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Seasonal Histopathological Changes in Gill Structure of *Orecohromis niloticus* in Manzalah Lake, Egypt

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Abstract: *This study was carried out to identify the histopathological changes taking place in gill structure of cichlids; Orecohromis niloticus collected from three different fishing areas namely, Ginka, Beshteir and Deshdy in Manzalah Lake .This lake receives enormous quantities of industrial and agricultural wastes through different sources of drainage water from Bahr el-Bakar, Hados, Ramses and El Sirew drains.Histopathological observation showed hyperplasia, hypertrophy and fusion of lamellae, edema, telangiectasis and necrosis in gills of O.niloticus in Ginka and Beshteir subjected to more wastes than in Deshdy relatively far from the inflow of heavy polluted water.*

Keywords: *Manzalah Lake, O. niloticus, gill, Pollution, Hyperplasia, Hypertrophy, Telangietasis, Edema, necrosis.*

I. Introduction

Manzalah Lake represents the largest coastal lake in Egypt .Its longer axis (about 65 km) directed from northwest to southeast. Its greatest width is approximately 45 km and has an area of about 700 km2 [1]. It is situated along the Mediterranean Sea coast between the Damietta branch of River Nile in the west and Suez Canal in the east. It is highly dynamic aquatic ecosystem. Manzalah Lake receives water from different sources of drainage water from Bahr el-Bakar, Hados, Ramses and El Sirew drains. Nile fresh water comes from Damietta branch through El-Enania channel and inflow of seawater results from the exchange through the two openings at El-Gamel region. Lake receives a variety of pollutants from these drains which carry daily heavily polluted water through Baher El-Bakar drain, because of the high evaporation rate, the decreasing and insufficient mixing with the waters of the Mediterranean, the dilution of liquid wastes entering the lake is significantly poor.

Several expected problems relevant to water pollution became obvious and presumably one of the final goals is a must to reduce the level of pollution to those which can at least be tolerated and be suitable to fish life in Manzalah Lake. Industrial and agricultural waste waters carry nutrients such as phosphates and nitrates beside heavy metals and sediments. Despite the known effect of different pollutants on fish some fish species were able to tolerate zinc concentrations in their gills with values higher than 230 mg/kg [2]. Fishar [3] determined the impact of different abiotic variables of water and sediment quality on macrobenthos in El-Gamil basin. Necrosis, myolysis, lesion, and haemorrhage in most of the examined tissues as a result of the pesticide toxicity on *O.spilurus* were showed by [4]. The gill morphometry and haematology of juvenile chinook salmon, were studied by[5]while [6] showed the influence of lake chemistry and fish age on cadmium, copper, and zinc concentration in various organs of indigenous yellow perch (*Perca flavescens*). The study of the cortisol receptor immunoreactivity in the branchial epithelium of the Atlantic salmon was reported by [7]. The gross and Microscopic morphology of the gills of both *O. niloticus* and the catfish *Clarias gariepinus* was described by using light and scanning electron microscopy [8].

Study the effect of environmental conditions of Abu-Zabal Lake and histopathological alterations of testis and gills of *O. niloticus* and *T. zillii* showed by [9]. Mohamed [10] denoted that the metals were accumulated in different tissues and several histopathological alterations, including vacuolar degeneration with focal areas of necrosis in liver, proliferation in the epithelium of gill filaments and fusion of secondary lamellae, severe degenerative and necrotic changes in the intestinal mucosa as a result of the accumulated metals.

Tilapia is the most famous and important fresh water fish in Egypt; the domination of tilapias in Manzalah Lake was a result of declining crop of marine species, which enter the lake for feeding and growth [11].Four species of tilapia, *Oreochromis niloticus, Sarotherodon galilaeus, Tilapia zillii* and *Oreochromis aureus*, inhabit the Egyptian inland waters, the brackish Manzalah Lake. The dominant catch is *O.niloticus*

The present work aims to reveal the effect of different pollutants, toxic phytoplankton, heavy metals , industrial and agriculture drainage on gill of *O. niloticus* .Histopathological study shows to how extent this phytoplankton induce hypoxemia, hyperplasia in primary lamellae and necrotic changes of the secondary lamellar and damage of respiratory epithelium.

