E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(5): 315-328

www.fisheriesjournal.com
Received: 24-07-2019
Accepted: 28-08-2019

Mohamed I Nofal
General Authority of Fish Resources and Development (GAFRD), Manzala Fish Farm, Fish Breeding and Production, Dakahlia, Egypt

Viola H Zaki
Departments of Internal Medicine, Infectious Diseases and Fish Diseases and Management, Faculty of Veterinary Medicine, Mansoura University, Egypt

Nabil Abu-Heakal S Ahmed
Department of Physiology, Faculty of Veterinary Medicine, Mansoura University, Egypt

Abed-Aziz AF
Department of Biochemistry, Faculty of Science, Mansoura University, Egypt

Maha M Rashad
Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Cairo University, Egypt

Corresponding Author:
Mohamed I Nofal
General Authority of Fish Resources and Development (GAFRD), Manzala Fish Farm, (Fish Breeding and Production), Dakahlia, Egypt

Effects of heavy metal pollution on Nile tilapia in Manzala farm: Oxidative stress biomarkers and histopathological findings

Mohamed I Nofal, Viola H Zaki, Nabil Abu-Heakal S Ahmed, Abed-Aziz AF and Maha M Rashad

Abstract
Incidences of the outbreak have occurred among *Oreochromis niloticus* in July 2018 in Manzala fish farm. About 150 naturally intoxicated fishes were sampled from 3 ponds 50 fish from each pond and 50 fishes from Abbasa fish farm as control. Fishes were transported to the laboratory for studying clinical signs, postmortem, histopathological examination and making some laboratory investigations (measuring of physicochemical parameters, detection of heavy metals accumulation in fish organs as the liver, kidneys, gills, muscles, and brain). The results revealed that the highest mean values of zinc were found in gills > liver > kidney > muscles > brain respectively, but in case of copper the highest mean values were found in liver> brain > kidneys > gill > muscle respectively. In case of iron the highest mean values of iron were found in liver> kidneys > gill > muscle respectively. Also measure the antioxidant enzymes like SOD, CAT and GPx and concentration of MDA in liver, gills, muscles, kidneys, brain, blood hemolysate and serum where estimated. The levels of SOD revealed that a significant ($p \leq 0.05$) increase over controls was observed in the liver only, while CAT activity revealed that a significant difference ($p \leq 0.05$) increase over controls was observed in liver, kidney, blood hemolysate, and serum. On the other hand, activity of GPx significant differences ($p \leq 0.05$) over controls was observed in liver, gills, muscles, kidneys, and brain.

Keywords: Heavy metals, SOD, CAT, GPx, Nile tilapia

1. Introduction
Environmental pollutants, such as metals, pesticides, and other organics, pose serious risks to many aquatic organisms [1]. Fish is a good bio-indicator because it has the potential to accumulate heavy metals and other organic pollutants [2]. *Oreochromis niloticus* is an important species in commercial fisheries in the world promptly responds to environmental alterations [3]. Metals are major pollutants of aquatic ecosystems due to disposal of industrial effluents in the river of waste material, such as sewage sludge and dredge spoil. They are usually toxic at high levels and may accumulate in aquatic organisms as metals are not biodegradable or eliminated from ecosystems. Additionally, they may interfere in several metabolic pathways of cells and thereby induce different cellular responses depending on concentration and metal proprieties [4]. These responses may promote oxidative stress by catalyzing the formation of reactive oxygen species (ROSs) such as the superoxide anion, hydroxyl radical (OH), singlet oxygen ($O_{½}$) and hydrogen peroxide (H_2O_2), which may generate DNA alterations and peroxidation of membrane lipids initiating cellular degenerative process [5]. ROS can be detoxified by enzymatic and non-enzymatic cell defense systems that can be measured as biomarkers of xenobiotic mediated oxidative stress.
Defenses against ROS include scavenger compounds like glutathione (GSH) and metallothionein (MT), and antioxidants enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) [6]. Lipid peroxidation is one of the main indicators of oxidative damage. Thus, measurements of these indicators are a valid biomarker for monitoring free radicals, among the most important antioxidant enzymes whose activity changes with pollution, are catalase (CAT), glutathione peroxidase (GPx) [7] and superoxide dismutase (SOD) [8]. Copper and zinc play an important role in cellular metabolism acting co-factors in several important enzymes.

However, they can become toxic when elevated concentrations are introduced into the environment [9]. Also, nickel is an essential element at low concentrations for many organisms; it is toxic at higher concentrations [10]. As well as, zinc wastes have direct toxicity to fish at increased waterborne levels [11]. Histopathological evaluation is a sensitive tool intoxicant impact assessment to indicate the effect of toxicants on fish health and allows for early warning signs of disease and injury in cells, tissues, or organs [12]. Therefore, the study was focused mainly on the estimation of oxidative stress by measuring enzymatic and non-enzymatic oxidative stress compounds in blood, serum and different organs like brain, gills, muscles, kidneys, and liver of affected *O. niloticus*. Also will estimate the accumulated metals as Cu, Fe and Zn in different organs of *O. niloticus* as well as estimation of clinical signs and histopathological alteration of affected fishes during outbreaks and finally estimation of physicochemical parameters of Hadous and Manzala fish farm water supply for detection of water pollution comparatively with controlled ponds of Abbasa fish farm.

2. Materials and Methods

2.1 Fish Collection and area of study

Incidences of the outbreak occurred among Nile tilapia, *O. niloticus* on July 2018 in Manzala fish farm (Water supply from Hadous drain) collect about 150 fishes naturally intoxicated fishes 200 ± 10 g weight from 3 ponds 50 fishes from each pond and 50 fishes from Abbasa fish farm as control (water supply from river Nil), so divided the intoxicated fishes into 3 groups (group 1, 2 and 3) and transported into laboratory of fish diseases in faculty of Veterinary Medicine, Mansoura University for studying clinical signs, postmortem, histopathological and biochemical examination.

2.2 Physico-chemical examination of water samples

Water samples were collected from different parts from the 3 ponds in Manzala fish farm also from the Abbassa region as controlled during times of outbreaks. These water samples were collected in clean and dark brown coppered glass bottle for measuring of dissolved oxygen, pH, temperature, phosphorus, ammonia toxicity (NH_3), iron, copper, zinc, Nickle, hardness, and salinity these samples are transferred to the laboratory of fish diseases at Veterinary Medicine Mansoura University. The standard method for water quality control **[13]**, and then the non-ionized ammonia (NH_3) was calculated from total ammonia [14].

2.3 Clinical & postmortem examination

Clinical examination was performed [15], and postmortem examination [16].

2.4 Fish diets

Fishes were fed on a commercial fish diet containing 25% protein in pelleted form and extruding, fish was fed 3% of their body weight once a day.

2.5 Sampling

A- Blood samples

The blood samples were collected directly through the insertion of 23-gauge syringes with an acute angle from caudal vein ventral to the anal opening. They are divided into 2 parts; the first part was collected in a test tube free from EDTA and kept in room temperature till clot formation, then they were centrifuged to obtain serum at 3000 rpm for 10 minutes and kept at -20 °C. While the second part was collected utilizing EDTA (10%) to prepare erythrocyte lysates. Serum samples were used for the following determination of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), and Lipid peroxide (Malondialdehyde) (MDA) concentration.

B) Samples of tissues

Fishes were dissected after collection of blood samples to obtain gills, brain, liver, kidneys, and muscles which are stocked in 3 parts after washing by normal saline. The first part of liver, kidneys, brain, muscles, and gills were kept at -20 °C [17] for detection of SOD, CAT, and GPx activities, and MDA concentrations using spectrophotometer. The second parts of the liver, kidneys, brain, muscles, and gills were kept also at -20°C and transported to the Animal Health Research Institute, Chemistry department for atomic absorption detection of accumulated metals as Iron, Copper, and Zinc using atomic absorption (Techno sens AA 1.0.2.2.1). The third part of the liver, kidneys, brain, muscles, and gills was fully immersed in 20% formalin for histopathological examination and transported to the histopathological department in Veterinary Medicine, Cairo University.

For histopathological techniques, the obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin &eosin stain, for long-term examination, the stained slides are covered using Canada balsam and examined with a light microscope (OLYMPUS CX21), using a reference control tissue and photographed using a digital camera [18].

2.6 Detection of metals in fish organs

a) Digestion of fish samples

Muscle samples were digested according to the method applied by Agemain *et al*. [19].

b) Metals estimation

The detection and estimation of these metals collected from fish farms were carried out by using Atomic Absorption model (Techno sens AA 1.0.2.2.1) spectrometer with hydride system Thermo made in the UK (Solar Atomic Absorption spectrophotometer). Detection of metals in samples that were collected from Manzala fish farm made by Atomic Absorption Spectrometry (AAS) of Animal Health Research Institute, Dokki, Giza, Egypt. Pyrolytic coated graphite tubes with a platform were used for metals determinations by GFAAS**.**

2.7 Detection of oxidative stress compounds

By using a spectrophotometer (SPEKOL11 CARL ZEISS JENA) (Germany) by colorimetric method for the detection of oxidative stress compounds in the blood, serum, and tissues by using a commercially available chemical kit (Biodiagnostic Company, Cairo, Egypt). Spectrophotometer (Spekoll 11, Germany). The activities of SOD [20], CAT [21], GPx [22], and MDA levels was determined [23].

3. Results

3.1 Results of clinical signs of intoxicated *O. niloticus*

Intoxicated fish show loss of appetite, reduced growth, decreased activity with spasmodic movements & increased opercular movements, amounts of mucous on the body surface with dermatitis and discoloration of the skin. The gill chamber was congested, filled with mucus, congestion of the lamellae and suffocation with heavy mortalities.

Results of postmortem (PM) examination of intoxicated *O. niloticus*

There are no pathognomonic PM lesions due to pollution directly but the stress factors due to pollution make some alteration in internal organs.

