The light and electron microscopic evaluation on the effects of water pollution with some heavy metals in health status of Tilapia fish from Manzala lake, Egypt.

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Abstract
This study provides information on some heavy metal concentrations in water and Tilapia fish from Manzala lake and to evaluate the histopathological and ultrastructure effects of metals on fish. Manzala Lake recorded highest concentrations of mercury, cadmium and lead in the water that exceeded the maximum allowable permissible limits internationally. This was attributed to the large amounts of industrial and agricultural waste and sewage that drain the lake . Tilapia Fish collected from the lake recorded accumulation of heavy metals (mercury , lead and cadmium). The study also showed that the liver tissue has a great affinity for metals storage than muscle which represent a risk to the consumer.The levels of these metal concentrations in both water and fish exceed the permissible limit of WHO and international standards. Histopathological alternation of gills revealed proliferation in the epithelium of gill filaments, hyperplasia in primary and secondary lamellae , degenerative and necrotic changes, aggregations of inflammatory cells, hyperplasia in mucin producing cells.Histopathological alternation of kidneys showed marked glomerular, tubular and interstitial pathological alternation. Dark granules in the apical sections of the tubular epithelial cell in Semithin sections of kidney. Ultrastructure of the glomeruli showed rough endoplasmic reticulum underwent a progressive loss of structural integrity, mitochondria degeneration, increased number of lysosomal , marked clumping of chromatin contain intranuclear inclusions bodies . The liver demonstrated vacuolar, focal areas of necrosis of hepatocyte and aggregation of mononuclear inflammatory cells between the hepatocyt, hemorrhages, dilatation and thrombos. Ultrastructure of hepatopancreace demonstrated all cells were showed structural damage ,extensive proliferation and swollen of smooth endoplasmic reticulum, The rough endoplasmic reticula lacked their characteristic arrangement. Mitochondria were also found to lose their characteristic shape. An increase in the numbers of lysosomes and electron dense bodies. Nuclei showed heterochromatin randomly distributed, rupture of nuclear membrane and Inclusion bodies.

Key words: *Fish, heavy metals, histopathology, ultrastructure, Manzala Lake Egypt .*

Introduction

Environmental pollutions caused by industrial and domestic wastes are nowadays the greatest concern in public health **(Chiesa *et al*., 2006** and **Pandey *et al*., 2008)**. Heavy metals Toxicity are one of the main pollutants of the environment related to the biological activity . The physiological influence of metals on the organisms of human and animals is conditioned by the nature of metal, by the type of compounds and by the amount of them . Heavy metals are persistent environmental contaminants since they cannot be degraded or destroyed. Many heavy metals are urgently necessary for functioning of the body of humans and other living organisms in small amounts and belong to the range of nutrients. Others, when passed on to the living organisms cause poisoning or death **(Danielyan, 2010)**. Thus contaminants change water quality and can cause serious damages to the organisms, including fish **(Jemal *et al*., 2002** and **van der Oost *et al*., 2003)**. Pollution of the aquatic environment by inorganic and organic chemicals is a major factors posing serious threat to the survival of aquatic organisms including fish. Fish is used as bio-

indicators of pollution in Manzala Lake **(Saeed and Shaker, 2008)**. The Egypt's northern Delta Lakes include Lake Manzala are an important natural resource for fish production in Egypt. Until 1991, Manzala was contributed the more country's total fish production, but at present time this has decreased **(Gafrd, 2006)**. Extensive research programs have been carried out to investigate the tilapia biology and fisheries in the inland waters of Egypt, including the northern Delta Lakes **(Shakweer and Abbas, 1996; Khallaf *et al*., 1998** and **El-Moselhy, 1999)**.

Histopathological alterations can be used as indicators of the effects of various pollutants on the organism including fish, and reflection of the overall health of the entire pollution. According to studies by **Mohamed (2009)** shown that the exposure of fish to pollutants, agricultural and industrial chemicals, were resulted in several pathological changes in different tissues of fish. Hepatic lesions, including neoplasms, focal lesions, degenerative or necrotic lesions are commonly detected in fish from contaminated environments (Myers *et al*., 1998). Continuous environmental and occupational heavy metals exposure can lead to chronic nephropathy, however, many experimental studies showed that several heavy metals caused renal failure associated with severe histopatholgical and physiological alterations **(Abdel-Moneim and Said, 2007; Kutlubay and Ogˇuz, 2007; Massanyi *et al*., 2007; Obianime and Roberts, 2009; Suradkar *et al*., 2009** and **Soudani *et al*., 2010)**.

Materials and Methods

Study Areas

Manzala Lake is a coastal shallow lake, which consists of 30 basins It is situated east of the Nile's Delta between the Damitta branch of the Nile River and the Suez Canal. The Mediterranean Sea is immediately north of the narrow coast, which separates the two water bodies. The sampling localities were selected close to the most important communities around the Lake and to the areas where the discharge of different types of pollutants occur, which negatively affect the Lake's condition and human health in the area. Manzala Lake in receives sewage, agricultural and industrial wastes from the surrounding Governorates which increase the lake pollution.

Collection of Samples:

Water and fishes were collected from five different locations of lake . forty Tilapia fish from each site . Immediately after collection , Body condition and any macroscopic lesions were recorded.

I -Biochemical Analysis:

1- Preparation of the samples for analysis:

Water samples preserved in a refrigerator till analysis for target heavy metals and The collected fish sample were kept under freezer -18° C until analyzed. Samples from , liver and muscles were weighted from each and dried in a hot air oven (100-105°C) till the wet tissues reached to a constant weight. According to **AOAC (2006)**. Moisture content of each sample was calculated . So our results are related to the dry weight One gram was taken from the dried grinded sample into a flasks . 5ml of nitric acid was added . The digestion flasks were kept overnight ,and then putted on the hotplate at 80°C until all the tissues were dissolved and the brown fumes of NO_3 escaped ,filtered and diluted till 25 ml with distilled water. A blank was carried out in the same way.

2-Preparation of the standard solutions :

All metals were determined against aqueous standards . standards prepared at dilution 0,05 and 1 ppm. the target elements analyzed were Mg, Cd, and Pb. Stock standard solution (Merck, Germany) of each element were used to prepared calibration solution to obtain calibration curves .

The metal analysis of samples were carried to the central laboratory of the faculty of Veterinary Medicine Assuit University using a ZEEnit 700 P Atomic Absorption spectrophotometer. The content of heavy metals are Results were expressed mg/kg dry weight (ppm) of the tissue.

II -Pathological Examination:

Tissue samples were collected after postmortem examination from kidneys, liver and gills. These samples were fixed in formalin 10% for at least 24-72hrs, then washed with water, dehydrated, cleared, and embedded in paraffin wax. Section of 5-7 microns were stained with Heamatoxylin and Eosin, Hematoxylin and eosin stain for histological exam. according to **Bancrofts *et al*. (1996)**.

III- Transmission Electron Microscopic Study

Liver and kidney tissues greater than 2 cm long were cut into smaller pieces of approximately 3 x 3 mm and then fixed in 4 percent glutaraldehyde. Toluidine Blue For the staining of semithin sections .and were examined under light microscope . subsequently ultrathin sections (500-800A) were obtained from selected areas in the semethin sections, stained with uranyle acetate and lead citrate according to **Reynolds (1963)**. The sections were examined and photographed with JEOL transmission electron microscope (J.E.M.- 100 Cx) operating at 80 Kv , Assuit University Electronmicro-center.

Results

Heavy Metals Contents in Water :

Analysis of examined water samples for lead ,cadmium and mercury showed increase in mean concentrations of these metals, as following 0.18500 ppm for lead, 0.03100 ppm for cadmium and 11.33 ppb for mercury. Table 1.

Heavy Metals Contents in Fish tissues:

Analysis of lead , cadmium and mercury levels in liver tissues of examined fish revealed 0.574 ppm, 0.0068 ppm and 2.070 ppb respectively. While mean concentrations of lead, cadmium and mercury in fish muscles presented by 0.1492 ppm, for lead, 0.0035 ppm for cadmium and 0.396 ppb for mercury. Table 1.

Table (1). Levels of some heavy metals in the lake water and organs (liver and muscles) of examined fishes.

	Pb (ppm)	Cd (ppm)	Mercury (ppb)
Water	0.18500±0.2928	0.03100±0.03205	11.333±0.3493
Liver	0.57400±0.05475	0.00680±0.00095	2.070±0.4440
Muscles	0.14925±0.07942	0.00352±0.00055	0.3960±0.0916

The pathological examination:

Post-mortum examination:
Fish were moderately small in size. The intestine got darker, swollen, the tissues of liver and kidney were fragile. The gross lesions mainly confined to the kidney which was dark or with hemorrhages.

