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Snails and Fish as Pollution Biomarkers in Lake Manzala and Laboratory B: Lake Manzala Fish

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***Abstract*—**This work aimed to examine *Oreochromis niloticus* fish from Lake Manzala in Port Said, Dakahlya and Damietta governorates, Egypt, as a bio-indicator for the lake water pollution through recording alterations in their hematological, physiological, and histopathological parameters. All fish samples showed a significant increase in levels of alkaline phosphatase (ALP), creatinine and glutathione-S-transferase (GST); only Dakahlya samples showed a significant increase ($p<0.01$) in aspartate aminotransferase (AST) level and most Dakahlya and Damietta samples showed reversed albumin and globulin ratio and a significant increase in γ-glutamyltransferase (GGT) level. Port-Said and Damietta samples showed a significant decrease of hemoglobin (Hb) while Dakahlya samples showed a significant decrease in white blood cell (WBC) count. Histopathological investigation for different fish organs showed that Port-Said and Dakahlya samples were more altered than Damietta. The muscle and gill followed by intestine were the most affected organs. The muscle sections showed severe edema, neoplasia, necrotic change, fat vacuoles and splitting of muscle fiber. The gill sections showed dilated blood vessels of the filaments, curling of gill lamellae, severe hyperplasia, edema and blood vessels congestion of filaments. The intestine sections revealed degeneration, atrophy, dilation in blood vessels and necrotic changes in sub-mucosa and mucosa with edema in between. The recorded significant alterations, in most of the physiological and histological parameters in *O. niloticus* samples from Lake Manzala, were alarming for water pollution impacts on lake fish community, which constitutes the main diet and the main source of income for the people inhabiting these areas, and were threatening their public health and economy. Also, results evaluate the use of *O. niloticus* fish as important bio-indicator for their habitat stressors.

***Keywords*—**Lake Manzala, *Oreochromis niloticus* fish, water pollution, physiological, hematological and histopathological parameters.

I. Introduction

THE fishes in aquatic environments may be exposed to a myriad of substances at the same time produced by different kinds of anthropogenic activities. Biological changes in fish that are related to the exposure or to the effects of contaminants are called biomarkers and their use has led to good results in environmental risk assessment [1]. Biomarkers are increasingly recognized tools for the assessment of pollution impacts in the marine environment worldwide and some had been already incorporated in environmental monitoring programs [2]. Biomarkers can be characterized as functional measures of exposure to environmental stressors which are usually expressed at the subcellular level of biologic organization; occur before other disturbances, such as disease, mortality, or population changes; and thus they may offer early warnings of pollution impacts [3], [4].

Fish blood is sensitive to pollution. Changes in the blood profile indicate alterations in metabolism and biochemical processes of the organism under the effect of various pollutants and make it possible to study the mechanisms of these pollutants [5]. Also, certain serum constituents can be used as markers for tissue damage [6]. Many biochemical alterations due to metal toxicity were reported, like increase in serum total protein and albumin levels [7], [8], alterations in AST, ALT and ALP activities [9], [10], as well as increase in urea and creatinine levels [11], [12]. The major protein of the blood is albumin which plays an important role in transporting a wide range of physiological and exogenous ligands, regulating blood colloid osmotic pressure [13]. Albumin may change its characteristics in responses to fish physiological status, environmental factors; pathological processes and parasites invasion [14], and chemical toxicants and long-term pollution exposure [15], [16]. Oxidative stress and genotoxicity biomarkers have been applied in several fish species for pollution assessment in harbors and coastal areas influenced by industrial discharges [17]-[21].

Also, many studies used histopathological alterations as biomarker to record the impact of exposure to metals on fish health either experimentally [22], [23] or in their environment [24]. One of the great advantages of using histopathological biomarkers in environmental monitoring is that they allow examination of specific target organs, including gills, kidney, and liver that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish [25]. Also, the alterations found in these organs are normally easier to identify than functional ones [26], and serve as warning signs of animal health damage [27]. Furthermore, histopathological changes produced by pollutants in organs and tissues can occur before they produce irreversible effects on the biota. So, histological methods can be used in conjunction with other parameters and/or ecotoxicological bio indicators as an early warning system for the survival of the species, as well as for environmental protection.

This work aims to examine *O. niloticus* fish collected from Lake Manzala as a bio-indicator for lake pollution through recording the alterations of the Physiological; liver and kidney

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functions and oxidative stress enzymes; hematological and histopathological parameters.

A. Study Area

Lake Manzala is the largest of the four brackish coastal lakes fringing the Nile Delta. It is bordered by Suez Canal from east, Damietta branch of the River Nile from the west and the Mediterranean Sea from the north. The lake connected to the Mediterranean Sea via three outlets, permitting exchange the water and biota between the lake and the sea. These outlets are El-Gamil, El-Boughdady, and the new El-Gamil [28]. Lake Manzala receives about 7500 million cubic meters of untreated industrial, domestic and agricultural drainage water, discharged annually into the lake through several drain; Bahr El-Baqer Drains (domestic and industrial sewage), Hadous, Ramsis, El-Serw and Faraskour Drains (agricultural effluents). This amount of water was reduced to about 4000 million cubic meters after the construction of El-Salam Canal [29]. Lake Manzala occupies the second polluted level after Lake Mariut, [30].

Lake Manzala attracts the attention of many scientists because of its important economic aspects. Several investigations have been carried out concerning its ecosystem. These studies dealt with different environmental aspects of the lake including geological aspects, hydrological regime, physicochemical properties, bacterial indices, phytoplankton composition, benthic invertebrates and fishery status [31]-[38]. Not only great efforts were needed for recovery of the purity and healthy of this lake, but also an additional information was needed to provide a data base for optimal fisheries and water quality status that help the proper management of the lake.

II. Materials and Methods

A. Sampling

During 2013, samples of *O. niloticus* were collected with the help of professional local fishermen from eight sits in Lake Manzala, (Fig. 1). The weight and length of the collected fish samples from the lake and the purchased fish from the governmental fish aquaculture "El-Qanater El-Khairya" were determined.

Fig. 1 Map of Lake Manzala, Egypt

B. Hematological and Biochemical Biomarkers

Four hematological and thirteen serum biochemical parameters; liver and kidney functions and antioxidant enzymes; were determined in *O. niloticus* fish samples collected from different the eight studied sites of Lake Manzala and El-Kanater El-Khairya fish aquaculture as a control group.

C. Hematological Biomarkers

Fish blood is collected by cardiac puncture from the heart ventricle by inserting needle perpendicular to the ventral surface of the fish in the center of an imaginary line between the anterior most parts of the base of the pectoral fins. Blood samples were taken from the caudal vein of the fish using heparinized tubes. The whole blood was immediately used for the estimation of red blood cells count (RBCs), Hb packed cell volume (PCV) and WBCs count.

D. Biochemical Biomarkers

1) Determination of Liver and Kidney Functions

Some other blood samples were collected and left to coagulate for 15–20 min at room temperature and then centrifuged at 3000 rpm for 10 min to separate serum then samples were stored at -20 °C till analysis. Fish serum samples were biochemically assessed for AST, ALT, ALP, glucose, urea, creatinine, total bilirubin (TB), total protein (TP), albumin (A) using biosystem autoanalyzer, Bachmann at Theodor Bilharz Institute hospital laboratories.

