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Physiological and histological alterations in fishes induced by pollution in Lake Nasser and the potential human risk assessment

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ABSTRACT

Lake Nasser ecosystem has undergone many changes that decline species diversity; the lakes' fisheries also have experienced a decrease during the last 10 years disturbing its prospect for sustainability. Therefore, this study aimed to evaluate and compare the effect of water contaminants on *O. niloticus* and *S. galilaeus* inhabiting different locations of Lake Nasser. Heavy metals (HM) accumulation in the liver, gills and muscles of both O. *niloticus and S. galilaeus* generally arranged in the following order; Fe> Mn> Zn >Cd> Cu. The metal pollution index (MPI) in the studied organs pursued the order: liver > gill > muscle. While the hematological and biochemical indices of *O. niloticus* and S. *galilaeus* exhibited a significant alteration due to inhabiting stressful conditions. Moreover, pathological changes were observed in muscles of both fish species from different sampling sites. The present study opined that *S. galilaeus* are more resistant than *O. niloticus* to environmental stress, while *O. niloticus* are more sensitive to aquatic pollutants. Moreover, there was a significant alteration in fish health, as well as adverse health effects, for habitual consumers of both fish species from S4.

INTRODUCTION

Lake Nasser is one of the longest man-made lakes in the world; it was formed after the construction of Aswan High Dam. Furthermore it considered the fundamental water bank of Egypt; which provides more than 95% of freshwater needs (**Ali *et al*., 2016**). The total surface area is 6276 km^2, 5237 km^2 represents Lake Nasser and 1039 km^2 in the northern part of Sudan is known as Lake Nubia (**Heikal, 2010**).

The lake bank is extremely irregular with several terminal extensions known as khors that is representing about 79% of total lake surface. Khors are the favorite habitat for fish; therefore Khors are important in fish production (**El-Shabrawy and Dumont, 2003**). Many previous studies investigated the ecology and morphology of Lake Nasser (**Latif, 1984; Khalifa *et al*., 2000; El-Shabrawy, 2014)**. The Water quality of the Lake Nasser is influenced by many factors such as; water levels, thermal stratification, water circulation and pollutants (**El-Shabrawy, 2014**).

Lake Nasser fisheries have undergone a steady decrease during the last 10 years obscuring its potential for sustainability. In **1976, Latif** recorded 58 species in Lake Nasser. However currently, the fisheries depend only upon a limited number of species which are adapted well to the new conditions and indeed become the main species in the Lake (**Khalifa *et al*., 2000**). Moreover, **Bishai *et al*. (2000)** reported that, the eutrophic state of the lake discriminated locally, vertically and seasonally, as the phytoplankton crop increased southwards. Likewise, **El-Far *et al*. (2020)** recorded only thirty-four fish species in the Lake where six of them were dominant, namely; *Oreochromis niloticus*, *Sarothrodon galilaeus*, *Coptodon zillii*, *Lates niloticus*, *Alestes baramoze*, and *Hydrocynus vittitus*, also reported a dramatic change in the lake ecosystem and species diversity, that Some species are now limited to the southern side of the lake, while others have disappeared completely. Fish are directly affected by their environment, only a thin epithelial membrane is the barrier between fish blood and water. Fish are very vulnerable to water chemical and physical changes which alter their blood components (**Çelik *et al*., 2012**).

Heavy metals (HM) generally exist in aquatic environments at low levels but the anthropogenic activities have elevated their levels, which stimulate environmental concern in lakes (**Ntakirutimana *et al*., 2013**). HM are non-biodegradable; they are not expelled from water due to self-purification, as soon as they are discharged into water systems, they adsorbed on sediment, accumulate in aquatic animals and get into the food chain (**Loska and Wiechula, 2003**), consequently fish absorb and accumulate HM from water and diet in tissues.

Blood parameters are pathophysiological indicators of the whole body, which is known to display pathological changes before morphological symptoms of toxicity or diseases. Biochemical characteristics of blood are the most important tool to diagnose fish health when exposed to pollutants or stress conditions (**Suvetha *et al*., 2010**).

To our knowledge, no study has been conducted to investigate the impact of Lake Nasser pollution on the fish health and human risk assessment. Therefore, this study aimed to evaluate and compare the effect of water contaminants on *O. niloticus* and *S. galilaeus* inhabiting different locations of Lake Nasser, through the hematological, biochemical and histological investigations.

MATERIALS AND METHODS

Area of study

Study area: Sampling was carried out in March 2019 from two selected khors of Lake Nasser, El-Ramla and Toushka (**Table 1**). Two sites were selected for each khor, in the entrance and middle of khors.

Sample collection

Samples of *O. niloticus* and *S. galilaeus* were collected from different sites by the assistant of local fishermen during March 2019, with average weight 30±10 g for *O.*

niloticus and 361±100g for *S. galilaeus*. Blood samples were collected by caudal severance, and separated into two aliquots. The first aliquot was collected in tubes with EDTA and stored at 4°C, for hematological assessments, while the other part was left to clot for 30 min at room temperature, the clotted samples were then centrifuged at 1000 x*g* at 4°C for 10 min, and the obtained serum was stored at −20°C for further analysis.

Table (1): Sampling sites and GPS data of Lake Nasser.

