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Accumulation of heavy metals in tilapia fish species and related histopathological changes in muscles, gills and liver of *Oreochromis niloticus* occurring in the area of Qahr El-Bahr, Lake Al-Manzalah, Egypt

by

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Abstract

Accumulation of metals (Fe, Zn, Cu, Mn, Pb, Cd and Ni) in the muscles and gills of *Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zillii* was assessed based on seven locations in the Qahr El-Bahr area in Lake Al-Manzalah. The average accumulation of metals was in the following order: *Tilapia zillii* > *Oreochromis aureus* > *Oreochromis niloticus*. To determine the risk of fish consumption, the estimated daily intake, carcinogenic, non-carcinogenic and relative risk indices were calculated. The results indicate that the intake of individual metals through the consumption of fish is almost safe for human health, whereas the intake of combined metals poses a high potential health risk to consumers. Assessment of carcinogenic risk of Cd and Ni from the consumption of tilapia species indicates that consumers are at risk of cancer. The contribution of Pb and Cd to the overall relative risk index ranged from 34% to 41%. Of the three species studied, *Oreochromis niloticus* is relatively safe for consumption as it poses the least health hazard, while *Tilapia zillii* is more predisposed to accumulate metals in its tissues. Histopathological changes were observed in the muscles, gills and liver of *Oreochromis niloticus* as a result of heavy metal accumulation in these organs.

Key words: Lake Al-Manzalah, tilapia species, heavy metals, gills, muscles, liver, histopathology

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1. Introduction

Lake Al-Manzalah is considered one of the most important outlets for inland fisheries in Egypt and is estimated to account for about 38.02% of the total fish catch from the Nile River Delta lakes. It is considered to be the second major source of fish after Lake El-Burollus. The importance of Lake Al-Manzalah fishery relies on two main targets, i.e. it is a source of protein for human consumption and a provider of employment (El-Bokhty 2010). There are five coastal lakes along the northern coast of Egypt, connected with the Mediterranean. They represent important fishing resources in Egypt. Due to human activity, these lakes are severely environmentally degraded (Abdel-Satar et al. 2017).

Lake Al-Manzalah is considered the largest and most productive lake in Egypt. Due to the increase in agricultural, municipal and industrial wastewater discharge, the fish production and water quality status of the lake have degraded (Ahmed et al. 2006; Goher et al. 2017).

Since the early 20th century, there have been continuous changes in hydrological, chemical and biological characteristics of the lake resulting from increased freshwater supplies associated with municipal and agricultural wastewater disposal (Abdel-Satar & Goher 2009). Lake Al-Manzalah is connected with five drains through its western and southern shores. These drains discharge their effluent into the lake, which affects its water quality (Abdel-Satar 2001, 2008; Ali 2008). Approximately 7500 million cubic meters of untreated industrial, domestic and agricultural drainage waters are discharged into the lake annually through the drains such as Bahr El-Baqer (industrial and domestic wastewater), Ramsis, Hadous, Faraskour and El-Serw (agricultural wastewater; Abu Khatita et al. 2015). The pollutants and wastes discharged from the drains affect the entire area of the lake (El-Naggar et al. 2016).

Several studies have addressed the ecology of Lake Al-Manzalah. These studies covered the lake water quality, hydrological system, geological aspects, benthic invertebrates, phytoplankton composition, bacterial indices, and fishery status of the lake (Yacoub et al. 2005; Abdel-Satar 2008; Abdel-Satar & Goher 2009; El-Refaie 2010; Hamed et al. 2013; Mehanna et al. 2014; Zahran et al. 2015; Orabi & Osman 2015; Hegazy et al. 2016; El- Shafei 2016).

Contamination with metals in aquatic systems has drawn particular attention due to their persistence, toxicity and biological accumulation (Zahran et al. 2015). Heavy metals enter the human body through different paths, such as the food chain, and pose both non-carcinogenic and carcinogenic health risks (Mohanta et al. 2020). Fatal diseases such as renal tumor, nephritis, osteoporosis, cancer, nasopharyngeal congestion, increased blood pressure associated with cardiovascular diseases, and malfunctions of different body systems are caused by heavy metals (Mohanta et al. 2020). Determination of heavy metal levels in environmental biota is an essential process in assessing the human health risk resulting from the presence of these contaminants in food. Pollutants can be categorized as non-carcinogens and carcinogens, and are found mainly in fish (Yu et al. 2014). Risk assessment is one of the fastest processes required to evaluate the impact of hazards on humans. The risks can be divided into non-carcinogenic and carcinogenic effects. The non-carcinogenic risk is based on the Hazard Quotient (HQ), while the carcinogenic risk is based on the Target Cancer Risk (TCR; Markmanuel & Horsfall Jnr. 2016).