2) Determination of Antioxidant Enzymes

The fishes were dissected and the livers were removed, washed in an ice cold 1.15% KCL solution, blotted and weighed. Fish liver samples were then homogenized with 0.15% M of KCL using electrical mortar. The resulting homogenates were centrifuged at 2500 rpm speed for 15 mins and the supernatant was decanted and stored -20 °C until analysis [39]. The antioxidant enzymes Catalase, GST and GGT were assayed using Spectrophotometer in fish liver extracts.

E. Histopathological Biomarkers

Fish were dissected and tissue specimens of the muscle, gills, liver, kidneys and gonads were immediately isolated and fixed in 10% neutral formaldehyde for 24 hrs. After fixation, the tissues were washed in 70% ethyl alcohol to get rid of excess fixative and then dehydrated through ascending grades of ethyl alcohol. The specimens were cleared in xylene for 15-20 min and infiltrated with and embedded in paraffin wax. The paraffin wax block was sectioned at the thickness of 5-6 µm. Sections were stained with Harris' hematoxylin and eosin.

F. Statistical Analysis

The data were subjected to One-way analysis of the ANOVA variance followed by Duncan's multiple range tests, in statistic package for social science 22.0 (SPSS Inc. Chicago, Illinois, USA) to determine the significance of differences at a $p<0.05$ were considered statistically significant. The results were expressed as means ±SE error.

III. Results

A. Samples

Fish samples collected from Dakalya governorate in Nasayma site have weight and lengths ranged between 37.6 and 28.6 g & 10.8 and 12.4 cm, Matarya site 73.5 and 39.5 g & 13.6 and 15.5 cm, Gammalya site 288 and 41.5 g & 15.3 and 24.1 cm and El-Dlea site 54.2 and 42.6 g & 12.5 and 13.8 cm. Fish samples collected from Port-Said governorate in Kobry El-Lansh have weight and lengths ranged between 71.1 and 41.2 g & 12.8 and 15.5 cm and Kaar El-Bahr site 57.3 and 26 g & 11 and 14 cm. Fish samples collected from Dameitta governorate in Sayala have weight and lengths ranged between 42.5 and 12.1 g & 8.5 and 19.5 cm and in Annaneya site 53.5 and 40.9 g & 13.5 and 14.6 cm. Control *O. niloticus* from the fish aquaculture have weight and lengths ranged between 193.5 and 205 g & 20.3 and 29.9 cm.

B. Hematological Biomarkers

A significant decrease of Hb was noticed in samples collected from Port-Said and Damietta while no significant decrease in RBC count was noticed in all examined fish samples. Samples from Dakahlya is the only sample which showed significant decrease in WBC count while samples from other sites showed no significant decrease or increase (Table I)

C. Biochemical Parameters

1) Liver and Kidney Functions

Samples collected from the River Nile showed a significant increase ($p<0.01$) in AST, ALT, glucose, urea, TP, A and a slight decrease in A/globulin (G) (AG ratio). Samples from Port-Said and Dakahlya showed significant increase ($p<0.01$) in AST and ALP, urea and creatinine while samples from Damietta showed a significant increase in glucose (Tables II, III). TB levels showed a significant increase in samples from Port-Said (El-Khankak), Dakahlya (Nasayma), and Damietta (Annanya). TP decreased in all field samples showing its maximum change in samples from Port-Said (El-Khankak). The highest AG ratios, 6.17 and 4.39 were detected in samples collected from Port-Said (Kobry El-Lansh) and Dakahlya (Nasayma), respectively, as a result of low level of G, while the lowest ratio, 0.59, was in samples collected from Dakahlya (Matarya) (Table III).

2) Antioxidant Enzymes

Results of the examined antioxidant enzymes; CAT, GST and GGT; in liver extracts of the studied Lake Manzala fish samples are presented in Table IV. All fish samples showed a significant increase in (GST) activity and the most altered samples ware from Nasayma site (Dakahlya) (1110 folds as compared to control). The most altered samples were from Gammalya site (Dakahlya), showed significant increase (47 folds as compared to control) and significant decrease in CAT activity (-6 folds as compared to control).

TABLE I
Blood Hematological Parameters, RBCs, Hb, PCV and WBC, of *O. niloticus* Fish Samples Collected from Different Sites in Lake Manzala in Port-Said Dakahlya, and Damietta Governorates

Sites of collection	Parameter	Hb		RBCs		PCV		WBCs	
	Treatments	g/100ml	% of change	10^6/mm^3	% of change	%	% of change	10^6/mm^3	% of change
Kanater Farm	Control	11.5±0.8		2.5±0.09		24±0.5		4.5±0.09	
Port-Said	Bahr El-Bakar	6.85±0.4	-40	1.8±0.02	-28	15.96±1.35	-34	4.45±0.28	-1
	Kobry El-Lansh	7.66±0.36	-33	1.9±0.04	-24	17.82±1.27	-26	5.24±0.36	16
	El-Khandak	8.36±0.43	-27	1.74±0.03	-30	16.27±1.22	-32	4.56±0.17	1
	Kaar El-Bahr	7.63±0.28	-34	1.65±0.02	-34	16.2±1.04	-33	4.25±0.24	-6
Dakahlya	Matarya	8.32±0.27	-28	1.68±0.02	-33	18.04±1.3	-25	4.95±1.45	10
	Dogo	8.25±0.52	-28	2.06±0.05	-18	17.65±0.93	-26	3.64±0.16	-19
	Deshda	8.52±0.53	-26	1.88±0.07	-25	16.93±1.3	-29	5.2±0.27	16
Damietta	Sayala	7.62±0.34	-34	1.36±0.03	-46	17.7±0.86	-26	4.93±0.41	10

TABLE II
AST, ALT, ALP, GLUCOSE AND CREATININE LEVELS IN SERUM OF *O. NILOTICUS* FISH SAMPLES COLLECTED FROM DIFFERENT SITES IN LAKE MANZALA IN PORT-SAID DAKAHLYA, AND DAMIETTA GOVERNORATES

Parameters		AST (U/ml)		ALT (U/ml)		ALP (IU/L)		Creatinine (mg/dl)		Glucose (mg/dl)	
Treatments		Level	% of change	Level	% of change	Level	% of change	Level	% of change	Level	% of change
Control		30.0±0.1		5.0±0.0		7.0±0.1		0.09±0.006		53.0±0.1	
Nile	El-lWarrak	278.0±26**	827	20.5±1.5**	310	7.5±1.5	7	0.15±0.065	67	159.5±6.5**	201
Port Said	El-Khankak	196.0±14.2**	553	26.0±3.0*	420	27.0±2.6**	286	0.13±0.005*	44	68.5±5.6	29
	Kobry El-Lansh	85.0±7.9*	183	13.5±6.5	170	49.0±3.8**	600	0.28±0.060*	211	41.5±2.9*	-22
Dakahlya	Nasayma	70.0±6.1*	133	6.0±3.0	20	35.0±2.1**	400	0.28±0.155	211	37.5±6.5	-29
	Gammalya	16.0±4*	-47	1.0±0.0*	-80	15.5±1.5*	121	0.26±0.025*	189	38.0±6.0	-28
	Matarya	160.0±25*	433	11.5±4.5	130	27.0±2.3**	286	0.60±0.032**	567	90.0±3.8**	70
	Deshda	258.5±6.4*	762	4.5±2.5	-10	13.5±2.5	93	0.18±0.050	100	127.0±3.6**	140
Damietta	Annanya	53.5±15	78	3.5±0.5	-30	22.5±5.5	221	0.01±0.00*	-89	89.0±3.0**	68
	Sayala	18.5±6	-38	6.5±1.5	30	14.5±2.5	107	0.07±0.045	-22	73.5±2.5**	39