Histopathological examination:
Gills:
The epithelial cells cover both the primary and secondary lamellae showed congestion , oedema, hemorrhage ,rupture of lamellar epithelium ,secondary lamellar disorganization (fig. 1a). The gill arch, especially at the bases of the gill filaments, showed numerous mononuclear leukocytic infiltration (fig. 1b&c). Proliferation in the epithelium of gill filaments . fusion and focal desquamation of the epithelial lining of the secondary lamellae (fig. 2a). Dilation and congestion in blood vessels of gill filaments were observed (fig.2a). The filament region constitutes edema with intense lamellar vasodilatation. The tips of epithelium showing hyperplasia and proliferation of in mucin producing cells , degenerative and necrotic changes in the epithelium of gill filaments , edema in secondary lamellae and gill filaments, aggregations of inflammatory cells (fig. 2b).

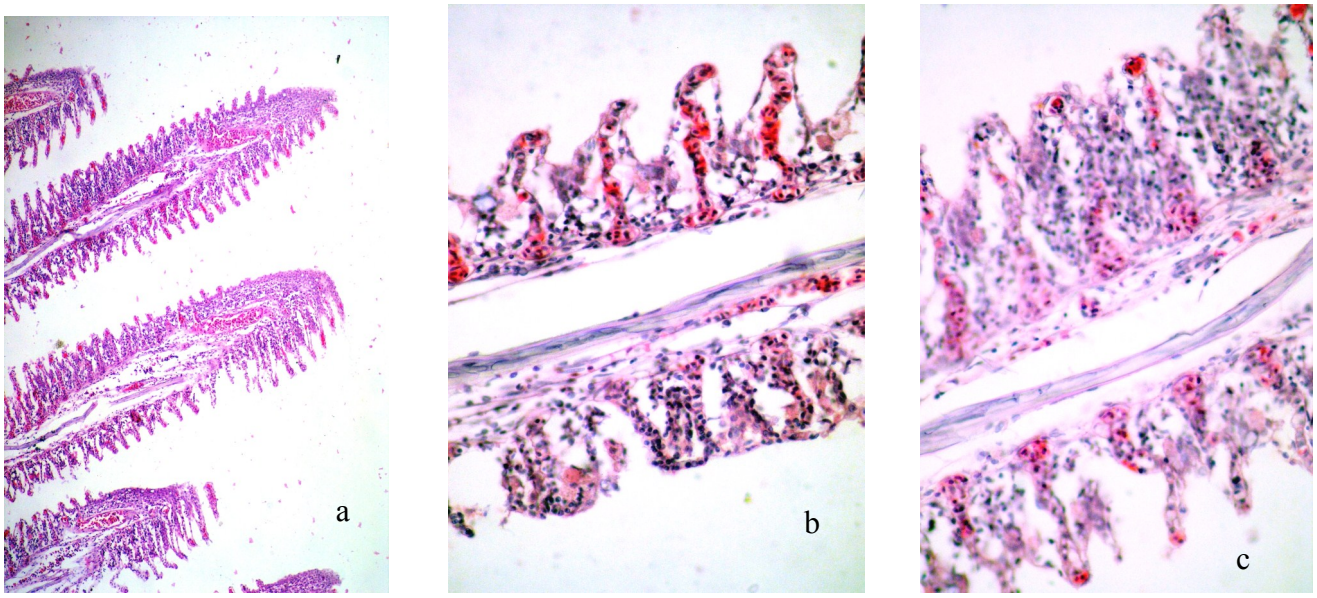

Fig. (1). Gills of fish (aX1,bX25&cX40) showing congestion , fusion of secondary lamellae, hyperplasia in secondary lamellae , proliferation in the epithelium of gill filaments and secondary lamellae , proliferation of mucous cells , oedema in secondary and gill filaments , aggregations of inflammatory cells in gill filaments H&E.

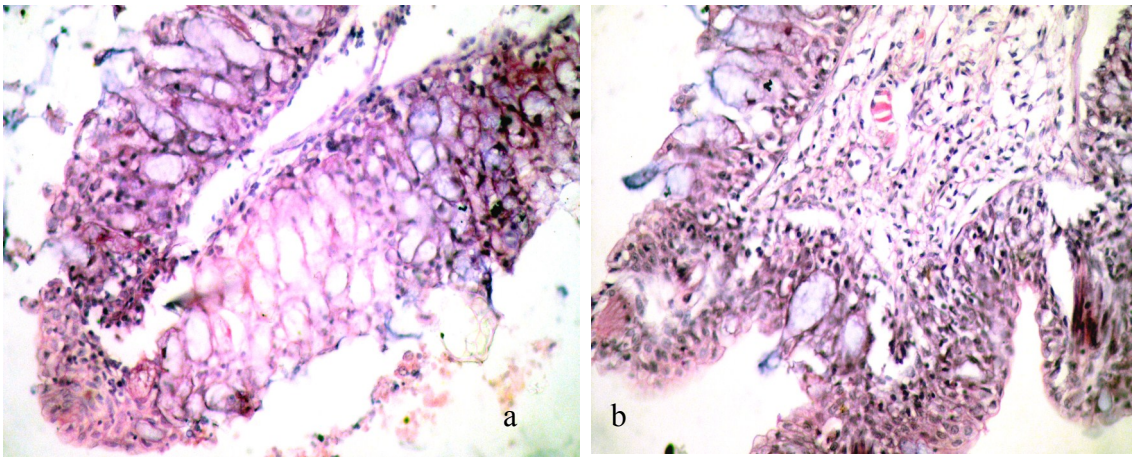

Fig. (2). Gills of fish showing (a &b) the tips of villi epithelium showing hyperplasia in mucin producing cells , degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae, aggregations of inflammatory cells in gill filaments , dilation and congestion in blood vessels of gill filaments.(X40) H&E .

Kidney:

The histopathological alterations in kidney from showed marked glomerular, tubular and interstitial alternations. Glomerular alternations revealed dilation of Bowman's space, some glomeruli represented hypercellularity and proliferation of mesangial cells (fig .3a), with hemorrhage and necrosis in glomeruli. oedema in Bowman's capsules and dilation in renal blood vessels (fig. 3c).

The lining renal epithelium showed degenerative changes mainly hydropic degeneration, with swelling and detachment of some lining epithelium . some tubules showed dilatation and contain cellular casts , focal necrosis was observed in the renal proximal tubule with karyolysis and cytolysis and the brush border was reduced or not present (fig. 3c). focal mononuclear inflammatory cells infiltrating renal tubules , interstitial hemorrhagic (fig. 3b) and chronic inflammation replaced urinary tubules with proliferation of fibrorus connective tissue were evident (fig. 3b). Swelling in the lining epithelium of the renal tubules with narrow lumen. The kidneys showed interstial oedema, dilatation and congestion of blood capillaries in posterior kidney (fig. 3c).

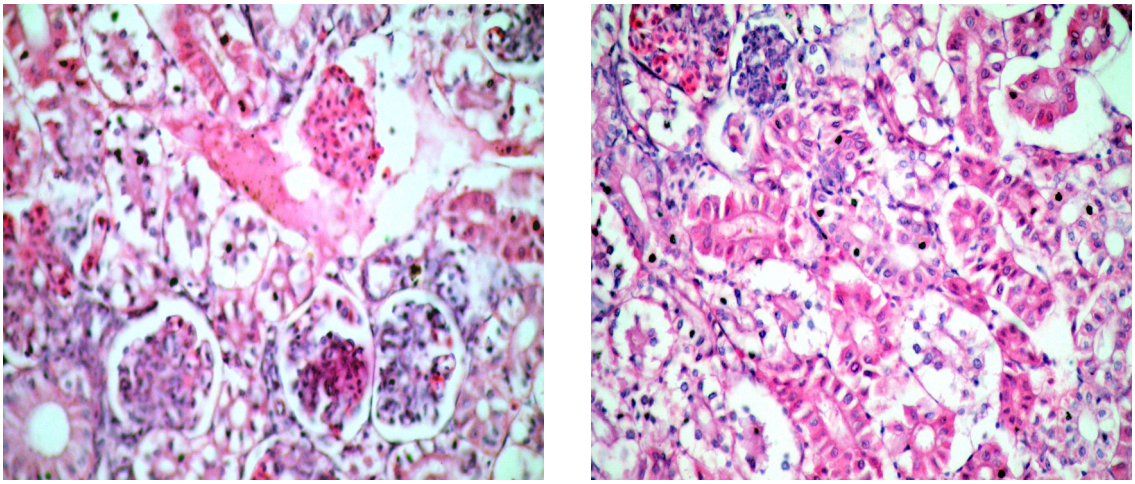

Fig. (3). Kidney of fish showing (a) Hypercellularity, proliferation of mesangial cells of glomeruli , expansion of space inside the Bowman's capsules , glomerular tufts with haemorrhage, necrosis , interstitial oedema . (b) Kidney of fish showing glomerular tufts with haemorrhage, hydropic degeneration , cytoplasmic vacuolation of tubular epithelial cells of the proximal convoluted tubules, dilatation ,hypertrophy of epithelial cells infiltration of mononuclear cells . Focal cellular necrosis, (X40) H&E.