Fish are considered one of the most important biomonitors in the aquatic ecosystem for assessing heavy metal pollution (Abou El-Gheit et al. 2012). Furthermore, fish are at the top of the food chain and can accumulate metals that are transferred to humans through consumption of fish, causing acute or chronic diseases (Al-Yousuf et al. 2000).

Accumulation of pollutants disrupts the physiology of fish tissues. The endpoint in assessing the risk of pollutants in the environment is the microscopic examination of target tissues through histopathological parameters (Fatima et al. 2015). Histopathological changes can be used as indicators of the impact of various anthropogenic pollutants on organisms and as a measure of the overall health of the entire aquatic ecosystem (Saad et al. 2011). Harmful effects of pollutants can be manifested in fish tissues before consequential changes in the external appearance and behavior of fish (Mahboob et al. 2020). The exposure of fish living in Lake Al-Manzalah to different types of wastes (industrial, agricultural, and sewage) resulted in several pathological changes in different fish organs (Tayel et al. 2014; Mahmoud & Abd El Rahman 2017).

The objectives of this research were: a) to determine the level of metals in the muscles and gills of *Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zillii* collected from Qahr El-Bahr in Lake Al-Manzalah, b) to assess the non-carcinogenic and carcinogenic risks associated with the consumption of tilapia fish by humans, and c) to evaluate the histopathological changes in the muscles, gills and liver of *Oreochromis niloticus*.

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2. Materials and methods

2.1. Site description

Lake Al-Manzalah is located between longitudes 31°45′E and 32°22′E and latitudes 31°00′N and 31°35′N (Fig. 1). The lake is bordered by the Mediterranean Sea to the north and northeast, by Dakahlia and Sharkia to the south, by the Suez Canal to the east and by the Nile Branch of Damietta to the west (Hossen & Negm 2016). Three outlets connect Lake Al-Manzalah with the Mediterranean Sea allowing the exchange of water and biota between the lake and the sea: El-Boughdady, El-Gamil, and new El-Gamil (Elewa et al. 2007). The lake is also connected with the Suez Canal at El-Qabouti, a few kilometers south of Port Said, and with the Nile's Damietta Branch by El-Inaniya, El-Ratma and Souffara canals (Sallam & Elsayed 2018).

The area of Qahr El-Bahr, located in the city of Port Said, is a semi-isolated area of Lake Al-Manzalah by the International Ring Road and 30 June road. It has an area of about 12.6 km^2 and is fed through the El-Qabouti Channel, a narrow opening that connects the seawater with the study area, which is considered one of the deepest part of the lake. Fish samples were collected at seven sites in September 2018. The distribution of heavy metals in the water and sediment of Lake Al-Manzalah is presented in Table 1.

2.2. Collection of samples

Samples of three tilapia species (*Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zillii*) were collected at seven sites in the area of Qahr El-Bahr, Lake Al-Manzalah. Every site was represented by 20 fish specimens of each species (*Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zillii*). The detailed information on the collected fish is listed in Table 2.

Figure 1
Map of Lake Al-Manzalah, Egypt (source: Google Earth 2020)

Table 1
Distribution of heavy metals in water and sediment of Lake Al-Manzalah (Goher et al. 2017)

Metal	Lake water		Lake sediment	
	Unit	Value	Unit	Value
Fe	$\mu g\ l^{-1}$	216.7–862.4	$mg\ g^{-1}$	8.50–15.36
Mn		8.7–34.6	$\mu g\ g^{-1}$	96.4–362.4
Zn		22.2–58.4		15.3–108.3
Cu		4.3–15.0		5.6–19.0
Pb		7.9–74.7		5.7–63.5
Cd		1.0–3.7		1.09–4.10
Ni*		13.99–51.54		10.01–62.73

*after Elmorsi et al. (2015)

Table 2
List of fish species, total length and size of tilapia fishes caught in Qahr El-Bahr, Lake Al-Manzalah

Species	Total length (cm) Mean ± SD	Body weight (g) Mean ± SD
Oreochromis niloticus (*O. niloticus*)	23.51 ± 1.12	265.2 ± 52.6
Oreochromis aureus (*O. aureus*)	22.36 ± 0.98	252.4 ± 48.3
Tilapia zillii (*T. zillii*)	13.11 ± 1.45	95.5 ± 15.6

