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Physico-Chemical Evolution, Gill Mda Concentration And Histology Of Tilapia Exposed To Mixed Effluent In Okrika River, Rivers State, Nigeria

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ABSTRACT
The physico-chemical evaluation and histological studies on Tilapia (*Oreochromis niloticus)* exposed to mixed effluent (industrial, domestic and municipal) from Okrika River were investigated. Tilapia samples were collected at about 500 meters from point of entry of mixed effluent into the River (downstream) and about 1.5 kilometers from the point of entry of mixed effluent into the River (upstream) while Tilapia from a fish pond affiliated to Rivers State Sustainable Development Authority (RSSDA) was used as control. Malondialdehyde (MDA) concentration was assayed in the gill homogenates in the Tilapia fish blood serum.
It was observed that the biological oxygen demand (BOD), total dissolved solids (TDS), total suspended solids (TSS), conductivity, chromium and cadmium were significantly higher in concentrations in the Okrika River exceeding FEPA regulations in Nigeria. Aside chromium whose concentration was more upstream of the river, BOD, TDS, TSS, conductivity and cadmium show more concentration downstream of the river. Results showed an increase in gill MDA concentration upstream samples (0.00±0.00 to 1.51± 0.24nm/mg), downstream samples (1.51±0.24 to 2.32±0.66nm/mg) and the control samples (2.32±0.66 to 2.70±0.89nm/mg). No significant change was also observed in gill MDA concentration of both downstream and upstream samples when compared with the control. Histology of the gill showed vacuolar degeneration, focal areas of necrosis and aggregation of inflammatory cells between the hepatocytes. From the investigation, the mixed effluents discharged into the river are toxic to the marine environment.
Key words: Physico-chemical, Histological, Effluent, Malondialdehyde and Tilapia.

INTRODUCTION

Pollution of the aquatic environment is a predominant form of pollution since the majority of chemical pollutants are disposed into seas, rivers, lakes and wetlands. These aquatic environments are home to a vast diversity of organisms and are also of enormous direct importance to humans, providing essentials such as water, food as well as transportation and other forms of economic opportunities.

Okrika River is an estuary from the Atlantic Ocean cutting across Abuloma and Woji towns of Port Harcourt in Port Harcourt Local Government Area of Rivers State, Nigeria. Abuloma and Woji are important towns as they are integral parts of Trans-Amadi Industrial Layout in Port Harcourt City. Okrika River however, is the chief source of life to the lower class inhabiting its coastline.

Aquatic food is constantly relied upon as source of protein and economic power through to the public. It therefore becomes imperative to highlight the integrity of the water quality and aquatic life following the enormous anthropogenic activities in and around the river.

Nile *tilapia* is one of the most important commercially cultured *tilapia* species indigenous to Nigeria (Ayoola, 2008). Tilapia is distinguished by its adaptation to living in fresh, brackish and nearly saline water, and can survive in partially polluted water (Zyadah, 1997). It is less sensitive to most toxic substances than most aquatic species. Any toxicant that affects tilapia would most likely be toxic to other aquatic organisms (Murungi & Robinson, 1987).

The use of molecular biomarkers, such as lipid peroxidation products, antioxidant enzymes and cytrochrome P_{450}, which are measurable internal indicators of changes in organisms at the molecular or cellular level is very important to assess the effect of the broad spectrum of industrial, agricultural, commercial and domestic chemicals (xenobiotics) being disposed into the aquatic environment as they may pose a threat to aquatic organisms and human health indirectly (Valavanidis and Vlachogianni, 2008). Histopathology provides a rapid method to detect the effect of irritants in various organs (Johnson *et. al.,* 1992). This research focuses on the effects of aquatic pollution from discharged mixed effluent in Okrika river on Tilapia (*Oreochromis niloticus)* using some selected biochemical biomarker and histopathologic assay of gills.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

A total of fifteen adult Tilapia (*Oreochromis spp.)* fish of both sexes with a mean average weight of 88.24 ± 26.41g were used for this study. The fishes were caught alive with hook from Okrika River and the Rivers State Sustainable Development Agency (RSSDA) assisted fish farm by random sampling and transported to the laboratory in a well aerated big plastic container with water.

EXPERIMENTAL DESIGN

The Tilapia fishes were assigned into three groups consisting of five (5) fishes each.

Control group: Tilapia fishes obtained from the RSSDA-assisted fish farm

Downstream group: Tilapia fishes obtained from about 500 meters from point of entry of mixed effluent into the Okrika River

Upstream group: Tilapia fishes obtained from about 1.5 kilometers from the point of entry of mixed effluent into the Okrika River.