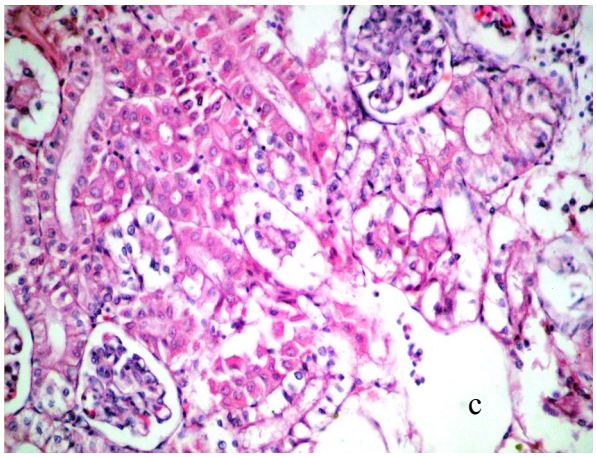

Fig. 3(c): Kidney of fish showing proliferation of glomeruli, sever degenerative and necrotic changes in the renal tubules with focal areas of necrosis (H&E X40).

Semithin section of the kidney showed marked degenerative changes in the kidney (fig. 4. a). The glomeruli revealed dilated space of Bowman's capsule(fig.4.b). vacuolar degenerative changes in epithelial cells of tubules and apical sections of the tubular epithelial cells contain dark granules (fig. 4. d & e). In some cells, bi-lobed nuclei, internuclear inclusion like demonstrated as homogenous translucent dark mass (fig.5. a & b) number of apoptotic bodies were increased, several miotitic division demonstrated in the tubular cells (fig. 5. c&d,) . vacuolar degeneration in the epithelial cells lining the renal tubules with hyperemic capillaries and hemorrhage, (fig.5. e).

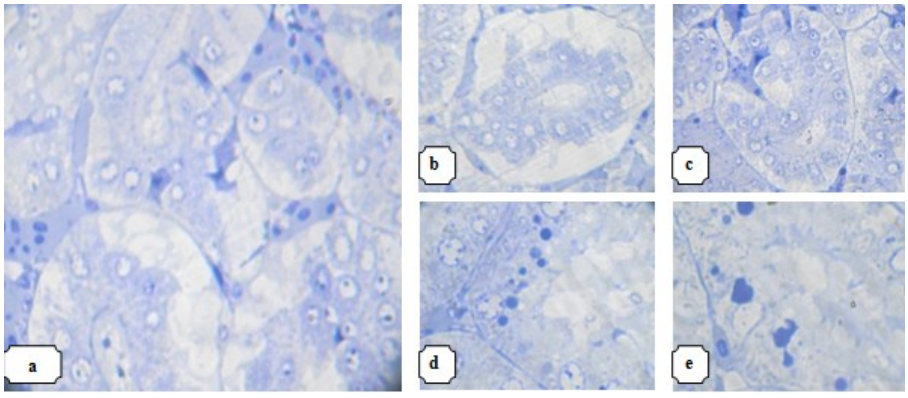

Fig. 4 (a) Semithin section showing Tubular cells degeneration (X40). (b) The glomeruli with diliated space of Bowman's capsule(X40) . (c) Epithelial cells of renal tubules showing vacuoles and hyperplasia. (X40). (dX40&eX100) Epithelial cells of kidney tubules with degenerative changes and dark granules in the apical sections of the tubular. Toluidine blue .

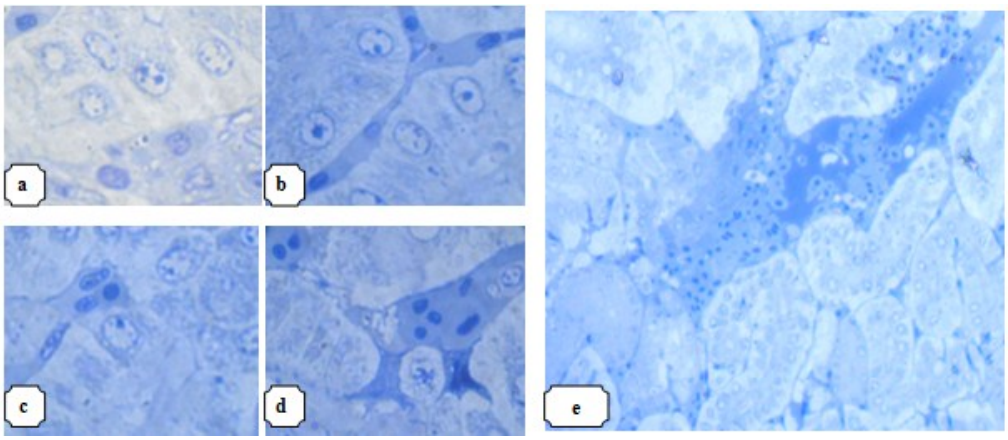

Fig. (5). (a&b) Semithin showing internuclear inclusion bodies like demonstrated as homogenous translucent dark mass X100 . (c&d) apoptotic bodies, mitotic division in the tubular cells(X100). (e) hemorrhage between renal tubule. X40, Toluidine blue .

Transmission Electron microscopic study of the kidney: Ultrastructure of the glomeruli showed mesangial matrix appeared to be increased and the mesangial cells characterized by numerous spinous cytoplasmic processes, and numerous dense bodies, clefted or segmented nuclei (Fig.6). The endothelial cells of the glomerular capillaries were swollen and lost their fenestrace, pyknotic nuclei. (Fig. 6). The podocyte nuclei were irregular outline, deposition of electron dense material in the podocyte, Foot processes were disorganized and in several cells the foot processes lost leaving fragment parts (fig.6b&d). The basement membrane become thickened from place to place with homogenous appearance (fig. 6 b & e). Rough endoplasmic reticulum underwent a progressive loss of structural integrity which were distributed haphazardly throughout the cell(fig.6b&c). glycogen deposit distributed in cells (fig.6b). Total disintegration of the reticule was also noticed with detachment or totally absence of ribosomes (Fig.6a). Lysosomes were numerous contained dense bodies of varying size. Furthermore, the accumulation of electron dens material of different sizes was observed , (Fig.6a). different types of lysosomes autophagic vacuole. Fully formed autolysosome containing a mitochondrion undergoing digestion (Fig.6b) .