II. Materials And Methods

Three distinguished stations were selected for samples collection taking into consideration the effect of Bahr el-Bakar drains on the selected area (Fig.1).

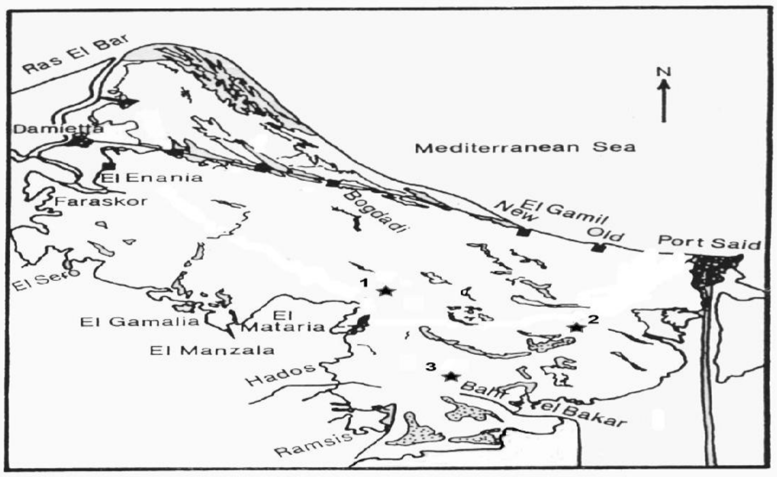

Fig. (1): A map showing the main drains and the sampling location sites of lake Manzalah (★).
1. Deshdy 2. Beshtier 3. Ginka

Deshdy (I), in the western side, on open area, Beshtier (II), in the middle of the eastern side of the lake affected by the drainage and seawater coming through the Lake-sea connection.Ginka (III), near the mouth of Bahr el-Bakar drain.

Samples of *Oreochromis niloticus* were seasonally collected alive during the period from September 1998 to October 1999. About 1260, 1547 and 1059 fish samples, were collected from three sides during the four seasons.

The physiochemical parameters (pH, dissolved oxygen, salinity and water temperature), were determined by using pH meter (Hanna instrument), field refractometer S 28 for dissolved oxygen, salinometer for salinity and ordinary thermometer (0-100^{o}C with scale of 0.2^{o}C) for water temperature.

Gills samples were detached from the fish after recording total fish length (cm), weight (gm) and directly fixed in 10% formalin. After fixation, samples of gills were preserved in 70% alcohol. Fixed tissues were dehydrated in ascending grades of alcohol, cleared in xylene and finally embedded in paraffin wax. Sections of 5-7 μ stained with haematoxylin and eosin were examined by light microscope (Leitz) supplied with camera.

III. Results

Deshdy station was shown to be less polluted than the other two stations, Beshtier and Ginka.Therefore, Deshdy was used as control group of fishes.

3.1 Environmental parameters: Environmental parameters were represented in table (1, 2) for the four seasons.

3.1.1 PH: In Deshdy, pH value fluctuated between (7.2±1.1) to (8.2±1.0).Meanwhile the maximum mean value was (8.6±9.0) in Beshtier in autumn and summer but the minimum mean value recorded (7.1±1.2) in Ginka in winter.

3.1.2 Dissolved oxygen: In Deshdy area the values of dissolved oxygen in winter and spring had the same value (3.5±1.5) mg/l, while the minimum value was (1.2±0.9) mg/l in autumn. In Beshtier, values of dissolved oxygen varied between (3.5±1.2) mg/l in summer as a maximum value to (1.5±1.2) mg/l in autumn as a minimum value. In Ginka, on the other hand, values of dissolved oxygen varied between (2.8±1.1)mg/l in winter as a maximum to (1.2±0.9) mg/l in autumn as a minimum value.

3.1.3 Salinity: The salinity in Deshdy was almost fluctuating between (4.0±2.0) ‰ as a maximum in winter and spring and minimum (2.5±1.6) ‰ in autumn. Meanwhile, the maximum recorded value of salinity was (6.0±4.1) ‰ in Beshtier in spring while the minimum (2.0±1.2) ‰ in Ginka in summer and autumn.