TABLE III
UREA, TB, TP, A, G AND A/G RATIO IN SERUM OF *O. NILOTICUS* FISH SAMPLES COLLECTED FROM DIFFERENT SITES IN LAKE MANZALA IN PORT-SAID DAKAHLYA, AND DAMIETTA GOVERNORATES

Parameter		Urea (mg/dl)		TB (mg/dl)		TP (g/dl)		A (g/dl)		G (g/dl)		A/G Ratio
Treatments		Level	% of change	Tissue	% of change	Level	% of change	Level	% of change	Level	% of change	
Control		3.23±0.24		0.20±0.01		3.1±0.01		1.9±0.01		1.2±0.01		1.58
Nile	El-Warrak	6.4±0.0**	98	0.20±0.00	0	2.2±0.15*	-29	0.9±0.10**	-53	1.25±0.05	4	0.72
rt Said	El-Khankak	7.5±1.1*	132	0.80±0.04**	300	1.3±0.40*	-58	0.95±0.25*	-50	0.35±0.25*	-71	2.71
	Kobry El-Lansh	8.6±0.0**	166	0.25±0.15	25	1.7±0.25*	-45	1.45±1.08	-24	0.235±0.76	-80	6.17
kahly	Nasayma	2.1±0.0*	-35	0.40±0.00**	100	1.8±0.55	-42	1.45±0.65	-24	0.33±0.57	-73	4.39
a	Gammalya	5.35±1.05	66	0.05±0.05	-75	2.3±0.15*	-26	1.4±0.15*	-26	0.85±0.00	-29	1.65
	Matarya	7.5±1.1*	132	0.10±0.10	-50	2.6±1.35	16	0.95±0.25*	-50	1.6±1.35	33	0.59
	Deshda	6.4±0.0**	98	0.10±0.00**	-50	2.5±0.40	-19	1.2±0.20*	-37	1.3±0.20	8	0.92
mietta	Annanya	5.4±1.05	66	0.90±0.07**	350	2.2±0.50	-29	1.0±0.20*	-47	1.2±0.30	0	0.83
	Sayala	6.45±2.15	100	0.20±0.00	0	2.0±0.90	-35	0.85±0.45	-55	1.15±0.45	-4	0.74

TABLE IV
CATALASE (CAT), GST AND GGT IN LIVER OF *O. NILOTICUS* FISH SAMPLES COLLECTED FROM DIFFERENT SITES IN LAKE MANZALA IN PORT-SAID DAKAHLYA, AND DAMIETTA GOVERNORATES

Parameters		CAT (U/g)		GST (U/g)		GGT (U/g)	
Treatments		Level	% of change	Level	% of change	Level	% of change
Control unexposed		0.83±0.005		0.35±0.16		549±24	
Port-Said	Kobry El-Lansh	0.81±0.012	-2	1.94*±0.45	454	520±73	-5
	Kaar El-Baharr	0.83±0.025	0	3.42*±0.78	876	470±123	-14
Dakahlya	Nasayma	0.79±0.00	-4	4.24**±0.11	1110	642±23	17
	Gammalya	0.78*±0.008	-6	1.14*±0.13	225	808*±0	47
	Matarya	0.82±0.007	-1	2.22**±0.24	533	687±58	25
	Deshda	0.79±0.066	-4	1.89**±0.14	440	409±152	-25
Damietta	Annanya	0.84±0.007	2	3.06*±0.0	774	726±140	32
	Sayala	0.80±0.023	-3	2.04**±0.29	481	526±83	-4

3) Fish Histopathology

The histological studies indicated that the fish *O. niloticus*, collected from reference site from EL-Qanater EL-Khyria farm have a normal architecture while several histopathological changes were seen in different organs of the fish - collected from different sites in Lake Manzala.

a) Muscles

Fig. 2 (a) shows the normal structures of the muscles bundles. Several histopathological alterations were seen in the muscles of *O. niloticus*, included degeneration in muscle bundles with neoplasia and cysts between them and focal areas of necrosis. Also, large fat vacuoles and atrophy of muscle bundles were observed. Edema and splitting of muscle fibers were also seen. Alterations were more severe in the muscle of studied fish collected from Port-Said and Dakahlya governorates.

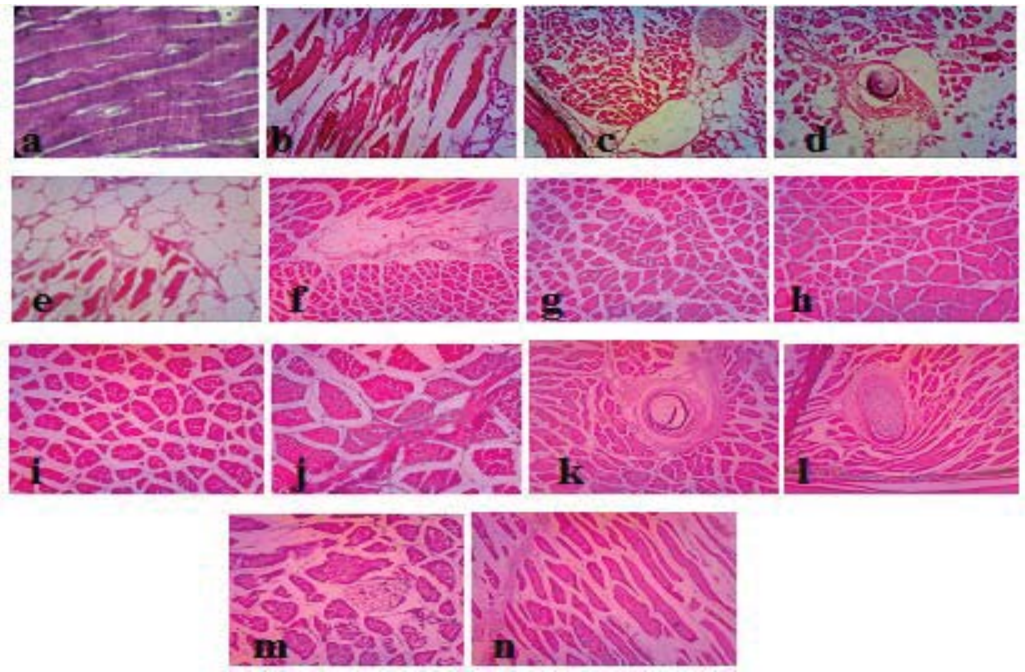

Fig. 2 Normal and altered histological structures of *O. niloticus* muscle: Muscle of fish showing normal structure (a), muscle of fish showing severe edema (b) Dakahlya (X100), fatty degeneration and neoplsia (c) (X100) Port-Said, necrotic change and cyst of parasite, Port-Said (d) (X100); large fat vacuoles and severe edema (e) (X100), necrotic change (f) (X100), vacuolar degeneration of muscle fibers (g) (X100) Port-Said, mild splitting of muscle fibers, edema and splitting of muscle fiber (h, i)(X100) Damietta, severe edema and splitting of muscle fiber (j) Port-Said; edema, necrotic change and cyst of parasite (k) Dakahlya; neoplasia (l,m), edema (n) Dakahlya

