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Eco-Toxicological Studies of Water and Their Effect on Fish In El Manzalah Lake.

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Abstract

This study was designed to express the impact of physico-chemical parameters including water temperature, pH, dissolved oxygen, ammonia, nitrite, nitrate and heavy metals (Fe, Mn, Zn, Cu and Cd) in El Manzalah Lake water on histological properties of *Clarias gariepinus* and *Mugil capito* living in this water as well as accumulation of heavy metals in these fish muscles. Water and fish samples were collected from the investigated areas during Spring season (2015). The results revealed changes in water quality and heavy metals as well as occurrence of histopathological alterations in selected organs (liver, kidney and spleen). So that it is necessary to treat the drainage water before discharging to the lake to protect fish and human from danger of pollution.

Keywords: El Manzalah Lake , *Mugil capito* , *Clarias gariepinus* , Heavy metals, Hematology , Histopathology .

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INTRODUCTION

El Manzalah Lake is considered one of the most important sources of inland fishery in Egypt. It is the largest lake of the four brackish coastal lakes fringing the Nile Delta. It receives annually about 4000 million cubic meters of untreated industrial, domestic and agricultural drainage water, discharged annually into the lake through several drains; Bahr El-Bakar Drains (sewage and industrial), Hadous, Ramsis, El-Serw and Faraskour Drains (agricultural effluents) [1]. While, the physico-chemical parameters are the most important principles in the identification of quality of the water for any aquatic ecosystem [2]. Consequently, fish can be considered as one of the most significant biomonitors in aquatic system for the estimation of metal pollution concentration [3]**.** Meanwhile, histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms [4]**.**

Therefore, the aim of this study is to investigate the impact of physico-chemical properties of water and heavy metals (Fe, Mn, Zn, Cu and Cd) as well as accumulation of this metal in fish muscle and histological structure of liver, kidney and spleen of *Clarias gariepinus and Mugil capito* fish inhabiting the water of El Manzalah Lake.

MATERIALS AND METHODS

Area of study

El Manzalah Lake lies between 310 45\, 320 15\ E and 310 00\, 310 35\ N.). Water and fish samples were collected from the lake at discharge point of Bahr El- Bakar (domestic and industrial wastes **I)** and El-Serw drains **(**agricultural wastes **II) (Fig.1).**

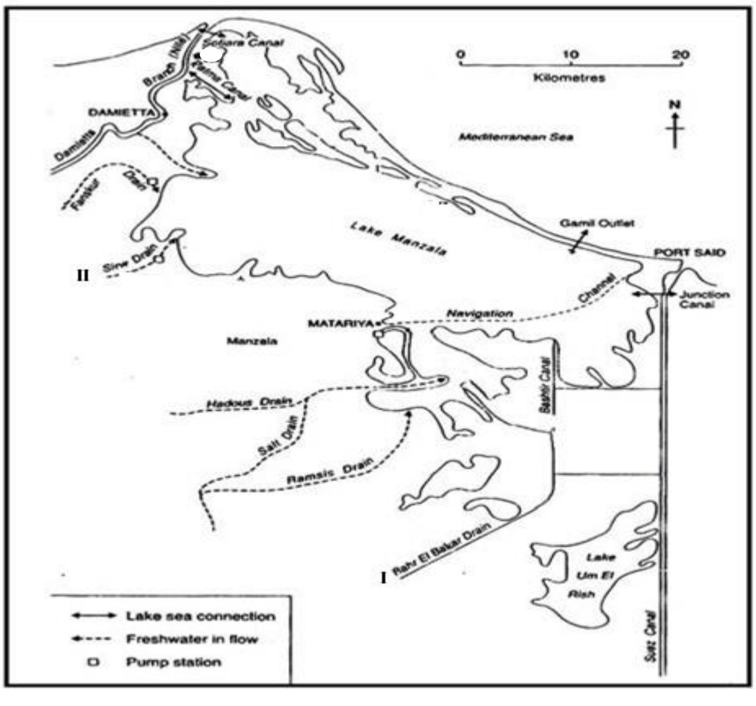

Fig.(1) : Location of sampling sites in El Manzalah Lake
(I) :Bahr El-Bakar drain (domestic and industrial wastes)
(II) :El- Serw drain (agricultural wastes)

Field observations

The water samples from El Manzalah lake were collected during spring 2015 to measure water temperature (ºC) by a dry mercury thermometer, and pH by pH meter model (Orion Research Ion Analyzer 399).

Laboratory analysis

Water samples

Another water sample was kept in one liter polyethylene bottle in ice box for analysis in the laboratory. The dissolved oxygen(DO) content analysis, concentration of ammonia(NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) were determined using the colorimetric techniques according to the method described by [5].

Measurement of heavy metals in water

Analysis of the heavy metals (Fe, Mn, Zn, Cu and Cd) in water was determined according to [6]. The results were expressed in µg / l.

Fish samples:

Accumulation of trace elements in fish muscles:

The collected muscle samples of *C. gariepinus* and *M. capito* fish were transferred to beakers and placed in a drying oven thermostatically regulated at 105 °C overnight. A sample of 1g/dry weight of each muscle sample was taken from fish specimens. These samples were digested according to the method described by [7]. The concentrations of iron, zinc, manganese, copper and cadmium in the muscle of the selected fish were measured by Atomic Absorption (I.C.P. plasma 400). Results were expressed in µg /g dry weight of the tissue.

