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Original Article

Eco-histopathological Studies on *Oreochromis niloticus* fish living in Damietta Branch in Egypt

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ABSTRACT

The aim of this study is to assess the physicochemical characteristics of water quality of El-Kanater El-Khayria, Benha and Talkha stations at Damietta branch in Egypt. The impacts of pollution on the histological structure of skin and muscles and gills of *O. niloticus* fish inhabiting these stations have been investigated. Water and fish samples were collected seasonally from investigated area during the period from spring 2012 to winter 2013. The results revealed changes in water quality that have negative impact on the histological structure of selected organs of the studied fish. So, it is necessary to treat the drainage water before its discharging into the Damietta branch to protect fish and human beings from the dangers of pollution.

Key words: Water quality, histology, muscles, gills, Damietta branch.

INTRODUCTION

The River Nile is considered as one of the most important and longest (680 Km) rivers in the world. The River Nile towards El-Kanater El-Khayria is bifurcated at north of Cairo into two branches, Rosetta and Damietta branches embracing the Delta in between [1, 2].

These branches are subjected to two sources of pollution. The first is a pointed source that refers to the contaminants that enter water way by pipe or dish such as sewage and industrial wastes. However the second is non- pointed source that refers to contaminants that enter water way by diffusion such as agricultural wastes [3]. Damietta branch extends from down the stream Delta barrage at 26.5 Km behind El- Roda Gouge station to the Mediterranean Sea with length about 245 Km [4]. Along Damietta branch, there are Talkha fertilizer plant , Kafer Saad electric power station, Delta milk, Edfine factories, besides to the domestic wastes discharged from neighboring villages along Damietta [5].

Water pollution occurs when it has pathogens (due to microbial pollution) and toxins (due to non-microbial pollution) present in water in dangerous amount exceeding than permissible limit [6]. Although water pollution is usually caused by human activities, polluted water has harmful impact on human health directly by drinking it or indirectly by eating the polluted fish [7].

Sewage contains high levels of biochemical oxygen demand and nitrogenous compounds. Also, ammonia and nitrite, in particular, are serious toxicants to fish. High biochemical oxygen demand causes the decrease in dissolved oxygen, while low dissolved oxygen lowers the lethal concentration for various toxicants [8].

Consequently, wastes altered the water quality of the River Nile via uncontrolled inputs [2]. The changing of the physico-chemical characteristics of the River Nile water has negative impact on the aquatic organisms, including fish [9].

Fish is one of the most important aquatic organisms greatly affected by the toxicants. This is reflected in function and structure of the different organs [10].

Tilapia are considered as one the most common Genus of Cichlidae, widely spread throughout Africa, south America and both the middle and far East. *Oreochromis niloticus* fish is selected for our investigation because of its economic importance

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and representing a high percent of the total catch each year along the River Nile. It is considered as a well marketable fish and has tolerance for a wide range of environmental condition, salinity and pollution and shows little susceptibility to diseases [3]. The histopathological studies are considered as direct evidence referring to any adverse effects on fish [11]. The most important fish organs affected by water pollution are skin, muscles and gills.

Therefore, the aim of this study is to assess the physicochemical characteristics of water quality of El-Kanater El-Khayria, Benha and Talkha stations at Damietta branch in Egypt. The impacts of pollution on the histological structure of skin, muscles and gills of *O. niloticus* fish inhabiting these stations have been investigated.

MATERIALS AND METHODS

Water and fish samples were collected from El-Kanater El-Khayria, Benha and Talkha stations (Talkha city, Talkha electrical power station, down Talkha electrical power station) during four seasons from spring 2012 to winter 2013.

Water sample analysis: The water samples from the studied areas were collected to measure water temperature, as well as electrical conductivity, by using of the conductance bridge (YSI Model 32, SCT Meter). The pH of water was measured in the field using pH meter.

Another water samples were kept in one liter polyethylene bottle in ice box to be analyzed in the laboratory. The dissolved oxygen content was performed by azide modification and biological oxygen demand by incubation 5 days methods. Concentrations of ammonia, nitrite and nitrate were determined using the colorimetric techniques chloride according to the method described by APHA [12].

Histological studies: Skin and muscles and gills samples obtained from *Oreochromis niloticus* fish were carefully removed then fixed in neutral formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sections of 5 microns were cut using Euromex Holand microtome. Sections were mounted on clean slides, staining was carried according to Barnet et al. [13] using haematoxylene and eosin method. Then the slides were examined microscopically.