3.1.4 Water temperature: Water temperature of Deshdy was (29±1.3)˚c in summer, while (14.7±1.3)˚c in winter and spring. On the other hand, water temperature in Ginka was fluctuating between (29.5±1.8)˚c as a maximum in summer, and a minimum of (14.9±2.0)˚c was recorded in winter. In Beshtier, the maximum of (28±1.5)˚c was observed in summer, while the minimum mean water temperature was actually recorded (14.5±1.3)˚c in winter.

Table (1): pH, dissolved oxygen, salinity and water temperature in three stations during Winter and Spring in Lake Manzalah

Parameter	Winter			Spring		
	Ginka	Beshtier	Deshdy	Ginka	Beshtier	Deshdy
PH	7.1 ± 1.2	7.2 ± 1.1	7.2 ± 1.1	8.0 ± 1.5	8.1 ± 1.5	7.8 ± 2.3
Dissolved oxygen mg/l	2.8 ± 1.1	3.4 ± 1.2	3.5 ± 1.5	2.3 ± 0.9	2.6 ± 1.0	3.5 ± 1.5
Salinity ‰	3.0 ± 1.0	5.0 ± 2.0	4.0 ± 2.0	4.0 ± 2.0	6.0 ± 4.1	4.0 ± 2.0
Water temperature ^oc	14.9 ± 2.0	14.5 ± 1.3	14.7 ± 1.8	18.6 ± 3.3	20.7 ± 2.4	14.7 ± 1.8

Table (2): pH, dissolved oxygen, salinity and water temperature in three stations during Summer and Autumn in Lake Manzalah

Parameter	Summer			Autumn		
	Ginka	Beshtier	Deshdy	Ginka	Beshtier	Deshdy
PH	7.7 ± 1.7	8.6 ± 0.9	8.2 ± 1.0	8.2 ± 1.0	8.6 ± 0.9	8.0 ± 1.2
Dissolved oxygen mg/l	1.5 ± 1.2	3.5 ± 1.2	1.3 ± 1.0	1.2 ± 0.9	1.5 ± 1.2	1.2 ± 0.9
Salinity ‰	2.0 ± 1.2	5.0 ± 1.4	3.0 ± 1.2	2.0 ± 1.8	3.0 ± 1.5	2.5 ± 1.6
Water temperature ^oc	29.5 ± 1.8	28 ± 1.5	29 ± 1.3	21 ± 3.0	21 ± 3.0	22 ± 6.0

3.2 Histological results of the effect of different pollutants on the gills of *O.niloticus*

The structure of gill filament revealed lamellae originated on both sides of filament lamellae consisting of centrally located pillar cells which support the epithelial layer and delimit blood lacunae. It composed of primary gill filament and laterally originating secondary lamellae with clear wide interlamellar spaces in between. The secondary lamellae include respiratory epithelial cells and pillar cells as shown in figure (2).

Fish samples collected from polluted stations Beshtier, Ginka and less polluted station Deshdy showed a chronic inflammatory process in distal region of the gill filaments with epithelial hyperplasia. Also, the rigidity of mucus in fish from such polluted station was increased in autumn and winter, similar changes of tilapia gills sections were observed in autumn, spring and winter, and moderate to severe hyperplasia of primary lamellar epithelium were shown in figure (3).

Such pathological changes are relevant to strong irritant reaction at the gill surface and osmotic distress. In figure (4&5) curling of lamellae was observed in fish samples collected from Beshtier in autumn and winter and shrinking. Gill hyperplasia has been regarded as a common sign of chronic toxicity caused by various chemical pollutants. In figure (6) shorting in secondary lamellar and the outer epithelium layer left of secondary lamellar. Also hemangietic of blood vessel was observed.

In figure (7) the interlamellar space was completely filled with hyperplasic epithelium and completely blocked. In figure (8) gills included hypertrophy of secondary lamellar cells hyperplasia of primary lamellar cells and blocking of interlamellar spaces and severe gill epithelial damage including hypertrophy and fusion of secondary lamellae.Telangiectasis were observed in figure (9).

The hyperplasia of epithelium leading to reduction in the length of the respiratory lamellae and a consequent decrease in respiratory surface area, followed by blocking of the water passage were observed in fish samples collected from Deshdy, Beshtier and Ginka in autumn, winter and spring, respectively (Fig.10).