Histopathological studies:

Liver, kidney and spleen of *C. gariepinus* and *M. capito* fish living in the water of El-Manzalah Lake were removed, fixed in 10% neutral formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in pure paraffin wax and cut in 4-6µm thick sections by microtome. Sections were stained with Harris Hematoxylin and Eosin method cited by [8]. Consequently, these sections were examined microscopically, photographed by using a microscopic camera.

RESULTS AND DISCUSSION

Water analysis:

The parameters of water analysis including water temperature, pH , dissolved oxygen, ammonia, nitrite, nitrate are shown in table **(1)** and heavy metals (Fe, Mn, Zn, Cu, Cd) in table **(2)** as described by [9].

Temperature (ºC):

Water temperature is an important parameter in the aquatic environment as it effects on growth rate, development, activity, reproduction processes and susceptibility to diseases [10, 11]. About 35 °C is considered as the maximum tolerance for most survival of aquatic life [12]. In the present study, water temperature was (28.2 ºC) at Bahr El Bakar drain station **(I)** while, it was (26.1 ºC) at El-Serw drain station **(II).** The changes of water temperature may depend on the variations in air temperature, latent heat of evaporation and different sampling times and seasons [13,14].

Hydrogen ions concentrations (pH):

Water pH plays an important role in biodiversity of fish fauna in the aquatic environment. The optimum pH value of fish production ranges from 6.5-9.0 while pH 4.0 is the acidic death point, at pH 4.0-5.0, no reproduction occurs, at pH 4-6.5 slow growth and pH 11 is the alkaline death point of water [15]. The pH value (7.5) was recorded at station **(II)** while the value (7.2) was observed at station **(I).** The increase in pH values at site **(II)** may be attributed to the dense of vegetation and phytoplankton, which were accompanied by photosynthetic activity and consumption of CO_2 [16,17]. Meanwhile, the discharge of effluents loaded with large amount of organic acids cause decreasing in pH value [18,19].

Dissolved oxygen (DO):

Dissolved oxygen is a very important factor to the aquatic organisms which decompose organic materials essential for respiration and enables completion of biochemical process [20,21]. Also, the susceptibility of fish to toxicity by chemicals increases at low oxygen concentration [22].

In the present study, there was depletion in oxygen at site I while the value it at site II was1.6 mg/l. The decrease in DO might be due to the elevation of water temperature and the increase in oxidative processes of organic matter [23]. Moreover, the depletion of DO may be attributed to exhaustion of dissolved oxygen for oxidation and decomposition of huge organic matter and domestic wastes discharged into the selected station [24,25].

Ammonia ($NH4^+$):

Ammonia and nitrogen concentrations more than 1 mg/l have been given as indicator of organic pollution and can be toxic to aquatic species if they are higher than 2.5 mg/l [26,27].

The values of ammonia in present study ranged from 3.9 mg/l at station (**II**) to 10.4 mg/l at station (**I**) . It has been observed that ammonia increased with increasing in temperature (28.2ºC), and depletion in oxygen(0.0mg/l) at station **I**, as well as to discharging of sewage wastes and their decomposition into organic matter [28].

Nitrite ($NO2^-$):

Nitrite is the intermediate oxidation state of the nitrogen. So, nitrite content in water may be an important indicator to the state of equilibrium between oxidation and reduction processes, making up the nitrogen cycle [29,27]. The values of nitrite ranged between 43.1 µg/l at station (**I**) to 145.9 µg/l at station (**II**). The low values of nitrite may be due to biological reduction of nitrite during the hot seasons into cellular amino, organic and fatty acids necessary for the growth and reproduction by the photosynthetic activity of plankton [30] as well as fast conversion of NO_2^- by nitrobacteria to NO_3^- [31]. On the other hand, the high nitrite level may be attributed to decomposition of organic matter present in the waste water where nitrosomonas bacteria oxidize ammonia to nitrite by denitrification process [28].

Nitrate ($NO3^-$):

Nitrate is the most stable form of nitrogen in aquatic environment in the absence of oxygen[32]. It also represents the highest oxidized form of nitrogen and used as essential nutrient for phytoplankton growth [33]. The values of nitrate fluctuated from 154.1 to 166.4 µg/l at station **(I) & (II)** respectively. The low values of nitrate concentration might be attributed to the uptake of nitrate by natural phytoplankton [34,35]. The increase of nitrate levels might be attributed to sewage wastes at two studied drains and low consumption of phytoplankton as well as the oxidation of ammonia by nitrosomonas bacteria and biological nitrification [36,14]. On the other hand, the increase of nitrate value at station **(II)** may be attributed to the extensive use of nitrate fertilizer in agricultural lands [37].

Table (1): Physico-Chemical parameters at El-Manzalah Lake during Spring, 2015.

Stations / Parameter	I	II	Permissible limits WHO,(1993)
Water temperature(ºC).	28.2	26.1	(25-35)
pH	7.23	7.59	-
DO (mg/l)	Not detected	1.68	6-14
NH_4^+ (mg/l)	3.903	10.40	≤ 1
NO_2^- (µg/l)	145.9	43.1	100
NO_3^- (µg/l)	166.39	154.16	25- 50

I : Bahr El-Bakar drain
II: El-Serw drain

Heavy metals:

Heavy metal salts in solution constitute a very serious form of pollution [38]. The major sources of these elements in water are composition of drilling mud and fluids, industrial effluents as well as discharging of municipal and domestic wastes into water [39]. Consequently, the determination of these metals is a primary target in environmental researches[40]. The elevation of metals concentrations in this study was confirmed by [41] who recorded highly significant concentrations of Cu, Cd, Pb and Zn in water samples from different El Manzalah Lake sites.