SAMPLE PREPARATION

The fishes where sacrificed immediately they arrived at the laboratory and weighed. A sterile scapel was used to make a neat slit on the dorsal portion from the heart to the gill and allowed to bleed; whole blood was collected into lithium heparinized bottle for enzyme/biochemical assay. The fish were then properly dissected and the liver was quickly removed with the aid of sterile forceps, washed in ice-cold saline to remove the adhering body fluid and then divided into portions. The first and largest lobe was put in ice cold normal saline for MDA assay, while the other was fixed in 10% formalin for preparation of histopathological studies. The fish gills were lastly removed after dissection, washed in ice-cold saline, divided into two parts and put in ice cold normal saline and 10% formalin respectively.

DETERMINATION OF WATER QUALITY PARAMETERS

Heavy metals were determined in water samples using a Perkin Elmer model 306 Atomic Absorption spectrophotometer (Detection limit of the heavy metal are shown in Table 1). The method used for the determination of physic chemical parameters was described by A.O.A.C. (2005). The results were tabled for interpretation.

HISTOLOGICAL EXAMINATION

The method of Baker and Silverton (1985) was adopted in the preparation of slices on previously fixed gill tissue for histological examinations. Following the decalcification, dehydration, impregnation, embedding and section cutting, the tissues were stained using Mayer's acid alum haematoxylin and eosin and mounted in natural balsam. The slides were then examined microscopically for histological changes.

STATISTICAL ANALYSIS

All data are presented as means ± SD of five determinations. The one way ANOVA was used to analyse the data. The results were considered significant at P values of less than 0.05 ($P<0.05$).

RESULTS

Physico-chemical Parameters of water sample from Okrika river.

As observed in Table 1, the biological oxygen demand (BOD), total dissolved solids (TDS), total suspended solids (TSS), conductivity, chromium and cadmium are higher in concentrations in Okrika River exceeding FEPA regulations in Nigeria. Aside chromium whose concentration is higher upstream of the river, BOD, TDS, TSS, conductivity and cadmium show higher concentration downstream of the river.

Table 1: Physico-chemical Parameters of water sample from Okrika River.

Parameters	Downstream	Upstream	FEPA	WHO
Temperature (^oC)	32.4±2.31	33.47±0.97	40.0	NG
Ph	6.60±0.53	6.51±0.31	6.0-9.0	6.5-9.2*
Dissolved Oxygen (mg/l)	6.0±0.5	6.27±0.25	5.0-9.5	NG
BOD (mg/l)	160.0±5.0	127.0±1.00	50.0	NG
COD (mg/l)	220.33±9.50	220.0±5.0	NS	NG
Total Phosphorus (mg/l)	0.44±0.04	0.29±0.01	5.0	NG
Total Dissolved Solid (mg/l)	14,580.0±72.11	13,000.0±500.0	2,000.0	1,500.0*
Chloride (mg/l)	8,133.33±152.75	8,200.0±200.0	NS	250.0
Total Suspended Solid (mg/l)	1,500±100.00	1,300.0±100.0	NS	NG
Conductivity (ws/cm)	12,926.67±110.15	12,593.33±46.19	1,500.0	NG
Chromium (mg/l)	1.05±0.01	1.79±0.08	0.05	0.05
Cadmium (mg/l)	0.41±0.07	0.413±0.05	0.005	0.003
Lead (mg/l)	0.24±0.01	0.226±0.03	0.5	0.01
Nickel (mg/l)	0.16±0.01	0.159±0.01	0.5	0.02

Values are mean ± standard deviation (n= 2) for downstream and upstream.
FEPA (1991): Federal Environmental Protection Agency; **NS**: Not Specified.
WHO's Guidelines, Geneva, 1993; NG: No Guideline.
*WHO Standards, 1971.

Gill MDA concentration

No significant change (p>0.05) was observed in gill MDA concentration of both upstream and downstream tilapia fishes when compared with control (Figure 2).