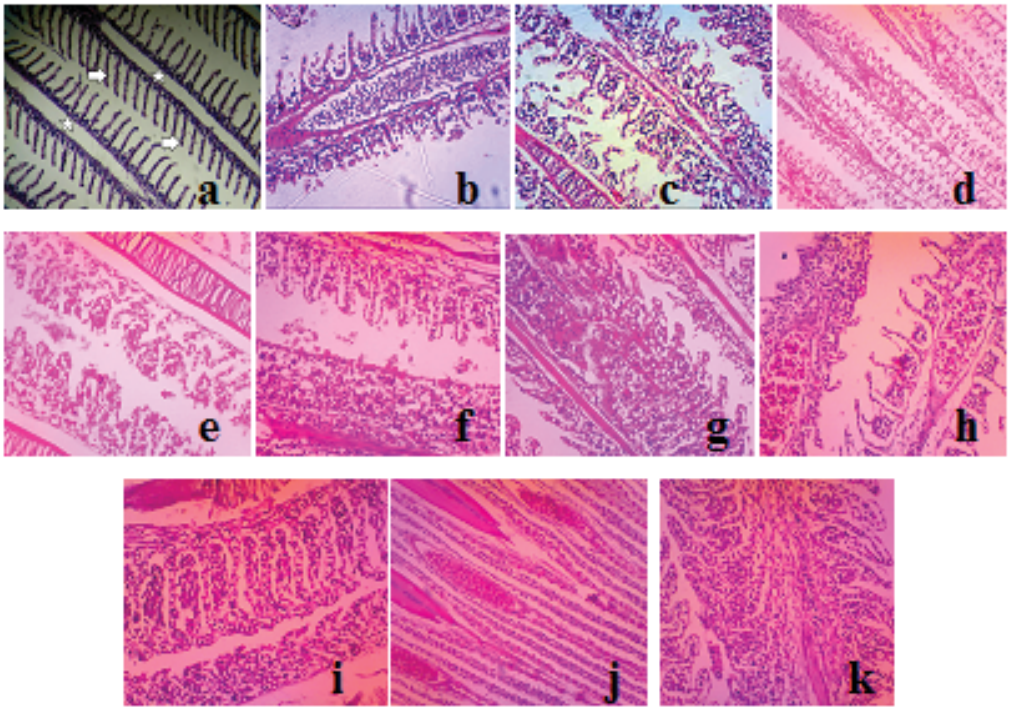

Fig. 3 Normal and altered histological structures of *O. niloticus* gills: Gill of fish showing normal structure, filament (star), primary lamellae (arrows) (a) (X100), dilated and congested blood vessel of the filament (b) Dakahlya (X100), epithelial lifting and curling of lamellae, Port-Said (c) (X100); severe edema and severe blood vessel congestion of filament (d) Port-Said (X100), necrotic change (e) Port-Said (X100), sloughing and epithelial lifting (f) Damietta (X100), severe hyperplasia and hypertrophied chloride cells (g) Port-Said; edema and severe blood vessel congestion of filament (h) Dakahlya. severe hyperplasia lead to fusion of gill filaments (i), severe blood vessel congestion of filament and sloughing (j) Dakahlya; curling of gill lamellae and severe hyperplasia (k) Dakahlya

b) Gills

The histological investigations indicated that the gills of control *O. niloticus* collected from the reference site (at Al-Qanater Al-Khairya station) have a normal architecture (Fig. 3 (a)). The histopathological alterations in the gills of *O. niloticus* were more or less similar between the three governorates but more severe in Port-Said and Dakahlya samples. It showed congestion in the blood vessels of gill filaments. Proliferative changes in the epithelium of gill filaments and secondary lamellae and separation of the epithelium of the secondary lamellae from the lamellar supporting cells in some gill filaments, curling of lamellae were noticed. Also, severe hyperplasia and hypertrophied chloride cells and necrotic changes in gill lamellae were seen. Besides sloughing in the epithelium were observed.

c) Liver

The histological structures of the liver of control *O. niloticus*, is shown in Fig. 4 (a). No abnormal histological features were observed. Several histopathological alterations were observed in the liver of the studied fish. Hydropic vacuolation and thrombosis formation were observed in some hepatoportal blood vessels. Also, focal areas of necrosis, aggregations of inflammatory cells and pyknotic nuclei were noticed between the hepatocytes. In addition, intravascular hemolysis in blood vessels and necrotic change of bile duct were observed. Moreover, destruction of some hepatoportal blood vessels can be noticed. The collected fish from Port-Said and Dakahlya governorates are the most affected.

d) Spleen

Fig. 5 (a) shows the normal histological structures of the spleen. The histopathological alterations in the spleen of fish included severe degenerative and necrotic changes with focal areas of fibrosis and hemorrhage, hemolysis and hemosiderin between parenchyma cells. Aggregations of inflammatory and melanomacrophage (MMC) cells were seen. Samples collected from Port-Said and Dakahlya governorates are most affected than others.

e) Kidney

Fig. 6 (a) shows the normal histological structures of the kidney. The histopathological alterations of the kidney were included atrophy, degeneration of the distal and collecting convoluted tubules. The cells of the kidney are destroyed; vacuolization, shrinkage and breakage of tissue, degeneration of tubular epithelium, hemorrhage between them and swollen nuclei can be also seen. In the same areas, the cell boundaries are disrupted and hence the cells become indistinct. Also, disorganization of glomerulus, pycnotic nuclei and edema in Bowman's capsules can be noticed. Generally, kidney in all governorates of Lake Manzala destroyed because it is one of the first organs to be affected by contaminants in the water.

f) Testis

Fig. 7 (a) shows the normal histological structures of testis of *O. niloticus*. The histopathological alterations of the testis included atrophy and necrotic changes in the cellular elements of the seminiferous tubules with focal areas of necrosis and degeneration in the wall of seminiferous tubules. The lumen of some seminiferous tubules filled with a large number of spermatids (Figs. 7 (d) and (e)). Fish samples of Dakahlya and Port-Said governorates are the most affected.

g) Ovary

Fig. 8 (a) shows the normal histological structures of ovary of *O. niloticus* contain large mature oocytes with good structure. The histopathological alterations of the ovaries showed severe oolytic changes like shrinkage of oocytes, which resulted in large interfollicular spaces and edema between ooytes. Most ovarian follicles were atretic as well as losing the typical round configuration and degeneration of follicles was observed. Damietta and Port-Said are the most affected governorates.

h) Intestine

Fig. 9 (a) shows the normal histological structures of the intestine. The histopathological alterations in the intestine of *O. niloticus* fish included severe degenerative and necrotic changes in the intestinal mucosa with necrotized and inflammatory cells aggregated in the intestinal lumen and fibrosis of villi, also edema between the intestinal submucosa and mucosa can be noticed. Port-Said and Dakahlya fish samples are the most affected.

