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Accumulation and risk assessment of heavy metals-induced biochemical and histopathological alterations in *O. niloticus* from Lake Nasser, Egypt

Nasr M. Ahmed, Sally M. Salaah*, Safaa I. Tayel
[1] National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt.
*** Corresponding author: sallysalaah84@gmail.com**

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ABSTRACT

Lake Nasser is the world's second greatest man-made lake and the main reservoir of fresh water in Egypt. There are no direct sources of heavy metal pollution in Lake Nasser. However, metals enter the lake ecosystem in many ways as erosion of the geological matrix, air deposition, anthropogenic activity, and from upstream the Nile's basin. The current study aimed to evaluate the physiological and histopathological effect of heavy metals on different organs of the Nile tilapia (*O. niloticus*) and assess the possible human health risk related to consuming fish from Lake Nasser. Fish were assembled from different khors of Lake Nasser (Bekheet, Medahib, Korosko, Sukar, and Yassen). Blood serum glucose, total protein, albumin, total lipids, ALT, AST, urea, uric acid, and creatinine, in addition to heavy metals accumulation (Fe, Mn, Zn, Cu, Cd, and Pb) in muscles, gills, and liver organs were determined in the current study. Also, these selected organs were histologically examined. The higher metal index was recorded in the liver followed by gills and muscles. Both physiological and histological parameters recorded a site-dependent response. The recorded data confirmed the negative effect of heavy metals on fish health and internal organs' function. Metals were accumulated in muscles, gills, and liver tissues according to the following order; Fe > Zn > Cu > Mn > Pb > Cd. However, the metal load in the muscles of *O. niloticus* was within the permissible limits for human consumption, and the hazard index recorded no adverse health effects on neither normal nor habitual consumers among all sampling sites.

INTRODUCTION

Lake Nasser is formed on account of the High Dam building in upper part of Egypt; it considers the second-largest non-natural lake in the world. The surface area and water level of the lake are depending mainly on the annual flow of water that comes from the Blue Nile from the Ethiopian highlands (Ahmed *et al*., 2020). Generally, Lake Nasser has an area of 5248 km^2 with an average width of 12 km and a mean depth of 25 m (maximum 130 m), since the lake's water volume varies every year and season, following the net amount of water received and stored by the High Dam. The lake has many secondary channels outsides the main channel, called Khors. These Khors have a surface area of about 4900 km^2 (about 79 % of the lake's total surface area and about 55% of the total lake volume) (Ebaid *et al*., 2010; Hegab *et al*., 2021).

According to CAPMAS (2019), the total lake fisheries production in Egypt is about 220,7 thousand tons, which represents about 10.8 percent of the overall fish production.

In Egypt, Lake Nasser has turned out to be one of the most significant fisheries (Nasr-Allah *et al*., 2016), accounting for 67.27 percent of Egypt's total inland lake catch in 2019. Constituting 67.3 percent of the entire capture for inland lakes in Egypt, However, Lake Nasser has experienced a decline in fish production and species variety (GAFRD, 2019)

Among different sorts of toxins in the aquatic environment, metals are the main hazard due to their wide cluster of compounds and pathways to harm the lake ecosystems. Subsequently, the source of metal pollution in Lake Nasser is chiefly the organic matter, which is produced as a consequence of anthropogenic activities and suspended in the floodwaters along the stream of Nile River through its basin countries, especially in Ethiopia (Farhat and Aly, 2018). Moreover, heavy metals are ecologically harmful owing to their devastating effect on the aquatic ecosystems' stability, noxiousness, endurance, and their high tendency to accumulate (Al-Taee *et al*., 2020). Heavy metals (HMs) can pose many harmful effects on the ecosystem, aquatic animals, and human health (Salaah and El-Gaar., 2020).

Fish are a cheap source of animal protein in Egypt. The tilapias are the most common cultured Cichlid fish species in Africa and the Middle East (FAO, 2004). On the other hand, Fish has been widely used as bio-indicators to assess the aquatic ecosystem health (Khalil *et al*., 2017). Fish are so sensitive to their environmental changes since only a thin epithelial membrane detaches the blood of the fish from the water. Hence, the hematological investigation is a reliable way to assess fish health in stressful conditions such as environmental pollution. Moreover, the histopathological study has been commonly used as a diagnostic tool to evaluate the efficiency of specific organs in fish exposed to pollutants. Furthermore, the alterations found in these organs are so much easier to identify (Salaah *et al*., 2018; Tayel *et al*., 2020).

The current study aimed to; (1) evaluate the impact of metal pollution in Lake Nasser on the biochemical indices and histology of gills, liver, and muscles of Nile tilapia (*O. niloticus*) fish as a bio-indicator, (2) assess the potential risk for human consumers.

MATERIALS AND METHODS

Lake Nasser is a major source of freshwater that is used in drinking, irrigation, fisheries hydroelectricity, and other domestic purposes. The lake is characterized by a large number of Khors, which are highly important for fish, especially tilapia fish which consider khors a preferred habitat for feeding and reproducing. There are about 86 khor in Lake Nasser with a total shoreline length of about 969.9 km, about 48 khor located on the eastern side and 37 on the western side (Latif *et al*., 1984).

The study area:

Fish were collected from five sites to cover the area of investigation. The location of the studied area is shown in (Table 1), during winter, 2020.

Table 1: The latitude and longitude of sampling stations at Lake Nasser (GPS).