Iron (Fe):

Iron is the most abundant important trace metal for the biological life. The distribution of iron in water system depends on dissolved oxygen and is usually present as insoluble $Fe(OH)_3$ as high concentration of oxygen leads to iron oxidation and subsequent hydrolysis to form insoluble $Fe(OH)_3$ [42].

There was observed increase in iron concentration at station **(I)** with the value of 1750 µg/l. On the other hand, the concentration at station **(II)** was 292.6 µg/l. The elevation of iron concentration may be attributed to the release of iron from the sediment during the dissolution of iron [18]. Also, the increase of it may be due to the breakdown of organic matter and dead microorganisms that release the metal into the water [43]. While, the low value may be attributed to the increase in dissolved oxygen which leads to oxidation of iron. This finding is in agreement with the results obtained by [44].

Manganese (Mn):

Manganese is considered to be an essential element in the biochemical cycle of ecosystem and usually present in well water as $Mn(HCO_3)_2$, $MnCl_2$ or $MnSO_4$ [45].

The results of manganese values varied from 44 µg/l at station **(II)** to150 µg/l at station **(I).** The major sources for manganese in air and water are iron, steel manufacturing and the burning of diesel fuel in the motor cars as cited by [46]. So, the engine boats which are distributed in El Manzalah Lake could be a reason for increasing the Mn in water. Also, the high concentration of manganese may be due to the breakdown of organic matter and dead micro-organisms with subsequent release of the metal into water [47]. While, the low concentration of manganese may be related to uptake of manganese by phytoplankton [48].

Zinc (Zn):

Zinc is an essential element for normal growth and reproduction of animals as well as for aquatic organisms [49]. On the other hand, it is a common pollutant found in several industrial effluents such as those produced by textile, mining, smelting, electroplating and motor [50].

The values of zinc concentration ranged between 29.4 µg/l at station **(II)** and 67 µg/l at station **(I)**.The high concentration of zinc may be attributed to domestic, sewage and agricultural wastes discharged into the lake and adsorption of zinc hydroxide which binds to organic matter[51]. Also, the engine boats which are found in El Manzalah Lake may be a reason for increasing the Zn concentration in the Lake water [46]. On the other hand, the low value of Zn may be related to the uptake of Zn by phyto and zooplankton [52]. Also, the decrease in zinc concentration is similar to iron in behavior; this may support the explanation attributed to the decrease of zinc concentration to its adsorption on $Fe(OH)_3$ sedimentation[39].

Copper (Cu):

Copper is an essential trace metal in small concentrations for several metabolic function in fish[53], but can exert adverse toxicological effects, when present in high concentrations in water. Also, it is widely used as a very effective algaecide and molluscicide [54,55].

The concentration of Copper ranged between 7.4 µg/l and 18 µg/l at stations **II** & **I**, respectively. The high concentration of copper at **(I)** may be due to sewage discharges where the domestic sources were the major contributors of copper in the environment [48]. On the other hand, the low concentration at **(II)** may be attributed to the uptake by phytoplankton[56] or may be due to the formation of CuO_2 that rapidly changed to amorphous Cu $(OH)_2$ and precipitated at the bottom which was supported by [57].

Cadmium (Cd):

Cadmium is an important toxic heavy metal pollutant. It may enter water as a result of industrial discharge or due to the deterioration of galvanized pipe[6] as well as known as renal toxicant.

The values of cadmium fluctuated from 2.0 µg/l to 15 µg/l at stations **II& I**, respectively. The high concentration of cadmium in present study may be due to the direct input of sewage and agriculture wastes [58]**.** While, the decrease of cadmium during spring may be attributed to the increase in water temperature which facilitate the precipitation of cadmium as carbonate into the bottom sediment as cited by [59]. Also, phytoplanktons play a more significant role in scavenging of cadmium than they play in the removal of other metals [43]**.**

Table (2): Heavy metals of water from El-Manzalah Lake during Spring 2015.

Stations / Metal	I	II	± SE	Permissible limits WHO, (1993)
Fe µg/l	1750	292.6	± 1030.5	≤1000 µg/l
Mn µg/l	150.0	44.0	± 74.953	5-50 µg/l
Zn µg/l	67.0	29.4	± 26.587	100 µg/l
Cu µg/l	18.0	7.4	± 7.495	100 µg/l
Cd µg/l	15.0	2.0	± 9.192	2 µg/l

I : Bahr El-Bakar drain
II: El-Serw drain
± SE: standard error

Accumulation of heavy metals:

Over few decades, there was growing interest to determine heavy metal levels in the aquatic environment and attention was drawn to find out concentration level of public food supplies particularly fish. Many investigations stated that there are direct relationships between heavy metals level in fish flesh and metals pollution level in aquatic environment as [60,61,31]**.** The concentrations of studied metals were shown in table (3).