Fish samples were regrouped according to their histopathological alteration degrees in different organs; mild (+), moderate (++ & +++) and severe (++++); then all studied hematological and biochemical parameters were reassessed in each group, results showed high significant difference than control reflection the correlation between different biomarkers and the high impacts on fish health (Tables V-VIII). Results in Table IX showed both muscle and gill were the most histopathologically altered among the examined fish organs.

TABLE V
HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN *O. NILOTICUS* FISH SAMPLES COLLECTED FROM LAKE MANALZA IN THE THREE GOVERNORATES; PORT-SAID, DAKAHLYA, AND DAMIETTA; REGROUPED ACCORDING TO THEIR HISTOPATHOLOGICAL ALTERATION DEGREES OF ORGANS; MILD, MODERATE, AND SEVERE

Effect degrees	Governorates	Hematological parameters (X±SE)			
		HB (g/100ml)	RBCs (10^6/mm^3)	PCV	WBCs (10^6/mm^3)
Control		11.5±0.8	2.5±0.09	24±0.5	4.5±0.09
Mild (+)	Port-Said	8.05±1.1[a]	0.50±0.05[a]	1.5±0.35[a]	1.2±0.67[a]
Moderate (++,+++)	Dakahlya	7.1±1.1[a]	0.18±0.15[a]	2.3±0.51[a]	1.3±0.31[a]
Severe (++++)	Damietta	5.9±2.1[a]	0.55±0.07[a]	2.1±0.70[a]	0.93±0.33[a]

[a]*p<0.01 significant decrease than Control*

TABLE VI
BIOCHEMICAL PARAMETERS; AST, ALT, ALP, CREATININE AND GLUCOSE; IN *O. NILOTICUS* FISH SAMPLES COLLECTED FROM LAKE MANALZA IN THE THREE GOVERNORATES; PORT-SAID, DAKAHLYA, AND DAMIETTA; REGROUPED ACCORDING TO THEIR HISTOPATHOLOGICAL ALTERATION DEGREES OF ORGANS; MILD, MODERATE, AND SEVERE

Effect degrees	Governorates	Biochemical parameters (X±SE)				
		AST (U/ml)	ALT (U/ml)	ALP (IU/L)	Creatinine (mg/dl)	Glucose (mg/dl)
Control		30.0±0.1	5.0±0.0	7.0±0.1	0.09±0.006	53.0±0.1
Mild (+)	Port-Said	140.5±11.05**	32.75±.75**	38.0±3.2**	0.21±0.032	55.0±4.3*
Moderate (++,+++)	Dakahlya	126.13±10.38**	5.75±2.5*	35.0±2.1*	0.33±0.06**	73.1±4.9**
Severe (++++)	Damietta	36.0±10.5**	5.0±1.0*	18.5±4.0**	0.04±0.02a	81.25±2.75**

***p<0.01 significant increase than control, * p<0.05 significant increase than control*

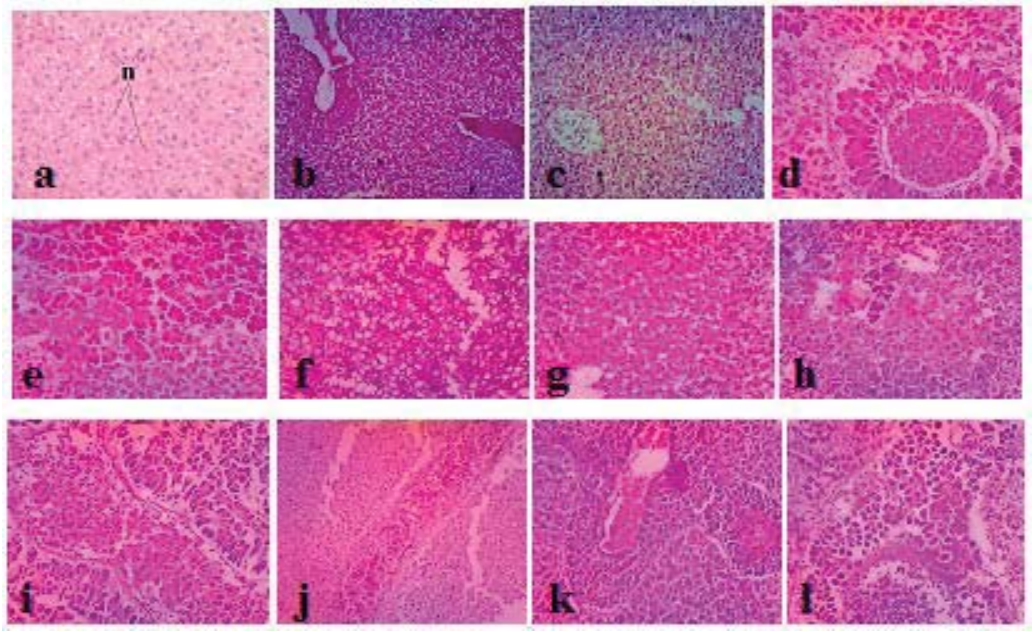

Fig. 4 Normal and altered histological structures of *O. niloticus* liver: Liver of fish showing normal structure (a) (X100), dilated veins and thrombosis formation in hepatoportal blood vessel (b) (X100 Dakahlya), hydropic vacuolation and focal area of necrosis (c) intravascular hemolysis in blood vessel and necrotic change (d) (X100) Port-Said; necrotic change (e) Port-Said (X100), hydropic vacuolation and fatty degeneration (f) (X100), hydropic vacuolation and hemosidrine (g) (100) Damietta; necrotic change of bile duct (h) (100) Port-Said; intravascular hemolysis in blood vessels (i, j) (X100) Dakahlya; thrombosis formation in hepatoportal blood vessel (k, l) (X100) Dakahlya

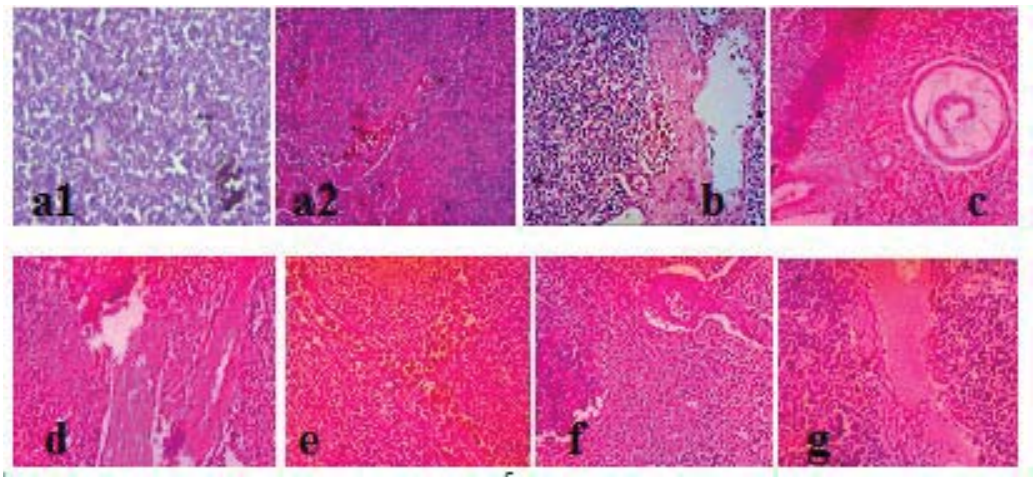

Fig. 5 Normal and altered histological structures of *O. niloticus* spleen: The normal histological structures of the spleen (a1); mild MMc and hemosidrine (a2) Dakahlya (X100), severe degenerative change and focal area of necrosis (b) Port-Said (X100), focal areas of fibrosis and focal areas of necrosis (c), Port-Said (X100); focal areas of fibrosis (d) Damietta; focal areas of hemosidrine (e) Damietta (X100), severe focal areas of fibrosis and MMC (f) Port-Said (X100), severe focal areas of fibrosis and MMC (g) Dakahlya (X100)

