Ass. Univ. Bull. Environ. Res. Vol. 2 No. 2, October 1999

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TOXOPATHOLOGICAL EFFECTS OF SOME ENVIRONMENTAL POLLUTANTS ON *OREOCHROMOUS NILOTICUS* GILLS

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ABSTRACT :

Total of 140 fish (5-10 g) (*Oreochroms niloticus*) were obtained from Assiut Univ. Fish Rearing Farm. Fish were acclimatized and divided into 7 groups (20 each). Fish groups were exposed to LC_{50} of copper sulfate (31.9 ppm), Baylucide (0.3125 ppm), Lead acetate (43.6 ppm), Sulfuric acid (LpH 4.075), Ammonia (0.53 mg/L), Upper incipionic Lethal Temp (UILT 35°C) and the seventh group was kept as control. The symptoms and post mortem findings were recorded. The gills were removed and prepared for scanning, semithin and transmission electron microscopic examination. Changes of different types were observed in the morphology of the covering epithelium in the gill filaments and secondary lamellae, the results were discussed.

INTRODUCTION :

Environmental pollution is one of the serious problems allover the world. Pollutants are considered the most deleterious agents to biological life. In Egypt the extensive use of chemical agents in agricultural activities in addition to their drainage in the River Nile has caused many problems to the aquatic life.

The exposure of fish to these pollutant hazards constitute one of the important factors responsible for the great losses of a good source of animal protein, which is one of the serious problems especially in the developing countries. Gills are the first and

the most sensitive organ exposed to these pollutants.

Fishes are exposed to many environmental pollutants as well as stress factors in the natural rearing condition in the River Nile. Some of these pollutants are copper sulfate moulluscicides which used for Bilharzial control, lead which comes from different sources, sulfuric acid which is the main industrial pollutant of superphosphate factory in Assiut [1]. Ammonia was considered as the direct cause of environmental gill disease (EGD) in fish being raised under intensive culture conditions [2], while stress factors as water temperature change and environmental pH are considered to be the indirect cause of EGD [3-5]. The main effects of EGD are reduced growth rate, reduced dietary efficiency and increased production costs, [6]. In this work it was intended to study the morphological changes in the gills of *Oreochroms niloticus* experimentally exposed to the above mentioned environmental pollutants and stress factors.

MATERIALS AND METHODS :

I -Chemicals :

1- Copper sulfate was obtained as pentahydrate powder (pure grade) from ADVIC laboratory chemicals, Cairo.

2- Bayluside was obtained from Bayer, Cairo Scientific office as wettable powder containing 70% active ingredient.

3- Lead acetate, sulfuric acid and ammonia were obtained from fluka as pure grade chemicals.

II –Fish :

Total of 140 fish (*Oreochroms niloticus*) were obtained from fish rearing unit in Assiut University ranged from 5-10 gm. Fish were acclimatized to the laboratory conditions for two weeks before experimental work. Tetramine fish feed (Tetra, Dr. Baensch Molle, West Germany) added twice daily ad libidum and withhold three days before introduction to bioassay to empty the gut [7].

III-Experimental design:

First group (copper sulfate: n=20) was exposed to the LC_{50} of copper sulfate 31.9 ppm, for 96 hours [8].

The second group (Bayluscide: n=20) was exposed to LC_{50} of Bayluscide, 0.3125 ppm, for 96 hours [9].

The third group (lead acetate: n=20) was exposed to LC_{50} lead acetate, 43.6 ppm, for 96 hours [10].

The fourth group (sulfuric acid: n=20) was exposed to Lph_{50} sulfuric acid, 4.075 for 96 hours [1].

The fifth group (Ammonia: n=20) was exposed to LC_{50} (0.53 mg/L) for 96 hours according to Arthur [11].

The sixth group (heat stress n=20) was exposed to UILT (Upper Incipionic Lethal

Temp.), 35°C for 96 hours [12]. Stress heat source was adjusted by heating and thermosetting the required temperature with embedded thermometer in water media.

The seventh group (n=20) was kept as control.