Site		Latitude	Longitude
KB	(Khor Bekheet)	22.348128	31.615439
KM	(Khor Medahib)	22.634756	31.869959
KK	(Khor Korosko)	22.594429	32.297566
KS	(Khor Sukar)	23.085833	32.671333
KY	(Khor Yassen)	23.646032	32.845305

During winter 2020, fish (*O. niloticus*) samples were collected from different Khors of Lake Nasser (Table 1, Fig. 1). Total 100 adult Fish (20 fish per site) with body length varied between 25.0 and 35.1 cm and body weight ranged from 190.8 to 545.0 g; blood samples were collected and stored for further assay.

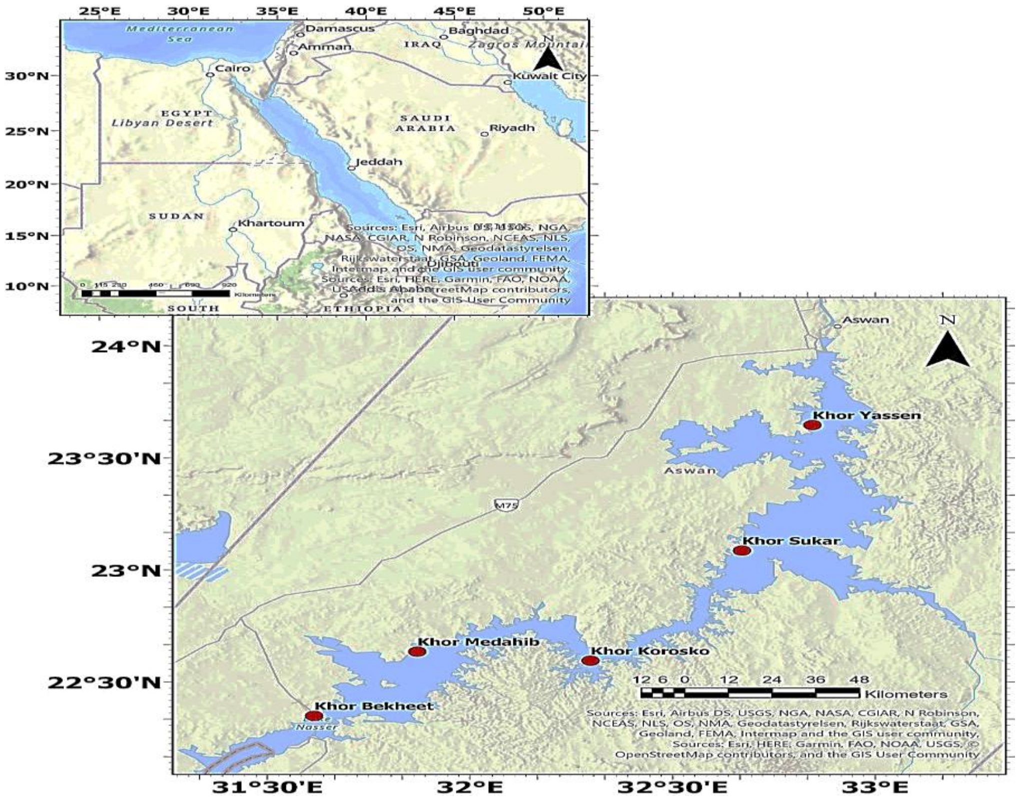

Fig. 1: A map of Lake Nasser showing sampling stations

The Biochemical indices:

Blood samples were obtained by severance of the caudal peduncle of fish and separated into two aliquots. The first aliquot part was collected in vials with EDTA and stored at 4°C for determine hemoglobin (Hb) content (Blaxhall and Daisley, 1973). The 2nd part of blood samples was collected (without EDTA) and centrifuged at 4000 *xg for* 10 minutes to obtain serum. Supernatant serum was collected using the micropipette model (Labystems K 33071) for the determination of serum glucose concentration according to Trinder (1969), total proteins and albumin using Gornall, *et al*. (1949) and Doumas*et al*. (1971) methods, respectively. Serum total lipids were estimated due to Frings and Dunn (1970). To determine triglycerides and cholesterol we used McGowan*et al*., (1983) and Roeschlau *et al*., (1974) methods, respectively. The serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase

(AST) were determined colorimetrically using commercial kits (Spectrum, Egypt) followed to the method described by Reitman and Frankel (1957). Both, serum urea and uric acid concentrations were measured enzymatically according to Tietz (1990). While serum creatinine was determined according to Tietz (1986).

Heavy metals Analysis:

Metals (Fe, Cu, Pb, Mn, Zn, and Cd) in different organs (muscles, gills, and liver) of *O. niloticus* were determined according to APHA (2005). Tissue samples were dried in oven at 105˚C and grinded into powder. One gram of each grinded sample was acid-digested by perchloric and nitric acids and heated (200 to 250°C). The clear solution was filtered through an acid-resistant 0.45 mm filter paper and diluted to complete 25 ml by deionized water. The residuals of heavy metals in fish tissues were quantified using an atomic absorption reader (SavantAA AAS with GF 5000 Graphite Furnace).

The Metal Pollution Index (MPI):

MPI of different fish tissues was calculated according to Usero *et al*. (1997).

Human health hazard assessment

Ingestion of food loaded with HMs may encourage many health hazards, since the content of HM in food is directly proportional to the absorbed dose through the human digestive system (USEAP, 1989).