Iron:

Iron is an important element in the earth crust as it serves more biological role than any other trace metal [6]. Its toxicity causes hemorrhagic gastroenteritis, diarrhea, liver necrosis and death due to hepatic coma [62]**.**

The values of Fe recorded at the muscle of *C. gariepinus* and *M. capito* were 108.90 - 127.10µg/g and 49.40 - 96.55 µg/g at site **(II)** and site **(I)** respectively**.** The iron accumulation in the muscle of both fish was higher than the permissible limit. This increase may be attributed to the increase of free metal iron concentration at studied areas and accordingly increased iron uptake by fish, as explained by [47]. In addition, these values were higher in fish collected from site **(I)** than that collected from site **(II);** this may be due to large amounts of sewage discharged from Bahr El-Bakar drain. On the other hand, the low values were recorded at site **(II)** for both fish; this may be due to decrease of iron concentration in the water which exhausted by phytoplankton as stated by [47,31] as well as precipitated into the sediment**.**

Manganese (Mn):

Manganese is an essential constituent for bone structure and function of enzymes. It is considered as micronutrient at low level as well as toxic at high level. Meanwhile, agrochemicals such as pesticide and herbicides release manganese and contribute to its accumulation in fish [63].

The present study showed that high values of Mn in *C. gariepinus* and *M. capito* were 63.10 µg/g and 55.10 µg/g at site **(I)** respectively while, the low values were recorded at site **(II)** as 60.20 µg/g for *C. gariepinus* and 40.20 for *M. capito*. These results are in agreement with [14,31]**.** Also, decreased oxygen concentration in water may lead to increase uptake of water with metal through gills[64].

Zinc

Is an essential element while, mining, smelting and sewage disposal are the major sources of zinc pollution while gills is the main entrance of zinc to the fish [65].

The values of zinc accumulation in the muscle of *C. gariepinus* were 59.80µg/g at site **(II)** and 60.30µg/g at site **(I).** While, the values in *M. capito* were 38.70 µg/g and 52.50 µg/g at site **(II)** and **(I)** respectively. The increase of zinc value may be due to high amount of sewage wastes discharged into water body as cited by [61,66]. Also, the increase of zinc in hot seasons may be attributed to the increase in metabolic rates, which result in increased heavy metals uptake as indicated by[67].

Copper

Is an essential trace metal in small concentrations for several fish metabolic functions (e.g. cellular respiration, neurotransmitter function and connective tissue biosyntheses [9].

Copper accumulation in the muscle of *C. gariepinus* in the present study were fluctuated from 7.1µg/g to 8.2µg/g at site **(II)** and **(I)** during spring respectively. On the other hand, the values in *M. capito* were 4.7 µg/g at site **(I)** and 4.6 µg/g at site **(II).** The values still in the permissible limit as recorded by National Health and Medical Research Council in Australia as standard concentration for human consumption (30 µg /g) as cited by [39]. The low accumulation value at fish muscle may be attributed to the decrease of cupper concentration in water as a result of uptake by phytoplankton[56].

Cadmium

Cadmium is a highly toxic non-essential heavy metal and it does not have a role in biological processes in living organisms. So, even in low concentrations, Cd could be harmful to living organisms [68].

The present study showed that cadmium accumulation in *C. gariepinus* fish muscle fluctuated between 1.5 µg/g and 2 µg/g at site**(II)** and **(I)** respectively while the values in *M. capito* were 0.90 µg/g at site **(I)** and 1.10 µg/g at site **(II).**

The low concentration of cadmium may be due to low tendency of fish to bioaccumulate or good ability to excrete Cd from the body while, the high levels of cadmium may be attributed to industrial and mining operations as well as the phosphate fertilizer which is considered as the main source of cadmium in the environment [69]**.** Thus, fish consumers may be vulnerable to toxicity of the metals [70]**.** The results showed that metals were more concentrated in *C. gariepinus* tissues than those of *M. capito*.

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Table (3): Heavy metals of *C. gariepinus* and *M. capito* from El Manzalah Lake during Spring 2015 & Recommended permissible limits of heavy metals in fish tissues (µg/g dry wt.) for human consumption, according to FAO, 1992

Parameters	Fish muscles (µg/g dry wt.)						Permissible Limit (FAO, 1992)
	C. gariepinus		± SE	*M. capito*		± SE	
	I	*II*		*I*	*II*		
Fe	127.1	108.9	± 12.86	96.55	49.4	± 33.34	30.00 µg/g
Mn	63.1	60.2	± 2.05	55.1	40.2	± 10.53	30.00 µg/g
Zn	60.3	59.8	± 0.35	52.5	38.7	± 9.75	50.00 µg/g
Cu	8.2	7.1	± 0.77	4.7	4.6	± 0.07	20.00 - 30.00 µg/g
Cd	2	1.5	± 0.35	1.1	0.9	± 0.14	2 .00 µg/g

