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Full Length Research Paper

Pathologic lesions in the gills of *Clarias gariepinus* exposed to sublethal concentrations of soap and detergent effluents.

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Investigations were carried out on the gill tissues of African cat fish (*Clarias gariepinus)* exposed to sub lethal concentrations (0.002, 0.003, 0.004, 0.005 and 0.006 mgl^{-1}) of soap and detergent effluents in a static renewal bioassay procedure for a total period of 52 days. The histopathological studies of the sectioned gills of this fish species showed marked histological alterations. The observed pathological lesions of the gills include infiltration of secondary lamellae, inter-lamellae hyper plastic lesion, oedema (an indication of ballooning dilatation in form of club deformation at the tip of secondary lamellae), congestion of the blood vessels, epithelial thickening and lifting, and necrosis of various degrees. The degree of all these recorded anomalies is concentration dependent. All the fishes held in the control stock showed inappreciable or no histological degradation as a result of stress in the course of handling while their staining patterns and cellular arrangement remained unaffected. Therefore, indiscriminate human exposure to such concentrations of this effluent is dangerous to health.

Key words:*Clarias geriepinus*, histological, sub lethal, soap and detergent, effluent and pathological.

INTRODUCTION

The rapid increase in human population and the proliferation of industries in Nigeria has resulted in discharge of large amounts of effluent and wastes into aquatic environments where they degrade the normal flora and fauna of the ecosystem. To date, there is paucity of information on the additive effects of heavy metal mixtures such as soap and detergent effluents in this part of the world.

Owing to the direct and continuous contact with the aquatic environment, fish gills which are organs for respiratory gas exchange, osmoregulation, excretion of nitrogenous waste products and acid base regulation, are directly affected by contaminants. Fish gill is very sensitive to physical and chemical alterations of the aquatic medium and to any change in the composition of the environment which is an important indicator of water borne toxicants. Acid and heavy metals pollution has been reported to alter cell structure and induce desquamation to lamellar epithelium and filament epithelium hyperplasia (Leino and McCormick, 1984). Soderberg et al., 1984; Crespo and Sala, 1986; Sinhaseni Tesprateed, 1987; Nowak, 1992; Risboug and Bastide, 1995; Ayoola, 2008a).

There is a close relationship between gill morphological alteration and stress (Peters and Hong, 1985) and several infectious agents have been described in association with proliferating gill diseases and gill necrosis (Kovacs-Gayer, 1984; Daoust and Ferguson, 1985). Gill morphology has been described as a good indicator of the water quality and the general health condition of cultured fish (Peters et al., 1984). Szakolczai, (1997) 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 epithetlium of the secondary gill lamellae was observed.

Most surface active agents (such as synthetic detergents) have been observed to induce oedema and this was also reported in the submission of Hunter and Ford, (1955) and Witter and Cottoned, (1956). Sub lethal level of detergents have been reported to induce gill damage

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and impaired active oxygen uptake (Lemke et al., 1963) and gill damage in form of hemorrhage has also been observed in *Gambusia affinis* exposed to Diquat (Tat-sing et al., 1983). The severity of damage to the gills depends on the concentration of the toxicants and the period of exposure [Karlson-Norrgan, et al., (1985); Mallat, (1985); Franchini, et al. (1994); Oliveira, et al. (1996); Olojo, et al., 2007; Ayoola, 2008]. Wide varieties of insecticides and other toxic by-product tend to accumulate in high concentration within it (Metelev et al., 1971; Ayoola, 2008a, b) and the organ suffers harmful effects.

C. gariepinus respires rapidly. It is very hardy since it tolerates both well and poorly oxygenated waters. It is widely cultivated and found in water bodies in Nigeria hence used as biological indicators in ecotoxicological studies (Wekler, 2000). Therefore, this study is aimed at investigating the possibility of histological alteration in the gills of *C. gariepinus* on exposure to sub lethal concentrations of soap and detergent effluents.

MATERIALS AND METHODS

Five hundred (500) species of *C. gariepinus* with the mean weight of 10.0 ± 0.4 g and standard mean length of 5.0 ± 0.25 cm were procured from a local fish farm in Ogbomoso, South-west, Nigeria, and was used for chronic toxicity test of soap and detergent effluent. The test organisms were brought to the Laboratory for acclimatization in plastic pool tanks for a period of two weeks. During this period, they were fed once daily with standard fish pellet while feeding was discontinued 24 h to the commencement of the definitive test.

Samples of soap and detergent effluents were collected from the discharging point of Global Soap and Detergent Industry, Ilorin, Kwara State, Nigeria. The sample was thoroughly mixed prior to refrigeration in the laboratory (Reish and Oshida, 1987) and their physicochemical characteristics were subsequently determined following the procedure of WHO (1988).