In all these groups the water hardness was 40-48 mg/l as Ca Co_3, pH value was 7.1-7.4 except for Sulfuric acid group. The temperature of water was 22°C except for heat stress group.

Tested Fish were observed allover the whole period of the experiment, Symptoms and gross lesions were recorded. Gill samples were taken from dead fishes and at the end of the experiment.

The samples were washed several times in cacodylate buffer and fixed in glutaraldehyde (5%) for either semithin section or scanning electron microscopy.

Preparation of semithin sections:

The previously fixed samples were trimmed to approximately 1×1× 2 mm blocks. These blocks were washed in cacodylate buffer (0.1 M, pH. 7.2) for 1-3 hours, and then postfixed in 1% osmiumtetraoxide for 2 hours. After repeated washing in cacodylate buffer (4 x 30 min) and dehydration in ascending gradients of ethyl alcohol up to 100% (30 min for every conc.), the specimens were first placed in propylene oxide for 60 minutes, then in pure epon 812 and incubated in a special polymerization incubator (one day at 35°C, one day at 45°C, and 3 days at 60°C). The blocks were trimmed with LKB ultratome. Semithin sections were obtained and stained with 0.25% toulidine blue for 2 minutes at 80°C and examined with light microscope.

Transmission electronmicroscopy (TEM):

Representative fields of semithin section were selected. Ultrathin sections (70 nm) were cut with a diamond knife using a Reichert OMVs ultramicrotome. They were mounted on copper grids and stained with uranyl acetate lead citrate stain [13]. The ultrastructural investigation was carried out with TEM (Joel Cx II).

Scanning electron microscopy (SEM) :

The fixed gill samples were processed for SEM according to the modified a tato method [14]. Samples were examined and photographed with SEM (JSM T 200) at 25 kv.

RESULTS :

Clinical signs and gross pathology:

- In the first group (copper sulfate):

At the beginning there were harried respiration manifested by increased rate of gill movement. Rapid and irritable movement of fish was recorded 30 minutes after dosing. Gelatinous layer of bluish red colour was detected covering the gill surface 2-6 h from exposure. Before death fish showed

unbalanced movement, laying down in the bottom of the aquarium with decreased respiratory movement. Grossly the fishes exhibited a dark skin. Red spots and heavy slimy bluish gelatinous layer of musin were obviously seen on the gill surface.

-In the second group (Bayluscide):

The obvious clinical signs on fish were recognized as dullness and restless ness. Grossly, there were congestion of the gills.

-In the third group (Lead acetate):

There was no detectable clinical signs or gross lesions.

-In the fourth group (sulfuric acid):

The clinical signs started as increased rate of gill cover movements (harried respiration), uncontrolled and irritable movement, laying down in the bottom of the aquarium with decrease respiratory movement. Heavy slimy bluish gelatinous layer of mucin were seen on the gill surface.

-In the fifth group (Ammonia):

The fish exhibit lethargy, reduced appetite and increased respiratory movement. Congestion was the only detectable gross lesion in the gills.

-In the sixth group (heat stress):

The fish showed restlessness, harried respiration, uncontrolled and irritable movement. There was congestion of the gills.

Histopatholoy :

Copper sulfate group:

There were hypertrophy and hyperplasia of the goblet cells in the epithelial layer of the gill filaments and secondary lamellae (Fig. 1, 2). The epithelial cells in the secondary lamellae showed necrobiosis manifested by vacuolar degeneration of the epithelial cells with pyknosis of their nuclei (Fig. 2, 3).

Bayluscide group :

The main prominent finding is the presence of many eosinophil granule cells (EGC) in the subepithelial layer of the gill filament (Fig. 4). Some of them were degranulated. The epithelial cells showed degenerative to necrobiotic changes. There was vacuolar degeneration of some epithelial cells with pyknosis of their nuclei (Fig. 5). In some cases, hemorrhages were seen in the subepithelial area around the gill ray (Fig. 6).

Lead acetate group:

There were no detectable pathological changes in the gills of this group.