Site	Location	Latitude	Longitude
S1	Intrance of khor Toushka	22° 32' 65,63" N	31°75' 56,73" E
S2	Middle of khor Toushka	22° 32' 58,48" N	31° 77' 95,32" E
S3	Interance of khor El-Ramla	23° 90' 61,11" N	32° 84' 75,34" E
S4	Middle of khor El-Ramla	23° 88' 07" N	32° 81' 42,73" E

Hematological indices:

Total red blood cells count (RBC) was done using an improved Neubaur hemocytometer (**Shah and Altindağ, 2004**). Hemoglobin (Hb) and Hematocrit (Hct) were determined using Cyanmethemoglobin and microhematocrit methods (**Blaxhall and Daisley, 1973**). Blood indices were calculated using the formulae mentioned by **Hrubec and Smith (2000)**.

Biochemical parameters:

Serum glucose levels were measured using colorimetric method described by **Tietz (1995)**. Total serum protein was assessed according to **Tietz (1994)**. Albumin was measured colorimetrically according to the method described by **Doumas *et al.* (1971)**. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity was measured according to **IFCC (1986)** Kinetic method. Serum creatinine was determined according to **Tietz (1986)**, while serum urea and uric acid were measured enzymatically according to **Tietz (1990)**. The total lipids were estimated using **Frings and Dunn (1970)** method. Serum potassium and sodium levels were estimated according to **Sunderman and Sunderman (1958) and Trinder (1951)**, respectively.

HM analysis:

portions of fish tissues (muscles, liver, and gills) were dissected for analysis from *O. niloticus* and *S. galilaeus*, separately. Dissected samples were transferred to Teflon beaker for acid digestion, as well as prepare the sample for heavy metal analysis according to the method described by **Ghazaly (1988)**. Fe, Mn, Zn, Cu, and Cd concentrations in fish tissues were analyzed by atomic absorption mode GBC SavantaAA AA with GF 5000 graphite furnace.

Metal Pollution Index (MPI).

MPI was calculated to indicate the overall metal load in fish tissues using the following formula according to **Usero *et al.* (1997)**:

$$\text{MPI} = (M_1 \times M_2 \times M_3 \times \cdots \times)^{1/n}$$

where M_n is the mean concentration of metal n (mg/kg dry weight) in the studied tissue.

Human Risk Assessment.

The risk assessment procedures were performed according to **USEPA (2000)** with abbreviation. The level of exposure caused by oral ingesting of HM in fish edible tissues was expressed by calculating the average daily intake of a specific chemical over a lifetime (ADD) using the following equation:

$$ADD\ (mg/kg/day) = C_m \times IR / BW,$$

where C_m is the mean metal concentration in fish muscle (mg/kg dry weight), IR is the ingestion rate (0.0312 and 0.1424 kg/day for normal and habitual fish consumers, respectively), BW is the body weight (assumed as 70 kg for normal adults) Risk was assessed by calculating the hazard index (HI; index of adverse health effects from intake of specific contaminant in food). HI is expressed as the ratio of the ADD to the oral reference dose of the metal according to the following equation proposed by **USEPA (2000)**:

$$Hazard\ Index = ADD / Oral\ RfD,$$

where oral RfD is the oral reference dose of the metal (mg/kg/day) based on the safe upper level of metal's oral intake for an adult human with average body weight of 70 kg. The oral RfD for Cu, Zn, Mn, Cd, and Fe is 0.04, 0.3, 0.14, 0.001, and 0.7mg/kg/day, respectively (**USEPA, 2015**). When HI $\geq$ 1.0, it may be assumed that adverse health effects are expected. The cumulative risk effect of all metals was calculated as the sum of HI values (**USEPA, 2002**).

Histopathological examination:

Immediately after dissection of the studied fish, parts of liver, gills and muscles were carefully removed and fixed in 10% formalin at 4°C, for 48 hours then the samples were dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned into five micrometers thick using Euromex Holland Microtome, and then stained according to Harris Hematoxylin and Eosin method. Finally, the sections were examined microscopically and photographed by a microscopic camera according the methods cited by **Tayel *et al*. (2018)**.

Statistical analysis

The results were expressed as Mean ± S.E of 6 fish, using a Microsoft Excel sheet on Windows 2010. The differences among treatments were analyzed using a one way Analysis of Variance (ANOVA) followed by a Duncan Multiple Range Test for multiple comparisons of the means. The statistical significance was set at $P < 0.05$ using SPSS for Windows version 23.0 (SPSS, Michigan Avenue, Chicago, IL, USA).

RESULTS AND DISCUSSION

Lake Nasser is the tank of fresh water in Egypt. However, there are no direct pollution sources of HM discharging into Nasser Lake. On the other hand, the lake receives HM via the anthropogenic activity of fishermen sewage, fishing boats, cruise ships, and others which disposed directly into the Sudanese Main Nile (**Darwish, 2013**).

Fishes are located at the top of the aquatic food chain, and they normally accumulate HM from food, water, and sediments (**Zhao *et al*., 2012**); therefore, fishes are major source of HM in food (**Sivaperumal *et al*., 2007**).

High accumulation levels of HM in fish tissues have induced sever adverse effects on human health (**Castro-González and Méndez-Armenta, 2008**), such as liver damage, renal failure, cardiovascular diseases, and even death (**Al-Busaidi *et al*., 2011**). Therefore, monitoring and management should be considered constantly to help the decision-maker to assess the health of aquatic environment of the lake (**Agrama, 2014**).

Metals are distributed unequally in fish tissues, but accumulate mainly in active tissues such as kidney, liver, and gill (**Yacoub and Gad, 2012**), while muscle exhibits lower HM levels due to its weak affinity of binding proteins and enzymes (**Papagiannis *et al*., 2004; Bayomy *et al*., 2015**). Fish accumulate HM through gills or ingestion of contaminated food; moreover, the accumulation of metals in the aquatic organism's tissues could be used as indicator for pollution, since it specific to species and mode of exposure (**Jarić *et al*., 2011**).