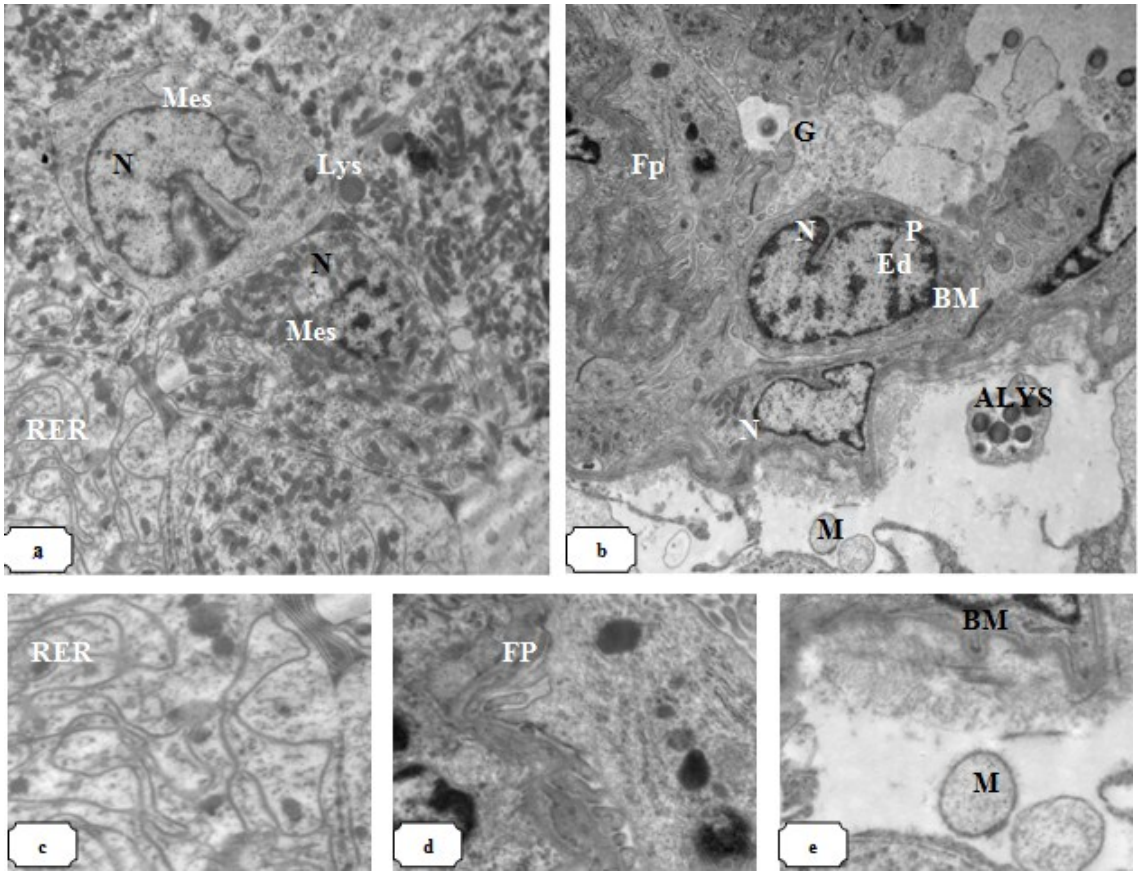

Fig. (6) a. Ultrastructur of fish kidney showing the glomerulus , mesangial cell (Mes) Rough endoplasmic reticulum (RER) numerous lysosomes contained dense bodies (Lys) (X 4800) . (B) The podocyte (P), disorganized Foot processes (Fp), endothelium cell (Ed), basement membrane(Bm) damaging Mitochondria (M), autolysosome (Alys) , glycogen deposit(G) X4800). (c) Rough endoplasmic reticulum(RER). (d) disorganized Foot processes (Fp). (e) Thickened basement membrane (BM) and damaging and swollen Mitochondria (M).

The most remarkable ultrastructural findings were degenerative changes of the the tubule epithelial cells. hyperplasia and proliferation of smooth endoplasmic reticulum. (fig. 7a&b). They were broken down into very small bits. Mitochondria1degenerated, swollen mitochondria matrix was also detected. (fig. 7 a & b). Lysosomes were numerous contained dense bodies of varying size. Ovoid bodies composed of dense osmiophilic material . Distal convoluted tubular cells showed an increase in the cytoplasmic density of some of the cells. Nuclei showed marked clumping of chromatin with aggregation of interchromatin material .The nucleolus was displaced peripherally (fig.7 c). Intranuclear inclusions of different sizes appeared a small electron-dense zone midway between the nucleolus and the nuclear membrane(fig.7c). There were partial loss of microvilli with electron pale material in lumen (Fig. 7c).

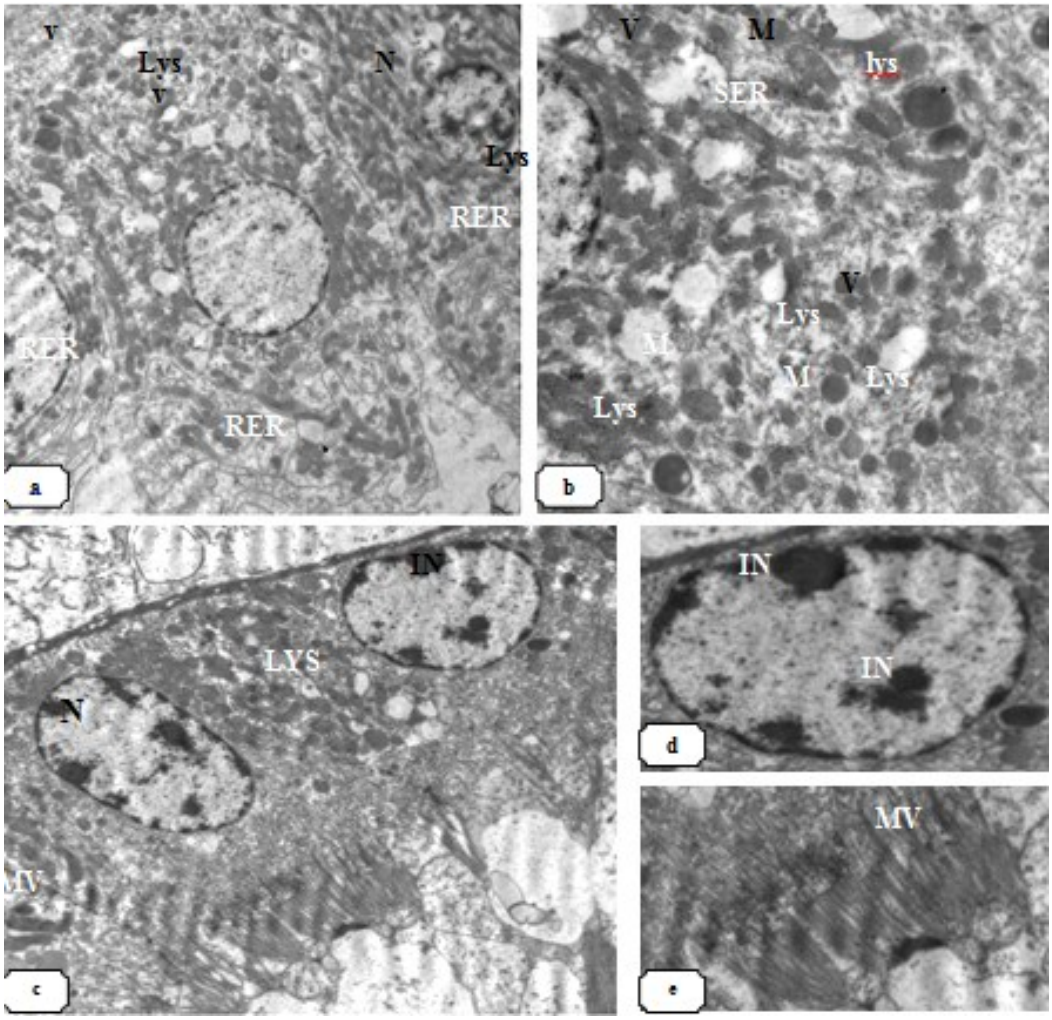

Fig. (7). (a X4800)&(b x10000) ultrastructural of proximal tubule of fish kidney showing Rough endoplasmic reticulum (RER) , mitochondrial proliferation (M), of lysosomal and abundancy of osmophilic bodies, (Lys) swollen smoth endoplasmic reticulum.(SER), vacuoles (v). (c X4800) distal convoluted tubule cells showing Nuclei (N) Intranuclear inclusions of different sizes(IN) . partial loss of microvilli(MV). (d) internuclear inclusions in distal tubule. (e) partial loss of microvilli .

Liver:

The most prominent lesions were distortion of cords hepatic (fig 8). cellular degeneration and vaculation of hepatic cells that, (fig8a&b). hepatopancreace of fish showed focal areas of necrosis, nuclear pyknosis, lysis, cytolysis in the majority of hepatic cells and inflammation around portal arch (fig. 8b&c). The central veins and the hepatic sinusoids were congested and widening of sinusoids could be seen. Focal mononuclear cell aggregates were seen in the hepatic parenchyma and portal areas (fig.8c). there was intercellular hemorrhage (fig. 8 d). Intravascular haemolysis in red blood cellswithvessels , hemorrhages in hepatoportal blood vessel (fig 8 d).