Estimated daily intake of HMs (EDI)

EDI for normal and habitual adult fish consumers was calculated over a lifetime according to Song *et al*., (2009).

Target Hazard Quotient (THQ)

According to USEPA (2011), the THQ is usually used to evaluate the non-carcinogenic hazard of each ingested metal using the reference dose (RFD) of metal cited by USEPA (2012).

Hazard Index (HI)

Calculate the possible risk related to ingestion of food contaminated with several metals (USEPA, 2011).

Target Cancer Risk (TR)

Generally, the TR is used to anticipate the carcinogenic hazard associated with the intake of certain metals such as Pb and Cd (USEPA, 2011).

Histological investigation

Muscles, gills, and liver samples obtained after dissection of *O. niloticus* were collected during winter 2020. Samples were precisely dissected out and fixed using 10% formalin; then the samples were dehydrated using ascending concentrations of alcohol. Then samples were cleared in xylene. Paraffin wax was used in embedding samples and the Euromex Holland microtome was used to cut tissues in 4-6 µm thick sections. The Harris hematoxylin and eosin method was used to stain the sections (Bernet *et al*., 1999; Hegab *et al*., 2020). Subsequently, these sections were examined microscopically and images were taken by a microscopic camera.

Statistical analyses

The data of biochemical parameters in blood serum were presented in mean ± standard deviation of ten fish. Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test. The differences between means were considered significant when $P \leq 0.05$.

RESULTS

Serum biochemical indices:

The hematological and biochemical profile in fish is a reliable index for assessment of fish health under metallic stress. The hemoglobin content was ranged between 13.99 ±0.65 at KM and 9.22 ±0.25 g/dL at KY. Our data exhibited a clear decline in hemoglobin at KK, KS, and KY as compared to KB and KM (Table 2).

According to Table "2", all the serum biochemical parameters recorded variations: glucose varied between 112.25±5.05 and 189.738±5.6 mg/dL, total proteins from 3.58±0.19 to 2.838±0.1 g/dL, albumin from 1.17±0.04 to 0.90±0.007 g/dL, total lipids from 1818.1±138.0 to 697.47±9.7 mg/dL, triglycerides ranged between 191.54±3.2 and 128.27±11.9 mg/dL, while cholesterol fluctuated from 159.77±11 to 110.3±5.7 mg/dL (Table 2).

Both ALT and AST varied between 23.35±2.4, 8.29±0.83 and 37.45±10.1, 21.06±1.5 IU/mL. likewise, urea, uric acid, and creatinine were ranged between "7.55±0.49 and 4.26±0.13", "3.72±0.2 and 2.20±0.04", and "9.23±0.16 and 1.45 ±0.36" mg/dL, respectively (Table 2).

Heavy metals (HMs) in *O. niloticus:*

Results of HMs residuals in the edible (such as muscles) and non-edible tissues (such as gills and liver) of *O. niloticus* collected from different sites of Lake Nasser were tabulated in Tables "3, 4, and 5". The recorded data documented the existence of heavy metals in all sampled fish tissues within different concentrations according to tissue type and sampling sites. The highest levels of heavy metals were recorded in the liver followed by gills, while muscles recorded the lowest HMs load among tested tissues. Metals were accumulated in muscles, gills, and liver tissues according to the following order: Fe > Zn > Cu > Mn > Pb > Cd. Gills from KB were the highest MPI followed by KY > KM > KS > KK, while the MPI of the liver was the most at KB > KY ≥ KS > KK > KM, muscles reported the highest MPI at KS > KY > KB ≥ KM > KK.

In muscles (Table 3), Fe concentrations varied between 45.0 mg/Kg dry wt. at KY and 21.6 mg/Kg dry wt. at KM. Mn recorded the highest levels at KY (0.9 mg/Kg dry wt.), while KB showed the lowest levels (0.4 mg/Kg dry wt.). Both Zn and Cu recorded their lowest levels at KB (2.6, not determined, and 0.3 mg/Kg dry wt., respectively), their highest levels were shown at KK and KS, (3.8 and 2.7 mg/Kg dry wt.), respectively. On the other hand, Cd was recorded only at KK (0.23 mg/Kg dry wt.), Pb levels ranger between 0.5 and 0.3 mg/Kg dry wt. at KY and KB.

Table "4" characterized the HMs levels in gills, where KY recorded the highest levels of Fe, Mn, and Pb (47.7, 0.95, and 0.50 mg/Kg dry wt., respectively), while KS recorded the highest Zn, Cu, and Cd levels (20.4, 4.9, and 0.24 mg/Kg dry wt., individually). Fe recorded its lowest level at KM (23.0 mg/Kg dry wt.), meanwhile, KB showed the lowest Mn, Zn, and Cu levels (0.6, 4.1, 0.2 mg/Kg dry wt., respectively). Both KM and KK recorded the lowest Pb levels 0.40 mg/Kg dry wt.

KY recorded the highest levels of most metals in the liver of *O. niloticus*, where Fe, Mn, Cu, Cd, and Pb recorded 49.7, 2.9, 6.5, 0.4, and 2.2 mg/Kg dry wt., respectively. While KS reported the maximum Zn level (25.4 mg/Kg dry wt.). Lowest Fe levels recorded at KM (40.1 mg/Kg dry wt.), KS showed the lowest levels of Mn, Cu, and Cd (1.6, 0.3, and ND mg/Kg dry wt.). Zn and Pd recorded their lowest levels at KY and KK (14.4 and 1.6 mg/Kg dry wt.), individually (Table 5).