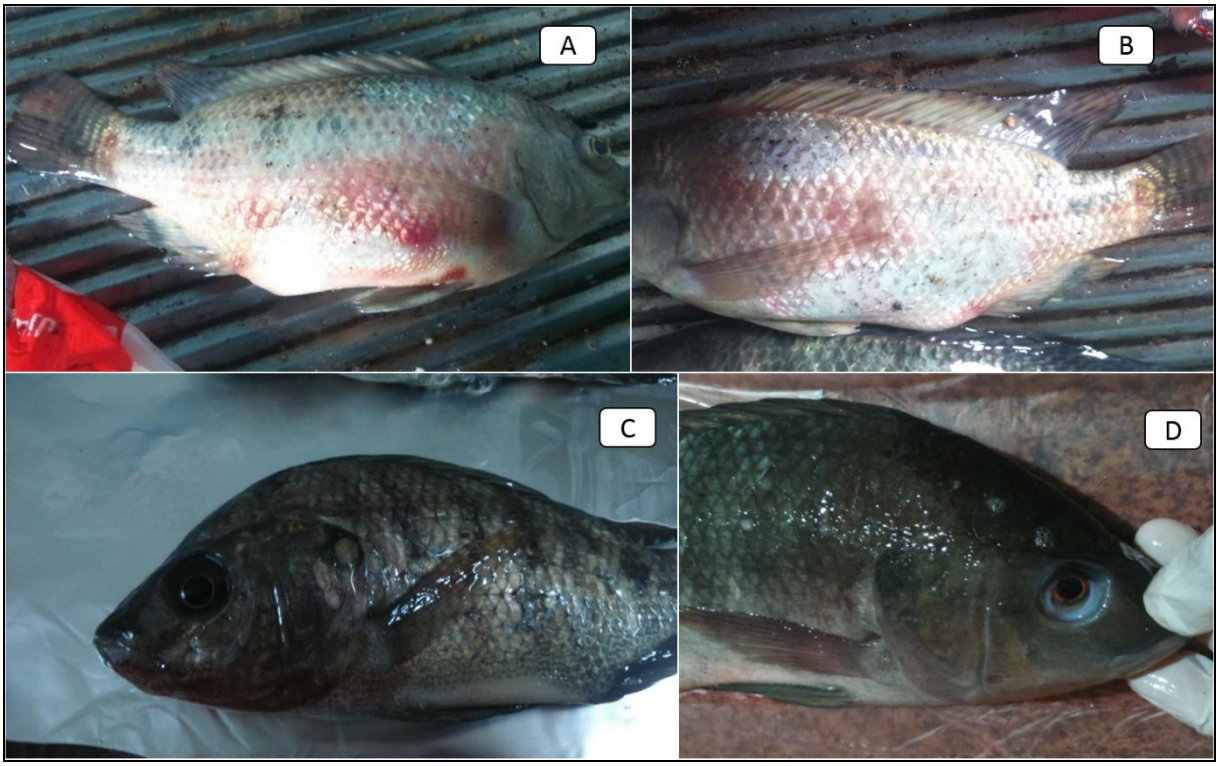

Fig 1: Intoxicated *O. niloticus* showed hemorrhagic patches on the skin and fins with large mucous (A & B), dark discoloration of the skin with large amounts of mucus (C), and emaciated and retarded growth fish (sunken eye) (D).

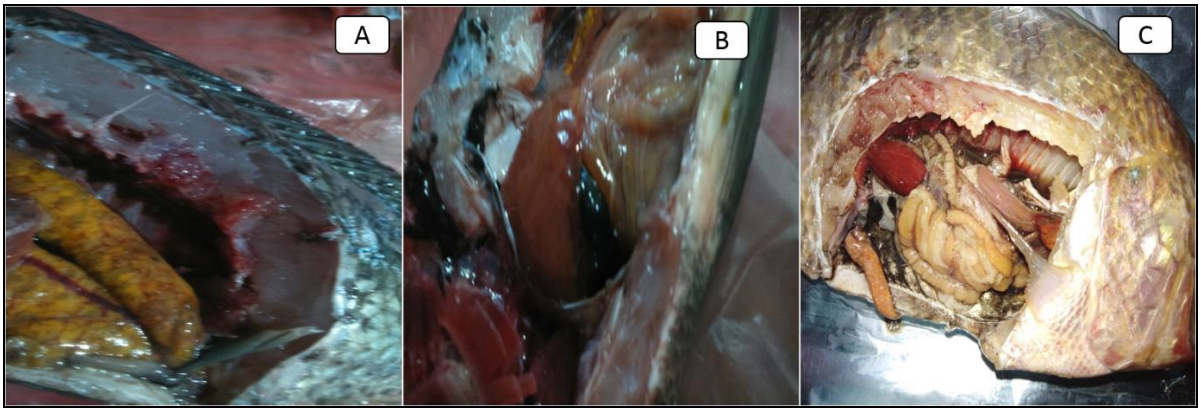

Fig 2: Intoxicated *O. niloticus* showed hemorrhagic inflammation of kidneys (A), pale discoloration and necrosis of the liver (B) and enlarged gall bladder and discoloration of the intestine (C).

Results of metals concentration in organs of intoxicated *O. niloticus*

Table 1: Zinc concentration (ppm) in tilapia tissues and organs (mean ± S.E).

Groups organs	**Control group** (Mean ± SE)	**Intoxicated group** (Mean ± SE)	**P-value**
Liver	0.48±0.04	2.47±0.12*	0
Gills	1.43±0.017	2.67±0.20*	0.003
Muscles	0.35±0.02	1.25±0.12*	0.002
Kidney	0.74±0.02	1.5067±0.21*	0.025
Brain	0.92±0.01	1.0667±0.06	0.106

Data are represented as mean ± SEM (standard error of the mean).

* indicate a significant variation between the two different groups by One-way ANOVA independent test at P≤ 0.05.

The concentration of zinc (ppm) in internal organs (liver, kidney, gills, muscles, and brain) in control and intoxicated samples were summarized in Table (1). The results in the highest mean values of zinc were found in gills > liver > kidney > muscles > brain respectively. Also, there is a significant difference between liver, kidneys, muscles, and gills (P≤ 0.05). A significant decrease in Zn concentration was observed in all organs in comparison with the permissible

limit.

Table 2: Copper concentration (ppm) in tilapia tissues and organs (mean ± S.E).

Groups organs	Control group (Mean ± SE)	Intoxicated group (Mean ± SE)	P-value
Liver	0.42 ± 0.01	2.2 ± 0.17*	0.001
Gills	0.25 ±0.02	0.3867 ± 0.05	0.082
Muscles	0.29 ± 0.005	0.3533 ±0.05	0.303
Kidney	0.64 ± 0.02	0.8467 ±0.10	0.117
Brain	0.7± 0.05	0.9267 ±0.03*	0.03

- Data are represented as mean ± SEM (standard error of the mean).
* indicate a significant variation between the two different groups by independent t-test at P≤0.05.

The concentration of copper (ppm) in the internal organs (liver, kidney, gills, muscles, and brain) in control and intoxicated samples were summarized in table (2). The results in the highest mean values of copper were found in liver > brain > kidneys > gill > muscle respectively. Also, there is a significant difference between liver, kidneys, muscles, and gills (P≤0.05). Significant decrease in Cu concentration was observed in all organs comparison with the permissible limit.

Table 3: Iron concentration (ppm) in tilapia tissues and organs (mean ± S.E).

Groups organs	Control group Mean ± SE	Intoxicated group Mean ± SE	P-value
Liver	4.92 ± 0.01	88.96± 5.62*	0
Gills	4.7 ± 0.11	8.8± 0.26*	0
Muscles	0 ± 0	2.15± 0.15*	0
Kidney	0± 0	17.2667± 0.12*	0

Data are represented as mean ± SEM (standard error of the mean).
* indicate a significant variation between the two different groups by independent t-test at P≤0.05.

The concentration of Iron (ppm) in internal organs (liver, kidney, gills, and muscles) in control and intoxicated samples were summarized in and Table (3). The results in the highest mean values of iron were found in liver > kidneys > gill > muscle respectively. Also, there is a significant difference between liver, kidneys, muscles, and gills (P≤ 0.05). A significant increase in iron concentration was observed in the liver, kidneys, gills, and muscles in comparison with the permissible limit.

Results of antioxidant enzymes in *O. niloticus*: -

Table 4: Effect of pollution on SOD concentration (U/g) in tilapia fish.

Groups organs	Control group Mean ± SE	Intoxicated group Mean ± SE	P-value
Liver	2340 ± 57.73	5377 ± 173.20 *	0
Gills	3750 ± 115.47	3555 ± 288.67	0.565
muscles	2970 ± 115.47	3300 ± 173.20	0.188
kidney	2390 ± 115.47	2205 ± 230.94	0.513
brain	2910 ± 57.73	2441 ± 230.94	0.12
blood hemolysate	3390 ± 57.73	3775 ± 404.14	0.399
serum	2440 ± 57.73	2578 ± 288.67	0.664

Data are represented as mean ± SEM (standard error of the mean).
* indicate a significant variation between the two different groups by independent t-test at P ≤ 0.05.

The results of the antioxidant activity level of SOD (U/g) due to pollution as shown in Fig. (6) and Table (4), revealed that a significant (p ≤0.05) increase over controls was observed in the liver only. However, no significant differences in SOD activity were observed in the gills, muscles, kidney, and brain.

Table 5: Effect of pollution on catalase concentration (U/g) in tilapia fish.

Groups organs	Control group Mean ± SE	Intoxicated group Mean ± SE	P-value
Liver	84.1 ± 11.54	457 ± 11.54 *	0
Gills	154.7 ± 2.30	159.35 ± 0.57	0.122
muscles	34.5 ± 2.30	36.18 ± 1.73	0.592
kidney	124.02 ± 2.30	356.55 ± 0.57 *	0
brain	36.34 ± 2.30	37.01 ± 0.57	0.792
blood hemolysate	51.2 ± 0.57	34.55 ± 0.57 *	0
serum	476.82 ± 2.30	259.445 ± 2.30 *	0

Data are represented as mean ± SEM (standard error of the mean).
* indicate a significant variation between the two different groups by independent t-test at P ≤ 0.05.