Protozoa was observed between interfilament spaces of lamellae in figure (11) meanwhile, hyperplasia was spread along the filaments, accompanied by dilation and congestion of the filament vessels and lamellar capillaries.

Trichodena was observed in gills collected from Beshtier between the ruptured lamellar epithelium and also severe hyperplasia resulted from the presence of protozoa caused fusion between the lamellae and sometimes detachment of some filament from the branchial arch occurred.

The gills which had been exposed to parasitic infestation or "Monogenea" showed hypertrophy of the epithelium in many secondary lamellae. These parasites caused the branchial lesions; epithelial separation in secondary lamellae and filaments as well as several other types of lesions showed in the gills of fish in Beshtier and Ginka more than in Deshdy during the four seasons as shown in figure (12).

In figure (13) completely destruction of cellular structure of lamellae as a clumped mass of cell in interlamellar space, living the supporting layer showing beginning of destruction .In addition, rupture of the branchial epithelium and necrosis was observed figure (14).

In figure (15) complete fusion of lamellar epithelium with neighbour filaments was observed in Ginka station in summer. Blood vessels were subject rupture in all parts and advanced necrotic changes were seen as well as lamellar fragmentation and dissociation also observed. Summer season gave the most progressive in the gills of fish collected through the four seasons as shown in figure (16).

The influences of algae on the death of fish showed extensive epithelial capillary separation and necrosis of both the filament and lamellar epithelia. The environmental hypoxia induced vascular distention, shortening of diffusion distance and swelling of red blood cells. The magnification of figure (16) showed telangiectasis and necrosis of respiratory epithelial cells of gill collected from Beshtier station in spring.

IV. Discussion

4.1 Manzalah Lake and its importance

Manzalah Lake is considered as an important source of fish production in Egypt for many reasons. Large amounts of drainage water from industrial and urban sewage contribute about 98% of the total annual inflow water to the lake [12] on the other side large quantity of the agricultural drainage water affects the environment of the lake, especially after the former inflow from the River Nile which ceased in 1965 after the Aswan High Dam construction [13] and El-Salam Canal project which affected the water budget of the lake.

The lake receives a variety of pollutants from different drains in particular; Beshtier and Ginka areas which represent the extension of Baher El-Bakar drain. This situation took place after the proposed project for the improvement of water quality of this heavily polluted drain. Deshdy on the other hand is considerably far from the drain's effect.

Cichlids are the most popular and highly economic fishes in most lakes in Egypt and play the essential role in Manzalah Lake fisheries [14]. Cichlids are represented by four dominant species, namely *Oreochromis niloticus, O. aureus*, *Tilapia zillii* and *Sarotherodon galilii*.

The pH value of water in Manzalah is always on alkaline side. The highest value was 8.6±0.9 in Beshtier during summer and autumn while the lowest one was 7.1±1.2 in Ginka during winter. The values of dissolved oxygen are widely varied from winter to summer and autumn in the three stations this may be attributed to the decrease in water temperature and coincides with increasing oxygen.

Prominent decrease of dissolved oxygen in Ginka station is influenced by the heavy load of organic material coming from the WWTP.The increase of dissolved oxygen during winter could be attributed to the continuous mixing of water by prevailing winds in this period of the year. The lowest dissolved oxygen is relevant to the bacterial decomposition of organic matters introduced to the lake in waters and sewage which consume most of dissolved oxygen.

Salinity $\%_0$ is the most important indicator of changes in Manzalah Lake water quality. High salinity was recorded in Beshtier is relatively connected to the inflow of sea water and the lowest one was recorded in Ginka which receives slightly fresh water from different drains. In Deshdy the highest salinity values were recorded in winter and spring.

The highest value of water temperature was 29.5 ±1.8 in Ginka during summer and the lowest one was14.5 ±1.3 in Beshtier in winter.