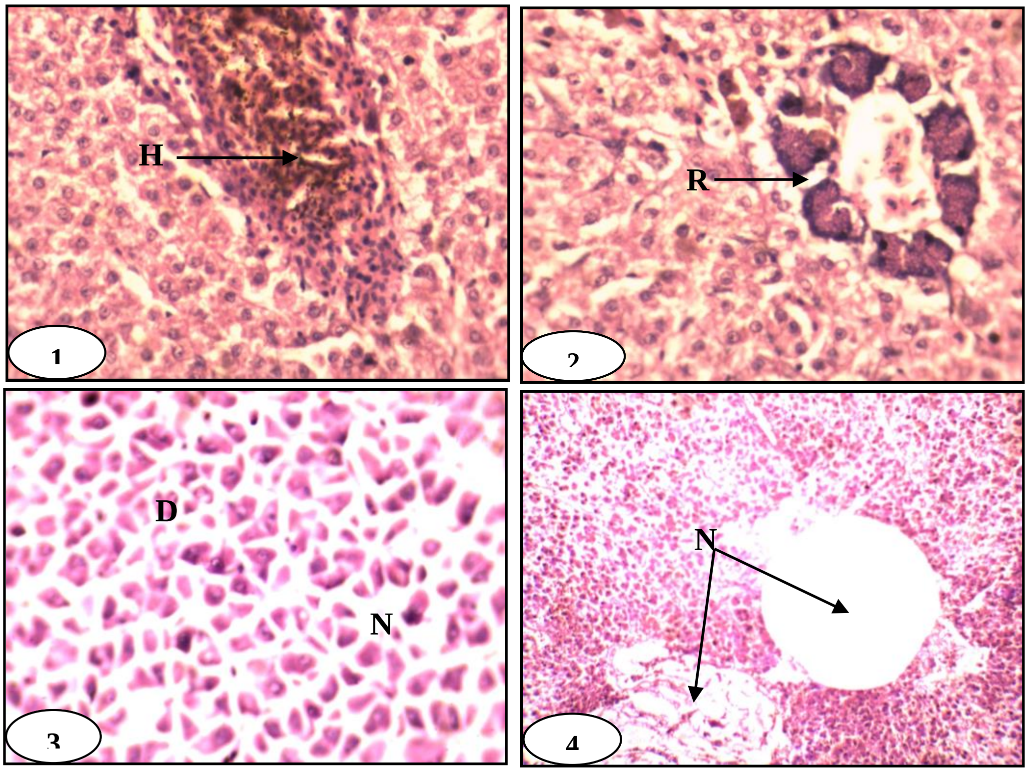
Histopathological structure :

Histopathology is an important field of fish health. Thus, histological alterations constitute excellent biomarkers of exposure to toxicants as they represent the result of biochemical and physiological changes. Therefore, histopathology has been used as a sub-lethal test for evaluating toxic effect of water pollutants on fish [71].

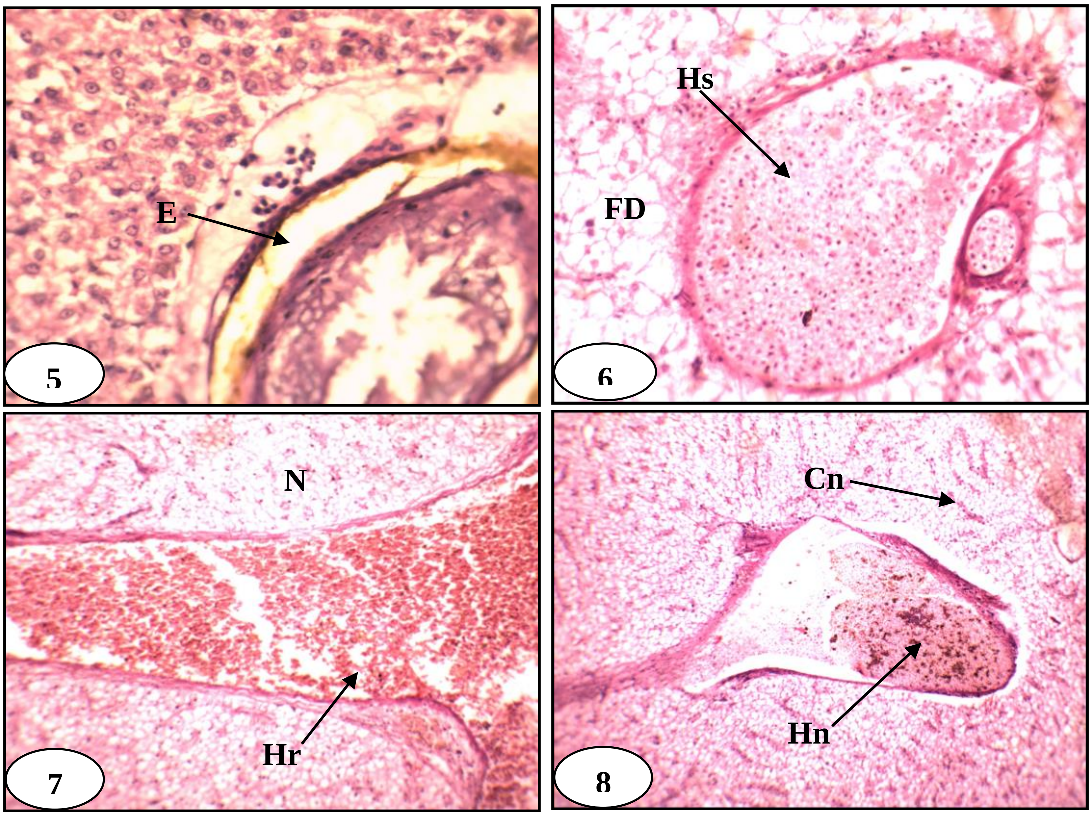
Liver:

Liver is the principle organ of detoxification in vertebrates and particularly in fish that responsible for the digestion, filtration and storage of glucose [40]. Meanwhile, fish liver is a good indicator of aquatic environmental pollution, where one of the important functions of the liver is to clean of any poisons or pollutants from the blood coming from the intestine[72]. The liver of *C. gariepinus* **(Figs. 1-4)** and *M. capito* **(Figs. 5-8)** from El Manzalah Lake showed many histopathological alterations**.** These alterations were necrosis and fatty degeneration in hepatocytes as well as the blood vessel showed hemosidrin, hemolysis, hemorrhage and edema in its wall and congestion in blood sinusoids.