The results of the antioxidant activity level of CAT (U/g) due to pollution as shown in Table (5), revealed that a significant difference (p ≤ 0.05) increase over controls was observed in the liver, kidney, blood hemolysate and serum. However, no significant differences in CAT activity were observed in the gills, muscles, and brain. The highest mean values of CAT activity (U/g) were highest in liver > kidneys > serum > gills > brain > muscles > blood hemolysate.

Table 6: Effect of pollution on G Px concentration (U/g) in tilapia fish

Groups organs	Control group Mean ± SE	Intoxicated group Mean ± SE	P-value
Liver	155.6 ± 2.88	428.9 ± 5.77 *	0
Gills	233.4 ± 1.73	78.06 ± 5.77 *	0
muscles	155.6 ± 2.88	97.5 ± 4.04 *	0
kidney	149.5 ± 5.77	103.7 ± 1.73 *	0.002
brain	194.51± 5.77351	58.35 ± 1.1547 *	0
blood hemolysate	77.8 ± 5.7735	68.07 ± 1.1547	0.174
serum	116.7 ± 5.7735	116.7 ± 2.3094	1

Data are represented as mean ± SEM (standard error mean).
* indicate a significant variation between the two different groups by independent t-test at P ≤ 0.05.

The results of the antioxidant activity level of GPx (U/g) due to the pollution are shown in Table (6). There is revealed that a significant difference (p ≤ 0.05) increase over controls was observed in the liver, gills, muscles, kidneys, and brain. However, no significant difference in GPx activity was observed in blood hemolysate and serum. The highest mean values of GPx activity (U/g) were highest in liver > serum > kidney > muscles > gills > blood hemolysate > brain.

Results of oxidative damage lipid peroxidation (MDA) in *O. niloticus*:

Table 7: Effect of pollution on MDA concentration (nmol/g) in tilapia fish

Groups organs	Control group Mean ± SE	Intoxicated group Mean ± SE	P-value
Liver	71.81 ± 0.57	314.3 ± 0.3 *	0
Gills	295.6 ± 1.15	341.49 ± 3.22*	0
muscles	30.9 ± 1.15	43.4 ± 1.73 *	0.004
kidney	290 ± 1.15	326.5333 ± 9.20 *	0.017
brain	170.17 ± 1.15	175.7 ± 2.88	0.15
blood hemolysate	53.2 ± 1.15	65.7333 ± 5.22	0.079
serum	52.4 ± 0.57	58.3 ± 2.30	0.068

Data are represented as mean ± SEM (standard error of the mean).
* indicate a significant variation between the two different groups by independent t-test at P≤0.05.

The results of the antioxidant activity level of MDA (nmol/g) due to pollution as shown in Table (7), revealed that a significant difference ($p \leq 0.05$) increase over controls was observed in liver, muscles, kidney, and gills. However, no significant differences in MDA activity were observed in serum, brain and blood hemolysate. The highest mean values of MDA activity (nmol/g) were highest in gills > kidneys > liver > brain > blood hemolysate > serum > muscles.

Results of the physicochemical parameter of water quality

The results of water quality due to pollution as shown in Table 9, revealed that there are increasing in PH of water of 3 ponds understudy in Manzala fish farm than the permissible limit**,** also than standard pond but the dissolved oxygen was very low, also ammonia, NH3, NH4, copper, hardness, iron, phosphate, and nickel were very high higher in the 3 ponds of Manzala fish farm understudy than permissible limit and controlled Abbassa pond. The alkalinity was higher in pond No.2 of Manzala fish farm only than the permissible limit and controlled one. Nitrite and Sulphide were higher in 3 ponds of Manzala fish farm than the permissible limit and controlled one.

Table 9: The comparative water parameters of Manzala and Abbassa farm.

Farms	Manzala ponds (Hadous water supply) (mg/L)				Abbassa hatchery (River Nile water supply) (mg/L)		Range Guideline (24)
Item	Water supply	Pond (1)	Pond (2)	Pond (3)	Water supply	Pond	
oxygen	3	3.5	3.7	3.4	5	7.5	≥ 4
PH	7.5	9	9.1	9.1	7.4	8.3	6.5-8.5 mg/l
Temp.	32	32.4	32.4	32.5	30 °C	31 °C	35 °C
Salinity	1.5	1.3	1.3	1.4	0.5	0.3	0-5 g / l
NH3	over range	over range	0.75	over range	Zero	0.01	0.02 mg / l
NH4	over range	over range	0.80	over range	Zero	0.02	0.03 mg/l
Copper	0.9	1.4	1.2	1	0.14	0.28	1.0 mg / l
Hardness	470	>500	>500	> 500	130	200	500 mg / l
Iron	3.4	2.05	2.3	1.4	0.1	0.2	0.30 mg / l
Phosphate	2	1.5	1.35	1.1	0.28	0.12	0.1 mg/l
Nickel	1.45	1.1	1.55	1.3	0.1	0.05	0.1 mg / l
Zinc	0.3	0.34	0.63	0.17	Zero	Zero	3 mg / l
Alkalinity	365	>500	420	495	125	185	500 mg / l
Nitrite	0.41	0.53	0.78	0.42	0.015	0.04	0.06 mg / l
Sulphide	0.06	0.1	0.4	0.13	0.02	0.06	0.003mg / l

Results of histopathological examination:

Histopathological finding in the liver of *O. niloticus*: -

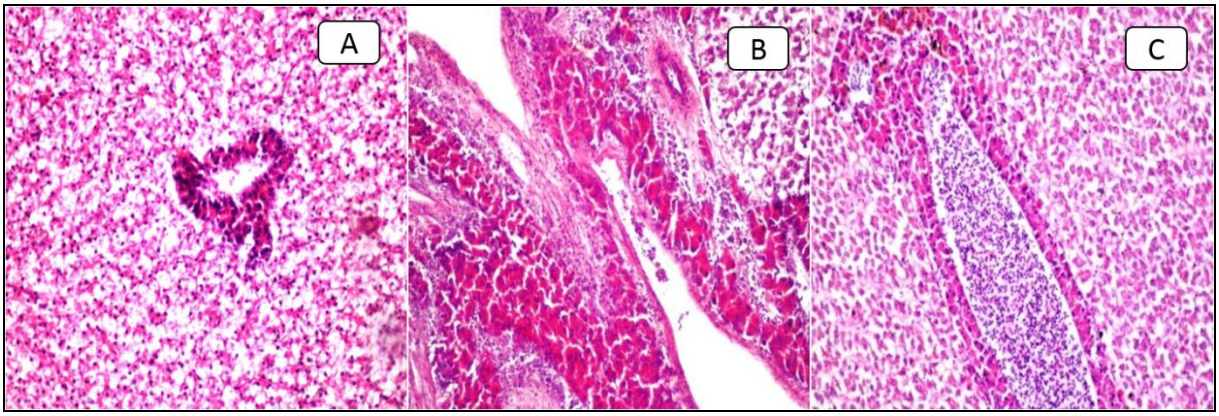

Fig 3: Histopathological finding in liver of *O. niloticus* (H &E x400) showing**: (A)** normal histopathological structure of the portal area containing portal vein and pancreatic structure and surrounded by hepatocytes of the parenchyma, **(B)** massive number of inflammatory cells infiltration in between the proliferated hyperplastic pancreatic cells at the portal areas, **(C)** Showing severs congestion of the portal vein.

Histopathological finding in the kidney of *O. niloticus*:

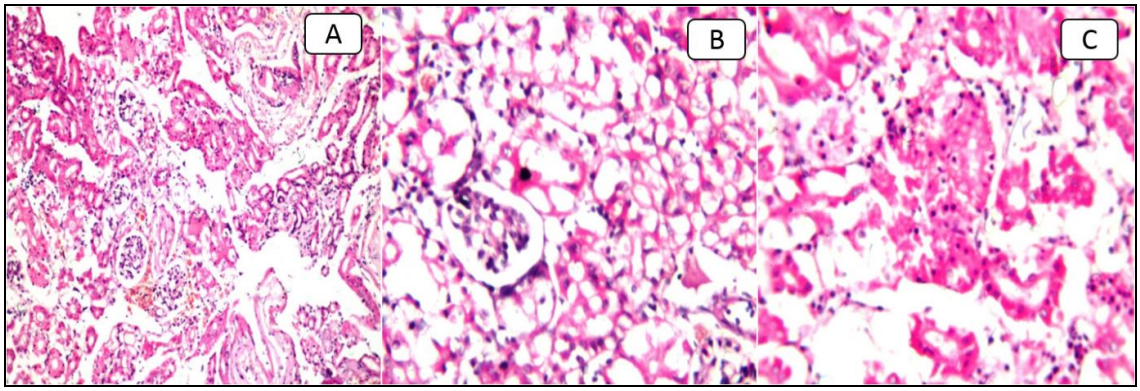

Fig 4: Histopathological finding in the kidney of *O. niloticus* (H & E X400) showing: (A) the glomeruli and tubules as normal histological structure, (B) vacuolized degeneration of the lining tubular epithelium in most of the tubules, and (C) coagulative necrosis in the tubular lining epithelium.

Histopathological finding in the gills of *O. niloticus*:

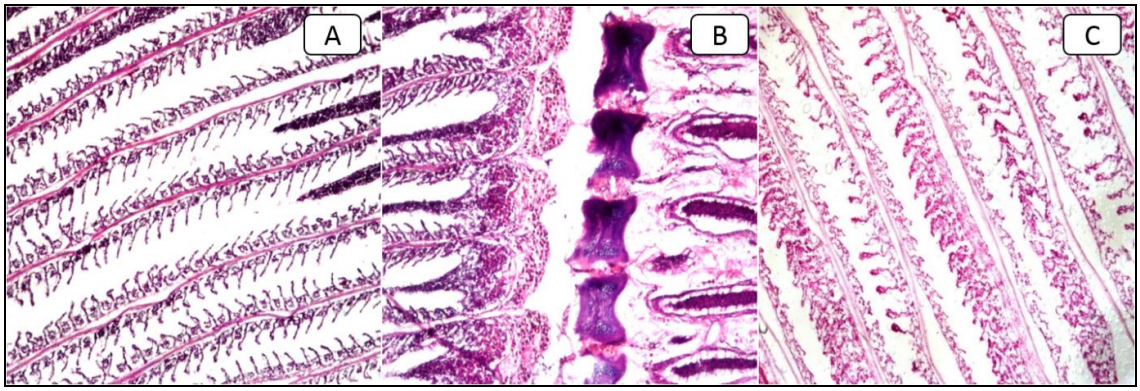

Fig 5: Histopathological finding in gills of *O. niloticus* showing; **(A)** normal histological structure of the filament with branching lamellae (H & E, X16), (B) normal histological structure of arch and base of filament with blood (H & E, X400), and (C) congestion with edema in the lamellae of the filaments (H&E x16).