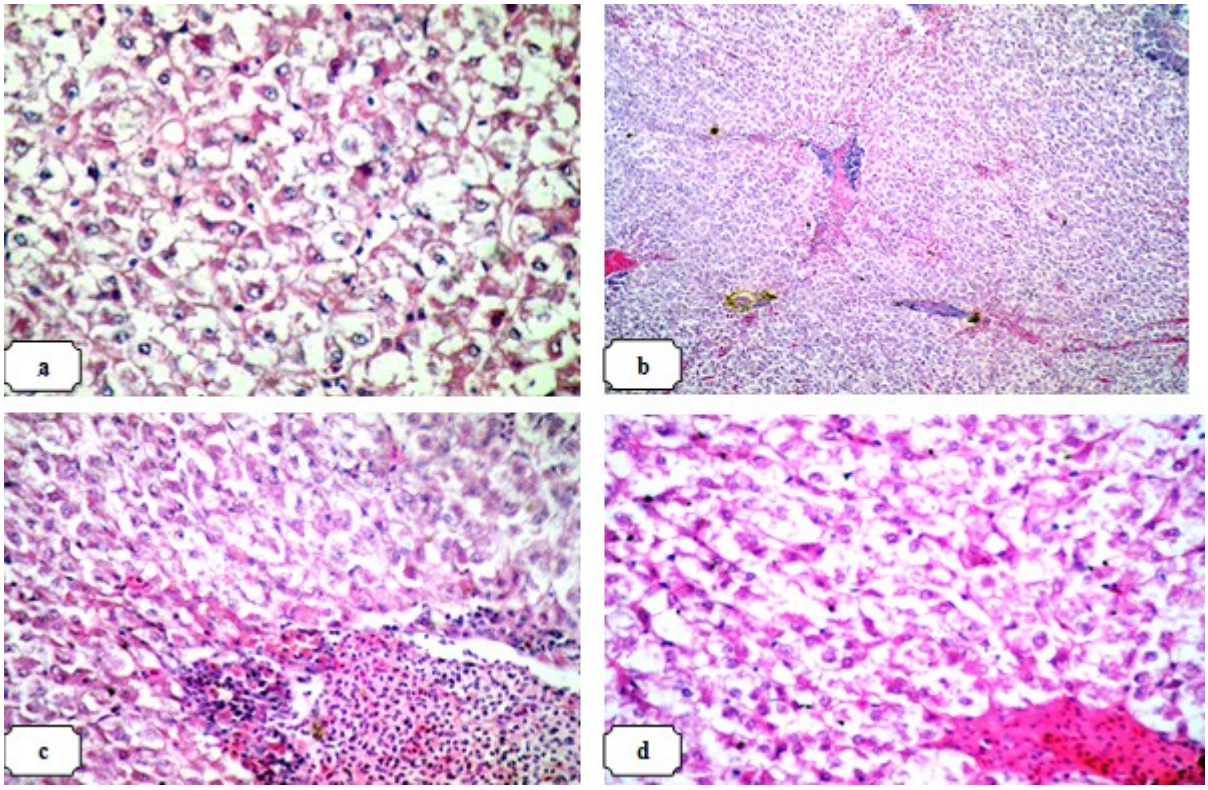

Fig. (8). Liver of fish showing (a X40) hydropic degeneration and vaculation of hepatic cells . (b X10) hepatopancreace of fish showing inflammation around portal arch. (c X40) aggregation of mononuclear inflammatory cells , Focal areas of necrosis, nuclear pyknosis, lysis , cytolysis. (d X40) haemorrhage in the hepatoportal blood vessel .

Transmission electron microscopic of hepatopancreace:

Hepatopancreatic tubule is lined by a pseudostratified epithelium that consists of five different cell types. All cells were showed structural damage . The ultrastructure of cells included damaged nuclei ,Nuclei showed heterochromatin randomly distributed in the nucleoplasm , thickening of the nuclear membrane from place to place were noticed and rupture of nuclear membrane in some cells (Fig. 9a&11f).many nuclei had lost their typical elliptical shape due to extensive shrinkage of the nuclear membrane denser distribution of chromatin (Fig. 9d).Totally disarrayed lipid droplets were distributed inside the cells (Fig.9a&b). Extensive proliferation and swollen of the smooth endoplasmic reticulum (Fig.9a,c,d&11a). the rough endoplasmic reticulum showed considerable alteration, it being disfigured and disintegrated in some cells(Fig.9a,c&d). The rough endoplasmic reticule were broken down into very minute pieces and were distributed haphazardly throughout the cells (Fig. 9a&d) while in others had the form of short and thin cisternae (Fig. 9c). In the central and basal region, the

rough endoplasmic reticulum had the form of concentric whorls(Fig.9a,10b &11b,c). The mitochondrial membrane was distended in some locations and partially degenerated in others (Fig.9b). The cristae of the mitochondria were swollen (Fig. 9d&11d). In some cases the mitochondria were found extremely swollen and totally devoid of cristae (Fig. 9a). Increased number of lysosomal related structures within the epithelial lining cells and aggregation of lysosomes (Fig.9a,b,d&11e). Furthermore, increasing numbers of secondary lysosomes with degraded cell organelles (Fig. 10a&11g). Large residual body-like structures were seen in the cells (fig.10b). vacuoles resulting in collection of electron dense materials was noticed (Fig. 10a&b). In addition occurrence of clusters of myelinated bodies (Fig. 10a&11h).

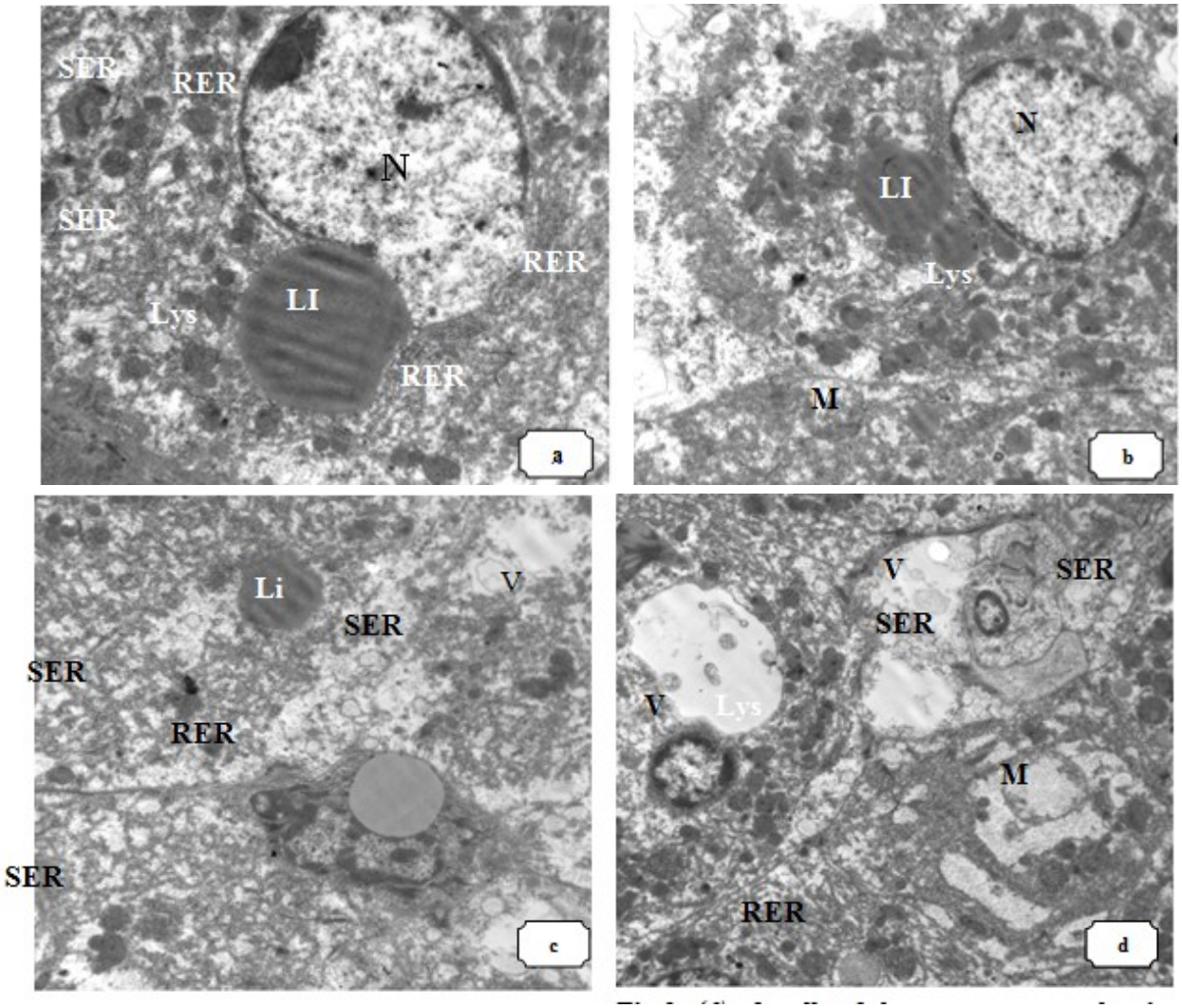

Fig. 9 (a). Ultrastructure of R cell of hepatopancreas showing extensive proliferation of the smooth endoplasmic reticulum (SER), degenerated rough endoplasmic reticulum(RER) , rupture of nuclear membrane and nuclei showed heterochromatin. large lipid vacuoles (LI) X7200. (B) M cell extensive proliferation of the smooth endoplasmic reticulum swelling of endoplasmic reticulum, swallow mitochondria (M), Increased number of lysosomal (Lys) X5800.