Statistical analysis: The comparison among means ± SE (standard errors) was tested for significance using one-way ANOVA analysis and Duncan's multiple range tests. The statistical analyses were calculated, using the computer program of **SPSS Inc**. (**2001 version 10.0 for Windows**) at 0.5 significance level.

RESULTS

The parameter of water analysis including water temperature, electrical conductivity, hydrogen ion concentration, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, ammonia, nitrite and nitrate, are shown in table (1).

Water temperature (T ºC): In the present study, the maximum value of water temperature (39.5 ºC) was recorded during summer at Talkha Electric power station, while the minimum value (16 ºC) was recorded during winter at El- Kanater El-Kahyria station.

Electrical conductivity (EC): The obtained data shows that the heighest value of EC (513 µmhos) was recorded during winter at down Electric power station. However the lowest value (283 µmhos) was recorded at El- Kanater El- Khayrea station during summer season.

Hydrogen ion concentration (PH): The present data indicates that the highest value (9.07) was recorded during autumn at Talkha Electric power station, while the lowest value (7.76) was recorded during autumn at El- Kanater El- Khayria station.

Oxygen studies:

A-Dissolved oxygen (DO): The obtained results showed that the minimal value (6 mg/l) was recorded at Talkha Electrical power station during summer while the maximal value (15.2 mg/l) was recorded at down Electrical power station during autumn.

B-Biochemical oxygen demand (BOD): The minimal value (3.2 mg/l) was recorded during winter at Benha station and the maximal value (6.5 mg/l) in summer and autumn at Talkha Electric power and down Electrical power stations, respectively.

C-Chemical oxygen demand (COD): The present results, showed that the highest value (8.5 mg/l) was recorded at El- Kanater El- Khayria station during summer season. However the lowest value (4 mg/l) was recorded at Talkha city station during winter.

Nutrient salts:

i-Ammonia (NH^{+}_{4}) : In general, , the maximal value (969.7 mg/l) was recorded during winter at down Electrical power station, while the minimal value (282.2 mg/l) was recorded during summer at the same above station.

*ii-Nitrite (**NO^{-}_{2}**):* The present investigation showed that the lowest value (1.5 µg/l) of nitrite concentration was recorded during autumn at Benha station while the highest value (43.1 µg/l) was recorded at down Electrical power station during winter.

iii-Nitrate (NO^-_3): The minimal nitrate value (31.7 µg/l) was recorded at El- Kanater El- Khayrea and Benha city stations during summer and winter, respectively and the maximal value (120.5 µg/l) was recorded at Talkha city station during spring.

Histopathological studies:

1-Skin and muscles: The normal structure of skin and muscles of *Oreochromus niloticus* showed the epithelial cell, epidermal, dermal, hypodermal and muscles layers. (Fig.1)

Several histopathological alterations were observed in muscles of *O.niloticus* during the four seasons in severe degree in Talkha station and hot seasons. The pathological findings include degeneration and accumulation of hemosidrin pigments in the epithelial layer. Necrosis was clear in the epidermal layer cells. Degeneration, necrosis and hemosidrin pigment in dermal layer cells. Also necrosis and hemosidrin pigment granules were obvious in the hypodermal layer cells. Meanwhile degeneration, necrosis, edema, parasitic form, hemorrhage, hemolysis and hemosidrin granules were detected in muscle fiber layer. (Figs.2- 4).

2-Gills: The normal structure of gills showed gills filaments that consist of the primary, secondary lamellae, the squamous epithelial cells, pillar cells and mucous cells (Fig.5).

The histopathological alterations in gills tissue were clear. Necrosis, hemorrhage, hemolysis and hemosiderin pigments were obvious in the primary lamellae. The histopathological damage in the secondary lamellae was more sever. Degenerative, necrosis was obvious in the tissue. Meanwhile fusion, curling, separation and pumb base and tip were detected. Hyperplasia of the cell, parasitic form, fibrosis, hemolysis and hemosiderin pigments were showed in secondary lamellae.Those lesions were more evident and severe in samples collected from Talkha station than those obtained from Benha and El- Kanater El-Khayrea stations and during hot seasons than cold seasons.(Figs. 6- 8).

