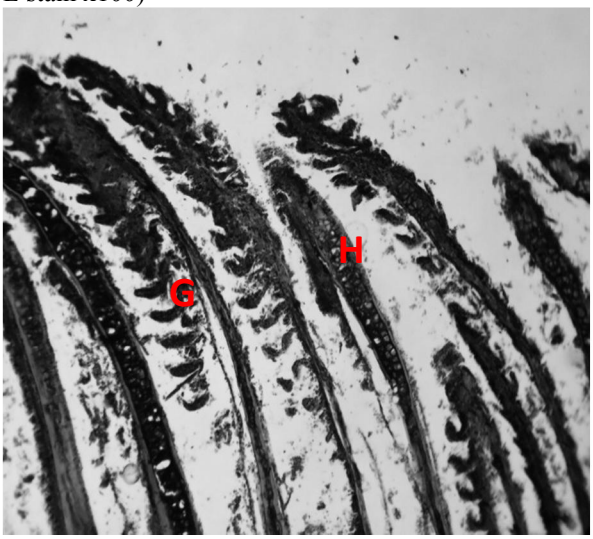

Fig.1C: Photomicrograph of gill epithelium of *C. gariepinus* exposed to 32 mg/L glyphosate. Necrosis (H), hyperplasia of epithelial cells at the base of the secondary lamellae and fusion (G) (H & E stain x100).

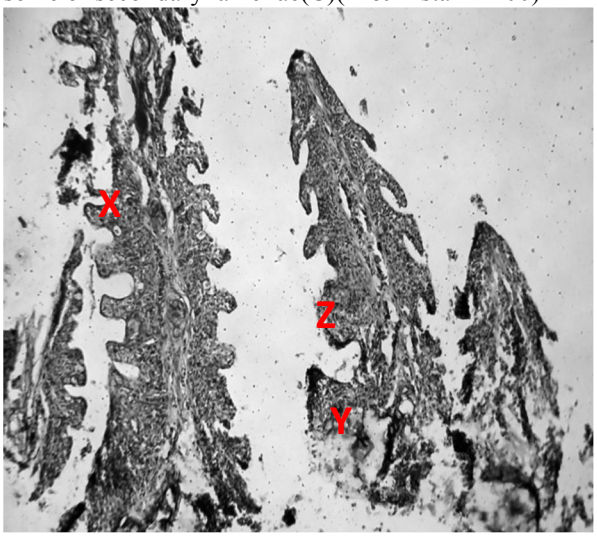

Fig.1D: Photomicrograph of gill epithelium of *C. gariepinus* exposed to 18 mg/L glyphosate. Hyperplasia of epithelial cells at the base of the secondary lamellae (X), Severe degeneration of lamellae (Y) and different degrees of lamellar fusion (Z) (H & E stain x100)

Fig.1 After 28 days of exposure to herbide

necrosis and oedema of the gills. Fish exposed to the 54 mg/L after the 28 day showed gill epithelial hyperplasia (Fig1b).

Fish exposed to 32 mg/L after the 7 day showed damage of some secondary lamellae and lamellar disorganization. After the 14 day of exposure, the gills showed congestion of lamellar and branchial blood vessels accompanied with telangictasis in some of secondary lamellae. Elastic cartilage of the filament, raised lamellar epithelium and interstitial oedema were observed in the gills of fish exposed to same concentration after the 21 day of experiment. Hyperplasia of epithelial cells at the base of the secondary lamellae characterized the gills after the 28day (Fig 1c).

Fish gill expose to the lowest concentration of 18 mg/L showed damage of some secondary lamellae and lamellar disorganization after the 7 days of exposure. Hyperplasia of epithelial cells at the base of the secondary lamellae and different degrees of lamellar fusion of gills were observed after the 14 days of exposure to same concentration. After the 21 days of exposure to the lowest concentration the gills showed severe hyperplasia in the epithelial lining the secondary lamellae, degenerative changes, necrosis and oedema of the gills . After the 28 day of exposure the gill of fish exposed to 18 mg/L showed gill epithelial hyperplasia (Fig 1d).

Gills are the first route of entry for pesticides into fish, and, in response to environmental changes, they may present adaptive strategies to preserve physiological function (33). Histological changes observed in gill tissue of all *C. gariepinus* exposed to glyphosate herbicide in this study include epithelial lifting, hyperplasia of the gill epithelium, lamellar fusion, vasodilatation and necrosis [Fig 1A-D] besides these changes, in post juvenile *C. gariepinus*, there were severe degeneration in secondary lamellar and lamellar telangectiases. This study agrees with the study carried out by (34) on the effect of glyphosate on Nile tilapia. He observed there were several alterations in the gills of the fish exposed to the herbicide. Reports from some authors also support the findings of this research that pesticides and other toxic agents have effects on fish gills(35, 33, 36). The histological changes noticed in the gills of *C.gariepinus* in this study agrees with that of (37). It was observed that hyperplasia, desquamation, and necrosis of epithelial, epithelial lifting, oedema, lamellar fusion, collapsed secondary lamellae and curling of secondary lamellae characterized the gills of *C.mrigala* exposed to various concentrations of dichlorvos.

Conclusion

The use of histopathological markers as endpoint in ecotoxicological studies should be encouraged as they show significant landmark in studying the health of organisms and water bodies.

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