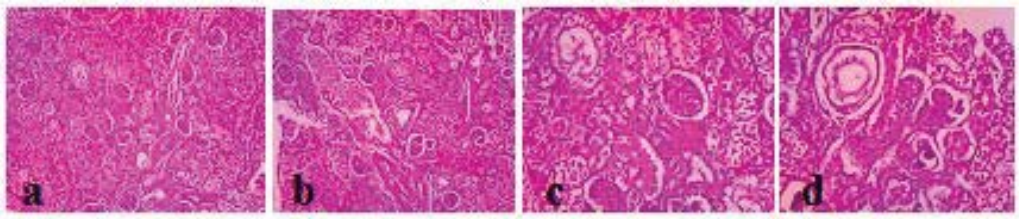

Fig. 6 Normal and altered histological structures of *O. niloticus* kidney: The normal histological structures of the kidney (a) (X100), degeneration, atrophy of renal tubules and hemorrhage between tubules (b), (c) severe degeneration of glomerulus and Pycnotic nuclei (d) (X100)

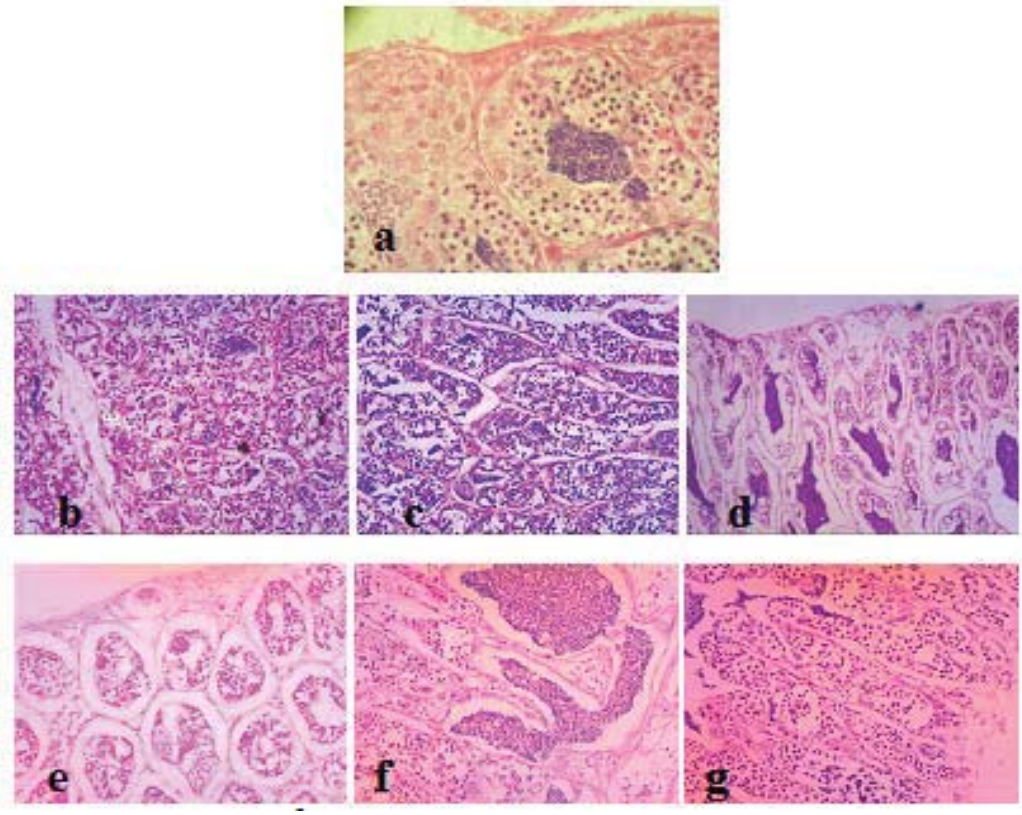

Fig. 7 Normal and altered histological structures of *O. niloticus* testis: The normal histological structures of the testis showing spermatogonia, different stages of spermatocytes and sperms filled the lumen of seminiferous tubules (a) (X100), severe degenerative and necrotic changes in the cellular elements of the seminiferous tubules (b) (X100), atrophy of cellular elements and edema (c) (X100) Dakahlya, severe edema and focal areas of necrosis (d), (e), (X100) Port-Said; focal areas of fibrosis (f, g) (X100) Dakahlya

TABLE VII
BIOCHEMICAL PARAMETERS; UREA, TB, TP, A, G AND A/G RATIO; IN *O. NILOTICUS* FISH SAMPLES COLLECTED FROM LAKE MANALZA IN THE THREE GOVERNORATES; PORT-SAID, DAKAHLYA, AND DAMIETTA; REGROUPED ACCORDING TO THEIR HISTOPATHOLOGICAL ALTERATION DEGREES OF ORGANS; MILD, MODERATE, AND SEVERE

Effect degrees	Governorates	Biochemical parameters (X±SE)					
		Urea	TB	TP (g/dl)	A (g/dl)	G (g/dl)	A/G Ratio
Control		3.23±0.24	0.20±0.01	3.1±0.01	1.9±0.01	1.2±0.01	1.58
Mild (+)	Port-Said	8.05±1.1**	0.50±0.05*	1.5±0.35[b]	1.2±0.67[b]	0.29±0.51[b]	4.44
Moderate (++,+++)	Dakahlya	7.1±1.1**	0.18±0.15[a]	2.3±0.51[b]	1.3±0.31[b]	1.02±0.53[b]	1.88
Severe (++++)	Damietta	5.9±2.1**	0.55±0.07**	2.1±0.70[b]	0.93±0.33[b]	1.18±0.38[a]	0.79

***p<0.01significant increase than control, [a]p<0.05 significant decrease than control; [b]p<0.01sinificant decrease than control*