Histopathological finding in the skin of *O. niloticus*: -

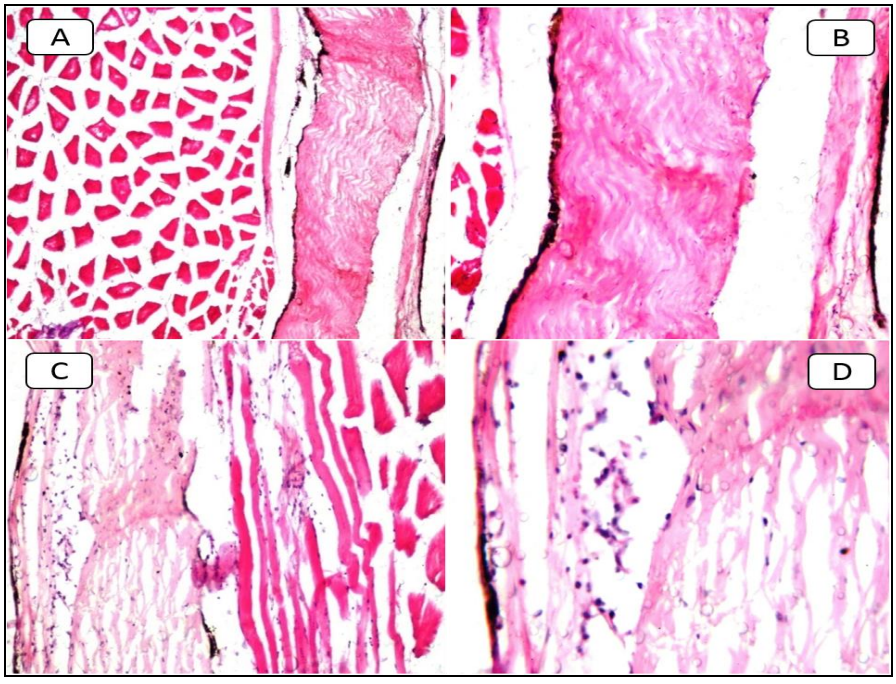

Fig 6: Histopathological finding in skin of *O. niloticus* showing; (A &B) normal histological structure of hypodermal, dermal and underlying musculature (H&E x400), (C) edema with inflammatory cells infiltration in hypodermal tissue (H&E x16), and (D) edema and inflammatory cells infiltration in hypoderm (H&E x400).

Histopathological finding in the skeletal muscles of *O. niloticus*

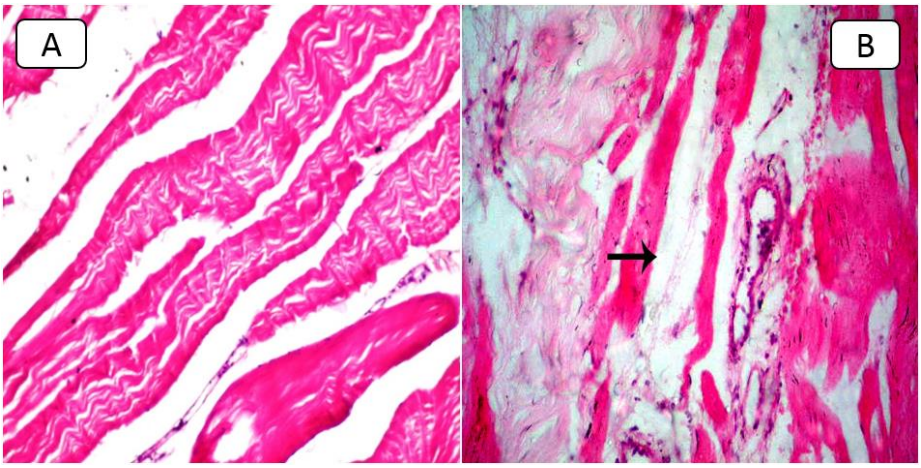

Fig 7: Histopathological finding in skeletal muscles of *O. niloticus* showing; **(A)** normal histological structure of the striated bundles (H &E, 400 x), and (B) muscle displays dilatation of blood capillaries in interstitial tissue with edema separating muscle fibers (arrow) (HE, 400x).

Histopathological finding in the brain of *O. niloticus*:

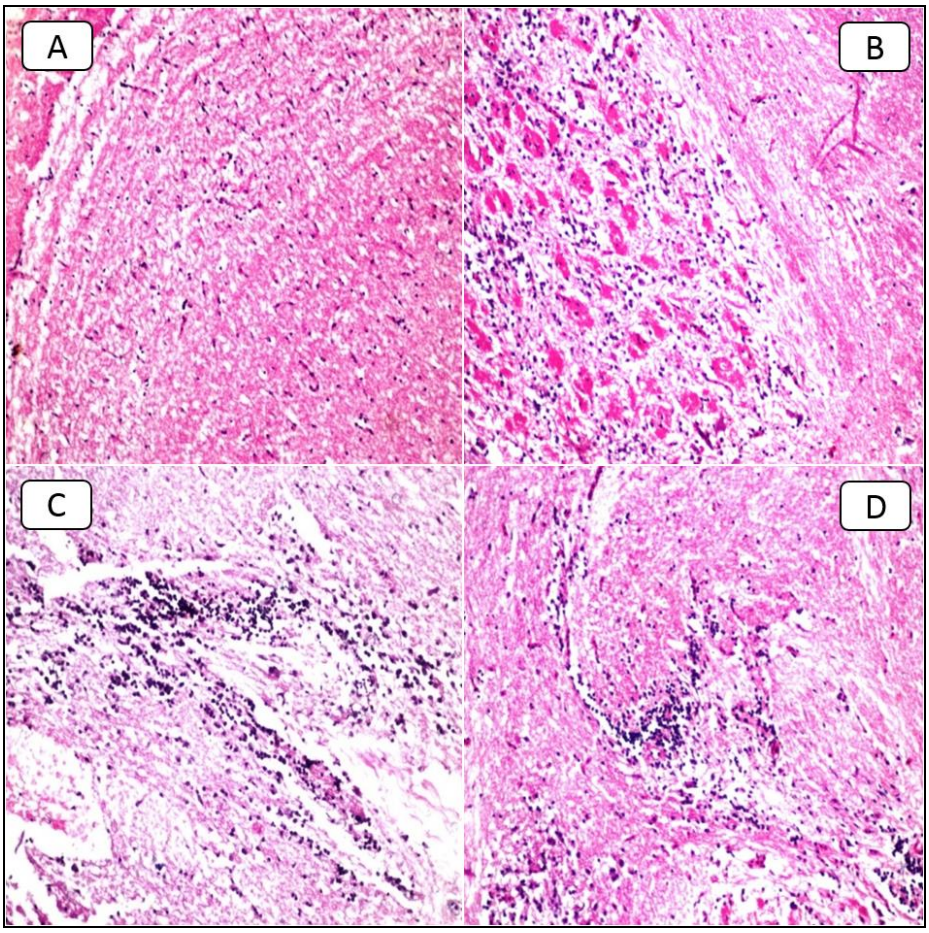

Fig 8: Histopathological finding in the Brain of *O. niloticus* showing; (A) normal histological structure of the cerebral cortex (H &E x400), (B) normal histological structure of stratum in cerebrum (H &E x400), (C) focal gliosis in cerebrum (H&E x400), and (D) focal gliosis in cerebrum (H&E x400).

4. Discussion

Water pollution is one of the principal environmental and public health problems facing Egypt and the Middle East region **[25].** *Oreochromis niloticus is* an important species in commercial fisheries in the world promptly responds to environmental alterations [3].

Regarding clinical examination in *O. niloticus*, there was loss of appetite, reduce of growth, decreased activity with spasmodic movements and increased opercular movements, amounts of mucous on the body surface with dermatitis and discoloration of the skin. The gill chamber was congested and filled with mucus, congestion of the lamellae, suffocation and finally death. These findings were like that of [26]. These are due that the gill surface of the fish tends to be alkaline, soluble ferrous iron can be oxidized to insoluble ferric compounds which then cover the gill lamellae and inhibit

respiration [27]. Also, the heavy metals like copper and zinc, individually or in combination with other metals, may exert a strong inhibitory effect on the cell division [28]. Finally, Numerous environmental stressors affect the liver and cause metabolic disturbances and structural damage, possibly leading to death [29].

Regarding the PM examination, there was hemorrhagic inflammation of kidneys due to damage, pale discoloration and necrosis of liver due to damage of liver cells, enlarged gall bladder and discoloration of intestine, blood-filled abdominal cavity. These findings were agreed with Abd El-Gawad [30].

In this study, the highest mean values of zinc were reported in gills followed by liver, kidneys, muscles, and brain respectively. Also, the values show a significant difference between liver, kidneys, muscles, and gills ($P \leq 0.05$) which was lower than the permissible limit. These results were agreed with Hogstrand [31] who reported that the main target of waterborne zn toxicity is the gills and liver also the highest mean values of copper were reported in the liver followed by brain, kidneys, gill, and muscle respectively. Also, there is a significant difference between liver, kidneys, muscles, and gills ($P \leq 0.05$) which were lower than the permissible limit.