Fig.9 (c). B cell of hepatopancreas showing hyperplasia , Swelling of smooth endoplasmic reticulum (SER),degenerated roughendoplasmic reticulum(RER) vaculation of cytosol (V) large lipid vacuoles (LI) 5800.

Fig.9 (d). f cell of hepatopancreas showing swelling of smoth endoplasmic reticulum(SER) , Mitochondrial swelling and vaculation(M), lyso som (Lys) shrinkage of the nuclear membrane(N), vaculation of cytosol(V) . 4800.

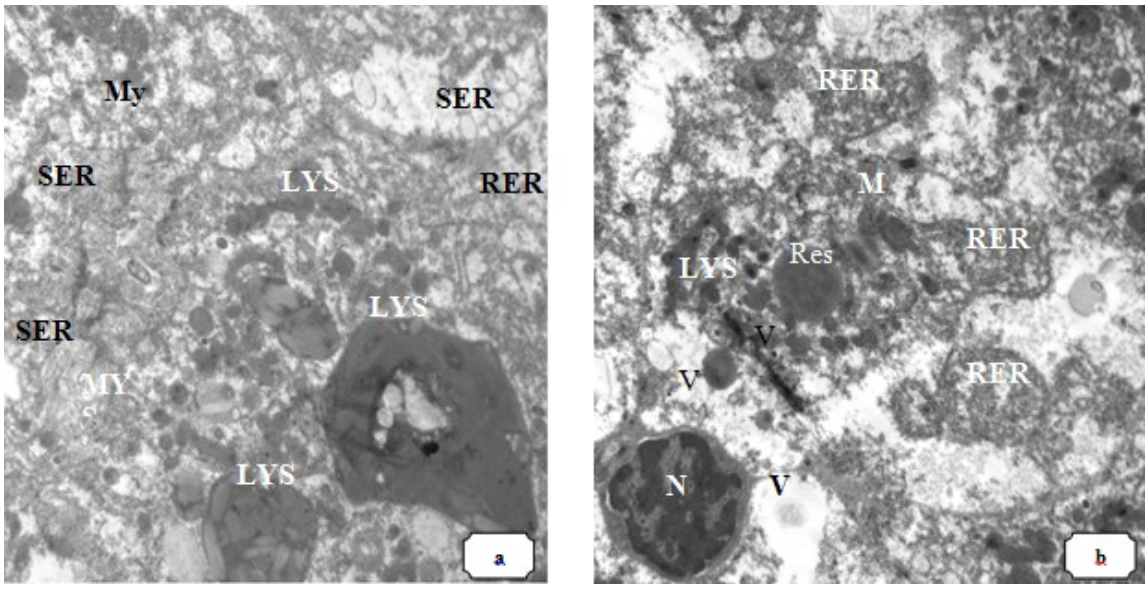

Fig. 10 (a) f cell hyperplasia and aggragation of smooth endoplasmic reticulum(SER) , Swallen and dilated rough endoplasmic reticulum(RER) , aggragation of lysosomes(Lys) , aggregations of myelinated bodies(My) x 5800.

Fig. 10(b) showing E cell of hepatopancreas showing vaculation of cytosol (V) ,swalling and degranulation of rough endoplasmic reticulum ,had the form of concentric whorls (RER), damaging Mitochondria(M) , lysosomal (Lys), presenceof residual body (Res) X5800.

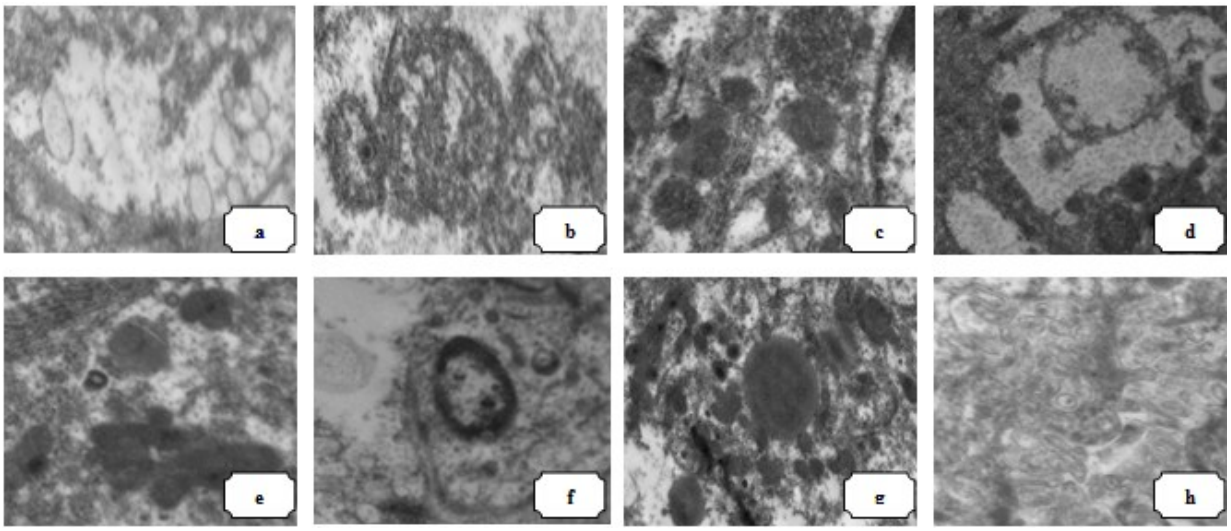

Fig. (11). Ultrastructure of hepatopancreas showing:(a) extensive proliferation of the smooth Endoplasmic reticulum, (b) degenerated rough endoplasmic reticulum. (c) degenerated mitochondria and swollen rough endoplasmic reticulum, (d) swallen and degenerated mitochondria, (e) lysosomal, and abundancy of osmophilic bodies (f) shrinkage of the nuclear membrane ,(g) presence of residual body ,(h) aggregations of myelinated bodies .

Discussion

As a result of industrial activities, the aquatic ecosystem has been highly heavy metals. This posses great stresses to aquatic organisms in particular and to the whole ecosystem in general. Investigation of heavy metal concentrations in fish and water compartment of the environment are an important aspect of the control of environmental pollution to counteract the human activities which progressively increase heavy metal concentration in the aquatic system. Pollution of Manzala lake occurs through the untreated sewage drainage from surrounding Governorates. Pollution of the

lake with many toxic substances occurs from the industrial wastes that drain into the lake (detergents, solvents, heavy metals, dyes, pigments, ... etc.). Pollution from agricultural practices is due to animal wastes, material eroded from the land, plant fragments, organic salts and minerals resulting from irrigation, herbicides and pesticides. High concentrations of the heavy metals in the lake water increase their concentrations in the edible plants and fishes and consequently in fishermen **(Abd ElWahab 1992** and **Osfor1998).**

Analytical findings of examined heavy metals levels in water samples revealed increase in lead, cadmium and mercury concentrations (0.18500, 0.0310 ppm and 11.33 ppb) respectively. The present results of lead and cadmium levels in water are more than results obtained by **Bashir *et al*. (2012),** who studied that evaluating the heavy metal levels in water and tissues of two commercial fish. The concentrations of Pb and Cd in these coastal waters ranged from (0.010_ 0.020 mg/L) and (0.010_ 0.030 mg/L) respectively. Also our results of lead level in the water samples more than results recommended by **Tabari *et al*. (2010)** who studied heavy metals (Pb and Cd) in fish, water and sediments sampled which showed 53.67 ppb for Pb. Our obtained results of lead levels in the examined water samples were above the permissible limit of WHO which is 0.01 ppm or united states environmental protection agency (US EPA) which is 0.05 ppm. Also our Water samples showed cadmium values more than the permissible limit of WHO which (0.003 ppm). The examined water samples showed values of mercury more than the permissible limits adopted by US EPA which is 2 ppb.