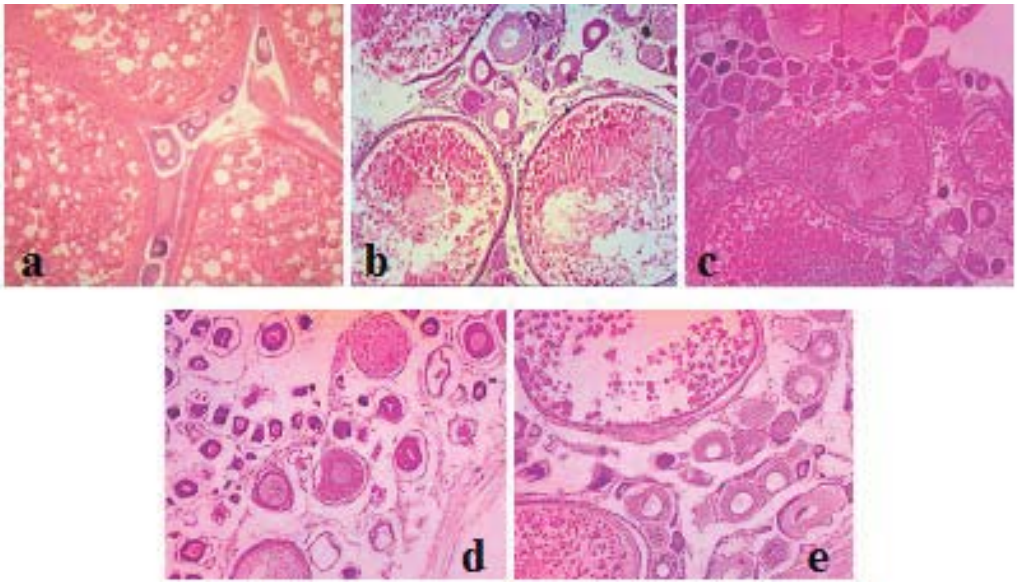

Fig. 8 Normal and altered histological structures of *O. niloticus* ovary: The normal histological structures of the ovary showing large mature oocytes (a) (X40), few atretic oocytes and large oocyte at second yolk stage (b) (X40) Port-Said, relatively degenerative change, edema and atretic young oocytes (c) (X40) Port-Said, severe degenerative change, edema and oocytes were absorbed (d), (X40) Damietta; most large oocytes were lose their typical round configuration (e) (X40), mild degenerative change, edema and large oocytes become atretic (X40) Port-Said

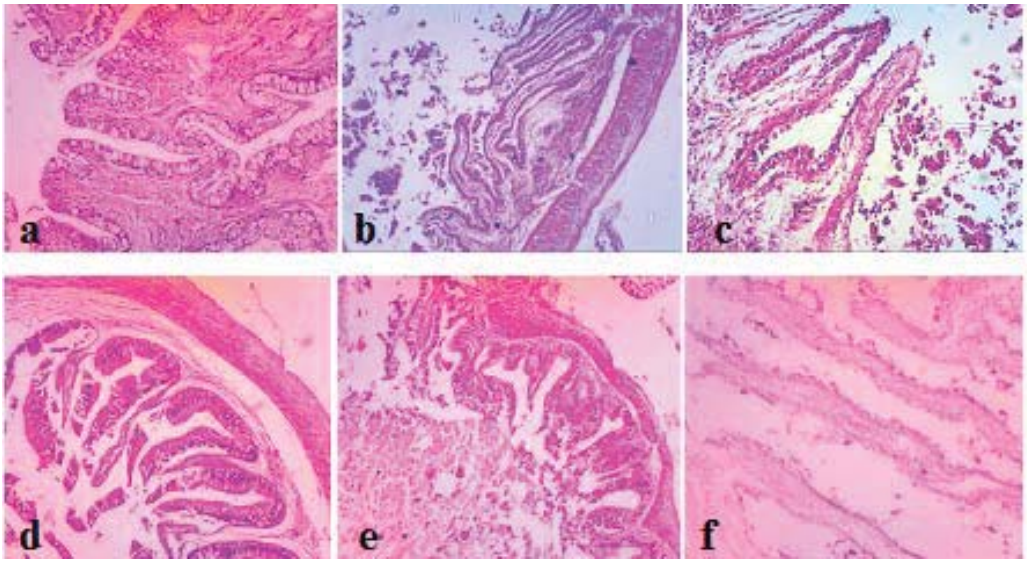

Fig. 9 Normal and altered histological structures of *O. niloticus* intestine: The normal histological structures of the intestine (a); disintegration the villi of mucosa layer (b) Dakahlya (X40), severe degenerative change and fibrosis of villi (c) Port-Said (X40), edema between mucosa and submucosa (d), Damietta (X100); aggregation of inflammatory cells in mucosa and submucosa (e) Port-Said (X100), severe degenerative change and fibrosis of villi (X40) Dakahlya

TABLE VIII
BIOCHEMICAL PARAMETERS; CAT, GST, AND GGT; IN *O. NILOTICUS* FISH SAMPLES COLLECTED FROM LAKE MANALZA IN THE THREE GOVERNORATES; PORT-SAID, DAKAHLYA, AND DAMIETTA; REGROUPED ACCORDING TO THEIR HISTOPATHOLOGICAL ALTERATION DEGREES OF ORGANS; MILD, MODERATE, AND SEVERE

Effect degrees	Govern-orates	Biochemical parameters (X±SE)		
		Catalase (CAT) (U/g)	GST (U/g)	GGT (U/g)
Control		0.83±0.005	0.35±0.16	549±24
Mild (+)	Port-Said	0.82±0.018	3.83±0.44**	556.0±98.0
Moderate (++,+++)	Dakahlya	0.80±0.02	2.37±0.16**	477.61±58.25b
Severe (++++)	Damietta	0.82±0.015	2.55±0.29**	626±111.5**

*p<0.05 significant increase than Control; **p<0.01 significant increase than Control; bp<0.01 significant decrease than Control

TABLE IX
HISTOPATHOLOGICAL ANALYSIS WITH FREQUENCY (%) OF ALTERATIONS IN FISH ORGANS FROM DIFFERENT SITES OF LAKE MANZALA

Fish organs / Effect degrees	Muscle	Gill	Liver	Spleen	Kidney	Testis	Ovaries	Intestine
Mild (+)	65%	78%	50%	24%	26%	10%	12%	5%
Moderate (++,+++)	75%	82 %	62%	32%	70%	22%	30%	40%
Severe (+++,++++)	82%	92%	----	------	----	-----	------	55%

IV. DISCUSSION

Recently, the pollution in Lake Manzala and its impacts on case studies of fishermen community and some aquatic organisms in the lake, snails, and fish were studied by [40], [41]. The authors showed that the collected water samples from Damietta sites showed the highest significant Cu & Cd concentration while Port-Said showed the highest Pb concentration and Dakahlia showed the highest Zn concentration. Also, the authors showed an increase in bioaccumulation of the heavy metals (Cu, Zn Cd, and Pb) in tissues of *O. niloticus* fish samples from Lake Manzala. Cu and Zn was most accumulated in liver of Port-Said fish samples (Kobry El-Lansh site). Cd and Pb were most accumulated in kidney of Dakahlya fish sample (Matarya site). This agrees with that reported in different tissues of several fish species exposed to pollution in the contaminated system in EL-Rahawy drainage canal [43], [44]. The authors [42] explained that heavy metal uptake by freshwater fish is taking place mainly via three routes namely, gills, skin and intestinal wall. In the present study, there is a significant elevation in the activities of serum AST, ALT and ALP in studied fish collected from Port-Said, Dakahlya governorates. Several investigations showed that these blood enzymes were highly increased in the fish treated with metals [45]-[47]. The blood enzymes increase reflect hepatic lesions leading to extensive liberation of the enzymes into the blood circulation [45] reported that the elevation of serum enzymes may be due to liver dysfunction. In addition, the increase of serum enzymes may be attributed to the hepatocellular damage or cellular degradation by metals, perhaps in liver, heart or muscle, [48]. Many histopathological alterations have been observed in the liver and muscle of the studied fish as those fish following exposure to Zn and Cd [49] which support the observed increase in serum enzymes activities. So, increased serum activities of ALP have been explained by pathological processes such as liver impairment, kidney dysfunction [50]; these results agree with the present study. The significance increased of G levels to meet the immunotoxic challenges lends evidence to the differential response of the constituent plasma protein. TP is used to evaluate protein metabolism. Hyper-proteinemia, seen with hemoconcentration and shock, contributed to the elevated osmolality [51] and nutritional status [52]. In addition, increased concentrations of TP can be caused by structural liver alterations reducing aminotransferase activity (as observed as a reduction of AST and ALT levels in this study) with concurrently reduced deamination capacity [53] and impaired control of fluid balance [54].

