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HEALTH STATUS OF *OREOCHROMIS NILOTICUS* IN FISH FARM IRRIGATED WITH DRAINAGE WATER IN EL FAYOUM PROVINCE, EGYPT

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ABSRTACT

Fish samples of *Oreochromis niloticus* (L.) were caught during year 2006 from a fish farm in El Fayoum Province for monitoring bioaccumulation of some heavy metals (Fe, Mn, Cu, Zn, Pb and Cd) in the liver, intestine, gills and muscles of the fish and their effect on the tissue organs. The results revealed that the concentrations of zinc and lead were higher than the permissible limit according to Food and Drug Administration (FDA) in the liver, intestine, gills and muscles of *O. niloticus*. The concentrations of copper and cadmium were higher than the permissible limit in the liver for copper and in the liver and intestine for cadmium. Several histopathological changes were observed in the liver, gills, intestine and muscles of the fish. The liver sections exhibited degeneration and necrosis of hepatocytes. The hepatic tissue was occupied with necrotic areas and ballooning degeneration. Microscopical examination of the fish gills showed edema in primary and secondary lamellae, complete fusion of secondary lamellae and telangiectasis (congestion with blood cells in the tips of secondary lamellae). The fish gills also suffered from severe hemorrhage. The hisopathological changes in the intestine included degeneration and necrosis of epithelial cells of mucosa, aggregation of inflammatory cells in submucosa and destruction of muscularis. Skeletal muscles exhibited different stages of degeneration, atrophy, necrosis and infiltration of inflammatory cells.

1. INTRODUCTION

Nile tilapia, *Oreochromis niloticus* (Linneaus), is one of the most important cultured species in Egypt (Mousa and Mousa, 1999). Aquaculture is considered as one of the most important sources of animal protein production. Countries that have overpopulation problems as Egypt have an increased demand for protein production of fishes (Magouz *et al.*, 1999).

Many fish farms were established around Lake Quarun in El Fayoum Province in earthen ponds. Few studies concerned with the water quality, zooplankton community structure and the effect of fertilizers on zooplankton assemblage, rearing fish and heavy metals distribution (Borhan, 1978; El Shebly, 1991; Mageed, 1996 and Ali and Abdel Satar 2005)

Heavy metals may enter the ecological system through anthropogenic activities, such as, sewage sludge disposal, application of pesticides and inorganic fertilizers as well as atmospheric deposition (Haiyan and Stuanes, 2003). The contamination of freshwater with heavy metals has become a matter of great concern over the last few decades (Canli *at al*., 1998). Heavy metals constitute a major problem because they are toxic and tend to accumulate in the body organs (El Ezaby, 1994 & Marzouk, 1994).

Fish are often at the top of the aquatic food chain and may concentrate large amounts of heavy metals. These metals accumulate differently in fish organs (Abou

Arab *et al.,* 1995; Gomaa *et al.,* 1995; Eiman and Zamzam, 1996; Yacoub, 1999; Kadry *et al.,* 2003 and Yacoub *et al.,* 2005).

The present study is initiated as a framework to seek an overview of the effect of heavy metals pollution in a fish farm in El Fayoum Province on some vital organs tissues (liver, gills, intestines and muscles) of *Oreochromis niloticus* (L.).

2. MATERIAL AND METHODS

2.1. Study area and fish sampling

About 1,000 feddans of fish farms, located at the southern region of Lake Quarun are present in El Fayoum Province. Tilapia and Mullet were the dominant fish species. Dayer El Berka Drain (The main water feeding source of all fish farms and in the same time received the output wastewater of the fish farm) and El Wadi Drain (used sometimes as a water feeder, when Dayer El Berka Drain water is dried). The fishes were fed with diet mainly formulated from fish meal, soybean meal, maize, rice bran, cotton seed oil and vitamins, mineral mixture, with different protein ratios ranged between 15-25%. The diets were offered twice daily at a rate of 4% of total fish mass in the pond (Metwally, 1999).

Random fish samples of *Oreochromis niloticus* (L.) were caught in the winter season during year 2006. The total lengths of *O. niloticus* (L.) fish samples ranged from 19.2 to 28.3 cm and total weights from 122.4 gm to 394.5 gm. The control group was collected from a Fish Farm in El Kanater El Khairya.