Table (3): Heavy metals concentrations (mg/kg dry wt.) in *O. niloticus* muscles from different Khors of Lake Nasser

Metals	KB	KM	KK	KS	KY	Guidelines (mg/kg dry wt.)
Fe	40.2	21.6	31.0	27.9	45.0	100 ^{AB}
Mn	0.4	0.5	0.8	0.85	0.9	1 ^{B}
Zn	2.6	2.7	3.8	3.7	3.6	75 ^{A}; 100 ^{B}
Cu	ND	1.9	2.1	2.7	0.5	30 ^{B}
Cd	ND	ND	0.23	ND	ND	0.2 ^{A}; 0.5 ^{B}
Pb	0.3	0.35	0.4	0.45	0.5	0.2 ^{A}; 0.5 ^{B}

$^{\mathbf{A}}$FEPA (2003); $^{\mathbf{B}}$ FAO/WHO (1989); ND; not detected

Table (4): Heavy metals concentration (mg/kg dry wt.) in *O. niloticus* gills from different Khors of Lake Nasser

Metals	**KB**	**KM**	**KK**	**KS**	**KY**	**Means**
Fe	43.8	23.0	35.0	38.8	47.7	37.66
Mn	0.6	0.7	0.8	0.8	0.95	0.77
Zn	4.1	11.7	13.3	20.4	5.9	11.08
Cu	0.20	2.1	3.3	4.9	3.3	2.76
Cd	ND	ND	0.23	0.24	ND	0.094
Pb	0.48	0.40	0.40	0.45	0.50	0.446

ND; not detected

Table (5): Heavy metals concentration (mg/kg dry wt.) in *O. niloticus* liver from different Khors of Lake Nasser

Metals	**KB**	**KM**	**KK**	**KS**	**KY**	**Means**
Fe	44.1	40.1	45.9	48.9	49.7	45.74
Mn	1.6	1.8	1.9	2.6	2.9	2.16
Zn	15.8	17.2	18.5	25.4	14.4	18.26
Cu	0.3	3.2	3.4	5.0	6.5	3.68
Cd	ND	0.2	0.40	0.35	0.4	0.27
Pb	1.8	2.1	1.6	2.0	2.2	1.94

ND; not detected

According to Table 6, KS recorded the highest MPI in muscles (1.88 mg/Kg dry wt.) followed by KY > KB > KM > KK, while KB documented the maximal MPI (4.39 mg/Kg dry wt.) in gills followed by KY > KM > KS >KK. the highest liver MPI was recorded at KS (4.95 mg/Kg dry wt.) followed by KY > Ks > KK > KM (Table 4). Among different sampling sites, KB recorded the highest mean MPI (3.74 mg/Kg dry wt.) pursued by KY (3.39 mg/Kg dry wt.), KS (3.29 mg/Kg dry wt.), KM (2.66 mg/Kg dry wt.), and KK (2.56 mg/Kg dry wt.).

Table (6): Metal pollution index (MPI) using organs of *O. niloticus* (mg/kg dry wt.) from different Khors of Lake Nasser.

	Muscles	Gills	Liver
KB	1.881	4.393	4.953
KM	1.809	2.753	3.426
KK	1.618	2.198	3.877
KS	2.544	2.634	4.707
KY	2.052	3.379	4.746

Table 7 represents the Target hazard quotient (THQ) of normal and habitual fish consumers. The THQ values in the present study for all studied heavy metals were less than 1 (THQ<1) in both consumers types. THQ handles each heavy metal individually, although contaminated food comprises several heavy metals, as in the present study, six heavy metals were detected in *O. niloticus* muscles therefore HI was calculated. HI is the mathematical summation of all calculated THQs for the fish muscles. HI is used as an indicator for public health concerns when exceeding 1 (Islam *et al*., 2014).

In the present study, no adverse health effects were expected among all considered sites (Table 7). According to the United States environmental protection agency (USEPA, 2012), both Cd and Pb are vigorous carcinogens. Cd was detected only at KK, while Pb was detected at all concerned sites from Lake Nasser. Thus, target carcinogenic risk (TR) was calculated for both.

Table (7): Target hazard quotient (THQ) and Hazard index (HI) posed by heavy metals in *O. niloticus* collected from different Khors of Lake Nasser

		KB	KM	KK	KS	KY
Fe	Normal	0.025	0.013	0.019	0.017	0.028
	Habitual	0.116	0.062	0.090	0.081	0.130
Mn	Normal	0.001	0.001	0.002	0.002	0.002
	Habitual	0.005	0.005	0.009	0.010	0.010
Zn	Normal	0.003	0.004	0.005	0.005	0.005
	Habitual	0.017	0.018	0.025	0.025	0.024
Cu	Normal	---	0.021	0.0234	0.030	0.005
	Habitual	---	0.096	0.106	0.137	0.025
Cd	Normal	---	---	0.102	---	---
	Habitual	---	---	0.467	---	---
Pb	Normal	0.044	0.052	0.059	0.066	0.074
	Habitual	0.203	0.237	0.271	0.305	0.339
HI	Normal	0.075	0.092	0.212	0.122	0.218
	Habitual	0.342	0.421	0.971	0.558	0.530

* Adverse health effects are expected (HI ≥ 1.0).