4.2 Histological examination of gills and effects of different pollutants

The fish respiratory system is remarkably flexible and capable of modification in relation to environmental changes because of the involved homeostic mechanisms [15]. Fish suffocation phenomenon in some areas of lake is generally supposed to be the direct cause of the fish death mainly caused by blooming of toxic plankton and heavy metals. Histologically examined gills clearly showed that certain phytoplankton and heavy metals induce hypoxemia which in turn causes hyperplasia in primary lamellae and necrotic changes of the secondary lamellar and damage of respiratory epithelium. As previously noted Beshtier and Ginka stations receive huge amounts of mixed waste water (partially treated) discharged from many sources; Baher el-Bakar, Bahr Hados and Bahr Ramsis. It was that gills collected from these stations showed a chronic inflammatory process in the distal region of the gill filaments, with epithelial hyperplasia.

Wastewater effluent in Manzalah contained some ammonia and urea levels. Severe ammonia toxicity caused edema, haematomas and general disruption of the respiratory epithelium [16]. Dependence on these findings, it has been proposed that one possible mechanism of ammonia toxicity is suffocation due to destruction of the respiratory surface [17& 18]. At the proximal parts of the lamellar epithelium swollen, hyperplasia and severe congestion of the lamellae was exhibited in Beshtier a result noted by [19] found the same results. Hyperplasic reaction may increase the epithelial thickness so as to retard or prevent the entry of toxic ions into the blood stream or to compensate for osmotic imbalance [20]. The pronounced hyperplasia leads to severe blocking of water passage in gills, and further more diminish the free surface of secondary lamellar cells and decrease the respiratory exchange surface.

Irritating action of ammonia inducing changes necrobiotic in character is responsible for tissue and organ necrosis. Furthermore Edema seems to implicate a protective and osmoregulatory manifestation of gills and this could result in inadequate gas exchange and consequently in a reduced diffusion capacity [21] gave the same result. Increased mucous secretion under toxic conditions is known to bar the entry of toxicants into the blood stream [22] probably interacting with the toxic ions, resulting in the formation of a film of coagulate mucous on the surface of the gills, or by providing a physical barrier for macromolecules. Waterborne pollutants in Manzalah Lake caused irreversible damage in the gill tissues of *O.niloticus* and this may lead to suffocation and finally death. This result agreement with Yacoub [23].

Abnormal features of gills including hypertrophy of secondary lamellar cells and hyperplasia of primary lamellar cells, besides swelling of the end of secondary lamellar cells was noticed in gills collected from Beshtier and Ginka during autumn and spring.

In conclusion, the histopathological changes in the gills of *O.niloticus* vary according to season and station .The severe situation of Manzalah Lake necessitates the thorough treatment of lake water to at least prevent more deterioration of the lake and also prevent damage in the gill tissues which this may lead to suffocation and death to fish.

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Figures (2 - 7): Light micrographs in the Gill of *Oreochromis niloticus* from Manzalah Lake

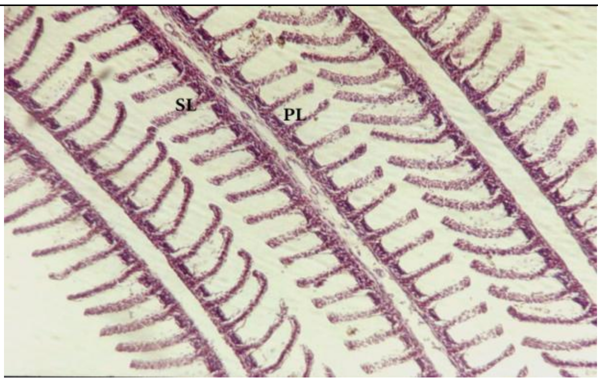

Fig. 2. Primary (PL) and secondary lamellar (SL). (10X).

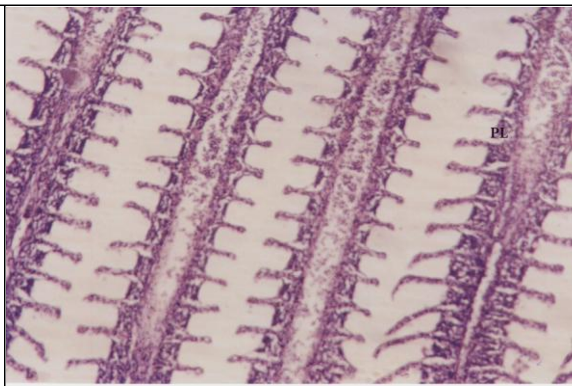

Fig.3. Moderate hyperplasia of primary lamellar (PL) and congestion with blood in it. (10X).