Sulfuric acid group:

The epithelial cells of the gill filaments and secondary gill lamellae undergo necrosis and sloughing. The capillary become denudated from epithelial covering (Fig. 7, 8). TEM showed that the necrosis begin by hydropic degeneration of the epithelial cells (Fig. 9).

Temperature group:

The secondary gill lamellae appeared swollen by SCM (Fig. 10). This swelling is due to hydropic degeneration of the epithelial cells (Fig. 11).

Ammonia treated group:

In SEM, there were thickening of the gill lamellae in comparison with the control one (Fig.12,13). This thickening is due to gill lamellae hyperplasia which lead to fusion of the secondary gill lamellae (inter lamellar occlusion (Fig. 14, 15). Another interesting lesion was the separation between the capillary and the epithelial layer (epitheliocapillary separation Fig. 16, 17). In addition there were degenerative process in the epithelial layer (Fig. 18). Eosinophile granule cells were also seen infiltrating the epithelial layer (Fig. 15, 18).

DISCUSSION :

It was generally accepted that gills are the first and the main route of entry of irritants in the fish in addition to its large surface area exposed to water. Consequently, absorption and binding of irritants to the branchial surface would lead to many pathological alterations.

Increase production of mucous with hyperplasia and hypertrophy of goblet cells in copper sulfate treated group were concerned mainly to the detoxification process and to dilute the irritant. This process was documented [15-17] who recorded a higher concentration of copper in the gills of fish exposed to sublethal copper concentration. Necrobiotic changes which were observed in the gill epithelium in copper sulfate treated group could be refered to the ion transporting membrane disturbance resulting in deterioration of the active transport process [18, 19]. Morever Cardheilac et al. [20] suggested that copper have binding ability to the gill tissue causing its damage with increase the production of mucous.

In the Baylucide exposed group, the main pathological lesions were vacuolar degeneration and necrobiosis of the gill epithelial covering. The presence of eosinophile granule cells infiltrating the epithelial layer pointed to the toxopathological effect of this compound to the gill tissue. The presence of hemorrhage in the subepethelial area was a manifestation of hypoxia induced by the necrosis of the epithelial cells, which interfere with the blood water exchange of oxygen [21].

Vacuolar degenerative changes, necrosis and sloughing of epithelial cells, observed in the sulfuric acid treated group are directly attributed to reduced oxygen permeability of gills caused by excess formation of mucous. This is in agreement with the results obtained by Daye and Grasida [22] they recorded a damage in the epithelium of the gill lamellae at pH level below 5.2 or above 9.0 in conjunction with hypertrophy of mucous cells. Mckenna and Dener [23] also reported that lower pH causes coagulation of mucous on the fish gill surface resulting in subsequent anoxia or respiratory failure.

The degenerative process, observed in the gill epithelium of fish in the heat stress group was attributed to the direct bad effect of increased temperature on the gill tissue. This could be also discussed on the bases of the concept given by Karwin-Kossakowski and Jezierska [24] who stated that, an elevation of water temperature was associated by an increase of oxygen requirements of fish and diminishes both available amount of oxygen in water and the efficiency of its binding with hemoglobin resulting in hypoxia.

In the sixth group, fish exposed to ammonia exhibited gill lamellar hyperplasia as well as progressive separation of the lamellar epithelium from the underlying endothelial cells of the capillaries. Hyperplasia in the gill lamellar epithelium are believed to reflect the mild irritant effect of ammonia and/or secondary to the degenerative effect of this agent. The recorded gill changes were also reported [21, 25, 26], who found that the common gill lesions induced by pollutants were necrosis, hypertrophy and hyperplasia of the branchial epithelium. Epitheliocapillary separation was recorded also in the gill lamellae after constant exposure of salmonids to ammonia level at and above 0.03 mg/liter [6]. The presence of eosinphile granule cells in the epithelial layer could be refered to the toxopathological effect of ammonia .

The results of these studies clearly indicated that, most of the used environmental stresses except lead acetate caused many pathological changes in the gills of Oreochromas niloticus which could be collectively named environmental gill disease. Studies of EGD will be continued evaluate the susceptibility of EGD-affected fish to aquatic bacteria and protozoa.