Five sub lethal concentrations of soap and detergent effluents were prepared in the order of 0.002, 0.003, 0.004, 0.005 and 0.006 mgl^{-1} in three replicates with control stock after series of preliminary screening tests following the technique of Solbe (1995). Fishes were fed with standard fish pellet at 5% of body mass once a day and (10) test medium were replenished at 24 h intervals with fresh test medium and were kept well-aerated; ten (10) number of fish specimen were maintained in each concentration tank. After 52 days, the test fishes were sacrificed for histopathological studies.

Parameters such as temperature, dissolved oxygen, pH, total dissolved solids, total dissolved oxygen, salinity and total alkalinity of the test solutions were measured at the peak of the experiment.

The gills of the test animals (fish) were excised keeping the filaments and rakers intact, rinsed in normal saline, fixed in 10% formallin for about 24 h at 4℃, dehydrated through series of graded alcohol, cleared in xylene, infiltrated with paraffin at 56℃, then embedded in paraffin wax (Luna, 1968). Thin section of the selected gill tissues of about 6 – 7 Nμm) was cut by means of a rotatory microtone, dehydrated and stained with haematoxylin and eosin. The sections were examined and photomicrographs using an Olympus BH2 microscope fitted with photographic attachment were taken. The prepared slides were used to describe the histological structure appearance, arrangement and their physiological conditions.

RESULTS

The selected physicochemical parameters of the test medium were recorded to be temperature, 33 - 38℃; Salinity, 7.5 mgl^{-1}; pH 8.7; total dissolved solids, 152.0 mgl^{-1}, biological oxygen demand (BOD) 1.2 $mgml^{-1}$ and dissolved oxygen (DO) - 1.80 mgl^{-1}.

In general, the most common tissue modification was the displacement of the epithelial layer of the secondary lamellae from the underlying connective tissue plate and oedematous condition. Mild cellular infiltration of seconddary lamellae, mild congestion of blood space and slight epithelial lifting was observed in the gill of the control experiment (Figure 1a). Across all experimental stocks, the observed pathologic lesions were; cellular infiltration of secondary lamellae, interlamellae hyper plastic lesion, cellular proliferation, oedema (an indication of ballooning dilatation in form of club deformation at the tip of seconddary lamellae), congestion of the blood vessels, epithelial thickening of both the lamellae and gill raker (Figures 1b, 2a, 2b 3a and 3b) coupled with the presence of mucin (depicting an inflammatory reaction) and necrosis, as observed in the gill tissues of the fishes held at highest concentration of soap and detergent effluent.

Therefore, all the pathologic lesions recorded in this study were basically concentration dependent and the damage done to the gills were irreversible.

DISCUSSION

This investigation revealed that the prominent features of the soap and detergent effluents showed a number of deviations from FEPA (1991) specification for maximum limit allowed for effluent discharge into water bodies. The concentrations of lead, iron and manganese in the effluent fell within FEPA (1991) specification, but their residual effects which may impair organs like the gills, liver, brain, kidney and genital organs cannot be ruled out. Low dissolved oxygen (DO), low biochemical oxygen demand (BOD), low dissolved solids (DS), high total suspended solid (TDS) and high alkalinity were also recorded which could be attributed to the high level of organic load in the wastewaters thus giving room for degradation of organic materials and this is in agreement with the submission of Adewoye and Fawole (2002); Adewoye et al. (2005).

Gills are the first target organ of several pollutants because of their very large interface area between external and internal fish environment, performing vital functions such as gas exchange and ion osmoregulation, gills are partially sensitive to adverse environmental conditions.