Table (8): Target cancer risk (TR) posed by heavy metals in *O. niloticus* collected from different Khors of Nasser Lake

		KB	KM	KK	KS	KY
Cd	Normal	--	--	3.8 E-05	--	--
	Habitual	--	--	1.77E-04	--	--
Pb	Normal	1.1 E-06	1.32E-06	1.51E-06	1.70E-06	1.89E-06
	Habitual	5.18E-06	6.05E-06	6.91E-06	7.78E-06	8.64E-06

TR is defined as; low (TR ≤ 10^{-6}); moderate (TR from 10^{-4} to 10^{-3}); high (TR from 10^{-3} to 10^{-1}); very high (TR ≥10^{-1}), and in the range from 10^{-4} to 10^{-6} is considered acceptable.

The histological analysis:

Several histopathological lesions were observed in the gills, liver, and muscles of *O. niloticus* fish obtained from the selected five khors during the period of study. Normally gill of the *O. niloticus* fish was almost consist of several cartilaginous arches. Each arch bears pairs of processes "Primary lamellae " which serve more for support of secondary lamellae than for respiration. From the side of which radiate a thin expansion, the so called "Secondary lamellae". Each secondary lamellae contains a thin walled gill sinusoid which performs the function of respiration, excretion and osmoregulation and surrounded by "Pillar cells". The secondary lamellae are covered with squamous " Epithelial cells " made up of specialized and undifferentiated cells. Among these cells the "Mucous" and " Chloride " cells are found. The chloride cells (ioncytes), present in euryhaline species only and are the primary osmoregulatory cells of fish. They are chiefly found at the base of both types of lamellae. This description was also reported by Tayel *et al*. (2013).

Many histopathological alterations were observed in fish gills including Degeneration and necrosis in epithelial cells of primary lamellae and curling as well as hemorrhage in secondary lamellae at KB. Hemorrhage in primary lamellae and degeneration along with necrosis in epithelial cells of primary and secondary lamellae at KB. Also, gills from KK recorded hemorrhage and in primary lamellae associated with degeneration and necrosis in epithelial cells of the primary and secondary lamella. Fish gills at KS showed hemorrhage in primary lamellae, necrosis in primary and secondary lamellae, and secondary lamellae swelling at KY, gills showed hemorrhage in primary lamellae as well as primary and secondary lamella epithelial cells necrosis (Plate I).

Normally liver of the *O. niloticus* fish was almost uniform in appearance as well as it was soft in consistency and uniformly dark red color. It is enclosed within a fibro connective tissue capsule. While these liver cells sample histologically appear forming a meshwork and they are arranged in a definite cord. Like pattern around well-defined sinusoid leading to central vein. The hepatic cells appeared as poly-hedral cells with central nuclei. Blood flows from branches of hepatic portal vein and hepatic artery through the sinusoids to central veins which empty into the hepatic vein, this structure also explained by Tayel *et al*. (2013).

Fish liver recorded various histopathological alterations such as Degeneration in hepatic cells and congestion in blood sinusoids at KB. Blood vessels dilation at KM. Anastomosis of blood vessels at KK. dilation and congestion of blood vessels at KS. Degeneration and hemosiderin in hepatic cells at KY in Plate (II). Normally muscles layer of the *O. niloticus* fish was composed chiefly of segmental myomeres. Each myomere is regarded as muscle and its fibers are parallel the long axis of the body Muscles showed various histopathological lesions including degeneration in muscle fiber of fish from KB and KM. Also, muscle displayed degeneration and necrosis in fiber at KK and KS along with edema between muscle fiber at KY (Plate III).