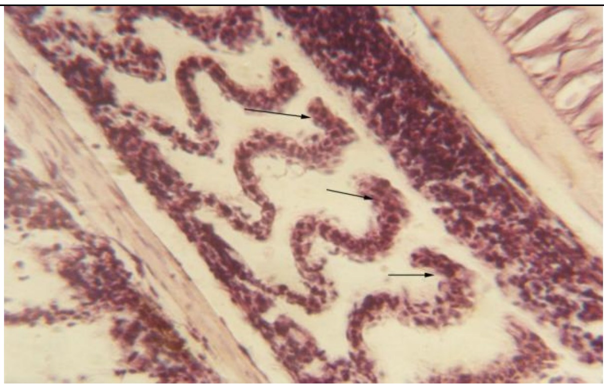

Fig. 4. Curling of secondary lamellae stained with haematoxylin &eosin. (see arrow) (40X).

Fig. 5. Necrosis (N) and the outer epithelium left the secondary lamellae (SL). (100X).

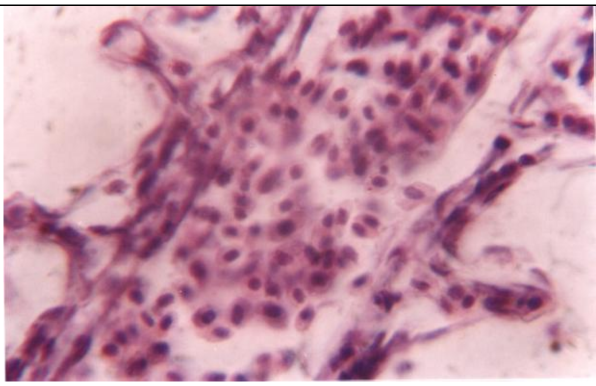

Fig. 6. Shorting of secondary lamellae and necrosis. (100X).

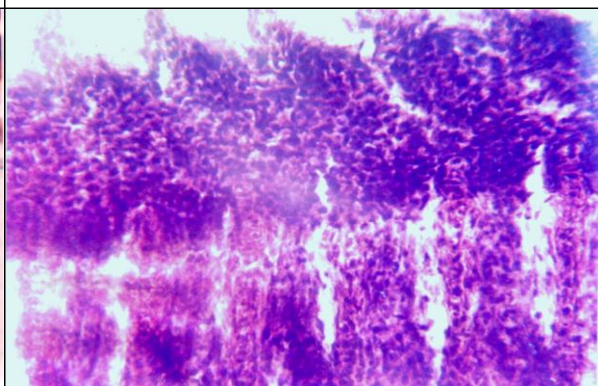

Fig.7. Completely fusion and blocked of secondary lamellae of gill filament.(100X).

Figures (8 - 13): Light micrographs in the Gill of *Oreochromis niloticus* from Manzalah Lake

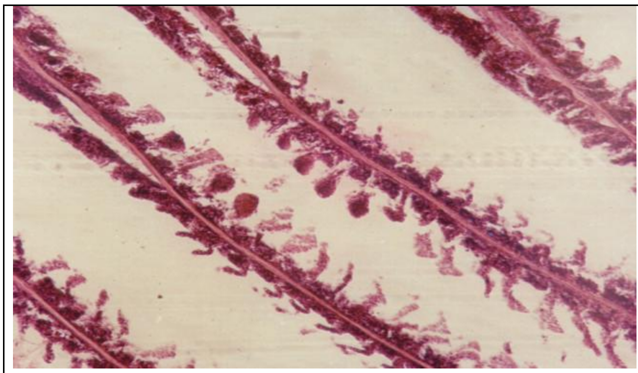

Fig. 8. Fusion and destruction of secondary lamellae, telangiectasis (T) (10X).