In this study, no recognizable changes were observed

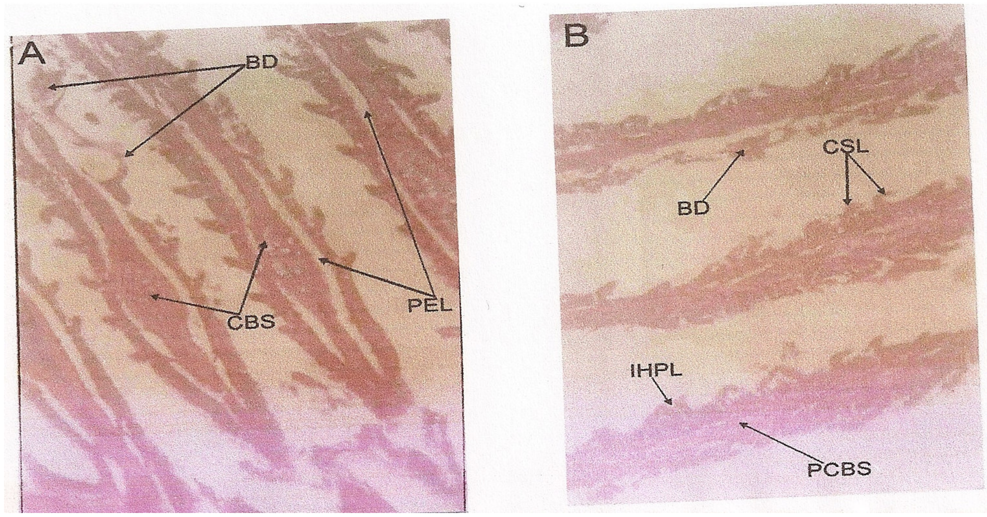

Plate 1. Light micrographs of gill filaments of C. gariepinus exposed to 0.000mg/l^{-1}and 0.002mg/$^{-1}$ concentrations of soap and detergent effluents; showing (A) The tissue changes was mild and characterized by mild ballooning dilatation (BD); Insignificant congestion of the blood spaces (CBS) Partial epithelial lifting (PEL); all these are indications of hyperplasia condition of the cells was noted to be due to stress. **(B)** Infiltration of the secondary lamellae (CSL); Interlamellae hyperplasia (IH); Partial congestion of the blood space (PCBS) by blood corpuscle is an indication of circulatory anomalies; and, Club shaped deformations at the tip of the secondary lamellae which is an indication of oedematous condition. All these were observed at the control experiment (0.000mgl^{-1}) and at lowest concentration (0.002mgl^{-1}) respectively.

in the gills of the control fishes Each gill consists of primary filament and secondary lamellae which conformed with the submission of Hassam et al. (2007); Anthonio et al. (2007) in their work on the histopathological changes in the liver and gills epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper, Ayoola, (2008a and b) where glyphosate herbicide was used on juvenile *O. niloticus* and Ayoola (2008). All pathological lesions observed in the gills of *C. gariepinus* in this study, are categorized under cellular infiltration, desquamation (displacement of epithelial cells), oedematous manifestation and cellular necrosis, these findings could be linked with increase in the activities of the test organisms exposed to the changing environment, diffusion distance from surrounding water to capillaries and at the same time an increase in the amount of tissue (blood corpuscles) in the blood spaces of secondary lamellae and this fact agreed with the opinion of Ayoola (2008 a and b); Anthonio et al., 2007; Olojo et al. (2005) where it was reported that such anomalies in the gill structure of *O. niloticus* on exposure to glyphosate herbicidal, waterborne copper and lead effects respectively. The cellular hypertrophic condition observed, led to a decrease in the respiratory capacity between the lamellae, impairs the diffusion of oxygen across the gills due to the swollen condition of the epithelium and decrease in free gas exchange which in turn limits the compensatory changes that makes organism in question to become unadaptive when the duration of exposure and the concentrations of the effluents exceeds biological tolerance limits, this result is in line with the submission of (Gardner and Yevich, 1970; Skidmore and Tovell, 1972; Wedemeyer et al., 1990 and Ayoola, 2008). Damages done to the gills indicated that the sub lethal level of this metals mixture caused impairment in gaseous exchange efficiency of the gills (Hassam et. al., 2007; Ayoola, 2008a and b).

Moreover, the presence of mucous-filled cavity (oedema) observed in the gill filaments of *C. gariepinus* may be considered as an ion trap, in a way to concentrate free elements from surrounding water between the neighboring secondary lamellae; However, the presences of white blood cells in the form of macrophages, lymphocyte and neutrophils aggregation in the oedematous cell clearly indicated an inflammatory reaction and cellular response of the fish to environmental contaminants (Zeeman and Brindley, 1981; Tan et al., 2000). The presence of mucous over the body surface of the test fishes was noted to gradually increased, this is a reflection of which reflected the hyperplastic glandular cells activity in the dermis of exposed fish; the concomitant appearance of inflammatory cells were also an indication of a secondary defense mechanism of the body against infections and this