The metal concentration and pollution index (MPI) of *O. niloticus* and *S. galilaeus* were given in **Table (2)**. The HM accumulation in liver, gills and muscles of both O. *niloticus* and *S. galilaeus* generally arranged in the following order; Fe> Mn> Zn >Cd> Cu.

According to **Table (2)** the levels of Fe, Zn, Mn and Cu were within the permissible limits in liver, gills and muscles of both fish species. While Cd levels exceeded the permissible limits in liver and gills, which are non-edible parts, in both *O. niloticus* and *S. galilaeus* (**WHO, 1989**). In the present study, the higher levels of HM was recorded at S4 in tissues from both fish species, that in line with **Hussein and El Shafi (2005)** who reported that the HM levels in the northern part of the lake were much higher than the southern part.

The MPI of the studied organs followed the order: liver > gill > muscle which gives a better idea about the target tissues for HM accumulation in *O. niloticus* and *S. galilaeus* fish. The results clearly indicate that each fish species, as well as their tissues, have different accumulating capacity of HM. Moreover, S. *galilaeus* recorded higher HM concentration and MPI in tissues than *O. niloticus* inhabiting the same site.

The hazard index (HI) calculations integrate both the HM level in the edible tissues of fish and the human consumption rate of these tissues to perform a risk classification. In hazard identification, available data on biological endpoints are used to determine if a material is likely to pose a hazard to human. Fish consumption data is necessary for estimating the human health impact of consuming chemically polluted fish (**Copat *et al*., 2012**). Based on the estimations used in this study (**Table 3**), the habitual consumption of both fish species recorded a health risk at S4 and at S2 for *S. galilaeus* only.

It is well recognized that both natural and man-made chemicals negatively affect fishes. Moreover, environmental pollution may change the count and structure of

blood indices (**Katalay and Parlak, 2004**). So the study of the hematological indices in fish are the most commonly used tool to diagnose fish health, as it's usually used for the detection of pathophysiological changes due to several stress conditions (**Nussey *et al*., 1995**).

Table (2): Metal concentrations (mg/kg dry weight) in tissues of *O. niloticus* and *S. galilaeus* from Lake Nasser and metal pollution index (MPI).

		O. niloticus						***S. galilaeus***					
		Fe	**Mn**	**Zn**	**Cu**	**Cd**	**MPI**	**Fe**	**Mn**	**Zn**	**Cu**	**Cd**	**MPI**
S1	**Liver**	39.3	24.35	1.13	0.04	0.45	1.82	39.8	25.26	2.12	0.06	0.52*	2.38
	Gill	25.05	8.41	0.34	0.04	0.47	1.07	35.22	9.58	0.53	0.05	0.47	1.25
	Muscle	23.65	5.8	0.68	0.04	0.44	0.92	28.81	6.61	0.37	0.06	0.32	1.03
S2	**Liver**	40.93	25.46	1.51	0.12	0.32	2.28	40.04	28.49	1.27	0.08	0.73*	2.45
	Gill	38.33	13.8	0.57	0.05	0.60*	1.58	47.383	12.38	0.65	0.05	0.61*	1.65
	Muscle	30.05	6.48	0.32	0.03	0.23	0.88	36.057	6.61	0.48	0.06	0.42	1.24
S3	**Liver**	45.7	26.55	1.7	0.06	0.99*	2.58	48.613	34.69	2.65	0.14	1.33*	3.88
	Gill	47.21	10.08	0.87	0.07	0.74*	1.84	41.78	20.93	1.19	0.08	0.66*	2.24
	Muscle	41.9	5.56	0.40	0.05	0.35	1.08	39.38	6.6	0.56	0.07	0.50*	1.38
S4	**Liver**	60.74	32.52	1.4	0.15	1.38*	3.58	69.73*	30.07	3.56	0.17	1.75*	4.69
	Gill	53.23	14.56	0.89	0.08	0.94*	2.23	60.10*	26.82	1.41	0.14	1.12*	3.25
	Muscle	43.22	6.39	0.51	0.05	0.48	1.29	44.02	7.6	0.82	0.06	0.5	1.56

*exceeded the maximum permissible limit (MPL) according to WHO (1989); 50, 40, 30, and 0.5 for Fe, Zn, Cu, and Cd, respectively.

Table (3): Hazard index (HI) and the cumulative risk effect for normal and habitual consumers of *O. niloticu*s and *S. galilaeus* from Lake Nasser.

		O. niloticus						***S. galilaeus***					
		Fe	**Cu**	**Mn**	**Zn**	**Cd**	**Cumulative risk effect**	**Fe**	**Cu**	**Mn**	**Zn**	**Cd**	**Cumulative risk effect**
S1	normal	0.015	0.0004	0.018	0.0004	0.209	0.243	0.018	0.0007	0.021	0.0004	0.143	0.183
	habitual	0.072	0.002	0.084	0.002	0.958	0.618	0.084	0.003	0.096	0.002	0.653	0.838
S2	normal	0.024	0.0004	0.021	0.0005	0.238	0.283	0.023	0.0007	0.021	0.0007	0.420	0.465
	habitual	0.111	0.002	0.094	0.002	0.484	0.693	0.105	0.003	0.096	0.003	0.854	1.061*
S3	normal	0.027	0.006	0.018	0.0005	0.156	0.2021	0.0250	0.0008	0.021	0.0008	0.242	0.2896
	habitual	0.122	0.003	0.081	0.002	0.712	0.921	0.114	0.004	0.096	0.004	1.105	1.323
S4	normal	0.028	0.0006	0.0203	0.0007	0.2006	0.4302	0.028	0.0007	0.024	0.001	0.232	0.2859
	habitual	0.126	0.003	0.093	0.003	0.915	1.14*	0.128	0.003	0.11	0.006	1.059	1.306*

∗HI ≥ 1.0, the point at which adverse health effects may occur

Table (4): The hematological indices of *O. niloticus* and *S. galilaeus* from Lake Nasser.