2.2. Methods of heavy metals analysis

Specimens of liver, intestine, gills and muscles were dried in an oven at 105°C for about 24 hours, and then ground to fine powder. A representative sample of 1 gm of each organ were digested according to the method described by Goldberg *et al.* (1993) in which concentrated nitric and perchloric acid (AR grade) with ratio of 5ml + 5ml were used in Teflon beakers on a hot plate, at 50°C for about 5 hours till complete decomposition of organic matter. The digested solutions were cooled to room temperature, filtered and diluted to a final volume of 50 ml deionized distilled water. The concentrations of Iron, Manganese, Copper, Zinc, Lead and Cadmium were measured by atomic absorption model (Perkin Elmer 3110, USA) with graphite atomizer (HGA-600) in Agricultural Research Center (Sediment, Water and Environment Research Institute). Results were expressed in µg/g dry weight.

2.3. Histological methods

Fish samples were dissected to obtain the tissues of liver, intestine, gills and muscles. The specimens of these organs were carefully removed and immediately fixed in 10% formalin, then dehydrated in ascending grades of alcohol, cleared in Xylene. The fixed tissues were embedded in paraffin wax, sectioned at 5 microns, stained with haematoxylin and eosin (H&E) and examined microscopically (Harris, 1900).

3. RESULTS AND DISCUSSION

The values of iron in the liver, intestine, gills and muscles of *Oreochromis niloticus* (L.) ranged from 463.1 µg/g to 55µg/g. Food and Drug Administration (FDA) recommended 0.5 mg iron daily dietary allowance supplied by 100 gm serving of fish muscles (Adeyeye, 1993a). The values of manganese ranged from 236.94 µg/g to 5.38 µg/g. Manganese functions as an essential constitute for bone system. Manganese is toxic only when present in high amount, but at low levels is considered as micronutrient (Fleck, 1976). The concentrations of iron and manganese in the intestine and gills were higher than their concentrations in the liver. The values of copper ranged from 53 to 2.12 µg/g (Table 1). National Health and Medical

Research Council in Australia recommended as standard concentration for human consumption is 30 µg Cu/g (Bernard, 1982). The concentrations of copper were in the permissible limit in all organs except for liver. The levels of zinc concentrations ranged between (22.8 – 11.22 µg/g). Koli *et al.* (1978) reported that zinc concentrations in muscle tissue of fish species from non polluted area, were less than 1ppm. The concentrations of zinc in all organs were significantly higher than the allowed level. The concentrations of lead ranged from 9.38 µg/g in the liver to 2.8 µg/g in the muscles (Table 1). Food and Drug Administration (FDA) recorded maximum permissible level (MPL) for lead is 2 ppm in fish muscles (Adeyeye, 1993b). The concentrations of lead in the fish organs of *O. niloticus* were higher than (MPL). The concentrations of cadmium ranged from 0.56 µg/g in liver to 0.18 µg/g in gills and muscles (Table 1). The values of cadmium slightly exceeded the permissible limit (0.5 µg/g) of National Academy of Science (Adeyeye, 1993b) in the liver and intestine. In previous study of heavy metals in muscles of *Mugil* sp. and *Tilapia* sp. in some fish farms in El Fayoum Province, Ali and Abdel Satar (2005) found that the concentrations of heavy metals ranged between 120- 63.6, 33- 20.5, 12.3- ND, 83.2- ND, 8.3- ND, 2.9- ND (ppm) in Fe, Mn, Cu, Zn, Pb and Cd respectively in the two species. The concentrations of heavy metals in the present study were approximately in the same range obtained previously by Ali and Abdel Satar (2005).

The present study revealed that, the concentrations of zinc and lead were higher than the permissible level in the liver, intestine, gills and muscles of *Oreochromis niloticus*(L.). The concentrations of copper and cadmium were higher than the permissible level in the liver for copper and in the liver and intestine for cadmium. Fish may absorb dissolved elements and trace metals from its feeding diets and surrounding water leading to their accumulation in various tissues in significant amounts and exhibit eliciting toxicological effects at target criteria (Mc Carthy and Shugart, 1990).

Table (1): Heavy metals accumulation in some selected vital organs of *Oreochromis niloticus* (L.) living in fish farm in El Fayoum Province (µg/g dry weight).