Estimated levels of lead in liver and muscle of examined fish were 0.57 and 0.149 ppm respectively. Analytical findings of lead levels in liver, and muscles revealed increase in lead concentration than many literatures studied by Bashir et. al (2012), who showed the lead concentrations in liver ranged from 1.1 to 1.5 µg/g while in muscles ranged from 0.1 to 0.2 µg/g .and **Copat *et al*. (2012)**, reported that lead concentrations in fish muscle tissue taken from Mediterranean Sea were, 0.32 µg/g. Also similar results obtained by **Ebrahimpour *et al*. (2011)** who stated that levels of lead fresh water fish species, were 5.4 µg/g in liver and 0.9 µg/g in muscles. Our obtained results of lead levels in liver and muscle of examined fish in harmony with **Tapia *et al*. (2012)**, Who estimated levels of lead in liver and muscle, the concentration in muscle tissue were generally lower than those found in the liver. Our obtained results of lead levels in the examined muscle samples were above the permissible limit of WHO which is 0.2 µg/g.

In the present study, the concentrations of cadmium in liver and muscle of examined fish were 0.0068 and 0.0035 ppm respectively. Analytical findings of cadmium levels in liver, and muscles revealed increase in cadmium concentrations than results obtained by Bashir, *et al*. (2012) concentrations in liver ranged from 1.01 to 0.694 µg/g while in muscles ranged from 0.048 to 0.088 µg/g . And **Copat *et al*. (2012)**, reported that cadmium concentrations in fish muscle tissue were 0.366 µg/g. Also **Malhat (2011)** showed that concentrations of cadmium in muscle of fish samples collected from El Menofiya Governorate, Egypt were 1.840 µg/g. Our obtained results revealed that cadmium concentrations in fish liver tissue were above than those found in muscle tissue . Cadmium concentration in liver is higher than in muscle. On the contrary, **Cyrille, *et al*. (2012)**, determined the concentrations of cadmium in gills, liver and muscle of tilapia fish,the cadmium concentrations in muscle tissue (1.19 - 5.18 µg/g) were above than those found in liver tissue (0.12 - 1.25 µg/g) . Our obtained results of cadmium levels in the examined muscle samples were above the permissible limit of WHO which is 0.2 µg/g.

Elghobashy *et al*. (2001) recorded higher concentrations of Cd and Pb in fish muscle and liver of Lake Borollus, whereas **El-Moselhy (1999)** recorded lower metal concentrations in fish organs from Lake Manzala than those in

the present study. **Saeed and Shaker (2008)** reported that Water and fish organs from Lake Manzala showed greater concentrations Cd and Pb recorded levels above the international permissible limits in water.

Analytical findings of mercury levels in liver, and muscles revealed 2.070 and 0.3960 ppb respectively. Our results consider more than results obtained by **Copat *et al*. (2012)**, reported that mercury concentrations in fish muscle tissue taken Mediterranean Sea were, 0.31µg/g. **Ramadan (2008)** studies the pollution levels and the toxicity status environment of Manzala Lake and reported that Hg showed the highest values and alarming toxicity levels and it is considered as one of the most hazardous pollutants in Lake Manzala.

The metals penetrate the epithelium of gills and develop oxidative stress in cells. The gills in the current study showed degenerative, necrotic and proliferative changes. Gills showed edema of the primary lamellae, hyperplasia, fusion and focal desquamation of the epithelial lining of the secondary lamellae were observed. According to **Mallatt (1985)**, the edema of the gill epithelium is one of the main structural changes caused by the exposure to heavy metals. Cellular proliferation in the gill epithelium is also observed in fish exposed to different pollutants as described by **Thophon *et al*. (2003)** Lifting, swelling, and hyperplasia of the gill epithelium could serve as a defense function, as these alterations increase the distance across which waterborne irritants must diffuse to reach the bloodstream. Lamellar fusion could be protective once it reduces the amount of vulnerable gill surface **area (Mallatt 1985)**. Also **Oliveira Ribeiro *et al*. (2002)** reported serious injuries in gills and olfactory epithelium. The changes found on gills were gluing and hyperplasia of secondary lamellae. This pathological change was caused by cadmium poisoning, and it is in conformity with the research findings of **Randi *et al*. (1996)**. **Mobarak and Sharaf (2011)** also reported similar histopathological changes in gill fish after lead acetate exposure.

Heavy metals accumulated in the kidney, liver and, gills induce oxidative stress in fish **(Oakes *et al*., 2003 and Farombi, 2007)**. The kidney is one of the first organs to be affected by contaminants in the water **Thophon *et al*. (2003)**. **Shore & Douben (1994)** and **Tanimoto *et al*. (1999)** remarked that the kidney is a critical organ affected by cadmium poisoning since the pathological changes are primarily observed on this organ.

In the present study kidneys showed marked glomerular, tubular and interstitial pathological alternation included necrosis and degeneration of the proximal tubular epithelium together with presence of of haemorrhage between renal tubules and edema in Bowman's capsules. Also, **Velmurugan *et al*. (2007)** observed necrosis of tubular epithelium, hypertrophied epithelial cells of renal tubules, narrowing of the tubular lumen, expansion of space inside the Bowman's capsules and contraction of the glomerulus in the kidney, while **Camago and Martinez (2007)** found cloudy swelling degeneration in the epithelium of renal tubules in the kidney .Tubular vacuolization, necrosis and dilation found in the present studies due to heavy metals were reported also by other workers **(Colle *et al*., 1980 and Karmakar *et al*., 1986)**. pathological proliferation of mesangial cells is followed by resolution involving mesangial cell apoptosis. Similar alterations were observed in kidney of Tilapia in several species of fish exposed to heavy metals and these alterations were described by **Oliveira Ribeiro *et al*. (2002) Jiraungkoorskul *et al*. (2003), Thophon *et al*. (2003)** and **Gupta and Srivastava (2006)**. **Geeth *et al*. (2005)** reported that Cadmium is a potent nephrotoxin that has been shown to induce apoptosis in some cells.

Semithin sections showed intranuclear inclusion bodies like, apoptotic bodies, miotitic division in the tubular cells .Epithelial cells of kidney tubules demonstrated vacuolar degenerative changes and apical sections of the tubular epithelial cells contain dark granules . Our result are in agreement with **Beširović1 *et al*.**

(2010) who reported that cadmium poisoning caused greater degenerative changes in the epithelial cells of the kidney tubules, the appearance of small dark granules in their cytoplasm. In several clinical situations and experimental models of injury to the renal glomerulus, pathological proliferation of mesangial cells was followed by resolution involving mesangial cell apoptosis. Similar alterations in kidneys were observed and these alterations were described by **Thophon *et al.* (2003)**. Accumulation of large quantities of lipid droplets has been observed in *M. dobsoni* exposed to mercury. Similar observations have been made by **Lowe (1988)** and **Moore (1988)**. **Goyer *et al*., (1970)** predicted that lead would have a profound effect in the nucleus because of the ability of metals to alter nucleic acid conformations. Further, lead is the most active metal in the formation of intranuclear inclusion bodies which is the consistent feature in lead-intoxicated animals. **Goyer (1971)** has proposed that the intranuclear inclusion bodies function to bind lead and to minimize its effects. Several observations and the present support this proposal, the appearance of the inclusion bodies, following lead exposure appears to depend on protein synthesis.

In present electron microscopic investigation showed hyperplasia and proliferation of smooth endoplasmic reticulum, mitochondrial proliferation was noticed and rough endoplasmic reticulum lost their characteristic arrangement. Lysosomes were numerous contained dense bodies of varying size, Ovoid bodies composed of dense osmiophilic material. A general negative effect exerted by metals on the membranes of both the rough and smooth endoplasmic reticulum is due to lipid peroxidation **(Buss and Gibson, 1979)**. According to **Cheville (1994)** extensive degeneration of the endoplasmic reticulum and detachment of ribosome is one sigen of cell injury causing stop functioning and leading to cell lyses . Extensive degeneration of the endoplasmic reticulum, as noticed in the present study would lead to malfunctioning of this organelle. According to **Viarengo (1985)** mercury, cadmium and copper are able to reduce the rate of protein synthesis by influencing the attachment of polyribosomes to the rough endoplasmic reticulum and probably damaging the ribosomes themselves . Detoxification and lipid synthesis are among the special functions of the smooth endoplasmic reticulum **(De Robertis and De Robertis, 1980)**.

George (1982) reported that metals accumulate in lysosomes by a combination of autophagy and generation of acidic groups within aging secondary and tertiary lysosomes. The formation of pathologically enlarged lysosomes was associated with membrane destabilization or increased permeability resulting in the release of degradative hydrolytic enzymes into the cytosol and also in lysosomal fusion with other intracellular vacuoles. The incidence of secondary and tertiary lysosomes as a conspicuous feature in the tubular cells suggests xenobiotic induced cellular pathology disturbing both structure and function **(Moore, 1985)**.