Water pollutants in Lake Manzala elevated serum glucose concentrations in chronic stress impact upon Tilapia fish (*O. niloticus*). Total serum proteins were useful in the diagnosis of fish disease. In this study, there was elevation in serum TP and A in fish *O. niloticus* collected from (Port-Said, Dakahlya and Damietta) due to activation of metabolic systems in response to Zn and Cd [49], degradation of the cellular material in the liver and relative changes in the mobilization of blood proteins, water loss in the serum and/or induction of protein synthesis in liver [55], [45], [8]. The present findings are in agreement with previous reports of increased level of serum proteins on exposure to metals [7], [8].

Also, bilirubin is the predominant bile pigment found in the circulation in fish derived from disruption of Hb [56]. In this study, the less value of bilirubin in *O. niloticus* was a consequence of the increased level of Hb. Bilirubin is a breakdown product of Hb and could be logically expected to decrease adversely with increasing levels of Hb. TB comes to the muscle and liver by binding it to the A and G in the serum through filtering by kidney. Serum bilirubin levels of fish can change with hepatic and nephritic diseases [57].

Serum urea and creatinine were useful in the diagnosis of renal function impairment, renal tubular necrosis, muscle tissue damage as well as impaired nitrogen metabolism, [58]. In the present study, serum urea and creatinine showed a significant increase. It is well known that the renal insufficiency or failure is usually associated with the decrease in urea and creatinine excretion, thus leading to its increase in serum as in fish samples collected from most Dakahlya sites. The authors [59] concluded that kidney damage may result in reduced renal blood flow with reduction in glomerular filtration rate, characterized by the increase in blood urea and creatinine [11], [12].

The severe damage of the gill epithelium in terms of necrosis and rupture may result in hypoxia, respiratory failure and problems in relation to ionic and acid-base balance, formed in fish exposed to metals [60], [61]. The lifting of lamellar epithelium may be due to the incidence of edema [62]. Edema with a lifting of lamellar epithelium serves as a mechanism of defense. Muscles of *O. niloticus* exposed to Lake Manzala pollution showed many histopathological changes, this agrees with the earlier finding, [63], alterations in the liver of studied fish have shown that vacuoles in the cytoplasm of the hepatocytes in fish samples collected from all governorates, contain lipids and glycogen, which is related to the normal metabolic function of the liver thus vacuolar degeneration, will result in a depletion of the glycogen reserves in the hepatocytes [27], [64], or will result in stress to fish because glycogen acts as a reserve of glucose to supply the higher energetic demand occurring in such situations [65].

Alterations exhibited in the liver of fish were dilation and congestion in hepatic sinusoids, hemorrhage, and coagulative necrosis. Furthermore, [66] reported that increased vacuolization of hepatocytes in fish exposed to contaminated water was a sign of degenerative process which itself suggests metabolic damage. Lesions such as focal necrosis have been reported to imply metabolic impairment in hepatocytes [67]. The spleen is one of the most important hematopoietic centers which face unfavorable consequences because of toxicants [68]. The histological examinations of the spleen of *O. niloticus* from Lake Manzala showed severe degenerative and necrotic changes with focal areas of fibrosis, hemorrhage, hemolysis and haemosiderin between parenchyma cells. Hemosiderin is a by-product of Hb breakdown and lipofuscin and ceroid are considered to be lipid pigments produced by the peroxidation of unsaturated fatty acids. Macrophage aggregates are focal accumulations of macrophages found in the spleen, head kidney, liver, and sometimes testis. They change in number, size, and pigment, and their function to localize products of tissue destruction [69]. The cells of the kidney of studied fish showed, destroyed; vacuolization, shrinkage, and degeneration of tubular epithelium, edema in Bowman´s capsule and swollen nuclei. The kidney of teleostean fish is one of the first organs to be affected by contaminants in water [23], and appears to be particularly sensitive to a variety of toxins due to the high renal blood flow, the ability to concentrate substances, and the biotransformation of the parent compound to a toxic metabolite [70]. Previous studies have shown that the most common alterations found in the kidney of fish exposed to water contamination are tubular degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman´s space [66], [67]. Exposure to metals frequently cause alterations in the tubules and glomerulus, such as was described by [23], for the perch (*Lates calcarifer*) exposed to cadmium; [71] found swollen Bowman´s capsule cells and melanomacrophages in the kidney of trout (*Salmo trutta*) and tilapia (*O. mossambicus*) exposed to mercuric chloride [72]. Fish testicular tissues from Lake Manzala showed pronounced decline in gonad activity of the studied fish which reflected by decreasing sperm and spermatids counting in ripe testes, ripe oocytes degeneration (atresia) including spermatogonia, of the seminiferous tubules and necrosis as these histological changes in testes tissue were progressively increased with the increasing degree levels of heavy metal accumulation, [73], and that resulted in permanent testicular damage which reduces the fish ability to reproduce by suppressing sperm production [74]. The reduction in the size and development of oocytes in the fish from Lake Manzala attributed to the affected vitellogenin and maturational enlargement of oocytes [75]. The toxicity of heavy metals was presented as a disruption in gonadal development, [76]. The intestine of the fish showed degeneration and necrotic changes in the intestinal mucosa and submucosa, atrophy in the muscularis and submucosa and aggregations of inflammatory cells in the mucosa and submucosa with edema between them. The authors [77] observed irritation and destruction of the mucosa membrane of the intestine, decrease absorption. The pathological lesions in the intestine are in agreement with those observed by many investigators about the effects of different toxicants on the fish intestine [74], [78]. Epithelial degeneration, inflammatory cells infiltration in the submucosa as well as submucosal edema was seen in the intestine of tilapia fish exposed to carbofuran [79]. The author [80], exposing fish *lethrinus elongatus* to crude oil, induced disturbance of general intestinal epithelium which may be due to depletion in carbohydrate contents. Similarly, glycogen and lipid depletion have been reported in *Fundulus heteroclitus* collected near an oil spill [81]. Also, the present expressive incidence of effects in fish organs and the significant biochemical disturbs supported by the foundations of [40], related to metal bioaccumulation, indicate the high impact of water pollution in Lake Manzala on fish health. In addition, the recorded high histopathological alterations in muscle, the most eatable part of fish, indicates the high risk of human exposure.

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