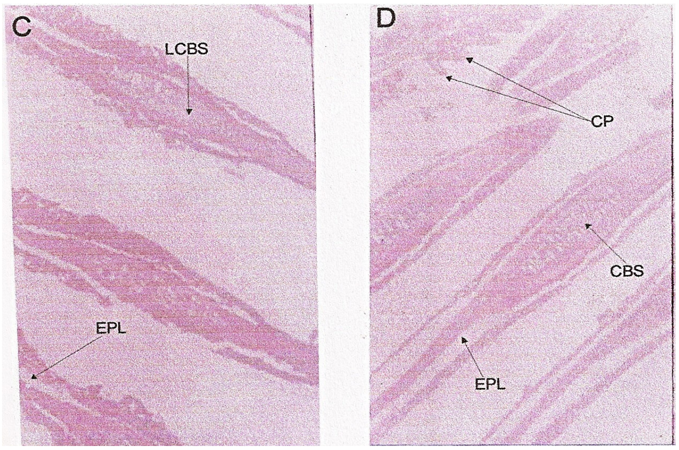

Plate 2. Light micrographs of gils filaments of C. gariepinus exposed to 0.003(C) and 0.004mgl^{-1}(D) concentrations of soap and detergent effluents. (C) Fairly pronounced congestion of the blood spaces by blood corpuscle (LCBS) (indicating circulatory anomalies); and, epithelial lifting at the base of the secondary lamellae (resulting from oedematous condition, macrophages aggregation or accumulation of white blood cell type). (D) Fairly pronounced cellular hyperplasia (CP) in the interlamellae region with marked outgrowth; Congestion of the blood spaces (CBS) and Epithelial lifting (EPL). These were observed at 0.003mgl^{-1} and 0.004mgl^{-1} respectively.

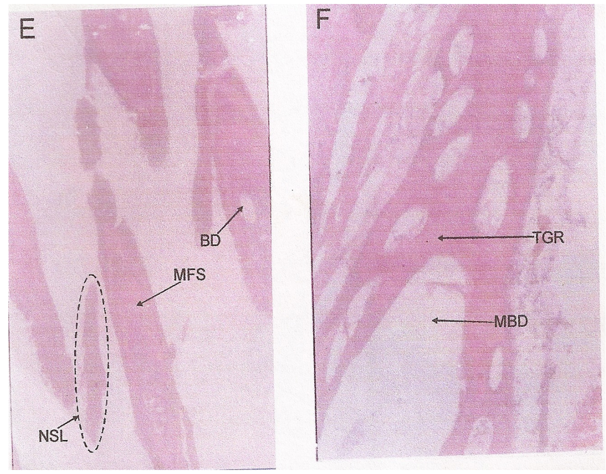

Plate 3. Light micrographs of gill filaments of *Clarias gariepinus* exposed to 0.005 (E) and 0.006 mgl^{-1} (F) concentrations of Soap and Detergent Effluents. (E) Total breakage of the secondary lamellae from the basement cells an indication of cellular necrosis; magnified ballooning dilatation (MBD) showing some degree of leukocyte or mucin filled space (MFS). (F) Magnified ballooning dilatation (MBD) of the secondary lamellae depicting higher degree of leukocyte or mucin filled space with thickening of the epithelial lining of the gill raker (TGR) on the gill arch; fusion of the secondary lamellae accompanied by aggregation of macrophages in the blood spaces of the gill lamellae resulting in the obliteration of the space between secondary lamellae and basement cells. All these are indications of cellular necrosis. 0.005 mgl^{-1} and 0.006 mgl^{-1} respectively.

this is in conformity with the submission of (Basanta and Subhas, 2000 and Ayoola, 2008). It may as well serve to protect the epithelial against both mechanical abrasion and infection as submitted by Olson and Fromm (1973) and Ayoola (2008b). The changes recorded in the gill structure of the test fish in this study were indicative of diminished oxygen supply to the test fish, resulting also in hypoxic respiratory response, though not lethal but not reversible.

Such histopathological changes of gills such as cellular hyperplasia and hypertrophy, epithelial lifting and increase in mucus secretions have been reported after the exposure of fishes to a variety of noxious agents in the water, such as pesticides, phenols and heavy metals in their mixtures, Ayoola (2008).

Conclusively, this study has revealed that, exposure of *C. gariepinus* adult to even low concentrations of soap and detergent effluents is enough to induce various toxicological effects in terms of histological degradations in gill structures. The damage induced on the gill of the test fish was found to be concentration and exposure period dependents and the pathologic lesion recorded in the gill structures of *C. gariepinus* were well pronounced mostly at higher chronic concentration of the effluents in question. It has been discovered that, toxic environmental conditions can result in two types of structural changes in tissues of the organisms. First, are the results of the direct toxic effect of the pollutant leading to degeneration and necrosis of vital organs usually at the cellular level. The second is the result of compensatory mechanisms that deal with the environmental stressor (in terms of handling, poison and so on) as observed in cellular hyperplasia (Hughes and Perry, 1976). In the present work, it seems that both types of structural responses are in operation, in that; at the control experiment, a mild hyperplastic condition of the cell was evident which is an indication of stress due to soap and detergent contamination; a situation that conforms with the submission of Hughes and Perry, (1976).

Therefore, it can be inferred that, indiscriminate discharge of soap and detergent effluents can induce damages to the tissue and organ, which might make the fish vulnerable to diseases and eventually lead to death of prominent edible species of the aquatic environment. Therefore, there is need for the adoption of proper effluent treatment technology which would ensure proper treatment of industrial effluent prior to their discharge into the environment.

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