	S1		S2		S3		S4	
	O. niloticus	***S. galilaeus***	***O. niloticus***	***S. galilaeus***	***O. niloticus***	***S. galilaeus***	***O. niloticus***	***S. galilaeus***
RBCs ($\times10^6$/ µL)	1.98 ±0.02 ^{a}	1.89 ±0.02 ^{a}	1.79 ±0.03 ^{b}	1.57 ±0.02 ^{b}	1.70 ±0.03 ^{c}	1.48 ±0.03 ^{c}	1.41 ±0.02 ^{d}	1.34 ±0.02 ^{d}
Hb (g/dL)	7.48 ±0.13^{a}	6.81 ±0.17^{a}	7.16 ±0.24^{a}	6.64 ±0.17^{ab}	7.01 ±0.22 ^{a}	6.56 ±0.19 ^{ab}	6.53 ±0.29^{b}	6.12 ±0.16^{b}
HCT (%)	22.65 ±0.25 ^{a}	21.36 ±0.43^{a}	19.61 ±0.23 ^{c}	18.07 ±0.30^{b}	20.48 ±0.30 ^{b}	16.90 ±0.24 ^{c}	16.71 ±0.31 ^{d}	16.01 ±0.27 ^{d}
MCV (fL)	87.69 ±0.95 ^{a}	89.72 ±1.19 ^{ab}	85.94 ±0.86 ^{a}	92.42 ±1.21 ^{b}	85.89 ±1.05 ^{a}	88.60 ±0.92 ^{b}	82.18 ±0.74 ^{ab}	87.01 ±0.72 ^{b}
MCH (pg/dL)	36.30 ±0.63 ^{a}	49.69 ±0.70 ^{a}	34.89 ±0.68 ^{ab}	41.18 ±0.67 ^{b}	34.76 ±0.64 ^{ab}	37.65 ±0.48 ^{c}	34.01 ±0.48 ^{b}	36.80 ±0.62 ^{c}
MCH C (g/dL)	36.68 ±1.32^{a}	35.06 ±1.46^{a}	31.94 ±1.37 ^{b}	30.06 ±1.45 ^{b}	31.22 ±1.17 ^{b}	30.10 ±1.08 ^{b}	30.53 ±0.85 ^{b}	26.14 ±0.84 ^{c}
WBCs ($\times10^3$/µL)	55.02 ±0.24 ^{c}	61.60 ±0.28 ^{c}	60.72 ±0.29 ^{a}	67.01 ±0.34 ^{ab}	59.21 ±0.28 ^{b}	67.22 ±0.38 ^{ab}	59.38 ±0.38 ^{b}	68.15 ±0.32 ^{a}

Data were presented as mean ± S.E (n=6 fish). Values within a row with different superscripts differ significantly (Duncan Multiple Range Test, P<0.05). RBCs, Red blood cells. Hb, Hemoglobin. Hct, hematocrit. MCV, mean corpuscular volume, MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration, WBCs, white blood cells.

In the present study, the statistical analysis of the hematological indices in *O. niloticus* and S. *galilaeus* exhibited a significant influence (**Table 4**). A reduction in RBCs, Hb, Hct and MCV was recorded in the blood of *O. niloticus* and *S. galilaeus* from polluted sites, compared to relatively less polluted site S1. Comparing sites, S4 recorded the lowest RBCs, Hb, Hct, MCV, MCH, and MCHC in both fishes, while S1 reported the highest values of latter variables. Conversely, WBCs count in both fishes was significantly increased (P<0.05) at S4, as compared to S1 which is recorded the least WBCs count. The present results find support from numerous studies which shown that the peripheral RBCs, Hb, Hct and MCV of fish decreased due to exposure to different pollutants (**Zutshi *et al*., 2010; Summarwar, 2012; Gupta and Chandra, 2014**).

The present reduction in RBCs, Hct and Hb as well as the WBCs enhancement may be attributed to the presence of HM, which found to decrease the RBCs count through RBC lysis (**Adekunle *et al*., 2007; Moharram *et al*., 2011)**. Also, **Katalay and Parlak (2004)** reported an alteration in the RBCs and WBCs of fish exposed to environmental pollutants. The present findings further support the hypothesis of **Moharram *et al*. (2011)** who reported an adaptive response of fish exposed to HM through increase WBCs count, hemodilution and impaired Hb synthesis, which eventually leads to hypochromic, macrocytic anaemic condition, and swelling of RBC, this attributed to hypoxic conditions demonstrated in the present study via gills degradation..

The investigation of biochemical constituents of blood is a fundamental tool in the physiological and pathological assessment of fish to understand the toxicological impacts of xenobiotics (**Li *et al*., 2011**). Blood glucose levels, protein, and lipid profile of fishes act as good biomarkers of fish exposed to HM under field conditions (**Javed and Usmani, 2013; salaah *et al*., 2018**). Serum glucose, total protein, albumin, and total lipids levels of *O. niloticus* and *S. galilaeus* are shown in **Table (5)**. In the present study, significant increase ($P<0.05$) was illustrated in mentioned serum metabolites in both fish species at different sampling site from Lake Nasser especially at S4.