The histopathological alterations in the liver of both studied fish could be a direct result of fertilizers, salts and sewage, which are entered to the lake with the drainage water as recorded by [28] who found the same histopathological changes in liver of *Mugil* species living in El Manzalah Lake. The presence of hemolysis and hemosidrin pigments may result from rapid and continuous destruction of erythrocytes by breakdown of hemoglobin and convert it into hemosidrin [38]**.** In addition, Fatty change in the liver was characterized by increasing lipid deposits and a consequent loss of sinusoidal spaces, this can be explained by a failure to mobilize lipid stores during exposure to toxins [73]. Meanwhile, necrosis of the hepatocytes may be due to cumulative effect of nutrient salts [74]**.**



Figures (1-4): Liver sections of *C. gariepinus* stained with H&E, showing: Degeneration(D), Necrosis (N) in hepatocytes. Hemosidrin (Hn) and Rupture (R) in blood vessels wall .



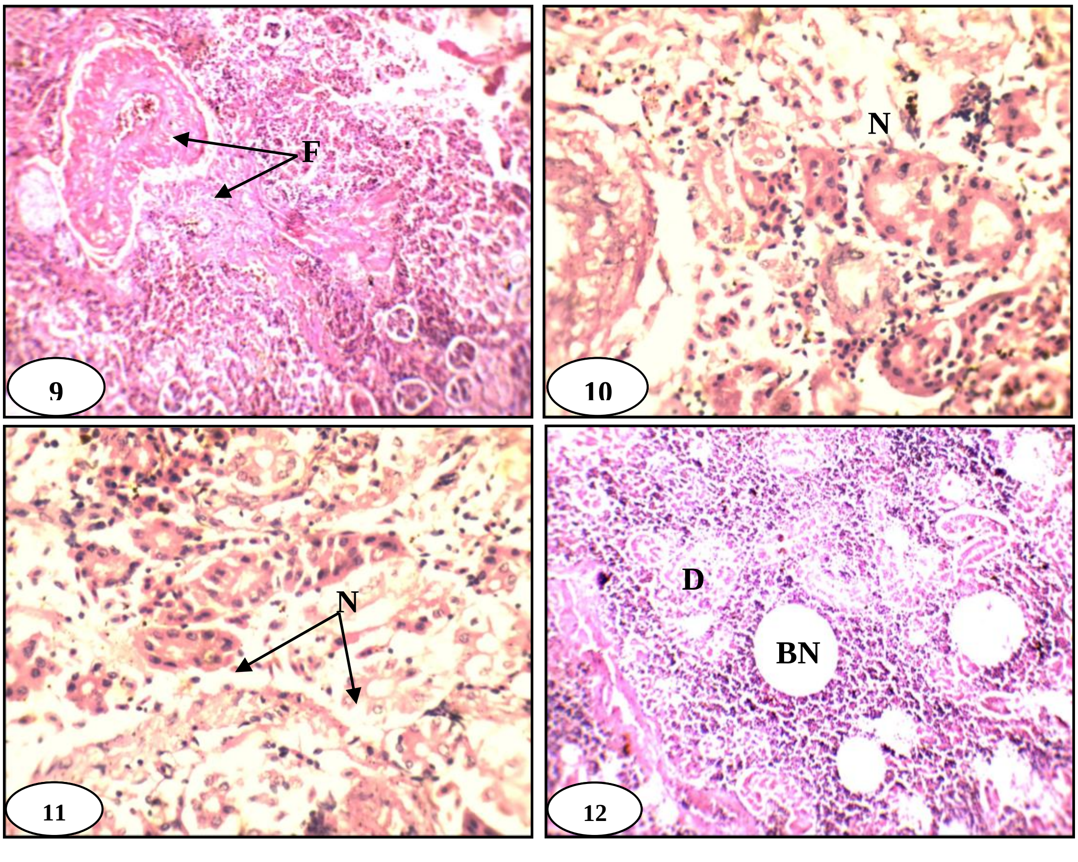
Figures (5-8): Liver sections of *M. capito* stained with H&E, showing: Necrosis (N)and Fatty degeneration (FD) in hepatocytes; Hemolysis (Hs), Hemorrhages (Hr), Hemosidren (Hn) and Edema (E) in blood vessels & Congesion (Cn) in blood sinusoids.

Kidney:

The teleostean kidney is the important organ for the maintenance of a stable environment with respect to water and salt, excretion and partially for the metabolism of xenobiotics [4] as well as for removal wastes from blood [75]. Normal kidney is composed of identical nephrons. Each nephron contains renal corpuscles that lead to renal tubules. The renal corpuscle contains vascular capillary glomerulus that is enclosed by Bowman's corpuscle. Within the nephron, the kidney contains hematopoietic tissue and blood vessels.