These results were an agreement with Kaoud and El-Dahshan [32] who recommended that the copper was shown distinct affinity to accumulate in the fish liver in high amounts. This is due that the liver is the most important organ in detoxification, and storage of heavy metals [33]. On the other hand, the highest mean values of iron were reported in liver followed by kidneys, gills, and muscle respectively, also there are significant differences were observed in liver and kidney, gills and muscles ($P \leq 0.05$) in comparison with permissible limit which was higher than the permissible limit. These results were agreed with Van Rensburg [34] who reported that the highest bioconcentration of iron in fish tissues was found in the liver and gonads, decreasing in brain, muscle. Additionally, the fish liver is the target organ for iron this is due to the liver act as an important organ for uptake, accumulation, biotransformation, and excretion of toxicant [35]. Also, the gills, liver, and kidney are commonly the primary target organs for many pollutants [36].

The most important antioxidant defense systems include antioxidant enzymes such as SOD, CAT and GPx [37]. It was found that SOD converts superoxides (O_2-) generated in peroxisomes and mitochondria to hydrogen peroxide [38]. The SOD catalyzes the breakdown of the superoxide anion (O_2^-) to water and hydrogen peroxide (H_2O_2) that were further detoxified by the CAT enzyme, the SOD & CAT system gives the initial defense in combating the oxygen toxicity [39]. In the present study, our data revealed that the high activity of SOD in blood hemolysate and serum combined with decrease activity of CAT in *O. niloticus* was agreed with [40]. Also, in this study, there was a decrease in the activity of SOD in gills kidneys and brain and these results documented by [41] who reported that excess production of ROS may also inhibit the SOD activity. In the present study indicate that low activity of SOD in gills, kidneys, and brain was agreed with Falfushynska *et al.* [42] who reported that low intensity, but prolonged effect of spontaneous sources of pollution can deplete SOD activity in fish tissues, also supported by Falfushynska and Stolyar [43] who suggested that there was weakness of antioxidant defenses at the chronically polluted industrial site.

CAT plays an important role in the cell redox equilibrium [44] where removes the hydrogen peroxide by converting it to water and oxygen [41]. In the present study, there was increased activity of CAT in liver and gills which agree with [45] who reported that Catalase activity was found in the liver and gills of carps from industrially polluted site as compared to the reference site due to the presence of higher peroxide concentrations [46]. Also, the increased activity of CAT can occur due to a high pollutant impact [47]. The decreased activity of CAT in this study was observed in blood hemolysate and serum this may be due to the binding of metal ions to –SH groups of the enzyme, increased hydrogen peroxide and/or superoxide radical [48]. The depletion of CAT in blood hemolysate and serum with an increment of SOD activity was reported in this study which agrees with [40]. The reduction in CAT activity leading to decreased ability to protect cells against H_2O_2 which leading to oxidative stress which has been reported by [49] because the removal of H_2O_2 by CAT is an important strategy of organisms against oxidative stress.

GPx catalyzes the reduction of both hydrogen peroxide and lipid peroxides, thus preventing the formation of free radicals formed by peroxide decomposition [50]. In this study, our data results indicate that there was increased activity of GPX in liver of *Oreochromis niloticus is* fish which agree with Hamed *et al.* [51] who reported that increase of GPx activity in liver of *O. niloticus* and *Clarias lazera* collected from industrial polluted sites when compared to control because Fish liver can be regarded as the body's detoxification organ and hence a target organ of various xenobiotic substances [52]. Also the present study revealed that decreased activity of SOD and GPX in some tissues as gills, brain and kidneys of the *O. niloticus* fish indicated that the abilities to protect against hydrogen peroxide were reduced and are not scavenged by these antioxidant enzymes which leading to oxidative stress and causing several damages cellular for the reason that the impairment in the radical formation due to accumulation of the high levels of H_2O_2, this could be associated to the O_2^- production or to the action of metals in enzyme synthesis [52]. Because GPx depletion promotes the generation of ROS and oxidative stress which affecting the functional and structural integrity of cell and organelle membranes. In our study, we noticed there is a compensatory relationship between catalase and GPX activities: low GPX activity was combined with high catalase activity that agrees with Godin and Garnett [53] because Catalase plays an important role in the cell redox equilibrium [45] where removes the hydrogen peroxide by converting it to water and oxygen [41].

LPO is one of the main indicators of oxidative damage. MDA is the final product of lipid peroxidation and their concentration gives direct evidence of the toxic process caused by free radicals [54]. An increase in MDA content in tissues can be used as an indicator of lipid peroxidation [55]. In this study, the data revealed that there was increased levels of MDA in all tissue organs, serum and blood hemolysate of *O. niloticus* fish which is agreement with findings of Avci *et al.* [56] who reported an increase in LPO in tissues of fishes exposed to petroleum hydrocarbons, the increase in LPO is due to an inhibitory effect on mitochondrial electron transport system leading to stimulation in the production of intracellular ROS [57].

The high level in MDA of the fishes from the Manzala fish farm compared with Abbassa farm suggests that the source of the Manzala farm was polluted this may be due to transition

metals (such as Cu, Ni, and Fe) cause peroxidation of membrane polyunsaturated fatty acids. This result in the synthesis of lipid peroxyl radicals which in turn produce many lipid degradation products such as MDA. Also, LPO, a complex process resulting from free radical reactions in biological membranes, forms lipid hydroperoxides that decompose double bonds of unsaturated fatty acids and destructs membrane lipids [58].

Low oxygen concentration in water interferes with the fish population, causing death and abnormalities in the offspring. Disturb the balance oxygen supply/demand influencing oxygen levels in tissues, which interfere with antioxidant defenses [59]. In this study the results were shown decreased in dissolving oxygen in all Manzala ponds from controlled one and from the range guideline [24], which was agreed with Vidal *et al*. [60] who reported that hypoxia increased the activities of catalase and glutathione peroxidase, that was shown in this study especially in liver, gills, muscles, kidneys, and brain according to catalase but according to glutathione peroxidase was showed in the liver only that is maybe due to hypoxia was found to increase oxidative stress levels by increasing protein carbonyls in the brain, liver and skeletal muscle relative to control fish which was supported by Lushchak *et al*. [61] who reported that hypoxia increased the of activities of SOD and catalase in liver of *Cyprinus carpio*. Additionally, in this study, there was an increased activity of SOD in the liver and lipid peroxidase in all tissues of hypoxic fish that was agreed with Lushchak and Bagnyukova [62]. Finally, fish under low-oxygen conditions (hypoxia, anoxia) increasing their antioxidant capacity to enhance their ability to quench ROS production upon return to normal oxygen concentrations [63].

The increase in temperature stimulates all metabolic processes in accord with known thermodynamic principles; it enhances oxygen consumption and, therefore, may increase ROS production as side products of intensified metabolism resulting in oxidative stress in fish [64]. Temperature and pH affect the catalytic efficiency and binding capacity of enzymes [65]. In this study, there was increased water temperature of Manzala ponds than that of controlled one, but lower than that of permissible limits. On the other hand, there was increased in water pH of Manzala ponds than that of controlled one and the range guidelines [24]. The present results were agreed with Osman *et al*. [66] who reported that the High levels of heavy metals during spring and summer could be attributed to the changes associated with higher water temperatures, which can cause higher activity and ventilation rates in fish where in this study there are high levels of heavy metals especially zinc, copper and iron.

Ammonia is a toxic metabolite and excess ammonia is known to trigger the operation of detoxification or utilization systems, chiefly by way for the formation of less toxic nitrogenous substances [67]. The excessive presence of NH_3 alters cellular metabolism, resulting in decreased cellular concentrations of ATP [68]. In this study our results revealed that there was increased in all levels of total ammonia, toxic ammonia (NH_3) and non-ionized ammonia (NH_4) in all ponds of Manzala fish farm from the controlled one and also from the range guideline by very high great ratio that was agreed with who reported that ammonia induce antioxidant defenses as (CAT), superoxide dismutase (SOD) in the liver of fish which supported by Hegazi *et al*. [69] who reported that Chronic ammonia exposure significantly increased SOD activity which was approved in our results where there are increased in SOD activity in liver, blood, and serum. On the other hand the present results were agreed with Sinha *et al*. [70] who reported that exposure fish to high environmental ammonia (HEA) resulted in induced production of H_2O_2, increased activity of CAT, increased lipid peroxide content (MDA), increased levels of GPx in the liver and no change of GST activity in the liver of goldfish and carp that is due to ammonia exposure can lead to oxidative stress in fish species.

Copper shows a distinct affinity to accumulate in the fish liver [71]. The oxidative stress was demonstrated to be induced by both dietary and waterborne exposure to high copper, or even by copper injection in fish [72]. In this study our results revealed that the copper was higher in pond 1 and pond 2 in Manzala fish farm than that of controlled one and that of range guideline but equal in pond number (3) of Manzala farm to range guideline. In the present, results have been agreed with Hansen *et al*. [73, 74] who reported that fish exposure to metal ions, particularly to copper, enhances the activities of primary (SOD, CAT, GPx) where there was increased of these antioxidant enzymes especially in the liver this is due to copper elevation induce cytotoxicity and enhanced ROS formation [75] also generate $(OH)^-$ through Fenton reaction.

In the present study our results revealed that there were increased levels of hardness in Manzala ponds over that of controlled one and also over the range guidelines [24] and increased activity of SOD, CAT and GPx in the liver which was agreed with (Saglam *et al*. [76] who reported that in the hard water there was increased concentration of copper in the water, increased activity of SOD, CAT and GPx in liver of *Oreochromis niloticus* fish this is due to hardness affects fish physiology and metal bioavailability and consequent metal uptake by fish [77] which leading to an oxidative stress due to production of ROS, so not only heavy metal induces oxidative stress but also hardness of water may cause the oxidative stress responses so the increase in SOD, CAT, and GPx activity may be related to cope with the increased oxidative stress caused by metal exposures.

Fish obtain iron from water by uptake across the gill epithelium and by intestinal uptake from food [78]. Because the gill surface of the fish tends to be alkaline, soluble ferrous iron can be oxidized to insoluble ferric compounds which then cover the gill lamellae and inhibit respiration.