Fig. (1): SEM of the gills showing an increase in the number and size of the goblet cells (G) in the gill lamellae X 1500.

Fig. (2): Semithin section of secondary gill lamellae showing an increase in the number of the goblet cells (G) with necrobiosis of the epithelial cell covering. Toulidine blue, 10 X 40.

Fig. (3): TEM of the epithelial covering the gill lamellae showing an enlargement of the goblet cell (G) and hydropic degeneration of other cells. Lead citrate uranyle acetate X 2750.

Fig. (4): Semithin section of the gill lamellae showing mild vacuolar degeneration of their epithelial covering and eosinophil granule cell infiltration (E) some of them were degranulated. Toulidine blue stain 10 X 100.

Fig. (5): TEM of the epithelial covering showing hydropic degeneration with nuclear pyknosis. Not also the presence of eosinophil granule cell (E). Lead citrate uranyle acetate X 2750.

Fig. (6): Semithin section of the gill lamellae showing hemorrhage (H) around the gill ray. Toulidine blue stain. 10 X 100.

Fig. (7): Semithin section of secondary gill lamellae. The capillaries were denuded from their epithelial covering. Teulidine blue stain. 10 X 100.

Fig. (8): SEM of the gill lamellae. The gill capillaries appear denuded from their covering (C). X 2500.

Fig. (9): TEM of the gill epithelium showing vacuolar degeneration with cytoplasmolysis of their organelles. Note also the sloughed epithelial cells (S). Lead citrate uranyle acetate X 2750.
Fig.(10): SEM of the gill lamellae showing swelling of the secondary lamellae. X 2500.

Fig.(11): Semithin section of the gill lamellae showing hydropic degeneration and swelling of the epithelial lining. Toulidine blue stain. 10 X 100.

Fig.(12): SEM of the gill lamellae of the control group. X 2500.
Fig.(13): SEM of the gill lamellae in the ammonia treated group showing thickening and fusion of the secondary gill lamellae X 2500.
Fig.(14): Semithin section of the gill lamellae from ammonia treated group showing hyperplasia of the gill epithelium. Toulidine blue. 10 X 10.

Fig.(15): High power of the gill lamellae showing fusion of the secondary gill lamellae. Note also presence of eosinophile granule cells (E). Toulidine blue stain 10 X 40.

Fig.(16): Semithin section of the gill lamellae from the ammonia treated group showing separation between the capillaries and the epithelial covering. Toulidine blue stain. 10 X 40.

Fig.(17): High power showing prominent epitheliocapillary separation. Toulidine blue stain. 10 X 100.

Fig.(18): TEM of the gill epithelium from ammonia treated group showing degeneration and lysis of cytoplasmic organelles in some epithelial cell. Note the presence of eosinophile granule cell (E). Lead citrate uranyle acetate X 2750.

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Ass. Univ. Bull. Environ. Res. Vol. 2 No. 2, October 1999

التأثيرات الباثولوجية لبعض الملوثات على خياشيم أسماك البلطى النيلى

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الملخص :

تم أقلمة عدد ١٤٠ من أسماك البلطى النيلى ٥-١٠ جم ، وقسمت إلى سبع مجموعات تم تعريض ستة منها إلى الجرعة نصف المميتة من كبريتات النحاس (٣١,٩ جزء فى المليون) ، مبيد القواقع بايلوسيد (٠,٣١٢٥ جزء فى المليون) ، خلات الرصاص (٤٣,٦ جزء فى المليون) حامض الكبريتيك بدرجة حموضة (٤,٠٧٥) ، أمونيا (٠,٥٣ ملليجرام لكل لتر) ، ولدرجة حرارة بدائية مميتة عليا (٣٥م) ، وتركت المجموعة السابعة كضابط للتجربة . وتم تسجيل الأعراض والصفة التشريحية ، وأخذت العينات وتم تجهيزها للفحص الميكروسكوبى الضوئى والمساح الألكترونى ، وتم تسجيل النتائج ومناقشتها .