Table (5): The biochemical parameters and blood electrolytes of *O. niloticus* and *S. galilaeus* from Lake Nasser**.**

	S1		S2		S3		S4	
	O. niloticus	***S. galilaeus***	***O. niloticus***	***S. galilaeus***	***O. niloticus***	***S. galilaeus***	***O. niloticus***	***S. galilaeus***
Glucose(mg/dL)	77.1 ±1.5^{d}	88.48 ±1.4^{b}	107.23 ±1.9^{c}	125.8 ±1.2^{a}	118.1 ±1.1^{b}	127.9 ±0.9^{a}	125.66 ±1.0^{a}	127.1 ±1.0^{a}
T.protein(g/dL)	4.1 ±0.13^{c}	5.1 ±0.1^{d}	4.98 ±0.12^{b}	6.06 ±0.09^{b}	4.73 ±0.16^{b}	5.97 ±0.17^{c}	5.78 ±0.17^{a}	7.83 ±0.23^{a}
Albumin (g/dL)	1.39 ±0.06^{c}	1.81 ±0.05^{c}	1.65 ±0.05^{b}	2.29 ±0.07^{b}	1.87 ±0.05^{ab}	2.11 ±0.9^{b}	2.02 ±0.06^{a}	2.60 ±0.9^{a}
T.lipids(mg/dL)	213.8 ±3.1^{d}	360.2 ±2.5^{d}	307.52 ±2.9^{c}	506.1 ±2.5^{c}	475.5 ±2.2^{b}	480.5 ±2.7^{b}	548.2 ±2.7^{a}	578.5 ±2.6^{a}
ALT (U/mL)	20.53 ±1.1^{b}	21.07 ±0.7^{c}	23.37 ±0.8^{c}	24.86 ±1.2^{c}	29.09 ±0.6^{b}	32.73 ±0.6^{b}	24.84 ±0.7^{a}	38.46 ±0.7^{a}
AST (U/mL)	7.09 ±0.4^{c}	6.64 ±0.5^{c}	10.54 ±0.6^{b}	7.62 ±0.5^{b}	13.18 ±0.4^{a}	6.73 ±0.3^{c}	15.69 ±0.4^{a}	14.45 ±0.4^{a}
Urea (mg/dL)	4.78 ±0.7^{b}	4.78 ±0.7^{b}	5.49 ±0.7^{ab}	5.49 ±0.7^{b}	5.04 ±0.3^{b}	5.04 ±0.3^{b}	7.07 ±0.5^{a}	7.07 ±0.5^{a}
Uric.acid(mg/dL)	1.54 ±0.1^{c}	1.61 ±0.07^{c}	1.70 ±0.1^{c}	2.67 ±0.07^{c}	3.45 ±0.08^{b}	6.10 ±0.16^{b}	5.24 ±0.08^{a}	8.19 ±0.31^{a}
Creatinine(mg/dL)	0.28 ±0.02^{d}	0.30 ±0.03^{b}	0.49 ±0.04^{c}	0.50 ±0.03^{a}	0.53 ±0.03^{b}	0.53 ±0.04^{a}	0.62 ±0.03^{a}	0.56 ±0.04^{a}
Potassium(mmol/L)	5.95 ±0.3^{b}	8.52 ±0.4^{b}	5.82 ±0.4^{b}	10.18 ±0.6^{b}	7.03 ±0.5^{ab}	9.64 ±0.6^{b}	8.46 ±0.4^{a}	12.61 ±0.4^{a}
Sodium(mmol/L)	22.46 ±0.9^{b}	21.36 ±0.8^{bc}	24.18 ±1.1^{ab}	20.03 ±0.5^{c}	23.07 ±0.8^{ab}	23.70 ±0.7^{b}	25.5 ±0.8^{a}	27.55 ±0.8^{a}

Data were presented as mean ± SE (n=6 fish). Values within a row with different superscripts differ significantly (Duncan Multiple Range Test, $P<0.05$). T.; total, ALT; Alanine aminotransferase, AST; aspartate aminotransferase.

The blood glucose level variations are used as an indicator of stress response in fish (**Kumar *et al*., 2016**). However, water pollutants may be attributed to the present hyperglycemia, since HM known to modulate the carbohydrate metabolism in fish and stimulates the synthesis of glucose from extrahepatic tissue such as proteins and amino acids (**Osman *et al*., 2010**). Moreover, animals inhabiting stressful environment produce more glucose for energy to cope with stress (**Jentoft *et al*., 2005**). Also the present

hyperglycemia_may refer to liver malfunction /damage or renal dysfunction which is opined by the present findings.

Serum proteins are the main contributors in HM transportation (**Bal *et al*., 2013**). Therefore, the impact of pollutants on serum total protein has been used to evaluate fish response to various stressors (**Hadi *et al*., 2009**). However, the major protein of blood is albumin which plays a critical role in the circulation of physiological, exogenous and endogenous chemicals, it also regulates blood osmotic pressure (**Baker, 2002**).Since the majority of serum proteins manufactured in the liver, according to the pathological finding of the present study, the increase in serum total protein and albumin reflects liver dysfunction which increases protein synthesis in liver due to HM pollution (**Osman *et al*., 2018; Salaah *et al*., 2018)**

The present hyperlipidemia in both fish species from polluted sites may attribute to the current reported disorder of liver function and lipid metabolism. Lipids are important constituent of cell membrane which maintains cell fluidity, so membrane damage by HM could be another possible cause of the hyperlipidemia (**Javed *et al*., 2017**). According to **Vaseem *et al*. (2013)** when exposed to stress of HM toxicity fish increased the lipid mobilization to compete the growing demand for energy, this is in accordance with the present hyperglycemic response.