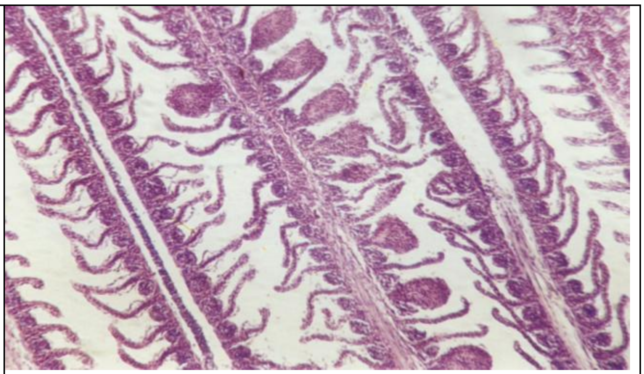

Fig.9. Telangectaisis and hyperplasia of primary lamellae (40X).

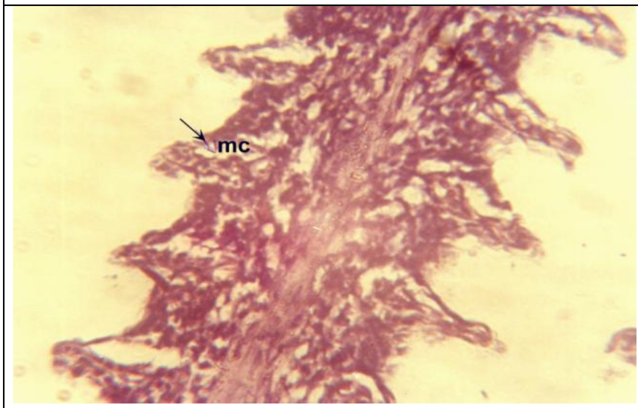

Fig. 10. Hypertrophied of secondary lamellae and hyperplasia of mucous cells (100X).

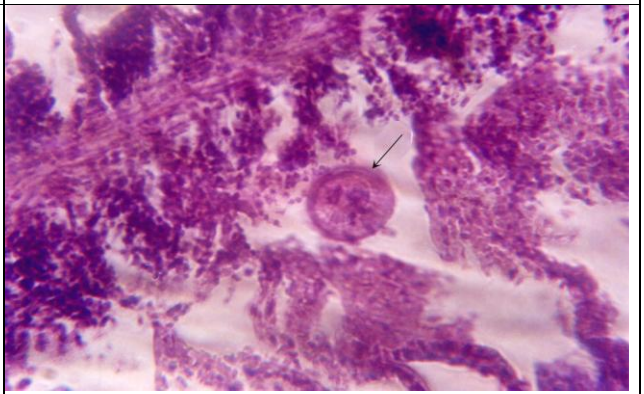

Fig.11. Hyperplasia of primary lamellar, destruction of secondary lamellae and *Trichodena* noticed between filaments. (see arrow)(100X).

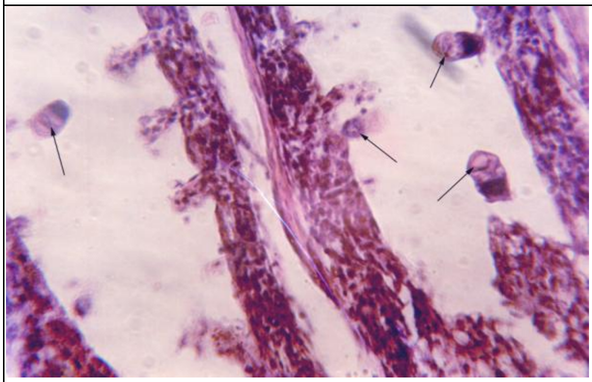

Fig. 12. Monogenia (Helminthes), necrotic changes; fragmentation, dissociation and blood vessel collapsed or ruptured. (see arrows)(40X).

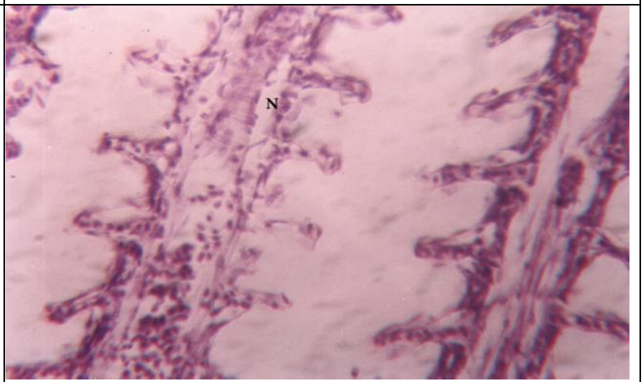

Fig. 13. Necrosis (N) and rupture of the branchial epithelium. (40X).