DISCUSSION

Temperature is a critical control parameter in the aquatic systems and it is a key parameter which influences the physical, chemical and biological transformation in the aquatic environment [3, 14]. The changes of water temperature in the present study may be depended on the variations in meteorological conditions, air temperature, latent heat of evaporation and the time of collecting samples during different seasons, the present results are in agreement with those reported by Abdo et al. [15] and El- Sayed [16]. The increase of temperature at station (IV) may be due to thermal pollution produced from Talkha Electric Power Plant at this area. This was confirmed by Abdo [17] at the same area.

Electrical conductivity is a measure of the ability of aqueous solution to carry an electric current. Generally, the high values of EC in the present study may be attributed to domestic and agricultural wastes that contain high amount of organic and inorganic constituents [2, 18].

PH is a measure of the concentration of hydrogen ions in the water**.** The increase in pH values during autumn season at station (V) in the present study may be due to the thermal pollution which leads to high density of vegetation and phytoplankton. This was accompanied by photosynthetic activity and consumption of CO_2 with elevation of pH value as recorded by Sabae [19]. The relatively lowest pH of the Nile water can be observed at station (I). This can be attributed to the discharge of effluents which loaded with a large amount of organic acids which agree with Ahmed [2]. It is noted that the relatively higher values are attained as the temperature decrease, which may be attributed to decrease of the photosynthetic activity and the amount of dissolved CO_2 gas in water as recorded by Abdel-Aleem and Samaan [20], El-Wakeel and Wahaby [21] and Saad et al. [22].

Dissolved oxygen is a very important factor to the aquatic organisms, because it affects their biological processes, respiration and oxidation of the organic matter in water and sediments [3]. The decrease of DO during summer at Talkha Electrical power station might be due to the elevation of water temperature and increase in the oxidative process of the organic matter [2, 23]. On the other hand, the increase of DO at site (V**)** may be due to the high solubility of oxygen at low water temperature, the velocity of wind action and air movement which allow a high transfer of oxygen across the air-water interface as well as an increase of photosynthetic activity by phytoplankton [22, 24, 25].

Biochemical oxygen demand is the amount of DO which is used to decompose the organic matter in water by microorganisms. It depends on several factors such as: temperature, concentration of organic matter and density of phytoplankton. Also it increases by increasing the COD as recorded by Tayel [1]; Mahmoud [10] and Siliem [26]. As a sequence, the increase of BOD in the present study may be attributed to the decomposition of high amount of organic matter by microorganisms which increased by the elevation of water temperature. These results are in agreement with that obtained by Tayel [1]; El-Sayed [16] and Al-Afify [27] While, the decrease of BOD during winter may be attributed to low photosynthetic activity and the low abundance of phytoplankton at this area as recorded by Ahmed [24].

The chemical oxygen demand is the total amount of oxygen required to oxidize all the organic matter completely in a site to CO_2 and H_2O [28]. The high values of COD in our study may be due to the effect of pollution by sewage and agriculture wastes as well as high load of organic matter and the low capacity of the water for self the purification [22, 29, 30]**.** On the other hand, reduction of COD observed in the present study may be due to the algal biomass which is capable of consuming organic material as recorded by Ghallab [31]. Ammonia is a common aquatic pollutant and is toxic to fish. It enters in the natural aquatic systems through industrial and agricultural wastes, and is also a natural product of nitrogenous organic matter breakdown [32]. The increase of ammonia in present work may be due to the large amount of organic matter outfalls and their decomposition of the organic matter exhausting the dissolved oxygen and producing high level of ammonia **as** recorded by Saad et al.[22]; Abdel-Satar [29] and sayed [33]. On the other hand, the decrease in the ammonia concentrations was related to the decrease in the biological activities of aquatic organisms and nitrification in the water column as investigated at site **(**V**)** which is confirmed by Saad et al.[22].

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate [34]. The low values of nitrite might be attributed to the fast conversion of NO^-_2 by nitrobacteria to NO^-_3- as recorded by Tayel [7] and Abdo [17]. On the other hand, the high nitrite level may be due to the decomposition of organic matter present in the waste water as reported by Tayel [1] and Saad et al. [22].

Nitrate ion is the final oxidation product of the nitrogen compounds in the aquatic environment [24]. The low values of nitrate might be attributed to the uptake of nitrate by natural phytoplankton and its reduction by denitrifying bacteria and biological denitrification [8, 22]. On the other hand, the increase of nitrate levels might be attributed to sewage wastes at Talkha city and the low consumption by phytoplankton as well as the oxidation of ammonia by nitrosomonas bacteria and biological nitrification as recorded by Saad et al.[22] and Abdo [30].