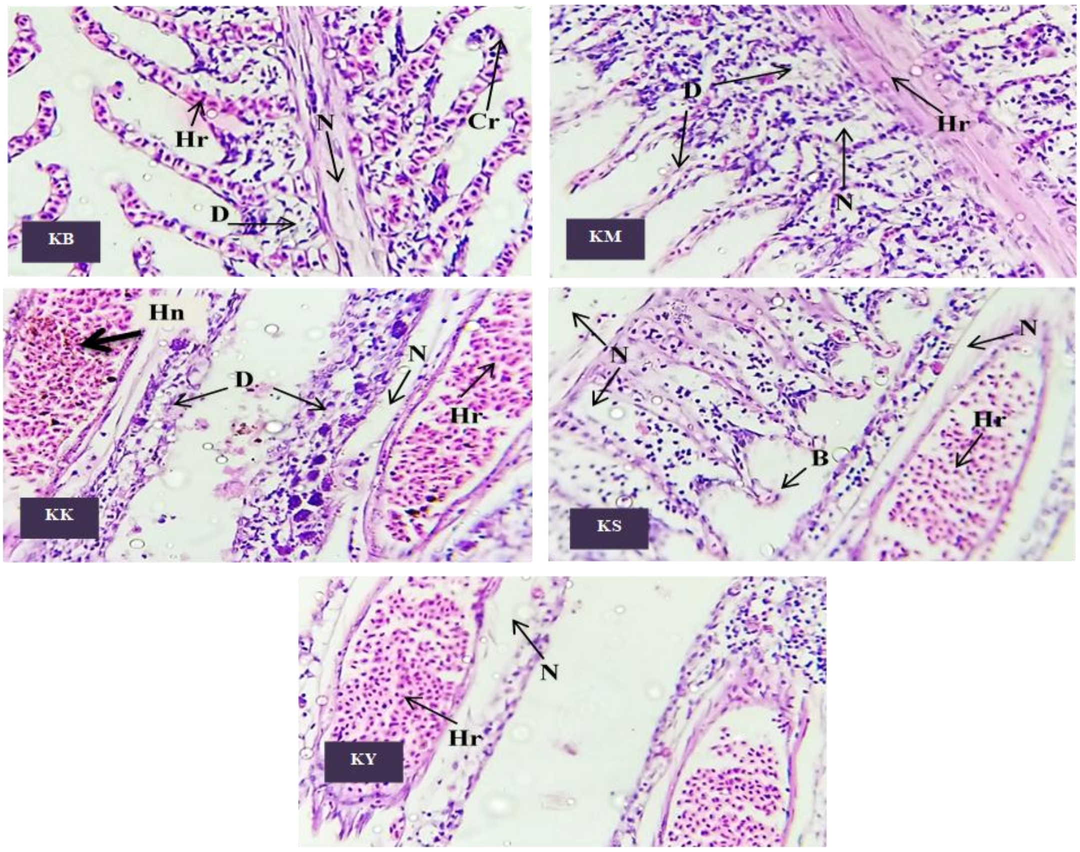

Plate (I): longitudinal section of gills in the Nile tilapia obtained from five khors of Lake Nasser: (KB) Khor Bekheet, degeneration (D) and necrosis (N) in epithelial cells of primary lamellae and curling (Cr) and hemorrhage (Hr) secondary lamellae. (KM) Khor Medahib, hemorrhage (Hr) primary lamellae and degeneration (D) and necrosis (N) in epithelial cells of primary and secondary lamellae. (KK) Khor Korosko, hemorrhage (Hr) and hemosiderin (Hn) primary lamellae and, Degeneration (D) and necrosis (N) in epithelial cells of primary and secondary lamella. (KS) Khor Sukar, hemorrhage (Hr) in primary lamellae, necrosis (N) in primary and secondary lamellae and swelling (S) in top of secondary lamellae. (KY) Khor Yassen, hemorrhage (Hr) in primary lamellae and necrosis (N) in epithelial cells of primary and secondary lamella, x 400. (H and E).

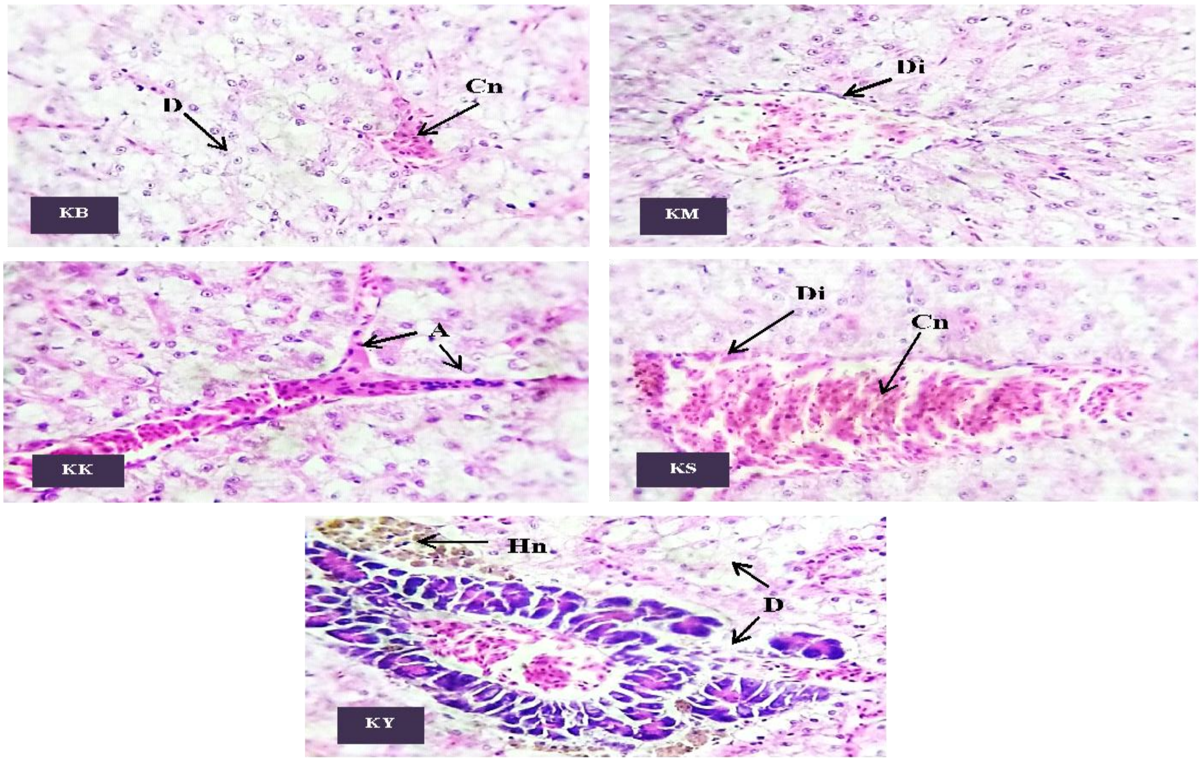

Plate (II): Liver section in the Nile tilapia obtained from five Khors of lake Nasser, (KB) Khor Bekheet, degeneration (D) of hepatic cells and congestion (Cn) in blood sinusoid, (KM) Khor Medahib, dilation (Di) of blood vessels. (KK) Khor Korosko, anastomosis (A) in blood vessels, (KS) Khor Sukar, dilation (Di) and congestion (Cn) in blood vessels, (KY) Khor Yassen, degeneration (D) and hemosiderin (Hn) in hepatic cells, x 400. (H and E).