In the present study there were increased levels of iron in all Manzala ponds over than that of controlled one and also over the range guidelines [24] which was agreed with Bagnyukova *et al*. [79] who reported that a strong positive correlation between lipid peroxidation products and the activities of catalase in liver indicated the possible up-regulation of the enzymes and iron this may be due to elevated production of superoxide anions which increase the release of free iron [80]. Also our results were agreed with Ruas *et al*. [81] who reported that significant increases in SOD activity and higher levels of LPO were observed in erythrocytes of fish with the highest levels when the concentration of iron in water was elevated that is due to the heavy metals iron present in polluted water of Manzala farm enter the fish, accumulates in fish tissues and exert toxic effect by the generation of ROS through their redox property thus oxidative stress experienced by these fish during such metal exposure is counteracted using antioxidant defense system.

Phosphorus is one essential major mineral to fish [82], in our study the concentration of phosphorus (po_4) in the water was very high which disagreed with Boyd [83] who reported that

the concentration of this element is low in freshwater. Thus, due to increased concentration of phosphorus in the polluted area leading to increased activity of SOD and CAT which responding more quickly thereby protecting organisms from oxidative stress [84].

Nitrite (NO_2^-) is commonly present as a contaminant in the aquatic environment and toxic to aquatic organisms. In the present study our results reveled that there was increased levels of nitrites in the three ponds under study of Manzala (pond1, 2and3) over than controlled one also over than the range guide line [24] also there was decreased activity of SOD and GPX also decrease concentration of GSH and MDA in the gills which were agreed with Rui Jia *et al*. [85] who reported that in gills, nitrite (0.4 and/or0.8 mM) apparently reduced the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) but increased the formation of malondialdehyde (MDA) this was due to potential routes for nitrite accumulation have been shown to occur across the intestinal and gill epithelium [86], also High concentration of nitrite is a potential factor triggering stress in aquatic organisms [87] because the toxic action of nitrite is the conversion of hemoglobin into methemoglobin, which is not able to carry oxygen resulting in the induced methemoglobinemia [88], all the previous were supported by several studies demonstrated that nitrite exposure in aquatic ecosystems could enhance the intracellular formation of ROS [89] where the ROS able to attack antioxidant defense system, leading to the loss of antioxidant components (SOD, GPx and GSH).

Hydrogen sulfide was known as a toxic pollutant, the main effects of sulfide poisoning are the loss of central respiratory drive due to lesions in the brain stem, and inhibition of cytochrome oxidase, leading to impaired aerobic energy metabolism [90].

In the present study our results revealed that there were increased levels of sulfide in Manzala fish farm ponds than that of controlled one and the range guideline [24], that result was agreed with Eissa *et al*. [91] who reported that decreasing solubility of oxygen also at high-temperature increase levels of ammonia, dihydrogen sulfide (H_2S) and alkalinity which dangerously affecting the fishes health also was agreed with Nofal and Abdel-Latif [92] who reported that water analysis during an outbreak in summer season in manzala farm revealed that there are increases in water temperature, iron, copper, nitrite, dihydrogen sulfide and decreasing in dissolving oxygen may due to increasing water temperature.

Histopathological changes in animals' tissues are reliable and direct indicators of environment stressors. It is also the easiest method for assessing both short- and long-term toxic effects [93]. The liver is the most organs associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply [94].

In the present study, our results revealed that there was a massive number of inflammatory cells infiltration in between the proliferated hyperplastic pancreatic cells at the portal areas and severs congestion of the portal vein while the controlled sample showing normal histopathological stricture which was agreed with Figueiredo-Fernandes *et al*. [95] who reported that the adverse effects of heavy metals were vacuolar hydropic, degeneration of cytoplasm in hepatocytes, which were finally necrotic and infiltrated with inflammatory cells that is due to exposure of Nile tilapia to sublethal levels of cu has been shown to cause histopathological alterations in liver [96]. Also, the cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular hemolysis observed in the blood vessels with subsequent stasis of blood [97].

In the present study the result of histopathological alteration of kidneys of *O. niloticus* fish due to pollution was vascular degeneration of the lining tubular epithelium in most of the tubules and coagulative necrosis in the tubular lining epithelium while controlled one was showing normal histological structure which was agreed with Schwaiger *et al*. [98] who reported that the histopathological alteration of kidneys of fish due to contamination was primarily of vacuolation, hypertrophy, and single-cell necrosis of tubular epithelial cells such as a granulomatous nephritis, accompanied by degenerative and necrotic changes of hematopoietic cells and of excretory renal tissue that results were supported by Salim and Kadhim [99] who reported that glomerular degeneration in Bowman's spaces; reduce hemopoietic tissue, peritubular edema, perirenal adipose tissue with inflammatory cells and vacuolation of cortical tubules this due to heavy metals toxicity can result in lower energy levels and damage to blood composition, kidneys, liver, and other vital organs [24] also kidneys are the metabolically active tissues, which possess high bioaccumulation ability [100].

The gills are more exposed to contaminated water and metals can penetrate through their thin epithelial cells [101], also the gills perform various vital functions (respiration, osmoregulation, and excretion) and have a large surface area in contact with the external environment. In the present study the result of histopathological alteration of gills of *O. niloticus* fish due to pollution was congestion with edema in the lamellae of the filaments of intoxicated samples while controlled one was showing normal histological structure of the filament with branching lamellae that result was agreed with Figueiredo-Fernandes *et al*. [95] who reported that exposure of Nile tilapia to sublethal levels of Cu has been shown to cause histopathological alterations in gills (edema and vasodilation of the lamellar vascular axis) also supported by Rosety-Rodríguez *et al*. [102] who suggested that when fishes suffer a more severe type of stress an inflammatory response could be occurred as lamellar aneurysms and blood congestion with dilation of marginal channels together with leukocyte infiltration all these results may be due to due to increase of ammonia, heavy metals, pH change and oxygen depletion.

In the present study the result of histopathological alteration of skin and muscles of *O. niloticus* fish due to pollution was edema with inflammatory cells infiltration in hypodermal tissue and dilatation of blood capillaries in interstitial tissue with edema separating muscle fibers that results were agreed with Tayel *et al*. [103] who reported that periphery inflammatory cells with some aggregate of glomeruli like under its connective tissue with infiltration of inflammatory cells, subcutaneous adipose tissue partly with fibrosis. A muscle with vacuolation, small blood vessels were in between, these alterations in skin and muscles may be attributed to inorganic fertilizers, ammonia, heavy metals and changes in water quality [104]. In the present study the result of histopathological alteration of the brain of *O. niloticus* fish due to pollution was focal gliosis in cerebrum of intoxicated fish while the controlled one shown normal histological structure of the cerebral cortex and striatum in cerebrum that results were agreed with Moustafa and El-Sayed [105] who

reported that brain of carp fish caught from area polluted with heavy metals showing gliosis (meningitis) which supported by Abdullah *et al*. [106] who reported the same result in *O. niloticus* at Qassim region, KSA. These results may because of copper on the brain which induces decreasing in muscarinic cholinergic receptor numbers in the brains though the mechanisms of action remain unclear. Also, the toxicant exposure of copper disrupted the brain serotonin and/ or dopamine levels [107] this explains the spasmodic movements of intoxicated fish before mortality. On the other hand, in this result, there was an increased level of MDA in the brain which indicates oxidative damage which induce a toxic process caused by free radicals, these explain the evidence of gliosis or meningitis.

5. Conclusions

Heavy metals and deteriorated water quality parameters caused a substantial toxicity signs in the exposed fish at Manzala.

6. References

1. Wood CM. Toxic responses of the gill. In: Schlenk, D., Benson, W.H. (Eds.), Target Organ Toxicity in Marine and Freshwater Teleosts. Taylor and Francis, London, UK. 2001, 1-89.
2. Ahmed AK, Shubaimi-Othman. Heavy metal Concentration in Sediments and Fishes from Lake Chini, Pahang, Malaysia. Asia network for scientific information. 2010; 1727-3048:93-100.
3. Vijayan MM, Morgan JD, Sakamoto T, Grau EG, Iwama GK. Food deprivation affects seawater acclimation in tilapia: hormonal and metabolic changes. J Exp. Biol. 1996; 199:2467-2475.
4. Monteiro DA, Rantin FT, Kalinin AL. Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinx, Bryconamazonicus (Spix and Agassiz, 1829). Ecotoxicology. 2010; 19:105-123.
5. Sanchez W, Palluel O, Meunier L, Coquery M, Porcher JM, Ait-Aissa S. Copper induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. Environ. Toxicol. Phar. 2005; 19:177-183.
6. Storey KB. Oxidative stress: animal adaptations in nature. Braz. J Med. Biol. Res. 1996; 29:1715-1733.
7. Zheng JL, Zhu QL, Wu CW, Zhu AY, Shen B, Zeng L. Zinc acclimation mitigated high zinc induced oxidative stress by enhancing antioxidant defenses in large yellow croaker *Pseudosciaena crocea*. Aquat Toxicol. 2016; 172:21-29.
8. Doherty VF, Ogunkuade OO, Kanife UC. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in some selected fishes in Lagos, Nigeria. American-Eurasian J Agric Environ Sci. 2010, 7(3):359-365.
9. Karan V, Vitorovic S, Tutundzic V, Poleksic V. Functional enzymes activity and gill histology of carp after copper sulfate exposure and recovery. Ecotoxicol. Environ. Saf. 1998; 40:49-55.
10. Magyarosy A, Laidlaw RD, Kilaas R, Echer C, Clark DS *et al*. Nickel accumulation and nickel oxalate precipitation by Aspergillus Niger. Appl Microbiol Biotechnol. 2002; 59:382-388.
11. Niyogi S, Wood CM. Interaction between dietary calcium supplementation and chronic waterborne zinc exposure in juvenile rainbow trout, *Oncorhynchus mykiss*. Comp Biochem Physiol C. 2006; 143:94-102.
12. Butchiram MS, Vijaya Kumar M, Tilak KS. Studies on the histopathological changes in selected tissues of fish *Labeorohita* to phenol, Jrnl. Envi. Biology. 2013; 34:247-251.
13. APHA. Standard Methods for Water and Wastewater Examination and Tests. New York, USA: APHA, American Public Health Association, 2005.
14. Boyd CE. Water Quality in Ponds for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama, 1990.
15. Austin B, Austin DA. Bacterial fish pathogens: Diseases in farmed and wild fish. Ellis Harwood Limited England, 1987, 250-262.
16. Amlacher E. Textbook of fish disease. T.F.H. Publicatios, Neatune city, New Jercy, 1970, 117-135.
17. Öztürk M, Özözen G, Minareci O, Minareci E. determination of heavy metals in fish, water and sediments of Avsar Dam Lake In Turkey. Iran. J Environ. Health. Sci. Eng., 2009; 6(2):73-80.
18. Banchroft JD, Stevens A, Turner DR. theory and practices of histological techniques. fourth Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo, 1996.
19. Agemain H, Sturtevant DP, Austen KD. Simultaneous acid extraction of six trace metals from fish tissue by holblock digestion and determination by atomic absorption spectrometry analyst, 1980, 105-125.
20. Nishikimi M, Roa NA, Yogi K. The occurrence of superoxide anion in the reaction of reduced phenazines methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 1972; 46:849-854.
21. Aebi H. Methods Enzymol. 105, 121-126 Fossati, P., et. al. (1980) Clin. Chem. 1984: 26:227-231.
22. Ohkawa, Hirosh, Ohishi W, Yagi K. Lipid peroxidation (malondialdehyde). Colorimetric Meth. Analyti. Biochem. 1979; (95):351-358.
23. Paglia WN, Valentine. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Paglia DE, Valentine WN. PMID: 6066618 [Indexed for MEDLINE]. MeSH terms. Chemical Precipitation, 1967.
24. WHO, Guideline for Drinking Water Quality. Health Criteria and Supporting Information, 1984; 2:63-315.
25. Anwar WA. Environmental health in Egypt. Int. J. Hyg. Environ. Health. 2003; 206(4, 5):339-350.
26. Svobodová Z. Water Quality and Fish Health. FAO, Rome, EIFAC technical paper No. 1993; 54:67.
27. Abbas HH, Zaghloul KH, Mousa MA. Effect of some heavy metal pollutants on some biochemical and histopathological changes in Blue tilapia, *Oreochromis aureus*. Egypt. J. Agric. Res 2002; 80:1395-1411.
28. Unyayar S, Celik A, Cekic FO, Gozel A. Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Viciafaba*. Mutagenesis, 2006, 77-81.
29. Brusle J, Anadon GG. The structure and function of fish liver. In: Munshi JSD and Dutta HM, editors. Fish morphology. Boston: Massachusetts. 1996, 77-93.
30. Abd El-Gawad AM, Histopathological studies on the liver and gills of Tilapia nilotica (*Oreochromis niloticus*) exposed to different concentrations of lead acetate and zinc sulphate. J Egypt Ger Soc Zool. 1999; 30:13-22.