The muscle is a good source of protein. Meanwhile, the muscular system constitutes the largest portion of the teleost body. Its functions in the overall body are locomotion, coordinated movement of skeletal elements, pumping of blood and peristaltic constriction of the visceral organs and their related structure [7, 18, 35]. The sever alterations in skin and muscles may be attributed to the effect of heavy metals [18, 36]**,** the parasitic infection [8, 37], the concentration of ammonia [1, 38] or to the inorganic fertilizers [10, 39].

Gills are the most delicate structure of the teleost body. They are also a multifunction organ involved not only in respiration but also in a variety of homeostatic activities such as osmoregulation, metabolism of circulatory hormones, nitrogen excretion and acid-base balance [2, 40].The malformation in gills may be due to the increase of ammonia, heavy metals, pH change, oxygen depletion and parasite forms. The increase of the turbidity of water polluted by sewage, industrial and agricultural discharge in investigated areas plays an important role on the histopathological alteration of gills. This observasion agree with Ibrahim and Tayel [40]; Yacoub et al. [41] and Ibrahim et al. [42].

Table (1): Physicochemical parameters of the River Nile water at the studied stations from spring 2012 to winter 2013.

Seasons	Spring					Summer					autumn					Winter					±SE
Stations	Mean					Mean					Mean					Mean					
Parameters	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	
Temperature (°C)	23	24.5	26	30	26	30	30.5	31	39.5	37	27.5	25.5	29	28	28	16	16.5	17	23.5	21	±2.73
EC (µ mohs)	350	364	388	458	418	283	294	328	335	321	408	405	435	431	442	469	457	497	492	513	±31.47
pH	8.67	8.61	8.26	8.34	8.31	7.95	8.3	8.2	8.95	8.9	7.76	8.79	8.69	9.07	9.03	8.63	8.66	8.72	8.61	8.48	±0.16
DO mg/l	8.9	8.9	6.9	6.5	7.4	7.8	7.4	6.4	6	6.5	10.8	14.8	14	12.8	15.2	13	13.2	11.6	8.5	9.5	±1.39
BOD mg/l	3.8	3.6	4.5	4.9	5.4	5.4	4.5	4.6	6.5	6.4	5	6.4	5.2	4.8	6.5	3.5	3.2	4.2	4.5	4.5	±0.45
COD mg/l	8	7.6	4.9	5.2	5.9	8.5	8	5	5.5	5.2	7	6.9	5.6	5.2	6.9	6.2	6.3	4	5.2	4.5	±0.57
Ammonia mg/l	470	425	561	520	516	329.5	371.3	306.9	291.7	282.2	450	417	501	505	512	469.2	589	857.9	892.3	969.7	±85.92
Nitrite µg/l	6.2	5.9	27.6	22.2	20.5	15.5	16.8	34.5	32.9	29.4	4	1.5	30.3	38.3	25.6	29.8	19	33.2	39.7	43.1	±5.55
Nitrate µg/l	76.2	72.5	120.5	105.8	100.6	31.7	50.8	37	36.5	41.6	49.6	54.9	58.9	52.3	60.1	59.1	31.7	59.1	79.7	78.9	±11.10

* Data are presented as means ± standard error (SE).

* Means followed by different letters in each column are significantly ($p<0.001$) different.