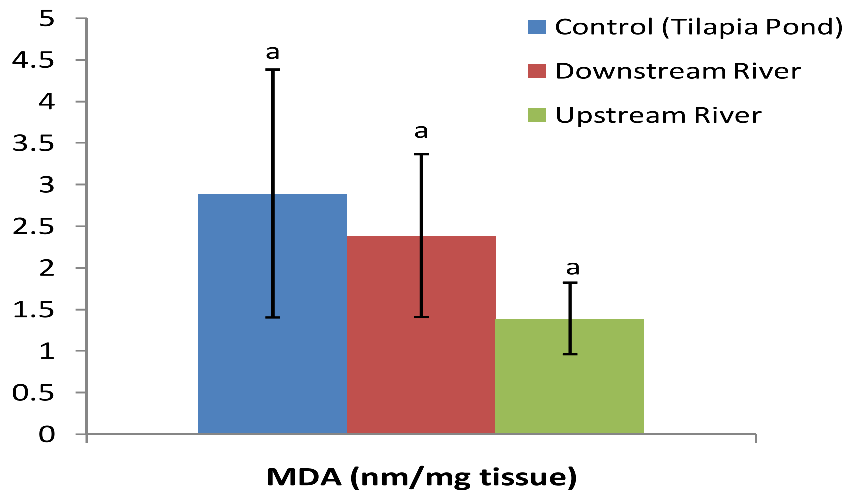

Figure 2: Effect of mixed effluent discharged in Okrika River on gill MDA of Tilapia fishes

Gill histopathology

Plate 1 shows the normal histological structures of the gill. No recognizable changes were observed in the gills of the control fish. Each gill consisted of a primary lamellar filament and secondary lamellae. Chloride cells were visible along the primary lamellar epithelium especially at the bases of secondary lamellae. The histological alteration in the gills of tilapia from Okrika River included proliferation in the epithelium of gill filaments and secondary lamellae, resulting in fusion of secondary lamellae (Plates 2-3), severe degenerative and necrotic changes in gill filaments and secondary lamellae and mucous proliferation (Plate 2a). Edematous changes characterized by epithelial lifting were observed in gill filaments and secondary lamellae (Plate 3b). Moreover, aggregation of inflammatory cells, dilation and congestion in blood vessels were observed in gill filaments. Atrophy of second lamellae was also noticed (Figure 6).