The most common alterations found in the kidney of *C. gariepinus* **(Figs. 9-12)** and *M. capito* fish **(Figs. 13-16)** collected from El Manzalah Lake were degeneration, necrosis, fibrosis in kidney tubules, Malpigian tubules and connective tissues. The histopathological alterations were recorded in both fish with different degrees of severity. These changes may be attributed to the fact that it is one of the principal site of detoxification in fish body as well as impaired blood supply due to toxic action of different pollutants in the lake, including heavy metals [28]**.**

On the other hand**,** [14] found that agricultural, industrial and sewage wastes caused renal injury in kidney of fish living in different parts of River Nile while, [76] found the same histopathological changes in kidney of *Mugil* species living in Lake Manzalah as a result of water quality changes. [77] found necrotic areas scattered in the hematopoietic tissue and renal tubules of the rainbow trout as a result of changes in water quality such as increase in pH, temperature.



Figures (9-12): Kidney sections of *C. gariepinus* stained with H&E, showing: Degeneration (D) & Necrosis (N) in kidney tubules and malpegian corpuscle; Balloon necrosis (BN) in hematopoietic tissue and Fibrosis (F) around blood vessel .

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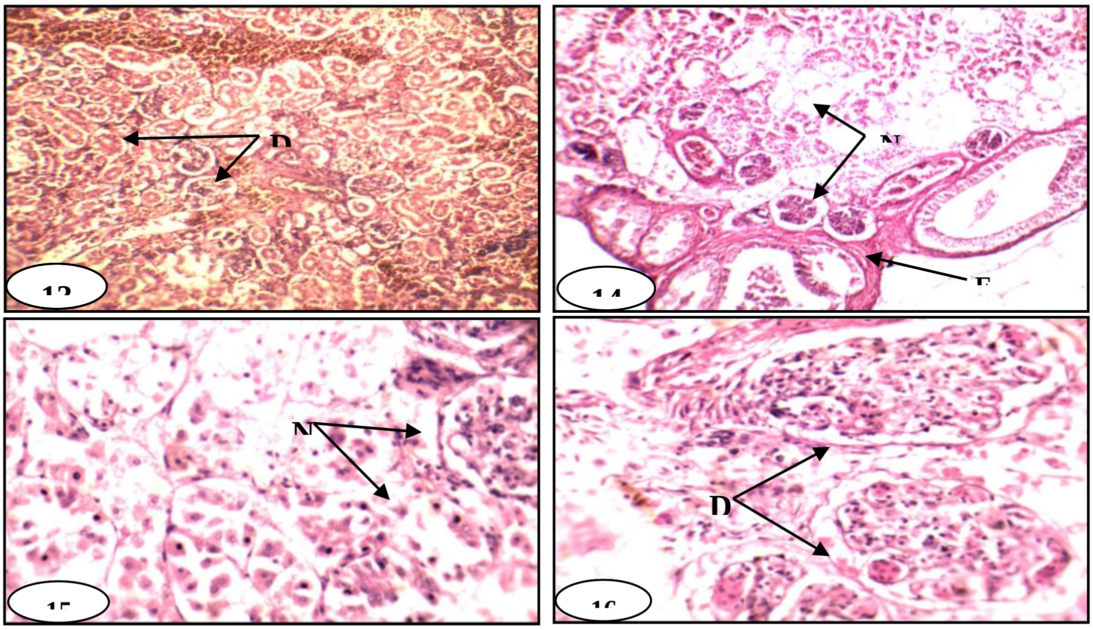
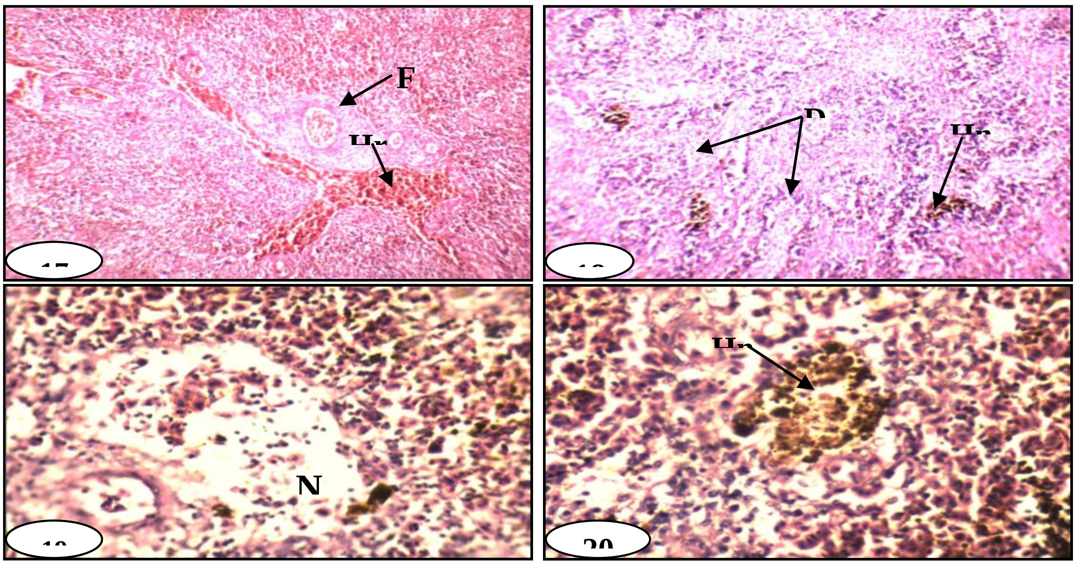