Organ / Metal	Liver	Intestine	Gills	Muscles	Mean±S.D.
Iron	74.32	463.11	82.40	55	168.71±196.6
Manganese	7.10	236.94	21.14	0.54	66.43±144
Copper	53.00	3.20	0.14	1.40	14.44±25.74
Zinc	11.22	22.80	11.42	12.78	14.56±5.54
Lead	9.38	7.30	3.40	2.80	5.72±3.15
Cadmium	0.56	0.52	0.18	0.18	0.36±0.21

The liver has been proposed as the critical target for toxicity in fish due to the role it plays in metabolism and detoxification (Sorensen, 1991). The use of histopathological biomarkers has been recommended for use in biomonitoring (Hinton, 1993). Microscopical examination of normal liver obtained from the fish farm of El Kanater Research Station showed that it is formed of connective tissue stroma and parenchymal cells (Fig.1). The stroma consists of thin capsule enclosing the liver tissue and trabeculae. The parenchymal cells are branched into the hepatic lobules. The liver plates are separated from each other by blood sinusoids. The connective tissue septae around the hepatic lobules contain branches of hepatic artery, hepatic vein, bile duct and lymph vessel. Each hepatic lobule drains in central vein (Groman, 1982). Liver of *Oreochromis niloticus*(L.) fish inhabiting fish farm in El Fayoum Province showed fatty degeneration of hepatocytes. The hepatic tissue was occupied with necrotic areas and ballooning degeneration. The pancreatic acini were dilated and congested with stagnant blood. Also, the blood sinusoids were dilated. The liver sections exhibited degeneration and necrosis of hepatocytes (Figs. 2 to 7). Mild to moderate vacuolar liver was noted in the present study. It has been suggested that such swelling of hepatocytes due to anoxia which is one of the main reasons for liver degeneration (Kamel and Fathalla, 1995). Gaber and Gaber (2006) showed extensive degeneration in the liver of *Tilapia zillii* and *Oreochromis aureus* in Lake Quarun.

Balasubramanian *et al.* (1999) stated that the liver of *Oreochromis mosambicus* under ambient urea stress exhibited various histopathological changes including nuclear pyknosis and necrosis leading to disintegration of hepatocytes.

Similar histopathological lesions were observed in liver of fish under the effect of different toxicants. Vacuolization and necrosis of hepatocytes of rainbow trout (*Oncorhynchus mykiss*) were observed after feeding with diets rich in oxidized lipids (Daskalov *et al.*, 2000) and fatty degeneration and necrosis in the liver of *Liza ramada* fish living in polluted water with heavy metals and pesticides in Lake Manzalah (Yacoub *et al*, 2005).

The fish gill is a multifunctional organ involved in respiration and homeostatic activities such as osmoregulation, metabolism, circulation of hormones, nitrogen excretion and acid base balance (Haaparanta *et al.*, 1997). They are among the most delicate structures of the teleost body and they have an external location so they are subjected to damage by irritant whether dissolved or suspended in the water. External irritant are the most frequent causes of significant gill pathological changes (Zaki and Saad, 1987). The normal gill consists of cartilagenous arch holding rows of filaments (Fig. 8). The filament consists of primary and secondary lamellae. The secondary lamellae include branchial epithelium consisting of a layer of one or two cells of interdigitating squamous epithelial cells. Also mucous secreting cells and chloride cells are found scattered between the lamellae (Groman, 1982). In the present study, the sections of gills specimens of *Oreochromis niloticus* (L.) from the fish farm exhibited edema in primary and secondary lamellae (Figs. 9 and 13) and hyperplasia progressed to complete fusion of secondary lamellae (Fig. 9). The fish gills showed telangiectasis (congestion with blood cells in the tips of secondary lamellae) (Fig. 10). The fish gills also suffered from severe hemorrhage in primary lamellae (Figs. 11 and 13), reduction of epithelial cells (Fig. 11) and degeneration of some epithelial cells in secondary lamellae (Fig. 12).