31. Hogstrand C. Zinc. Academic Press, New York, USA, 2011.
32. Kulac B, Atli G, Canli M. Investigations on the ATPase activities and cadmium uptake in freshwater fish *Oreochromis niloticus* following exposures to cadmium in ıncreased salinity. Turkish Journal of Fisheries and Aquatic Sciences. 2012; 12:861-869.
33. Malik N, Biswas AK, Qureshi TA, Borana K, Virha R. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. *Environ Monit Assess*, 2010; 160(1-4):267-276.
34. Van Rensburg EL. The bioconcentration of atrazine, zinc and iron in Tilapia sparrmanii (Cichlidae). M. Sc. Thesis, Rand Afrikaans University, South Africa, 1989.
35. Pedlar RM, Klaverkamp JF. Accumulation and distribution of dietary arsenic in Lake Whitefish (*Coregonus clupeaformis*). Aquat. Toxicol. 2002; 57:153- 166.
36. Cerqueira CC, Fernandes MN. Gill tissue recovery after copper exposure and blood parameter responses in the tropical fish Prochilodusscrofa. Ecotoxicol. Environ. Saf. 2002; 52:83-91.
37. Abdel-Moneim AM, Abu El-Saad AM, Hussein HK. Gill oxidative stress and histopathological biomarkers of pollution impacts in tilapia Oreochromis niloticus from Mariut and Edku Lakes (Egypt). Journal of Aquatic Animal Health. 2012; 24:148-160.
38. Otitoloju A, Olagoke O. Lipid peroxidation and antioxidant defense enzymes in *Clarias gariepinus* as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons. *Environment Monitoring Assessment*, 2011; 182:205-213.
39. Sheriff SA, Balasubramanian S, Baranitharan R, Ponmurugan P. Synthesis and *in vitro* antioxidant functions of protein hydrolysate from backbones of *Rastrelliger kanagurta* by proteolytic enzymes. Saudi J. biol. Sci. 2014; 21:19-26.
40. Huang DJ, Zhang YM, Song G, Long J, Liu JH, Ji WH. Contaminants induced Oxidative Damage on the Carp *Cyprinus carpio* Collected from the Upper Yellow River, China. *Environmental Monitoring and Assessment*. 2007; 128:483-488, ISSN 0167-6369.
41. Min EY, Ju-Chan K. Effect of waterborne benomyl on the hematological and antioxidant parameters of the Nile tilapia, Oreochromis niloticus. Pestic. Biochem. Physiol. 2008; 92(3):138-143.
42. Falfushynska H, Gnatyshyna L, Priydun Ch, Stoliar O, Nam YK. Variability of Responses in the Crucian Carp *Carassius carassius* from Two Ukrainian Ponds Determined by Multi-marker Approach, Ecotoxicology and Environmental Safety. 2010; 73:1896-1906, ISSN 0147-6513.
43. Falfushynska H, Stolyar O. Responses of Biochemical Markers in Carp *Cyprinus carpio* from Two Field Sites in Western Ukraine, *Ecotoxicology and Environmental Safety*, 2009; 72:729-36, ISSN 0147-6513.
44. Shi Y, Vaden DL, Ju S, Ding D, Geiger JH, Greenberg ML Genetic perturbation of glycolysis results in inhibition of de novo inositol biosynthesis. J Biol Chem 2005; 280(51):41805-10.
45. Lushchak, Oksana B Stoliar, Volodymyr I. Environmental Pollution and Oxidative Stress in Fish, Oxidative Stress - Environmental Induction and Dietary Antioxidants, Dr. Volodymyr Lushchak (Ed.), 2012; ISBN: 978-953-51-0553-4.
46. Liu H, Wang W, Zhang J, Wang XR. Effects of copper and its ethylenediaminetetraacetate complex on the antioxidant defenses of the goldfish, Carassius auratus. Ecotoxicol. Environ. Saf. 2006; 65:350-354.
47. Lushchak VI. Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comparative* Biochemistry and Physiology. Toxicology & Pharmacology: CBP, 2011; 153(2):175-190. ISSN 1532-0456.
48. Atli G, Alptekin O, Tukel S, Canli M. Response of catalase activity to Ag2+, Cd2+, Cr2+, Cu2+ and Zn2+ in five tissues of freshwater fish Oreochromis niloticus. Comp. Biochem. Physiol. 2006; 143C(2):218-224.
49. Papagiannis I, Kagalou I, Leonardos J, Petridis D, Kalfakakou V. Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece). Environ. J. 2004; 30: 357-362.
50. Scholz RW, Cook LS, Todhunter DA. Distribution of selenium-dependent and non-selenium-dependent glutathione peroxidase activity in tissues of young cattle. Am. J. Vet. Res., 1981; 42(10):1724-1728.
51. Hamed RR, Farid NM, Elowa SE, Abdalla AM. Glutathione related enzyme levels of freshwater fish as bioindicators of pollution. Environmentalist. 2003; 23:313-322.
52. Padmini E, Usha Rani M. Evaluation of oxidative stress biomarkers in hepatocytes of grey mullet inhabiting natural and polluted estuaries. Sci. Total Environ. 2009; 407:4533-4541.
53. Godin DV, Garnett ME. Species-related Variations in Tissue Antioxidant Status- I. Differences in Antioxidant Enzyme Profiles. *Comparative Biochemistry and Physiology*, 1992; 103(3):737-742, ISSN 1879-1107.
54. Sieja K, Talerczyk M. Selenium as an element in the treatment of ovarian cancer in women receiving chemotherapy. Gynecol. Oncol. 2004; 93:320-327.
55. Gultekin F, Akdogan M. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (*in vitro*), 2000; 74(9):533-538.
56. Avci A, Kacmaz M, Durak I. Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. Ecotoxicology and Environmental Safety, 2005; 60:101-105.
57. Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. Journal of Environmental Pathology, Toxicology and Oncology. 2001; 19:201-213.
58. Maiti AK, Saha NK, Paul G. "Effect of lead on oxidative stress, Na+K+ATPase activity and mitochondrial electron transport chain activity of the brain of *Clarias batrachus L*." Bulletin of Environmental Contamination and Toxicology, 2010; 672-676.
59. Oliveira M, Ahmad I, Maria VL, Pacheco M, Santos MA. Antioxidant responses versus DNA damage and lipid peroxidation in golden grey mullet liver: a field study at Ria de Aveiro (Portugal). Arch. Environ. Con. Tox. 2010; 59:454-463.
60. Vidal ML, Bassères A, Narbonne JF. Influence of temperature, pH, oxygenation, water-type and substrate on biomarker responses in the freshwater clam Corbicula fluminea (Müller). Comp. Biochem. Physiol. C. 2002; 132:93-104.
61. Lushchak VI, Bagnyukova TV. Hypoxia induces