Figures (14 - 16): Light micrographs in the Gill of *Oreochromis niloticus* from Manzalah Lake

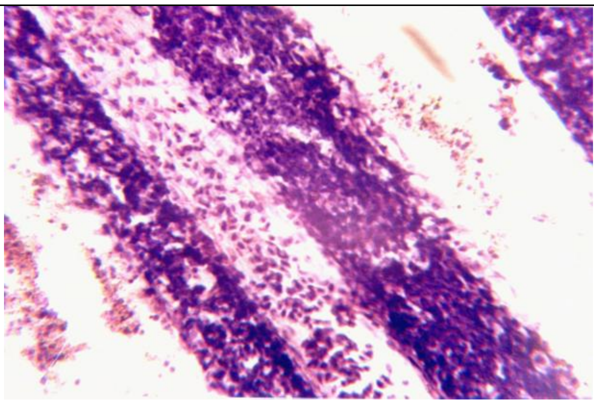

Fig.14. Haemangietic blood vessel and necrotic changes (100X).

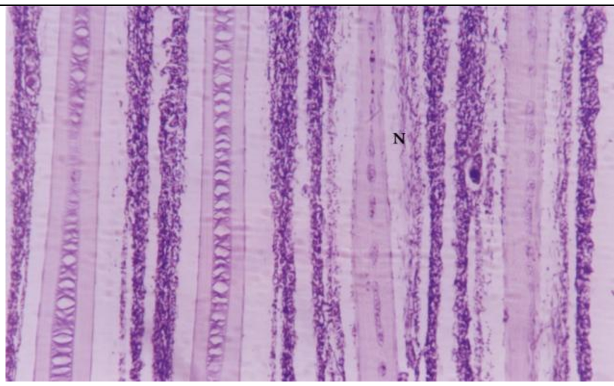

Fig.15. Completely destroyed of lamellar epithelium and infiltration of blood in primary lamellae (40X).

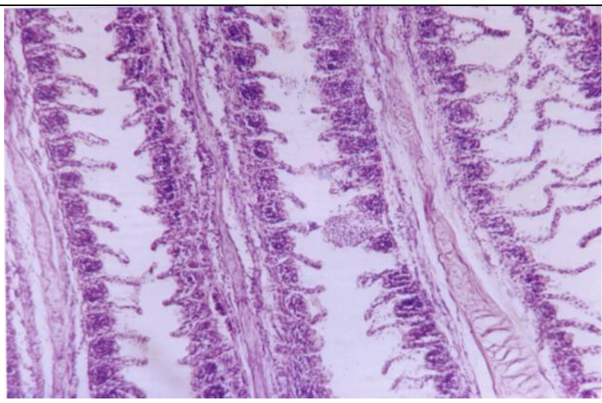

Fig. 16a. Edema, hypertrophy of epithelium in secondary lamellae necrosis (40X).

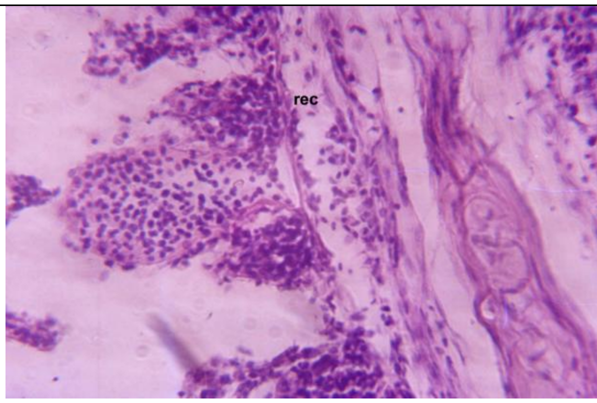

Fig. 16b. Rupture of secondary lamellae, hypertrophy of epithelium and necrosis of respiratory epithelial cells (rec) (100X).