According to **Viarengo *et al.* (1985)**, metals like mercury, copper and cadmium initially decrease the RNA synthesis. **Bubel (1976)** reported that cadmium had particularly adverse effects on the mitochondrial membrane . **George (1982)** reported active accumulation of metal cations by mitochondria, the organelles responsible for aerobic ATP production. **Burce *et al*., (1980)** showed mitochondrial swelling and the presence of increased numbers of lysosomes within renal proximal tubule cells. Nuclear alterations were the early histological alterations developed due to long-term intoxication. This confirms that the nucleus is the key factor in most cell disturbances to injurious agents **(King *et al*., 1983)** .

The liver showed degeneration of the hepatocytes, congestion of central vein and nuclear pyknosis in the majority of hepatic cells. These findings were apparent as the liver considered the organ of detoxification, excretion and binding proteins. The metal-binding proteins were present in the nuclei of hepatocytes suggested that the increase in the cell damages **(De Smet**

***et al*., 2001)**. Similar results were observed by **Van Dyk (2003) and Mela *et al*. (2007**). Pathological findings in liver included cytoplasmic vacuolation with partial nuclei hypertrophy in hepatocytes. It has previously been shown that anomalies such as irregular shaped hepatocytes and cytoplasmic vacuolation were also founded after exposure to heavy metals **(Fanta *et al*., 2003)**. The presence of hemosiderin a product of hemoglobin degradation (Khan *et al*., 1994) as a results of in internal bleeding in the liver tissues.

Hepatopancreas has been identified as a target organ for heavy metal pollution because it shows critical histopathological and ultrastructural alterations at very early stages of exposure to toxicants **(Johnston *et al*. 1998)**. According to **Sousa1 and Petriella (2007)** At the ultrastructural, R and F cells were the most damaged cells but the present finding revealed all type of cells were showed structural damage . In the present study, mitochondria are sensitive to many kinds of stressors and react very quickly with general pathological symptoms such as swelling, shrinkage, disruption of cristae. Injury to mitochondria may produce degenerative changes consisting of fragmentation and intense swelling were reported by **(De Robertis and De Robertis 1980).** Extensive disruption and disintegration of the endoplasmic reticulum observed may indicate serious alterations associated with heavy metal stress. The extent of damage seemed to be directly proportional to the concentration of toxicant **(Manisseril, and Menon 1995)**. The rough endoplasmic reticulum appeared vesiculated and dilated. Similar suggestions have been derived in heavy metal toxicity **(Triebskorn and Kohler,1996)**. RER proliferation and fenestration **(Braunbeck *et al*., 1989, 1990; Lester *et al*., 1993)** Similar findings were reported by **(Au *et al*., 1999)**. Furthermore, SER proliferation is generally regarded to be indicative of the induction of biotransformation processes **(Braunbeck *et al*., 1989, 1990)**. The route of detoxification leads to the production of metal-rich deposits or granules by possessing membranous whorls with myelin figures and residual bodies in the cell cytoplasm of the hepatopancreas . This indicates the fact that lysosomal breakdown of metallothionein was possibly operative. Presence of dilated membranous structure and myelin bodies has also been reported **(Kutlu *et al*., 2005)**. Accumulation of large quantities of lipid droplets has been observed. Similar observations have been made by **Lowe (1988), Moore (1988) and Sousa1 and Petriella (2007)**. A large increase in the number of lipid droplets in the cell cytoplasm reflected the decline of protein synthesis that accompanies hepatocyte injury which blocks the utilization of lipid-protein conjugation **(Cheville, 1994).** Similar finding were reported by Bi**agianti-Risbourg *et al*. (1996), Braunbeck (1998), Strmac and Braunbeck (2002). Sujatha *et al*. (2011)** reported accumulation of the metal within the residual bodies. In the present investigation the residual bodies takes several location with different sizes inside the cytoplasm.

The nuclear damage observed included damage of the nuclear membrane, and overall shrinkage resulting in the nuclei losing their characteristic shape **(Viarengo 1985**). Remarked that these changes would profoundly influence the normal functioning of the nuclei and metals can interact with nuclear proteins, altering the complex structure of chromatin or the catalytic activity of the enzymes involved in DNA and RNA metabolism .

Conclusion

It is concluded from this study that heavy metals in water and fish in Manzala lake exceed the permissible limit of WHO and international standards. Also, Environmental contamination of Lake Manzla induced several histopathological,ulrastructure alterations in the tissues of fish. Therefore, in view of these results, regular monitoring of levels is necessary to assess the impact of heavy metals in this aquatic system. Also sufficient effort should be carried out to achieve further reductions in heavy metal emissions to the aquatic system in order to minimize the risk of adverse health effects.

Consequently, it is recommended to coordinate different efforts to rescue Manzala Lake from the environmental pollution problems.

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التقييم بالميكروسكوب الضوئى والالكترونى على تاثير المياة الملوثة ببعض المعادن الثقيلة على اسماك البلطى ببحيرة المنزلة, مصر.

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*****قسم الباثولوجي – الاسكندرية.**

الملخص العربى

استهدف البحث تقييم المعادن الثقيلة فى انسجة اسماك البلطى ببحيرة المنزلة ومدى الارتباط بين تركيز هذ ه المعادن فى الانسجة والتغيرات البا ثولوجية. وقد أوضحت الدراسة النتائج التالية:سجلت مياه بحيرة المنزلة أعلى مستوى لهذه المعادن. ولقد فاقت تركيزات الزئبق والكادميوم والرصاص فى مياه بحيرة المنزلة الحد الأقصى المسموح به دوليا. وقد أعزى ذلك للكميات الكبيرة من المخلفات الصناعية والزراعية والصرف الصحى التى تصل إلى البحيرة عن طريق المصارف. أظهرت الدراسة أيضا تراكم العناصر الثقيلة الزئبق -الرصاص و الكادميوم فى أنسجة الأسماك المجمعة من بحيرة المنزله. كما أوضحت الدراسة أن أنسجة الكبد لها قابلية كبيرة لإختزان العناصربالمقارنة بالعضلات والتى تمثل خطورة على المستهلك وفاق تركيز العناصرفى عضلات الأسماك المجمعة من بحيرة المنزلة الحد الأقصى المسموح به عالمياً .. واظهرالفحص الهستوباثولوجي للخياشيم ، احتقان وارتشاح فى الخلايا الطلائية التي تغطي كلا من صفاحات الابتدائية والثانوية وانزفة . تمدد واحتقان في الأوعية الدموية من شعيرات الخيشومية. وجود تجمعات من الخلايا الالتهابية مع وزيادة في الخلايا المخاطية وظهور الفجوات من صفاحات الثانوية. وقد اظهرالفحص الهستوباثولوجي للكلى تغيرات في الكبيبيات والانابيب البولية والنسيج البيني. واظهر فحص قطاعات السميثن ان المعادن الثقيلة أظهرت عدد كبير من الحبيبات الموجبة للصبغة فى الجزء الاعلي للخلايا. وقد اظهرالفحص بالميكروسكوب الالكتروني جليا حدوث تغيرات واضحة فى خلايا الكبيبات و الأنابيب تتمثل فى انحلال الميتوكوندريا في جميع الخلايا اما االشبكة الاندوبلازمية فقد فقدت تركيبها كليا مع وجود العديد من اليسوزومات في بعض الخلايا متفاوتة الحجم ، وزيادة عدد المواد الليسوزومية المتبقية مع وجود جسيمات متبقية كبيرة. . وأظهرت النواة تكتل واضح للكروماتين فكانت تحتوى على جسيمات داخل النواة . اوضح الكبد احتوائة على فجوات دهنية منتشرة وبؤرتنكرزية تشتمل على تغيرات فى النواة من تغلظ وتفكك و انحلال خلوي ، وكانت التغييرات الأكثر تميزا فى الكبد ارتشاح للخلايا الالتهابية وحيده النواه . كما لوحظ ان فحص البنكرياس الكبدي أظهرحدوث تغيرات في جميع الخلايا و هذه التغييرات تتألف من انتشار واسع للشبكة الاندوبلازمية الملساء، اما الشبكة الإندوبلازمية الخشنة فقد وجدت متمددة ومجزأة، الميتوكوندريا فقدت الشكل الطبيعي، مع زيادة في أعداد الليسوزوم ، أعداد متزايدة من الليسوزوم الثانوية. اما التغييرات النووية فشملت تكتلات كروماتينية ، وعدم انتظام الغشاء النووي مع وجود تخللات نووية .