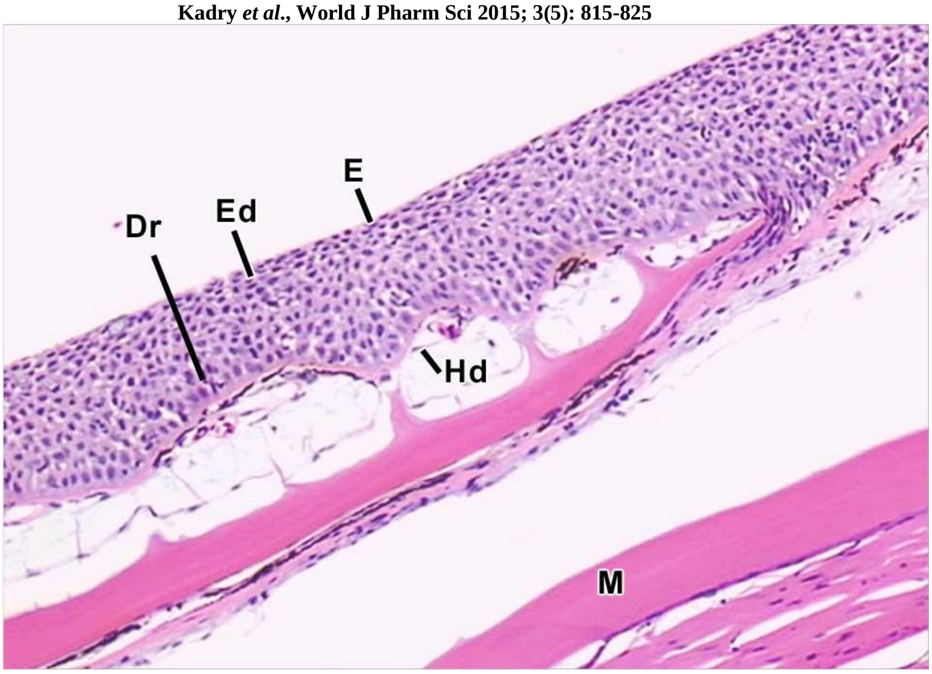


Figure (1):V.S. in skin and muscle of *Oreochromus niloticus* showing epithelial cell layer (Ep), epidermal layer (Ed), dermal layer (Dr) layer, hypodermal (Hd) and muscle layer (M). HE-X 400.

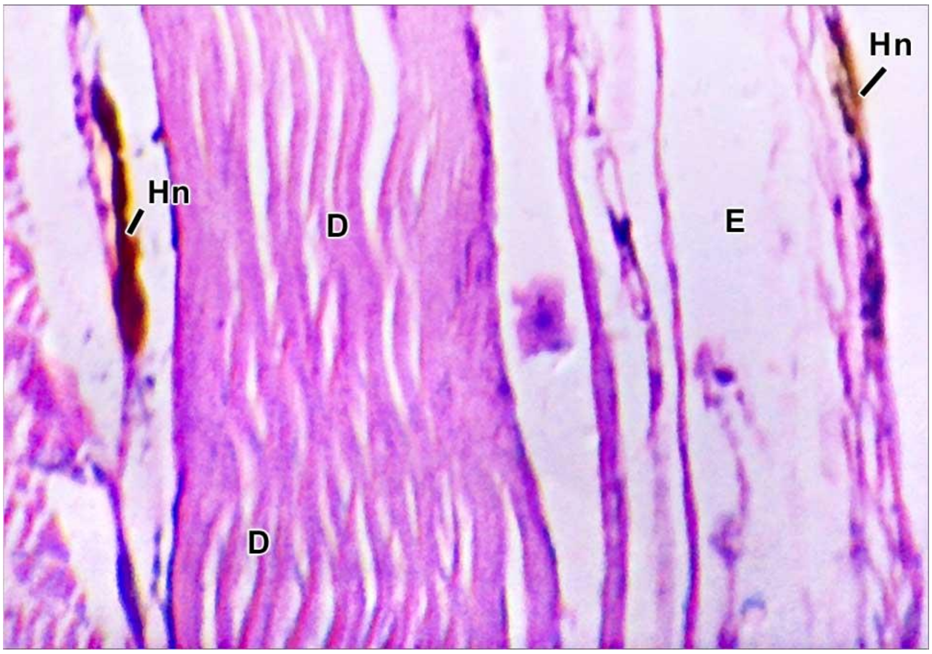


Figure (2):V.S. in skin of Oreochromus niloticus obtained from Talkha station showing: Hemosidrin pigment (Hn) in epithelial and connective tissue layers. Edema (E) in epidermal layer. degeneration (D) in dermal layer HE-X 400