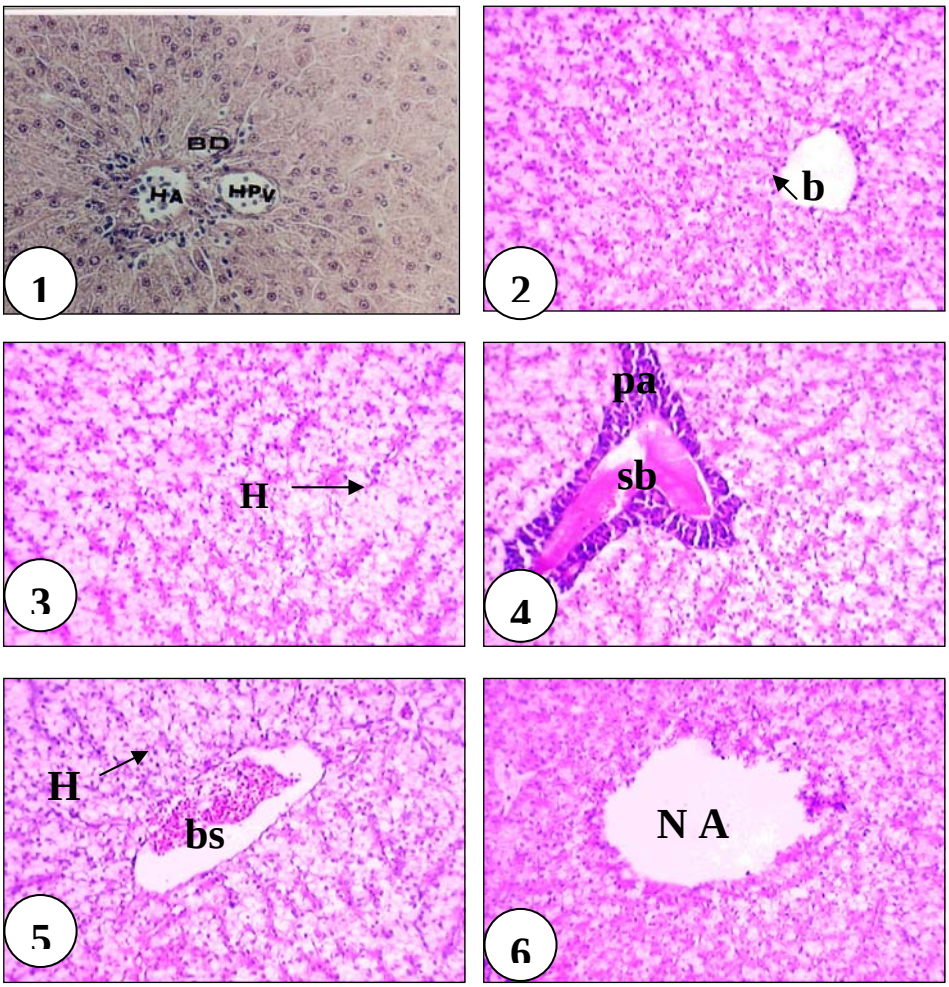

Plate I

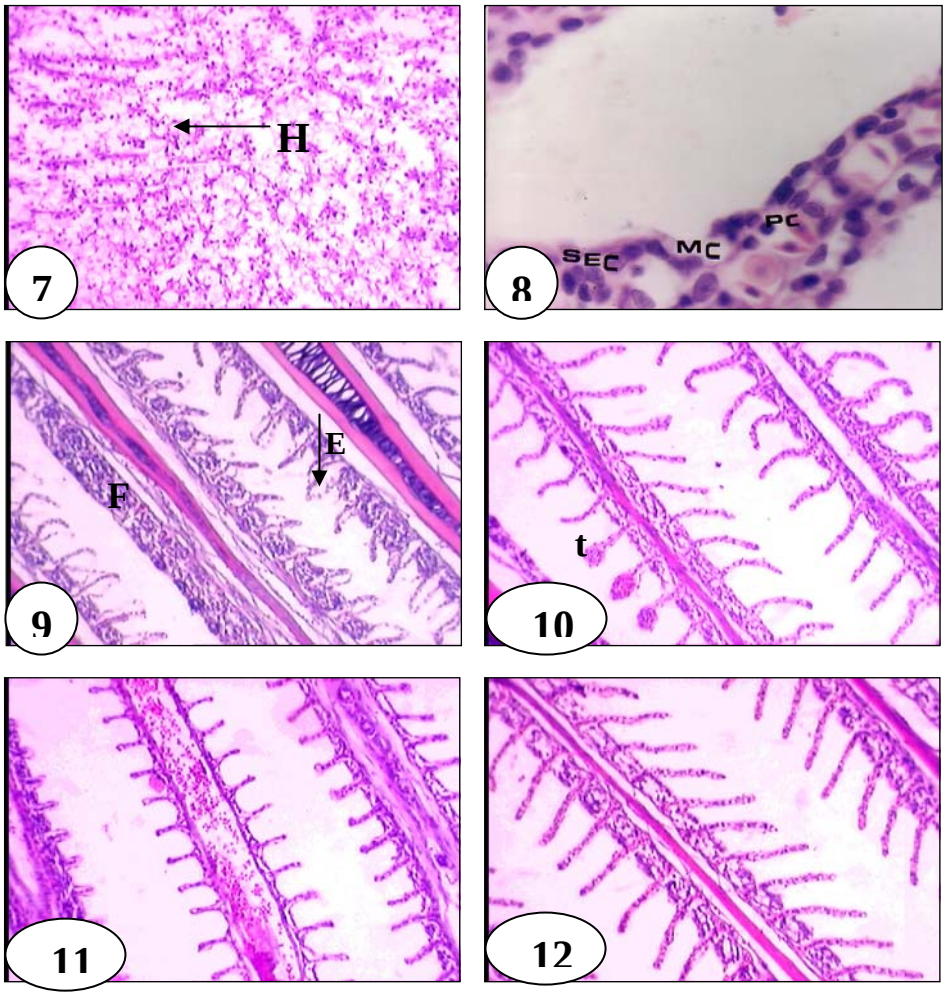

Plate II

Histopathological lesions noted in gill epithelium are largely nonspecific in nature, as each was detected under many different exposure conditions (Mallat, 1985). Kantham and Richards (1995) and Yacoub (2003) suggested that the gill hyperplasia may increase the epithelial thickness, so as to prevent the entry of toxic ions into the blood stream. These cellular proliferation in respiratory lamellar epithelium may lead to great disturbance of gas exchange and ion regulation for osmoregulation performed by the gills (Randal and Augustine, 1990).

Edema seems to implicate a protective and osmoregulatory manifestation of gills (Kantham and Richards, 1995) and this could result in inadequate gas exchange and consequently in a reduced diffusion capacity (El Feki, 1998). Fusion of adjacent secondary lamellae, one of the gill pathologies specially caused by cadmium, clearly implies a reduction in the respiratory surface (Randi *et al.*, 1996).

Gill lamellar telangiectasis is primarily caused by disruption of pillar cells, capillary distension occurs and blood accumulation may lead to further fibrosis (Roberts, 1981). This pathology was reported as an effect of heavy metals on *Clarias gariepinus* in River Nile (1999) as well as *Mugil cephalus* in Bardawil Lagoon (Yacoub, 2004). Ibrahim and Tayel (2005) get similar results in their study on the effect of heavy metals on the gills of *Tilapia zillii* inhabiting El Rahawy drain. They reported that the gills of *Tilapia zilli* suffered from hemorrhage in primary and secondary lamellae, hyperplasia, degeneration, necrosis and telangiectasis.

The structure of normal intestine is shown in (Fig. 14). It consists of four layers: an outer serosa, muscularis, submucosa and mucosa. The serosa covered the outer surface and was composed of a single layer of simple squamous epithelium. The muscularis was composed of muscle layers (longitudinal and circular). The submucosa was made up of areolar blood vessels containing eosinophilic granular cells. It folded to form a lamia propria, which supported the mucosa epithelium. The mucosa epithelium consisted of columnar epithelium lined with vascular lamina propria, and mucous-secreting goblet cells thrown into the villii (Groman, 1982). In the present study, histopathological changes in the intestine of *Oreochromis niloticus* (L.) from fish farm in El Fayoum Province included degeneration and necrosis of epithelial cells of mucosa (Figs. 15 and 16) and destruction of muscularis (Fig. 16). Reduction in villii and aggregation of inflammatory cells in submucosa (Fig. 17).