oxidative stress in tissues of a goby, the rotan *Perccottusglenii*. *Comparative Biochemistry and Physiology Part B*, 2007; 148:390-397.
62. Lushchak VI, Bagnyukova TV, Lushchak OV, Storey JM, Storey KB. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (Cyprinus carpio) tissues. Int. J Biochem. Cell Biol. 2005; 37:1319-1330.
63. Lushchak VI, Bagnyukova TV. Temperature increase results in oxidative stress in goldfish tissues. R. Antioxidants and associated enzymes. Comparative Biochemistry and Physiology Part C. 2006; 143:36-41.
64. Bagnyukova TV, Danyliv SI, Zin'ko OS, Lushchak VI. Heat shock induces oxidative stress in rotan Perccottusglenii tissues. J. Therm. Biol. 2007; 32:255-260.
65. Carvalho CS, Fernandes MN. Effect of copper on liver key enzymes of anaerobic glucose metabolism from freshwater tropical fish Prochiloduslineatus. Comp. Biochem. Physiol. 2008; 151A:437-442.
66. Osman AGM, Abd El Reheem AMA, AbuelFadl K, GadEl-Rab A. Enzymatic and Histopathologic Biomarkers as Indicators of Aquatic Pollution in Fishes. Natural Science. 2010; 2:1302-1311.
67. Begum G. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish Clariasbatrachus (Linn) and recovery response. Aquat. Toxicol. 2004; 66:83-92.
68. Costa WM, Glvez AO, Brito LO, Santos EL. Produço de ortofosfato, amônia, nitrito e nitrato no cultivo de Litopenaeus vannamei utilize ando diet as. com diferentesnveis de protena vegetal e animal. B. Inst. Pesca, Sao Paulo. 2008; 34(2):303-310.
69. Hegazi MM, Attia ZI, Ashour OA. Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure. Aquat. Toxicol. 2010; 99(2):118-125.
70. Sinha AK, AbdElgawad H, Giblen T, Zinta G, De Rop M *et al*. Anti-Oxidative Defences Are Modulated Differentially in Three Freshwater Teleosts in Response to Ammonia-Induced Oxidative Stress. PLoS ONE. 2014; 9(4):e95319.
71. Jezierska B, Witeska M. The metal uptake and accumulation in fish living in polluted waters. from book Soil and Water Pollution Monitoring, Protection and Remediation (pp.107-114),Chapter January 2006 *with* 1,863 Reads, DOI: 10.1007/978-1-4020-4728-2_6, Publisher: 2006, 1568-1238.
72. Baker RTM, Handy RD, Davies SJ, Snook JC. Chronic dietary exposure to copper affects growth, tissue lipid peroxidation, and metal composition of the grey mullet, Chelonlabrosus. Mar. Environ. Res. 1998; 45:357-365.
73. Hansen BH, Romma S, Softeland LI, Olsvik PA, Andersen RA. Induction and activity of oxidative stress-related proteins during waterborne Cu-exposure in brown trout (Salmo trutta). Chemosphere. 2006a; 65:1707-1714.
74. Hansen BH, Romma S, Garmo OA, Olsvik PA, Andersen RA. Antioxidative Stress Proteins and Their Gene Expression in Brown Trout (*Salmo trutta*) from Three Rivers with Different Heavy Metal Levels. *Comparative Biochemistry* and *Phisiology*, 2006b; 143(C):263-274, ISSN ISSN 1532-0456.
75. Bopp SK, Abicht HK, Knauer K. Copper-induced oxidative stress in rainbow trout gill cells. Aquat. Toxicol. 2008; 86:197-204.
76. Saglam D, Gülüzar Atli, Zehra Dogan, Emine Baysoy, Ceren Gurler, Ali Eroglu *et al*.: Response of the Antioxidant System of Freshwater Fish (*Oreochromis niloticus*) Exposed to Metals (Cd, Cu) in Differing Hardness Turkish Journal of Fisheries and Aquatic Sciences 2014; 14:43-52, ISSN 1303-2712.
77. Monserrat JM, Martinez PE, Geracitano LA, Amado LL, Martins CMG, Pinho GLL *et al*. Pollution biomarkers in estuarine animals: critical review and new perspectives. Comparative Biochemistry and Physiology, 2007; 146:221-234.
78. Bury NR, Walker PA, Glover CN. Nutritive metal uptake in teleost fish. J. Exp. Biol. 2003; 206:11-23.
79. Bagnyukova TV, Chahrak OI, Lushchak VI. Coordinated response of goldfish antioxidant defenses to environmental stress. Aquatic Toxicology. 2006; 78:325-331.
80. Emerit J, Beaumont C, Trivin F. Iron metabolism, free radicals, and oxidative injury. Biomedicine and Pharmacotherapy. 2001; 55:333-339.
81. Ruas CBG, Carvalho CD, Araujo HSS, Espindola ELG, Fernandes MN. Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river. Ecotoxicology and Environmental Safety. 2008; 71:86-93.
82. Pimentel-Rodrigues A, Oliva-Teles A. Phosphorus requirements of gilthead sea bream (*Sparus aurata* L.) juveniles. Aquac Res. 2001; 32:157e61.
83. Boyd CE. Craftmaster Printers Inc; 1981.
84. Dewez D, Geoffory L, Vernet G, Popovic R. Determination of photosynthetic and enzymatic biomarkers sensitivity used to evaluate toxic effects of copper and fludioxonil in alga Scenedesmus obliquus. Aquat. Toxicol. 2005; 74:150-159.
85. Rui Jia, Cen Han, Ji-Lin Lei, Bao-Liang Liu, Bin Huang, Huan-Huan Huo *et al*. Effects of nitrite exposure on haematological parameters, oxidative stress and apoptosis in juvenile turbot (*Scophthalmus maximus*). Aqua Tox., 09.016, 2015
86. Tomasso J. Environmental nitrite and aquaculture: a perspective. Aquacult. Int. 2012; 20:1107-1116.
87. Sampaio L, Wasielesky W, Miranda-Filho KC. Effect of salinity on acute toxicity of ammonia and nitrite to juvenile Mugil platanus. Bull. Environ. Contam. Toxicol. 2002; 68:668-674.
88. Madison BN, Wang YS. Haematological responses of acute nitrite exposure in walleye (*Sander vitreus*). Aquat. Toxicol. 2006; 79:16-23.
89. Jensen FB, Gerber L, Hansen MN, Madsen SS. Metabolic fates and effects of nitrite in brown trout under normoxic and hypoxic conditions: blood and tissue nitrite metabolism and interactions with branchial NOS, Na+/K+-ATPase and hsp70 expression. The Journal of experimental biology, jeb. 120394, 2015.
90. Beauchamp RO, Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA. A critical review of the literature on hydrogen sulfide toxicity. CRC Crit Rev Toxicol. 1984; 13:25-97.
91. Eissa AE, Zaki MM, Abdel Aziz A. Flavobacterium columnare/ Myxobolus tilapiae concurrent infection in the earthen pond reared Nile tilapia (*Oreochromis niloticus*) during the early summer. IBC Interdisciplinary Bio Central, 2010,

92. Nofal MI, Abdel-Latif HMR. Ectoparasites and Bacterial Co-infections causing Summer Mortalities Among Cultured Fishes at Al-Manzala with Special Reference to Water quality parameters. Life Science Journal. 2017; 14(6):72-83.
93. Hinton DE. Cells, cellular responses and their markers on chronic toxicity of fishes. In: Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives, Eds., Malins D.C. and G.K. Ostrander, Lewis Publishers, Boca Raton, 1995, 207-239
94. Van der Oost R, Beyer J, Vermeulen NPE. Fish Bioaccumulation and Biomarkers in Environmental Risk assessment: a Review. Environmental Toxicology and Pharmacology, 2003; 13(2):57-149, ISSN 0926-6917.
95. Figueiredo-Fernandes A, Ferreira-Cardoso JV, Garcia-Santos S, Monteiro SM, Carrola J, Matos P *et al*. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. Pesq. Vet. Bras 2007; 27:103-109.
96. Arellano JM, Storch V, Sarasquete C. Histological changes and copper accumulation in liver and gills of the Senegales Sole, Solea senegalensis. Ecotoxicol. Environ. Saf. 1999; 44:62-72.
97. Mohamed F. Impacts of environmental pollution in the southern region of Lake Manzalah, Egypt, on the histological structures of the liver and intestine of *Oreochromis niloticus* and *Tilapia zillii*. J. Egypt. Acad. Soc. Environ. Develop. 2001; 2:25-42.
98. Schwaiger J, R¨udiger Wanke, Stefan Adam, Michael Pawert, Wolfgang Honnen, Triebskorn R. Journal of Aquatic Ecosystem Stress and Recovery. 1997; 6:75-86.
99. Salim F, Kadhim S. Survey on histopathological changes in different organs of local fresh water in Basra Province. Journal of International Academic Research for Multidisciplinary. 2014; 2:10.
100. Heier LS, Lien IB, Stromseng AE, Ljones M, Rosseland BO, Tollefsen KE *et al*. Speciation of lead, copper, zinc and antimony in water draining a shooting range-Time dependent metal accumulation and biomarker responses in brown trout (*Salmo trutta* L.). Science of the Total Environment, 2009, 4047-4055.
101. Nwaedozie JM. The determination of heavy metal pollutants in fish samples from River Kaduna. J. Chem. Soc. Nigeria, 1998; 23:21-23.
102. Rosety-Rodríguez M, Ordoñe FJ, Rosety JM, Rosety A, Ribelles, Carrasco C. Morpho-histochemical changes in the gills of turbot, *Scophthalmus maximus* L., induced by sodium dodecyl sulfate, Ecotoxicology and Environmental Safety. 2002; 51:223-228.
103. Tayel I, Seham A Ibrahim, Soaad A. Mahmoud Histopathological and muscle composition studies on *Tilapia zillii* in relation to water quality of Lake Qarun, Egypt. Journal of Applied Sciences Research. 2013; 9(6):3857-3872.
104. Abou El-Gheit E, Abdo M, Mahmoud S. Impacts of Blooming Phenomenon on Water Quality and Fishes in Qarun Lake, Egypt International Journal of Environmental Science and Engineering (IJESE), 2013; 3:11-24.
105. Moustafa MZ, El-Sayed EM. Impact of Water Pollution with Heavy Metals on Fish Health: Overview and Updates Global Veterinaria. 2014; 12(2):219-231, ISSN 1992-6197.
106. Abdullah A, Mehana EE, Meki A. Evaluation of lead and cadmium levels in freshwater fish farms at Qassim region, KSA. Journal of Agricultural and Veterinary Sciences. 2008; 1(2):59-69.
107. De Boeck G, Nilsson GE, Elofsson U, Vlaeminck A, Blust R. Brain monoamine levels and energy status in common carp (*Cyprinus carpio*) after exposure to sublethal levels of copper. Aquat. Toxicol. 1995; 33:265-277.