Liver functions: ALT and AST are valuable biomarkers which frequently used in the diagnosis of damage caused by pollutants in several tissues, for instance liver, gills, and muscle (**De la Tore *et al*., 2000; Osman *et al*., 2010**).

Serum ALT and AST were significantly increased ($P<0.05$) in *O. niloticus* and *S. galilaeus* from S2, S3, and especially S4 when compared to S1. Similar increase have been observed in plasma and serum of fish exposed to HM due to the injury of hepatocytes which consequently caused the leakage of these cytosolic enzymes into the bloodstream (**Harvey *et al*., 1994; Salaah *et al*., 2018**).

The kidney is known to play a significant part in the detoxification and discharge of toxicants. Urea, uric acid, and creatinine are non-protein nitrogenous compounds, while creatinine is a waste product mainly from muscles breakdown, urea is the major metabolite from dietary protein and tissue protein turnover. While, uric acid in fish is formed from exogenous and endogenous purines then converted in the liver to urea and excreted by the gills (**Ajeniyi and Solomon, 2014**). Urea, uric acid, and creatinine are useful in diagnosis of renal dysfunction, muscle damage, and nitrogen metabolism impairment (**Murray *et al*., 1990**).

In the present study urea, uric acid, and creatinine concentrations were significantly increased ($P<0.05$) in blood serum of both fish species gathered from different sites of Lake Nasser, especially at S4. Such increase was reported previously in fish exposed to HM (**Abdel-Khalek, 2015**). The present azotemia caused by HM, which characterized by low blood flow and filtration rate, while diagnosed by high levels of serum urea, uric acid, and creatinine (**Chang *et al*., 1996**).

In fish, the sodium (Na^+) and potassium (K^+) are the main electrolytes; K^+ is the dominant in extracellular fluids and Na^+ in the serum and in other fluids. The electrolytes main function is to maintaining the acid-basic balance (**Tavares-Dias *et al*., 2008**). Furthermore, the homeostatic mechanism of fish is commonly trailed the aquatic ecosystem alterations, therefore electrolytes have been used as quantitative indicators of contamination in an ecosystem (**Brewer *et al*., 2001**). Disease, stress and gill injury usually alters osmoregulation in fish through affecting gill permeability of ions (**McDonald and Milligan, 1997**).

The levels of blood serum electrolytes (Na^+ and K^+) in the present study were significantly elevated ($P<0.05$) at S4 in both fish species, comparing to other sites. According to **Zaki *et al*. (2001)** higher Na^+ and K^+ levels may be a sign of kidney and gill damage, which may have impaired the osmoregulatory capacity in fish. We agree with this hypothesis, since blood electrolytes in the present study are in line with HM levels and the current pathophysiological changes in fish from different sampling sites.

Histopathological findings:

The liver is considered as the master of detoxification in vertebrates and particularly in fish. Liver is responsible for cleaning up the blood from xenobiotics. In the present study, liver of *O. niloticus* at S2 and S4 showed degeneration and necrosis in blood vessel and hepatocytes in Fig. (1, A-B). On the other hand, the results showed degeneration, necrosis, hemorrhage, and hemolysis in blood vessel, and degeneration hepatocytes in *S. galilaeus* collected from S2 and S4 (Fig. 1, C-D). Moreover, liver of *S. galilaeus* collected S3 cleared degeneration, balloon necrosis in hepatocytes, and dilation and hemorrhages in blood vessels (Fig. 1, E).

The gills participate in many important functions in fish, such as respiration, osmoregulation, and excretion. Gills are vulnerable to the water quality fluctuations as it's in direct contact with the external environment; also it considered the primary target of the contaminants (**Fernandes and Mazon, 2003**). Gills are formed of primary lamellae or gill filament and secondary lamellae, which found laterally from the primary lamellae. The surface of the gill lamellae is covered with epithelial cells running parallel along the surface. Injuries and pathological abnormalities frequent occur in gills due to xenobiotics whether dissolved or suspended in water which suppresses respiration by reducing the surface area (**Tayel *et al*., 2013**).

Gills of *O. niloticus* obtained from S2 and S4 showed degeneration and severe necrosis in primary and secondary lamellae and hemorrhage in secondary lamellae (Fig. 2, A-B). In *S. galilaeus* collected from S2 and S4, gills showed degeneration, severe necrosis and separation in both primary and secondary lamellae, as well as hyperplasia, which lead to fusion of filament and curling of lamellae epithelial cells illustrated in Fig. (2, C-D), also a sever hemorrhages in primary lamellae showed in gills of *S. galilaeus* from S4 (Fig. 2, E).

The muscular system is the main portion of the teleost body; it's responsible for locomotion, blood pumping and peristaltic constriction of viscera. On the other hand, fish would probably be invaded by micro-organisms when they have epithelial lesions in polluted water (**Kadry *et al.*, 2015)**.The present pathological changes observed in muscles of both fish species from different sampling sites including: Degeneration, necrosis, and edema in muscle fiber. In *O.niloticus*, degeneration and necrosis was observed in samples from S2 and S4 (Fig. 3, A-B). While, *S. galilaeus* collected from S2 and S4 showed dilation, hemorrhages in blood vessels, and fatty cells in hypodermal layer (Fig. 3, C-D), also showed necrosis in muscle fiber and fatty cells in hypodermal layer of fish samples from S3 (Fig. 3, E). However, the present study demonstrated that the fish inhabiting S4 (Khor El-Ramla) are more affected by water polluted (HM) than fish from other sampling sites. Moreover, *S. galilaeus* showed more severe cases than *O. niloticus*, this illustrated by higher level of HM bioaccumulation and MPI in *S. galilaeus* than *O.niloticus*.