Similar lesions were observed in the intestine under different exposure conditions; in *Heteropneusts fossilis* after exposure to cadmium (Sastry and Gupta, 1979), in *Claris lazera* subjected to crude oil (Mazhar *et al.*, 1987). Mohamed (2001) recorded that exposure of *Tilapia zillii* to different concentrations of phenol, led to intestinal histopathological changes included degenerative and necrotic changes in the intestinal serosa, muscularis, submucosa and mucosa in addition to proliferation in the villii and aggregation of inflammatory cells in submucosa and mucosa. Mohamed (2004) studied the effect of the insecticides Reldan and Lannate on the intestine of *Oreochromis niloticus*. Proliferation in the villii, resulting sometimes in obliteration of the intestinal lumen, beside, atrophy in some cases, were observed in the intestinal mucosa.

Kruatrachue *et al.* (2003) found several lesions in the villar region after two-week feeding on dietary cadmium. Many cells showed sloughing off of cell apices into the lumen. Blood vessels in the lamina propria were dilated and infiltrated with numerous lymphocytes. A slight vacuolation was observed in the submucosa.

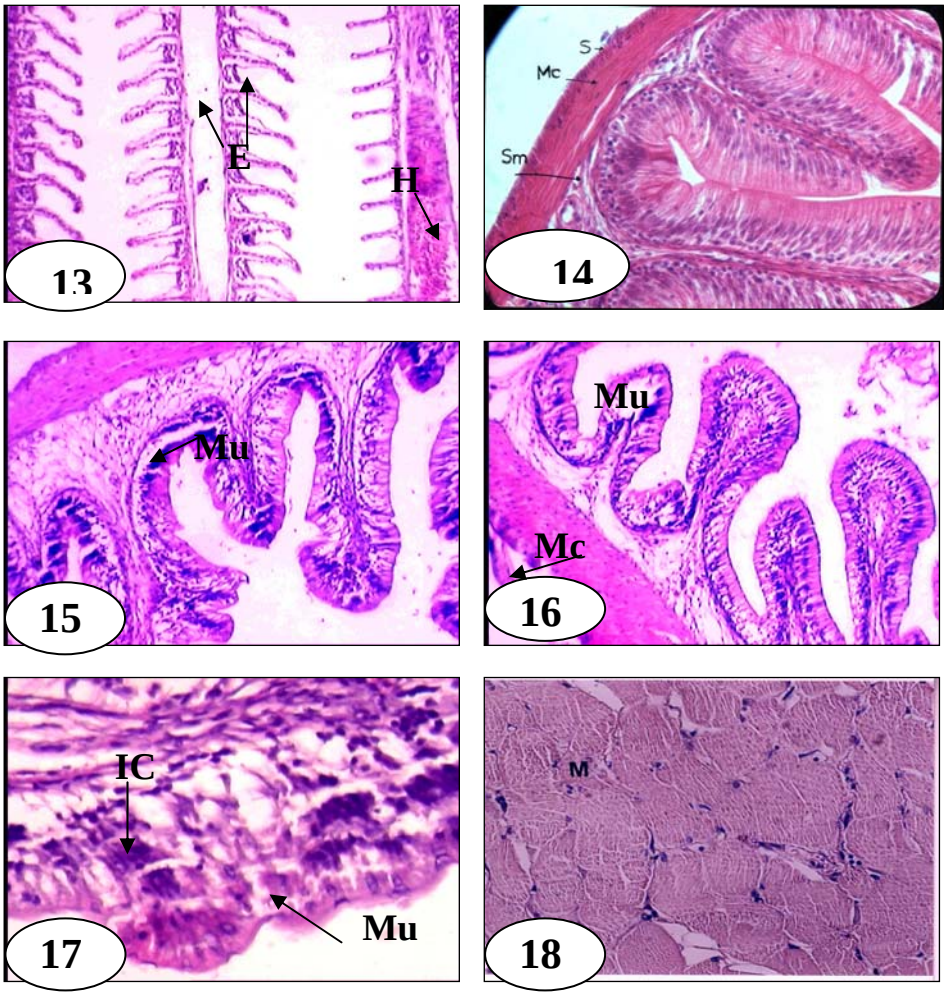

Plate III

Skeletal or striated muscle of fish is found exclusively in tissue subjected to voluntary control and composed of muscle fibers as shown in Fig.18. Its multinucleated fibers contain myofibrils. The myofibrils are composed of hundreds of myofilaments divided into thin actin and thick myosin elements. Oval nuclei are located peripherally beneath the sarcolemma of each muscle fiber. These fibers insert into broad sheets of collagenous connective tissue termed myoseptae (Groman, 1982).