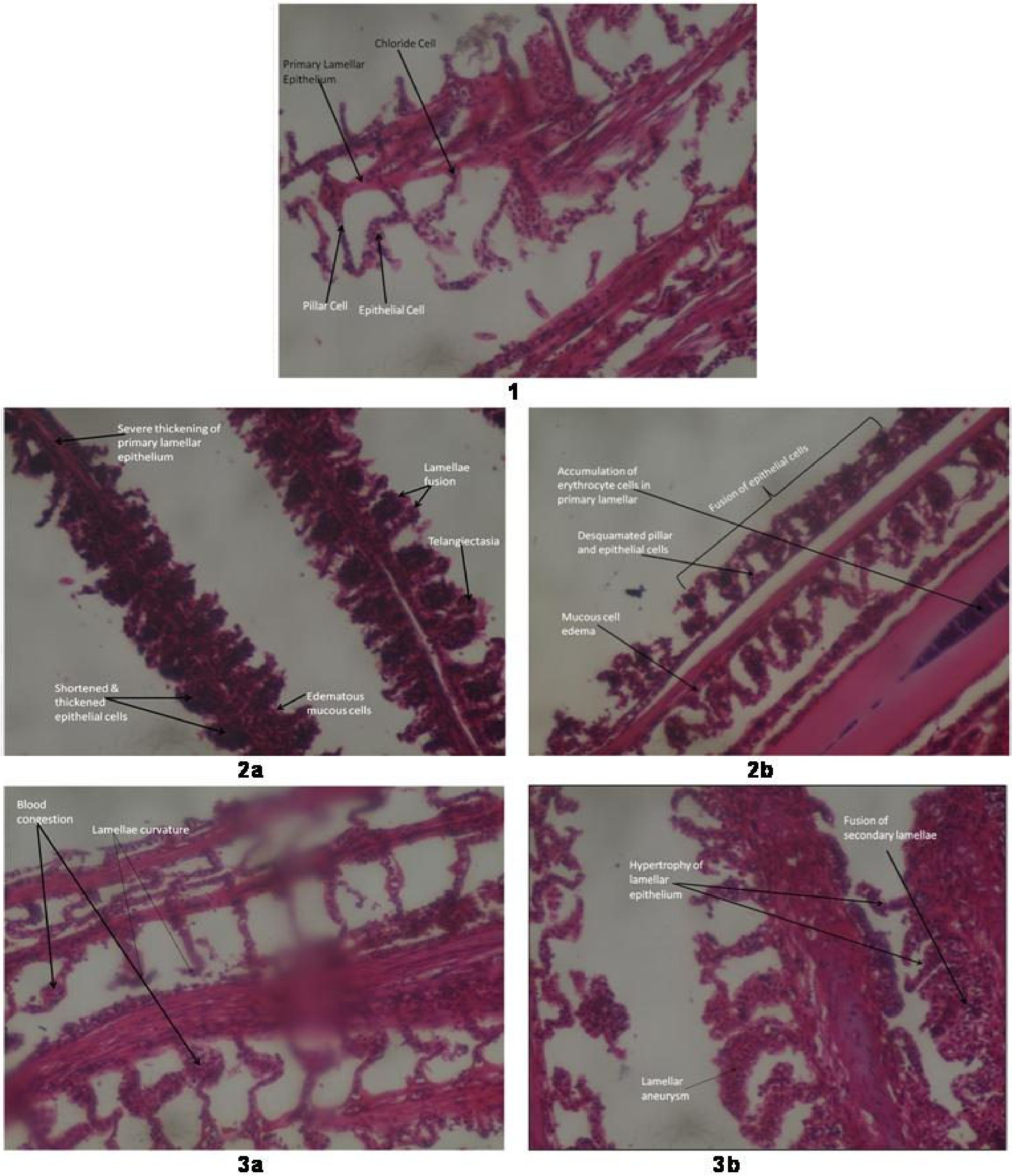

Figure 6: Effect of mixed effluent on gill histopathology of tilapia fishes

Plate 1: Tilapia pond (Control) showing normal appearance of primary lamellar epithelium, chloride cell, epithelial cell, mucous cell and pillar cell. **Plate 2a:** Tilapia Downstream showing telangiectasia at the tip of secondary lamellae and thickening of primary lamellar epithelium, fusion of secondary lamellae, extensive edema of the epithelial and mucous cells. **Plate 2b:** Tilapia downstream showing swelling and fusion of the secondary and primary lamellae, cellular infilteration and breakdown of pillar cells. **Plate 3a:** Tilapia upstream showing mild curvature of secondary and primary lamellae, separation of epithelial cells and fusion of adjacent

secondary lamellae. **Plate 3b:** Tilapia upstream showing mild edema of epithelial cells, hypertrophy and hyperplasia of mucous and chloride cells.

DISCUSSION

The presence of pollutants in water affects the physicochemical parameter of the water. Conductivity, chromium, cadmium, and total suspended solids were above specified limits (FEPA, 1991) in the Okrika River. The increase in conductivity concentration may be due to high concentration of TDS in the river which may be linked to the fact that effluents can introduce some reactions that precipitate more solids in the river. The physico-chemical parameters of the river in this investigation reveal some variations between upstream and downstream locations. TDS decreased upstream due to natural filtration (Ajao, 1990; Ayoola & Kuton, 2009). The biological oxygen demand (BOD) is above specified limit in both locations but much higher in the downstream samples. The high level of BOD may be due to increased effect of surface run-offs, soil erosion and effluent discharges into the receiving water body (Oyewo *et. al.,* 1999; Ayoola & Kuton, 2009). The uniformity of the river temperature may be linked to the regular tidal motion, which ensured the complete mixing of both locations. This is in agreement with Ajao (1990), Oyewo (1998) and Ayoola & Kuton (2009) in their works on Lagos lagoon. Temperature is a stable environmental factor in the shallow brackish environment of West Africa, and it is most unlikely that even its variation constitutes an important ecological factor in this area (Hill & Webb, 1958; Lenghurst 1958; Olaniyan, 1969). Similarly, pH is relatively stable in both locations. High seawater influx and strong tidal currents due to high buffering capacity of the system and effective flushing may cause the stable pH observed in this environment (Ayoola & Kuton, 2009). Lead, nickel, phosphate and dissolved oxygen (DO) are present within acceptable limits indicating sub-lethal pollution load.