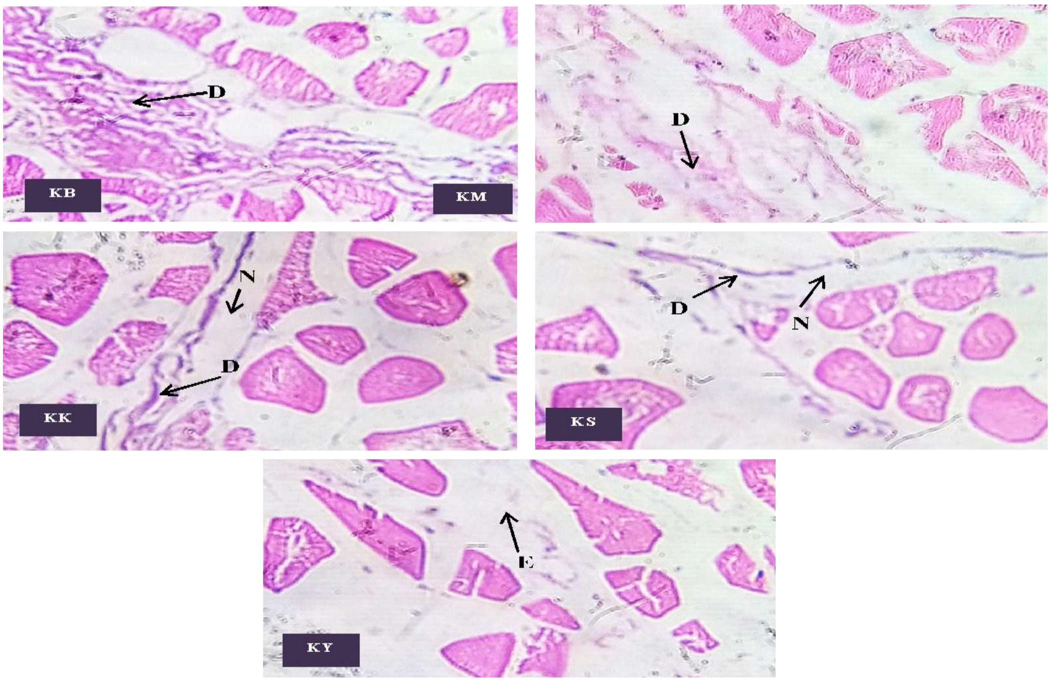

Plate (III): Vertical sections of *O. niloticus* fish muscles obtained from five studied Khors of lake Nasser: (KB) Khor Bekheet and (KM) Khor Medahib degeneration (D) in muscle fiber. (KK) Khor Korosko and (KS) Khor Sukar degeneration (D) and necrosis (N) in muscle fiber (KY) Khor Yassen, edema (E) between muscle fiber, x 400. (H and E).

DISCUSSION

Hemoglobin generally reflects the hematological changes of fish exposed to HMs (Essa *et al*., 2018; Vo *et al*., 2019). The decline in the content of hemoglobin was observed after HMs exposure. This reduction was recorded to be coinciding to the hemopoietic system damage (Ramesh and Ramachandra, 2021), due to hemorrhage and gills impaired, as confirmed by the present histopathological findings.

The serum biochemical indices were exhibited in a site-dependent manner. On one hand, the highest values of total proteins, albumin, total lipids, cholesterol, and creatinine were recorded at KB, while the highest values of ALT, AST, urea, and uric acid parameters were recorded at KY, but the highest glucose levels were recorded at KM.

On the other hand, the lowest levels of total proteins and albumin were recorded at KY, while glucose, total lipids, and creatinine recorded the least levels at KK, both triglycerides and cholesterol levels were the least at KY; however, ALT, AST, urea, and uric acid reported the lowest levels at KM (Table 2).

The study of serum biochemical indices has become an important tool in the assessment of fish health. Some biochemical indices used as a specific biomarker of fish exist in water loaded with metals (Salaah *et al*., 2018). The cited fluctuations of blood biochemical parameters in *O. niloticus* fish may be related to the presence of HMs in Lake Nasser (Ahmed *et al*., 2020a and Salaah, and El-Gaar, 2020).

Blood glucose level has been used as an indicator for environmental stress, HMs pollution affects the carbohydrate metabolism in fish and elevates the production of glucose from extra-hepatic tissues (Osman *et al*., 2010), which justify the documented hyperglycemia in fish accumulated more HMs (Table 2). Blood serum proteins are the primary carrier of HMs through the body (Bal *et al*., 2013), higher levels of both serum total proteins and albumin in fish from KB may be caused by the effect of chemical stress (Masaya *et al*., 2002; Salaah and El-Gaar, 2020). Although, lower levels of total proteins and albumin at KY may associate with a lower protein synthesis rate or high consumption rate of proteins as a source of energy in stressful conditions (Ahmed *et al*., 2020b).

In accordance with the present biochemical fluctuations, higher lipids in *O. niloticus* at KB confirmed the utilization of more energy to meet the stress induced by HMs and liver injury (Vaseem *et al*., 2013; Javed *et al*., 2017). Liver enzymes such as ALT and AST are usually used as a diagnostic tool for liver malfunction (Osman *et al*., 2010). Urea, uric acid, and creatinine were used to assess the kidney function in fish ((Ajeniyi and Solomon, 2014). Higher serum ALT, AST, urea, uric acid along with creatinine met the prementioned findings and confirm liver injuries and kidney dysfunction associated with exposure of fish to HMs (Tayel *et al*.,2014).