Muscle specimens of *Oreochromis niloticus* (L.) from fish farm in El Fayoum Province exhibited different stages of degeneration. Mild degeneration was observed in Fig. 19 and severe degeneration and fusion Fig. 20 in addition to necrosis (Fig.21). Large fat cells formed adipose tissue within the muscle fibers (Fig. 20). The muscle fibers were atrophied, ruptured and infiltrated with inflammatory cells (Fig. 22). These results agreed with that obtained by Holcomb *et al.* (1976) who reported that lead causes muscular atrophy in the brook trout *(Salvelinus fontinalis).* Ibrahim *et al.* (1998) noticed that degeneration and fragmentation in red and white muscles of *Gambusia affinis* treated with lead and cadmium have the potential to disturb the equilibrium and swimming of the fish.

Yacoub (1999) and Sitohy et al. (2006) reported degeneration as well as necrosis in muscles of *Clarias gariepinus* in response to effects of heavy metals in River Nile (at El Kanater and El Tebbin).

Kadry (2001) noticed degeneration, chronic inflammatory infiltration and prominent necrosis in cadmium treated *Clarias gariepinus*. Also, Yacoub *et al.* (2005) cited histological changes in the muscles of *Liza ramada* fish inhabited polluted water of Lake Manzalah including degeneration, shrinkage, atrophy and necrosis of muscle fibers.

4. CONCLUSIONS

Drainage water (the main water source of the fish farms in El Fayoum) is loaded with pesticides and inorganic fertilizers which contain heavy metals in their chemical compositions. These heavy metals accumulated in the vital organs of *Oreochromis niloticus* (L.) and caused many pathological lesions. So, we recommend treatment of drainage water before its entrance into fish farms.

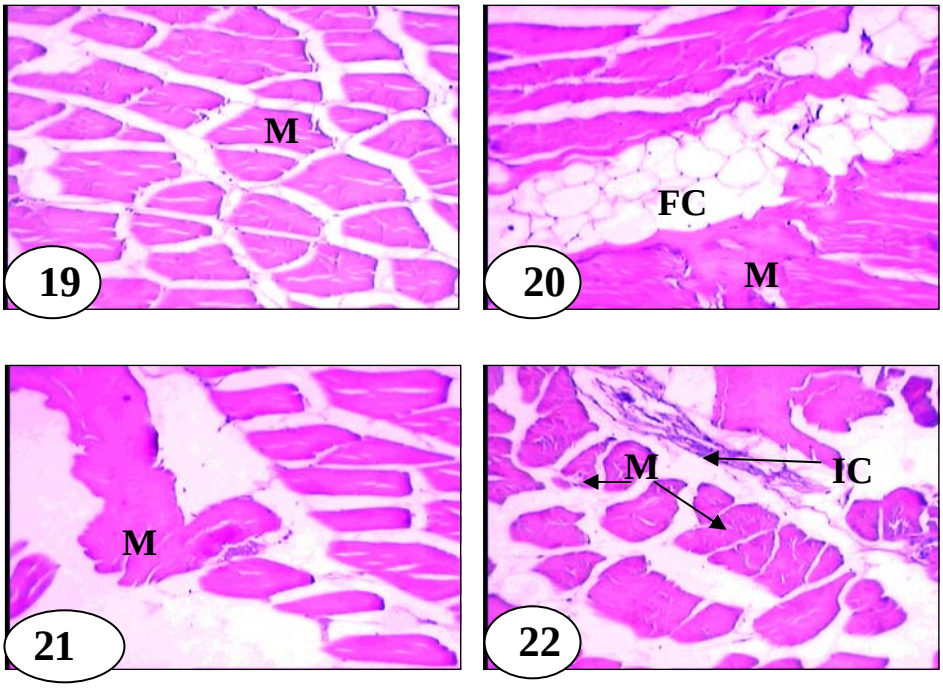

Plate IV

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EXPLANATION OF FIGURES

Plate I

(Fig.1): Liver section of control *Oreochromis niloticus* fish obtained from the fish farm of El Kanater Research Station, showing the bile ductile (DB), branches of hepatic portal vein (HPV) and hepatic artery (HA). (H&E, X400)