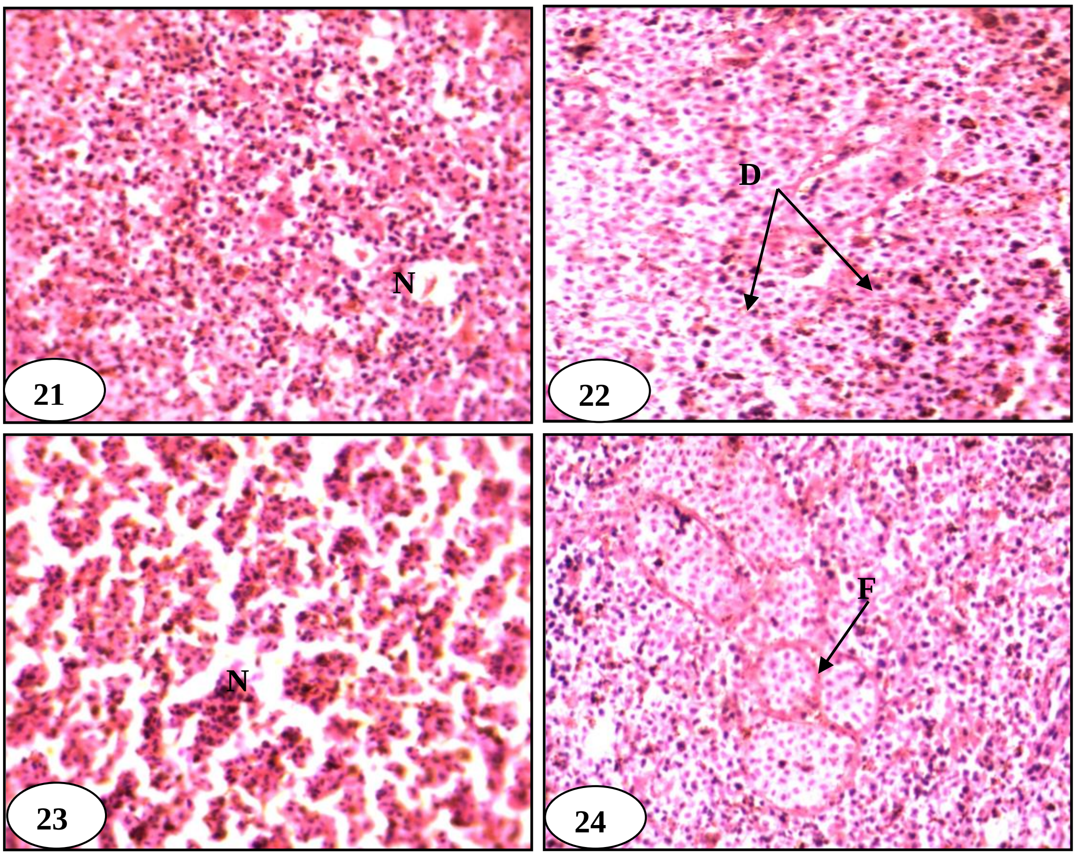
Figures (13-16): Kidney sections of *M. capito* stained with H&E, showing: Degeneration (D) & Necrosis (N) in kidney tubules and malpegian corpuscle , and Fibrosis (F) around blood vessel.

Spleen:

Spleen is a small red organ and important member of the body's immune and lymphatic system. Its functions are filtration of blood, producing & storage of red blood cells, removing old & abnormal erythrocytes and producing antibody against blood borne antigen [21]**.** The normal spleen of fish is enclosed with a capsule and consists of lymphatic laden area, white pulp surrounded by pink areas, the red pulp which contains large numbers of blood cells and sinusoids. Endothelial cells of the sinusoids are not in contact but are separated by slit like spaces. Red blood cells are broken in the red pulp [78]**.** The spleen of *C. gariepinus* **(Figs. 17-20)** and *M. capito* fish **(Figs. 21-24)** were represented by fibrosis around blood vessels, increase in white pulp and decrease in red pulp, degeneration between white and red pulp as well as hemorrhage hemosidrinosis in spleen tissue. These changes may be due to trace elements which were accumulated in the spleen from polluted water. Such explanations were recorded by[79,80,14]. The hemosidrin formation (accumulation) may be attributed to the increase in iron content as reported by [81].



Figures (17-20): Spleen sections of *C. gariepinus* stained with H&E, showing: Degeneration (D) & Necrosis (N) in red and white pulps, Hemorrhages (Hr), Fibroses (F) and Hemosidrinosis (Hn) .



Figures (21-24): Spleen sections of *M. capito* stained with H&E, showing: Degeneration (D) and Necrosis (N) in red and white pulps and Fibroses (F).

CONCLUSION

The results of this study supply valuable information about contamination of El-Manzalah Lake with many pollutants. The pollution may affect the utilization of the lake for fish production and may pose health hazards for consumers. Meanwhile, governmental efforts still needed to control environmental pollution in addition to treatment of drainage water before discharged into the Lake to improve the water quality.

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