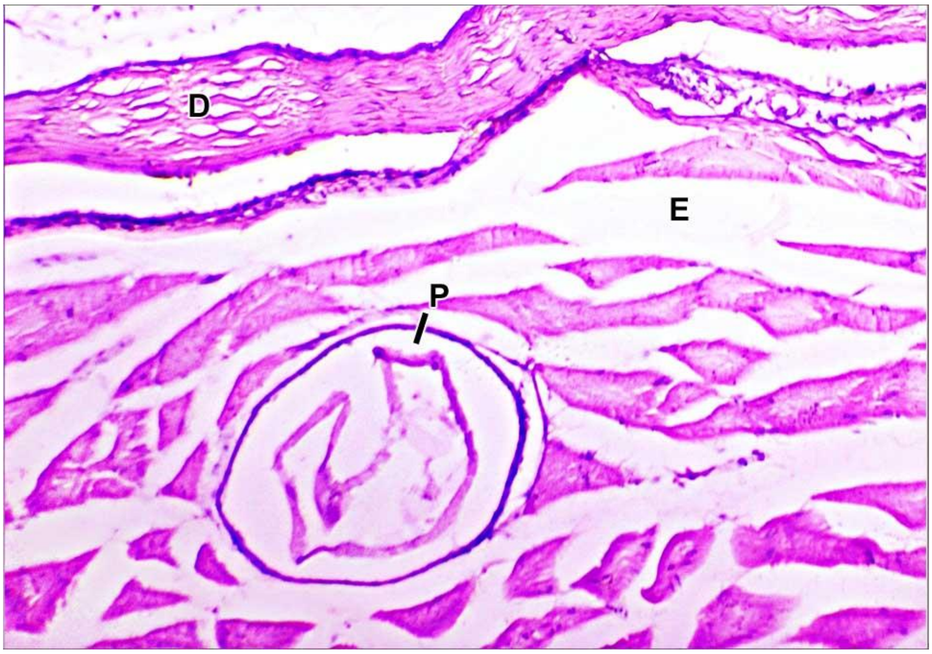


Figure (3):V.S. in skin of Oreochromus niloticus obtained from Talkha station showing degeneration (D) in dermal layer. Edema (E) and parasitic form (P) in muscle layer. HE-X 400.

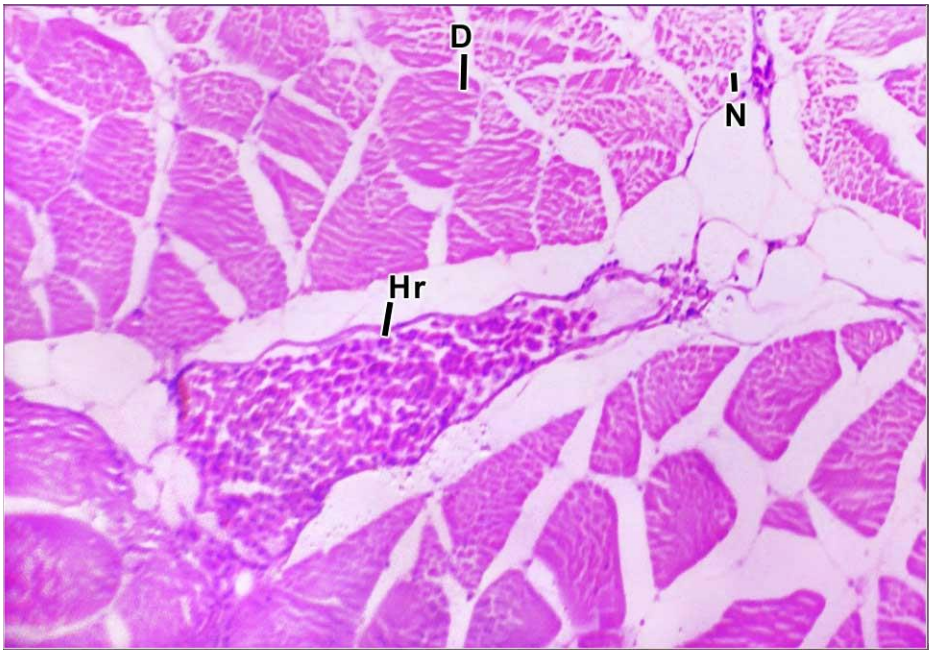
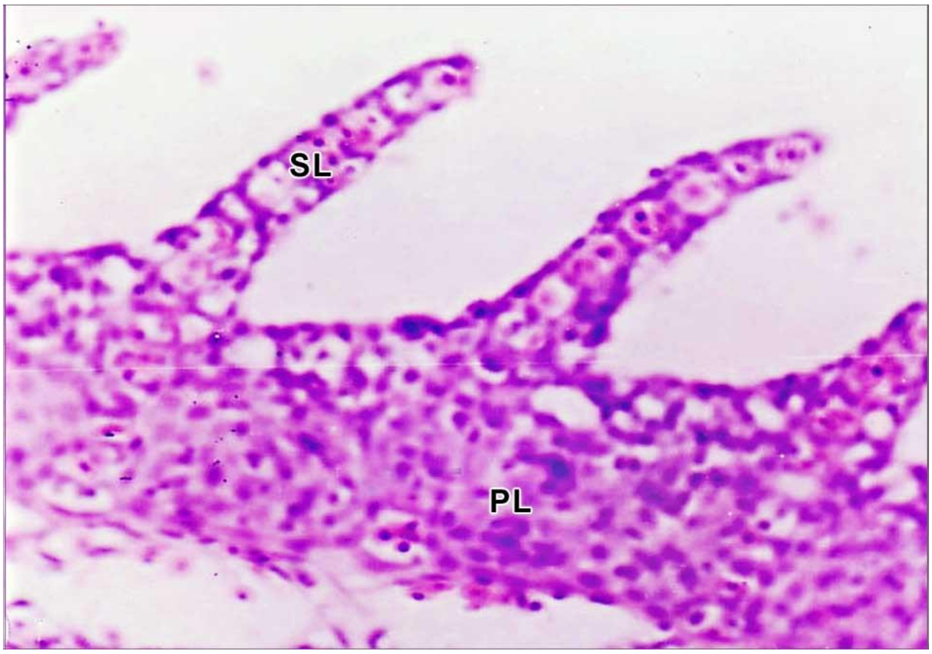