Human destructive influence on the aquatic environment is in the form of sub-lethal pollution, which results in chronic stress conditions that have negative effect on aquatic life (Mason, 1991). The result of the present study showed (the results says no significance, check) significant elevation in the concentration of liver MDA in Tilapia samples collected from downstream when compared with control. The elevated MDA observed may be due to the increased amount (above the FEPA 1991 specified limits) of chromium. Chromium is capable of redox cycling in which it accepts or donate a single electron producing reactive radicals and the resultant reactive oxygen species (Pratviel & Genevieve, 2012). The mechanism of action involves probably Fenton's reaction (Fenton, 1894) and the Haber-Weiss reaction (Haber and Weiss, 1932), in which hydroxyl radical is produced from reduced iron and hydrogen peroxide occasioned by stress induced environment. The hydroxyl radical initiates lipid peroxidation (Devasagayam *et. al.,* 2004). Therefore the presence of chromium in the river above tolerance level may enhance its absorption into biological systems of the Tilapia in an uncomplexed form (not in a protein or other protective metal complex) leading to significant increase in the level of lipid peroxidation (MDA) and oxidative stress. Conversely, there was no significant change in the level of lipid peroxidation upstream, which, apparently may be due to “dilution” of the xenobiotics present in the mixed effluent as it spreads across the river through tidal movement.

The present study also revealed that tilapia from Okrika river manifest histopathological changes in gill both upstream and downstream. It is possible that the pathological alterations in the tissues of tilapia could be a direct result of the heavy metals, pesticides, salts and sewage, which enter the river with drainage water (Mohamed & Gad, 2008; Ali *et. al.,* 2008; Ali & Fishar, 2005; Mansour and Sidky, 2003; Gupta & Abd El-Hamid, 2003; Sabae & Rabeh, 2000) including industrial and domestic effluents from the zone. In the upstream samples, there were mild necrotic changes involving vacuolar degeneration and aggregation of inflammatory cells indicating that pollutants in the river are dose-dependent. Also, edema, separation of the respiratory epithelium of primary and secondary lamellae with necrosis of lamellar epithelial cells and severe epithelial proliferation of secondary gill lamellae were evident following exposure of tilapia downstream. This observation is similar to findings of Hadi *et. al.* (2009), Omitoyin *et. al.* (2006), Aguigwo (2002), Smith & Haines (1995) and Yang & Albright (1992). Severe epithelial proliferation of secondary gill lamellae results as a response of the malpighian cells to xenobiotic irritation, as they migrate distally, often in the early stages, resulting in accumulation of cells at the leading edge of the secondary lamella, and progression of this migration leads to lamellar fusion and terminal lamellar clubbing (Hadi *et. al.,* 2007; Robert, 2001). Adjacent lamellae fusion (telangiectasis) as a characteristic pathological change of the gill associated with physical and chemical trauma was also reported by Robert (2001), and Soufy, *et al.*,(2007). It has been reported that toxicants at lower levels given for a prolonged time causes severe damage to the bronchial system of fish than to short term treatment (Ramesh, 1994). In the present study also, extensive cellular hyperplasia filling the entire inter-lamellar spaces, haemorrhage and complete damage of epithelium were more in tilapia fish caught downstream when compared to that of upstream and control tilapia fish. As observed, necrosis of gill epithelium of fish may be due to direct deleterious effect of the mixed effluent toxicants in the river. Lloyd (1960), Brown (1968) and Skidmore & Tovell (1972) reported that many noxious compounds and ions have been shown to damage the respiratory epithelium of gills. Damage of the gills indicated that the mixed effluent caused impairment in gaseous exchange efficiency of the gills. Similarly, the stressful behavior of respiratory impairment to the toxic effect of the mixed effluent on the gills was similar with the reports of Omitoyin *et al.,* (2006) and Aguigwo (2002) that herbicides impair respiratory organ, which may result to asphyxiation (Warren, 1977). A layer of edema has the primary effect of increasing the diffusion distance between blood and water and thus reducing the affected area to an essentially dysfunctional state (Wood *et. al.,* 1988). Gill epithelial swelling, complete desquamated lamellae and blood capillary pillar cells would also account for the impairment of oxygen-carbon dioxide exchange, and for the hypoxia (Wedemeyer, 1971). Swelling (edema) and desquamated epithelium of gills observed in the present study might have caused impairment of oxygen or carbon-dioxide exchange. Morgan and Tovell (1973) stated that lifting up of epithelium is a protective effect, resulting in increased water blood diffusing distance and hindered toxicant uptake. Ramesh (1994) reported that separation and lifting up of the epithelium might be a defense response of the fish in response to toxicants. A similar situation may prevail in fish from Okrika river in the present study thus finding the support from the observations of the above authors.

In conclusion, the mixed effluents discharged into the river is toxic to the marine environment as it results in oxidative stress inducing lipid peroxidation thereby elevating tissue damage via generation of reactive oxygen species (ROS) in body metabolism although the level of toxicity ameliorates as it spreads away from the point of discharge due to tidal motion.

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