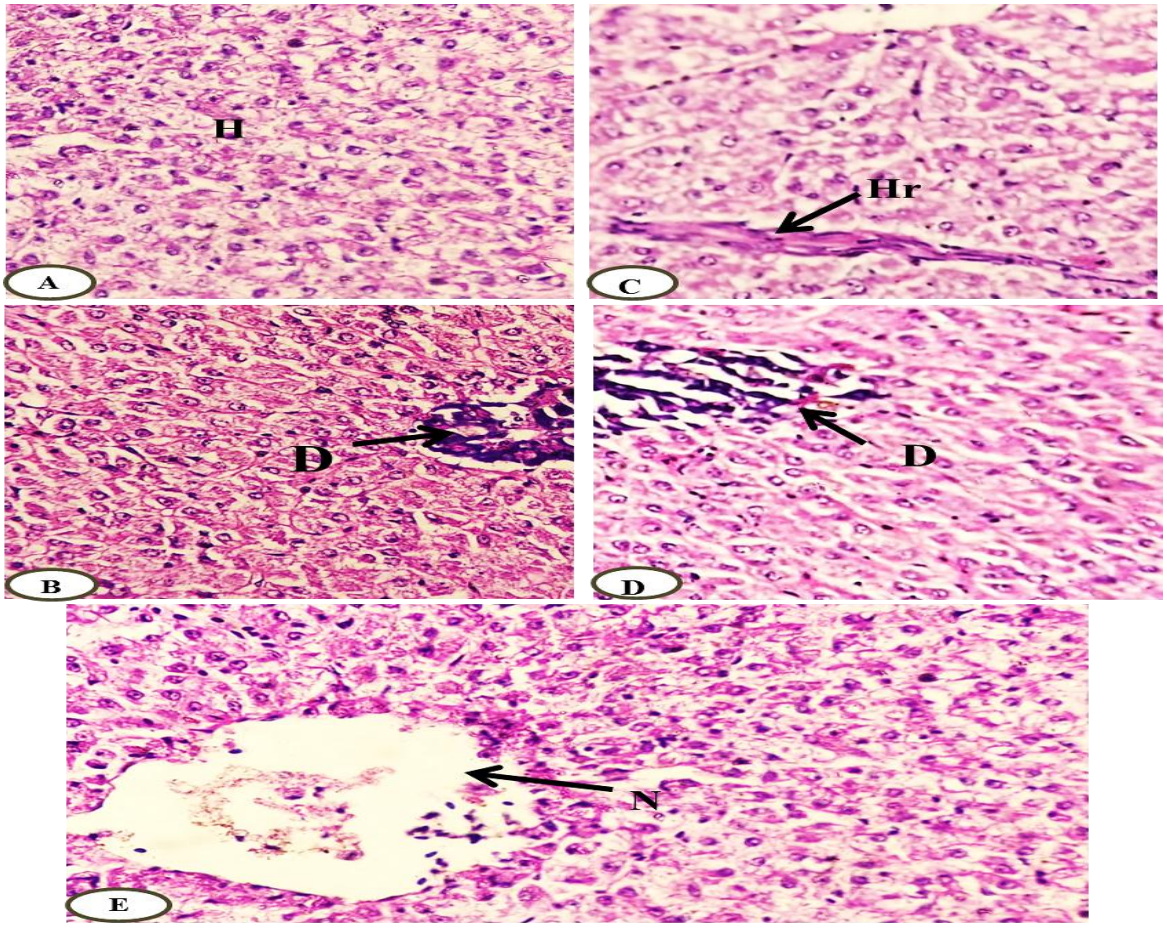

Figure (1): Photomicrograph of liver section of *O. niloticus* from S2 and S4 (A and B), *S. galilaeus* from S2, S3 and S4 (C, D and F). Hepatocytes (H), Necrosis (N), Degeneration (D), Hemorrhage (H).