Figure (4):V.S. in skin of Oreochromus niloticus obtained from Talkha station showing degeneration(D), necrosis (N) and hemorrhage (Hr) in muscle layer. HE-X 400.



Figure(5):L.S. of normal gill filaments, showing the primary (PL) and secondary (SL) lamellae. HE-X 400.

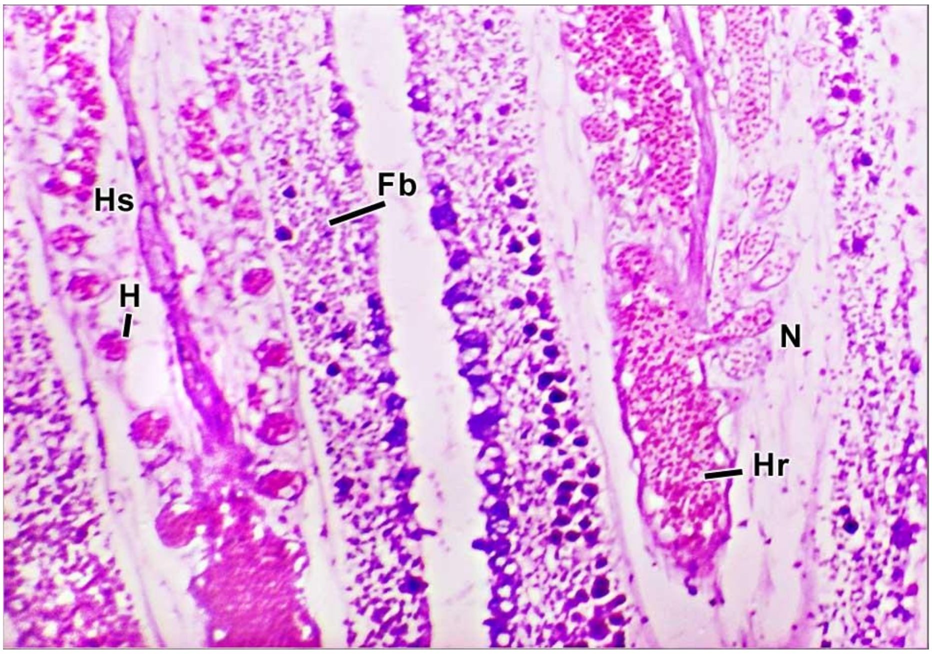


Figure (6):L.S. of gills of *Oreochromus niloticus* obtained from Talkha station showing necrosis (N), hemorrhage(Hr), hemolysis(Hs)in primary lamellae and fusion (Fu) in secondary lamella. HE-X 400.