(Fig.2): Liver section of *Oreochromis niloticus* fish obtained from the fish farm, showing ballooning degeneration within the hepatocytes. (H&E, X400)

(Fig.3): Liver section of *Oreochromis niloticus* fish obtained from the fish farm, showing fatty degeneration of hepatocytes (H). (H&E, X400)

(Fig.4): Liver section of *Oreochromis niloticus* fish obtained from the fish farm, showing degeneration of pancreatic acini (pa). The pancreatic ductile is filled with stagnant blood (sb). (H&E, X400)

(Fig.5): Liver section of *Oreochromis niloticus* fish obtained from the fish farm, showing dilation of blood sinusoid (bs) and fatty degeneration of hepatocytes (H). (H&E, X400)

(Fig.6): Liver section of *Oreochromis niloticus* fish obtained from the fish farm, showing large lucent necrotic area (NA) inside the hepatic tissue. (H&E, X400)

Plate II

(Fig.7): Liver section of *Oreochromis niloticus* fish obtained from the fish farm, showing degeneration and necrosis of hepatocytes (H). (H&E, X400)

(Fig.8): L. S. of gills of control *Oreochromis nilotcus* fish obtained from the fish farm of El Kanater Research Station, showing the secondary gill lamellae. Notice squamous epithelial cells (SEC), pillar cells (PC) and mucous cells (MC). (H&E, X1000)

(Fig. 9): L. S. of fish gills of *Oreochromis niloticus* from the fish farm, showing edema (E) in primary and secondary lamellae and hyperplasia progressed to complete fusion of secondary lamellae (F). (H&E, X400)

(Fig. 10): L. S. of fish gills of *Oreochromis niloticus* from the fish farm, showing telangiectasis (t) at the tips of secondary lamellae. (H&E, X400)

(Fig. 11): L. S. of fish gills of *Oreochromis niloticus* from the fish farm, showing severe hemorrhage in primary lamellae (PL) and reduction of epithelial cells in secondary lamellae (SL). (H&E, X400)

(Fig. 12): L. S. of fish gills of *Oreochromis niloticus* from the fish farm, showing degeneration of some epithelial cells (EC). (H&E, X400)

Plate III

(Fig. 13): L. S. of fish gills of *Oreochromis niloticus* from the fish farm, showing hemorrhage in primary lamellae (H) and edema in primary and secondary lamellae (E). (H&E, X400)

(Fig.14): T. S. of normal intestine of *Oreochromis niloticus* obtained from the fish farm of El Kanater Research Station, showing the layers of intestine: mucosa (Mu), submucosa (Sm), muscularis (Mc) and Serosa (S). (H&E, X400)

(Fig.15): T. S. of intestine of *Oreochromis niloticus* from the fish farm, showing degeneration and necrosis of epithelial cells of mucosa (Mu). (H&E, X400)

(Fig.16): T. S. of intestine of *Oreochromis niloticus* from the fish farm, showing necrosis of epithelial cells of mucosa (Mu) and destruction of muscularis (Mc). (H&E, X400)

(Fig.17): T. S. of intestine of *Oreochromis niloticus* from the fish farm, showing reduction of mucosal layer (Mu) and aggregation of inflammatory cells (IC) in the submucosa. (H&E, X400)

(Fig. 18): L. S. of normal muscles of *Oreochromis niloticus* obtained from the fish farm of El Kanater Research Station, showing the myomers(M). (H&E,X400)

Plate IV

(Fig. 19): L. S. of muscles of *Oreochromis niloticus* from the fish farm, showing mild degeneration of muscle fibers (M). (H&E, X400)

(Fig. 20): L. S. of muscles of *Oreochromis niloticus* from the fish farm, showing large fat cells (FC) within the muscle fibers, also degeneration and fusion of muscle fibers (M). (H&E, X400)

(Fig. 21): L. S. of muscles of *Oreochromis niloticus* from the fish farm, showing degeneration, fusion and complete necrosis of some muscle fibers (M). (H&E, X400)

(Fig. 22): L. S. of muscles of *Oreochromis niloticus* from the fish farm, showing atrophy and rupture of muscle fibers (M) and infiltration of inflammatory cells (IC). (H&E, X400)