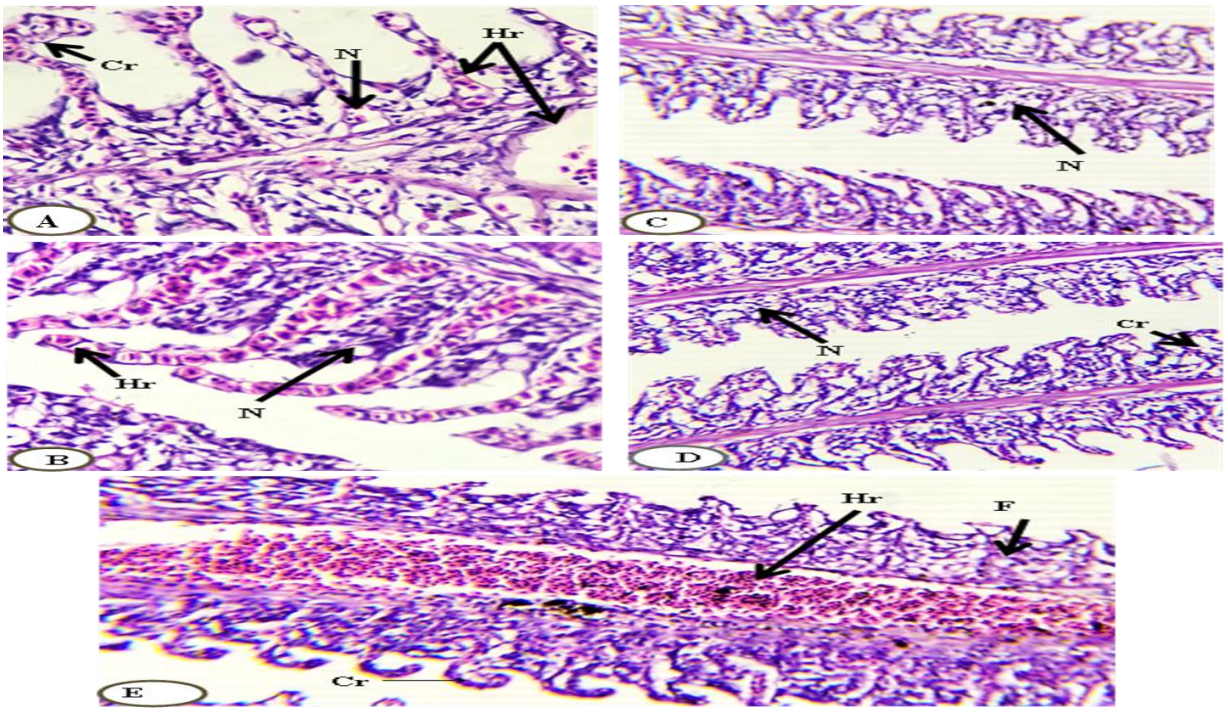

Figure (2): Photomicrograph of gill section of O. niloticus from S2 and S4 (A and B), S. galilaeus from S2 and S4 (C, D and E). Necrosis (N), Hemorrhage (H), Fusion (F), Curling (Cr).

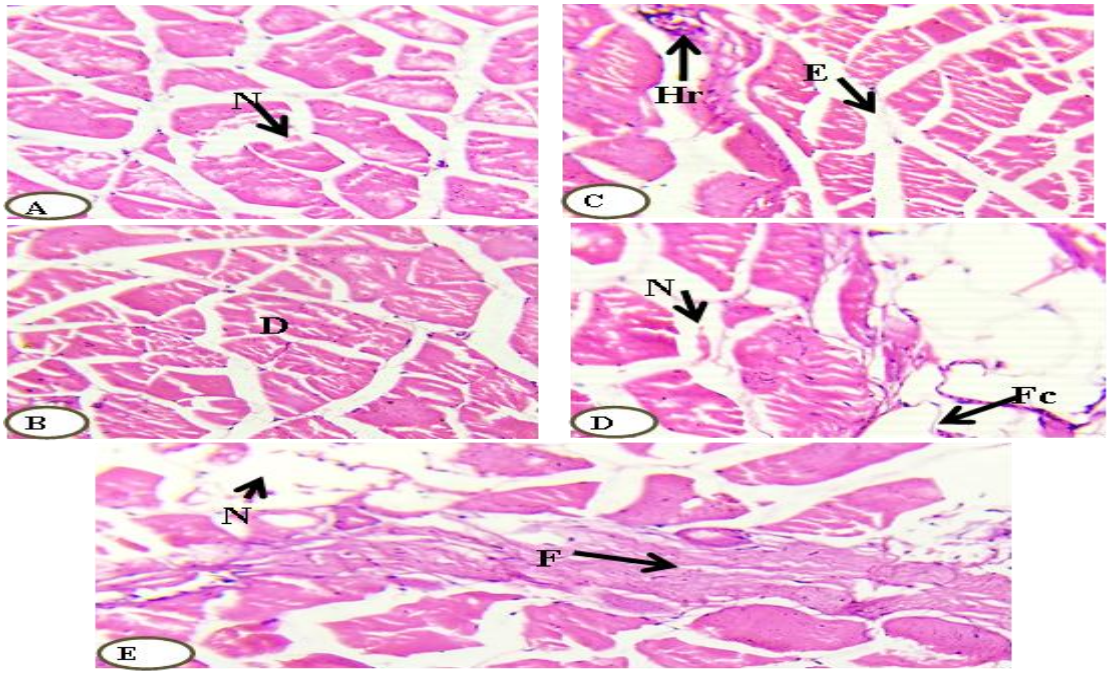

Figure (3): Photomicrograph of muscle section of O. niloticus from S2 and S4 (A and B), S. galilaeus from S2, S3 and S4 (C, D and F). Necrosis (N), Degeneration (D), Hemorrhage (Hr). Degeneration (D), Fatty cell (Fc).

CONCLUSION

The hematological and physiological alterations along with histopathological findings in *S. galilaeus* and *O. niloticus* from different sites of Lake Nasser indicated poor fish health and susceptibility to diseases. This may attribute to the dramatic change in fish production, distribution and diversity of fish in Lake Nasser. The present data supports the hypothesis that fish species is a critical concern in routine application for bio-monitoring scopes. Since *S. galilaeus* reported inferior hematological indices and higher MPI, as well as worst liver and kidney function and sever histopathological changes, when compared to *O. niloticus* collected from the same sites of Lake Nasser. This opined that *S. galilaeus* are more resistant than *O. niloticus* to environmental stress, while *O. niloticus* are more sensitive to aquatic pollutants. Moreover there was an adverse health effects for habitual consumers of both fish species from S4 (Middle of Khor El-Ramla).

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