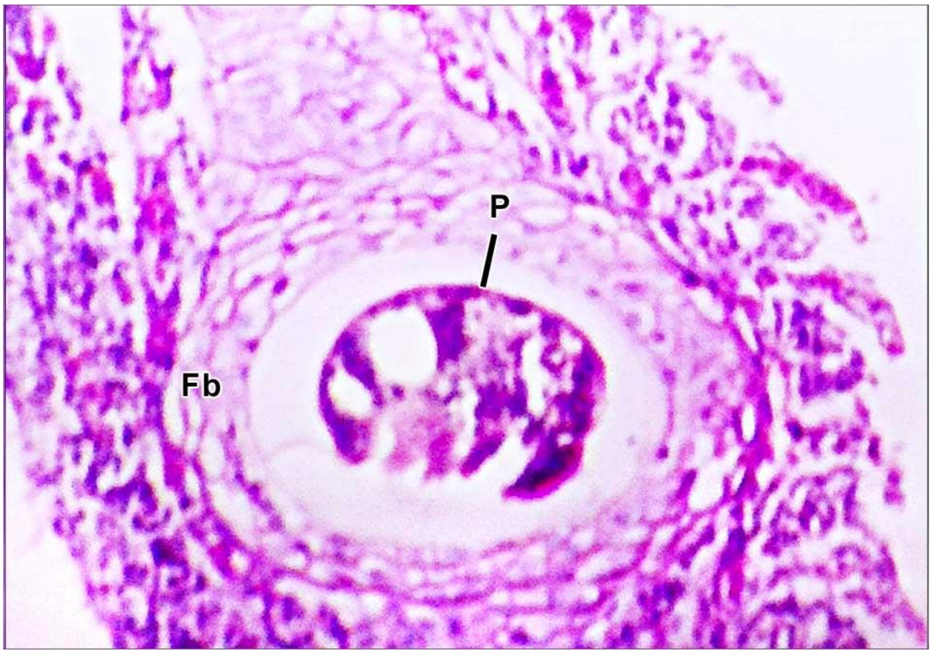
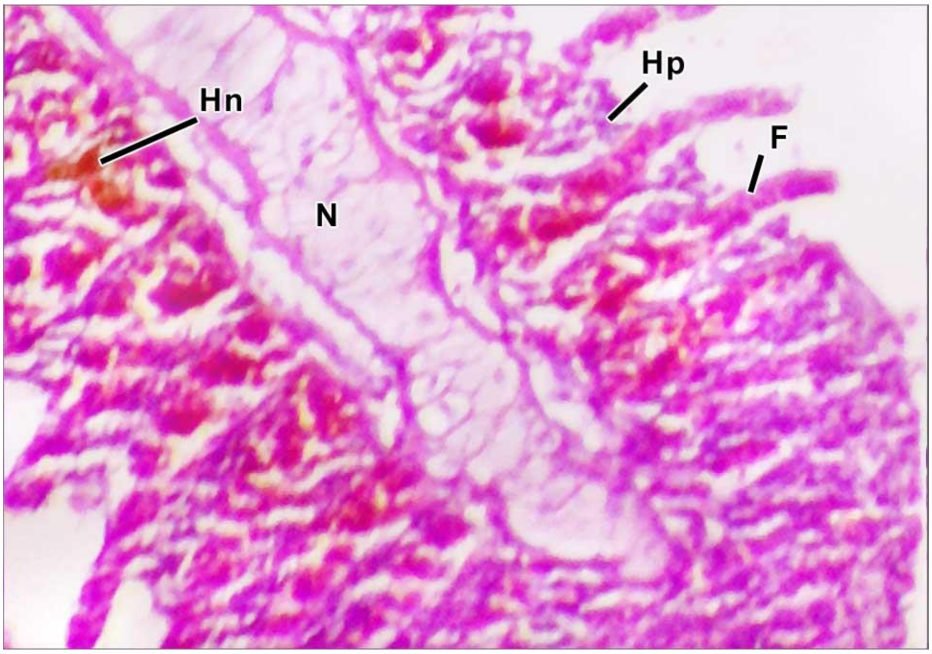


Figure (7):L.S. of gills of *Oreochromus niloticus* obtained from Talkha station showing fibrosis (Fb) and parasitic(P) form in secondary lamella. HE-X 400.



Figure(8):L.S. of gills of *Oreochromus niloticus* obtained from Benha station showing nerosis(N) of primary lamellae. Hemolysis(Hs), hemosidrin(Hn), hyperplasia(Hp)and fusion (Fu) in secondary lamella. HE-X 400.

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