HMs tend to accumulate in various tissues and food substances. Fish can pile up metals from water in many ways such as intake of polluted food and direct contact through their gills and skin (Jovanovic *et al*., 2011). The bioaccumulation of HMs in fish may serve as a bio-indicator of pollution (Nair *et al*., 2006 and Mohamed *et al*., 2020).

In fish, the capacity to accumulate HMs is affected by many factors such as fish species and nature of aquatic environments (Kalay*et al*., 1999), the metal availability, metal gradients, as well as other water physical and chemical parameters (Roméo*et al*., 1999). The outcomes of the present study demonstrate the accumulation patterns of heavy metals in different tissues of *O. niloticus* collected from different sites of Lake Nasser.

HMs concentrations reported a site and tissue-dependent behavior. HMs in the present study were irregularly distributed in fish tissues, higher levels were found in metabolically active organs such as; the liver and gills, meanwhile muscular tissue accumulated lesser HMs owing to its lower enzymatic activities and binding proteins (Papagiannis *et al*., 2004). Generally, the concentration of HMs in the gill follows the level where the fish subsist in water, while the concentration in the liver signifies the storage of metals (Rao and Padmaja, 2000). Therefore, among fish organs, gills are usually considered as an ecological indicator of water contamination (Obasohan *et al*., 2008; Yilmaz, 2009).

The liver recorded the highest HMs load and MPI, followed by gills, while muscles recorded the lowest MPI. This may be attributed to the liver's significant role in the storage and elimination of metals (Amiard *et al*., 2006). Moreover, high levels of metals especially Zn, Cu, and Cd were directly connected to metal-binding proteins such as metallothionines, which are involved in the metabolism of essential metals as well as protect cells against metals' toxicity (Gorar*et al*., 2012). Many authors recorded similar results of higher metals in the liver (Dural *et al*., 2007; Eisler, 2009; Zhao *et al*., 2012). Although, some fishes accumulated higher Cd in gills (Qadir and Malik, 2011). The present study is in agreement with Khalid (2004) and Eisler (2009) who reported a positive correlation between fish size and higher Cd levels in the liver than other tissues, as fish accumulate Cd all over its life.

The concentrations of both essential and non-essential metals in the muscles of *O. niloticus* collected from Nasser Lake were within the maximum permissible limits for human consumption (Table 3).

Muscle was specifically used in the present study to assess the health risk, as it is the only edible part of fish. Although heavy metals concentrations in *O. niloticus* muscles were within the recommended limits of FAO (1989), Cd at KK was slightly higher than FEPA (2003) guidelines.

The method of human health risk assessment is defined by the Environmental Protection Agency (EPA) as the possible adverse health impacts in humans exposed to chemicals in the contaminated environment recently or after time. The threat of HMs on human health is predicted using target hazard quotient (THQ), hazard index (HI), and target cancer risk (TR). These parameters' calculations use the quantity of pollutants intake and the exposure recurrent and span, as well as the oral reference dose and median body weight.

THQ is a ratio of heavy metal content in food to its RfD. THQ reveals possible non-carcinogenic hazards when exceeding 1. THQ is not a hazard pointer; it expresses the degree of concern (Harmanescu *et al*., 2011; Jovic and Stankovic, 2014).

On one hand, Cd in the present study recorded low cancer risk (3.8×10^{-5}) for the normal consumers, and moderate carcinogenic risk (1.7×10^{-4}) for one per ten thousand habitual consumers at KK. On the other hand, Pb showed low ($TR \leq 10^{-6}$) cancer risk for both consumers' types among sampling sites. TR give a rise to the maximal accepted limit of toxins exposure, which may induce cancers throughout a lifetime.

Many studies have considered the link between heavy metals intake and adverse health effects in humans (FDA, 2001; Powers *et al*. 2003; ATSDR, 2004; Sivaperumal *et al*. 2007).

The present histopathological alterations could be a direct result of pollutants discharged (such as; fertilizers, salts, and other pollutants) at these sites (Ahmed *et al*., 2020a; Tayel *et al*., 2014). While, the cellular degeneration may be attributed to oxygen deficiency as confirmed by the vascular dilation and intravascular hemolysis of blood vessels with subsequent stasis of blood (Bayomy *et al*., 2017). Liver lesions (degeneration and necrosis) could refer to the cumulative effect of nutrient salts or metals in the liver, which is an important de toxicant organ of endogenous waste products as well as externally derived toxins. Moreover, Hemolysis and hemosiderin pigments may result from a rapid and continuous degeneration of erythrocytes by the breakdown of hemoglobin and convert it into hemosiderin. Among all sampling sites, fish from KY showed the most histopathologic lesions (Tayel *et al*., 2018).

CONCLUSION

Both the biochemical indices and histopathological analysis confirm that fish at Khor Korosko, Khor Sukar, and Khor Yassen were exposed to stressful conditions induced by heavy metals. Although, the levels of heavy metals in muscles were within the permissible guideline for human. Cd in fish from Khor Korosko documented a possible health risk for habitual *O. niloticus* consumers. So, continuous health risk assessment of accumulative risk induced by metal contamination of fish is fundamental to maintain healthy fish and population.

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