2.3. Analysis of heavy metals

Specimens of muscles and gills of different fish species were dried in an oven at 105°C for about 24 h. The dried specimens were then ground to a fine powder. A representative sample of 1 g dry weight of muscles or gills was taken from fish specimens. The samples were digested according to the method described by Goldberg et al. (1983), during which concentrated nitric and perchloric acid (AR grade) in the 5:5 ratio was used in Teflon beakers on a hot plate at 50°C for about 5 h till complete decomposition of organic matter. The digested solutions were cooled to room temperature, filtered and diluted to a final volume of 50 ml with deionized distilled water. The concentrations of Fe, Pb, Mn, Zn, Cu, Cd and Ni were measured by an Australian GBC atomic absorption reader (model Savant AA-AAS) with a GF 5000 graphite furnace and expressed in mg kg^{-1} dry weight.

The precision of the analyzed metal values was controlled by including triplicate samples in analytical batches. Standard deviations for mean values of triplicate measurements were up to 5%, which was regarded as an acceptable precision. Practical quantitation limits for the analysis of heavy metals were in the range of 0.02–0.05 $\mu g\ g^{-1}$.

2.4. Health risk assessment

In the present study, the muscles of tilapia species were specifically selected for heavy metal analysis because they are the only edible tissue, therefore the level of toxicants present in this tissue is particularly relevant.

2.5. Estimated daily intakes (EDIs)

The estimated daily intake (mg kg^{-1} body weight) for heavy metals was calculated in fish muscle samples using the following formula (Shaheen et al. 2016):

$$EDI = \frac{(FIR \times Cm)}{BW}$$

where FIR is the food ingestion rate (g person^{-1} day^{-1}; 57 g in Egypt, FAO 2014), Cm is the metal concentration in fish muscle samples (mg kg^{-1}), and BW is the body weight of an adult assumed to be 70 kg.

2.6. Non-carcinogenic risk

The non-carcinogenic risk for each metal in the muscles was assessed by the hazard quotient (HQ) (USEPA 2000) using the following equation:

$$HQ = \left\{\frac{EF \times ED \times FIR \times Cm}{RfD \times BW \times AT}\right\} \times 10^{-3}$$

where EF is the exposure frequency, ED is the lifetime exposure duration (70 years), RfD is the oral reference dose (mg kg^{-1} day^{-1}), AT is the averaging time for non-carcinogens (365 days year^{-1} × 70 years) and 10^{-3} is the unit conversion factor. If the HQ is higher than 1, there is a potential health risk (Islam et al. 2017).

The overall potential for non-carcinogenic effects from all studied metals was assessed using the hazard index (HI). The hazard index was expressed as the sum of the hazard quotients (USEPA 2000) using the following equation:

$$HI = \sum_{i=1}^{n} HQi$$

2.7. Carcinogenic risk

The carcinogen risk was estimated using the target carcinogenic risk (TCR; USEPA 2000), which was derived from the intake of Pb and Cd using the following equation:

$$TCR = \frac{EF \times ED \times FIR \times Cm \times CPS_o}{BW \times AT} \times 10^{-3}$$

where CPS_o is the oral carcinogenic potency slope (mg kg^{-1} day^{-1}). The CPS_o values for Pb, Cd and Ni were 0.0085 (OEHHA 2009), 0.38 and 1.7 (USEPA 2011), respectively. The US Environmental Protection Agency set acceptable risk levels for carcinogens ranging from 10^{-4} to 10^{-6} (Atique Ullah et al. 2017; Miri et al. 2017).

2.8. Relative risk

The relative risk (RR) of pollutants, defined by Yu et al. (2014), can be helpful in identifying the most harmful metals. The RR was calculated according to the following equation:

$$RR = \frac{Cm}{RfD}$$

Human health risk from fish consumption should increase as the relative risk values increase (Yu et al. 2014).

2.9. Histological studies

Muscles, gills and liver of the fish *Oreochromis niloticus* collected from the area of Qahr El-Bahr were carefully removed and immediately fixed in 10% formalin for 48 h, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and cut with an Euromex Holland microtome into 4–6 microns. Sections were stained using the Harris haematoxylin and eosin (H&E) method (Saad et al. 2011). They were then examined under a microscope and photos were taken by a microscope camera.

2.10. Statistical analysis

The results were tested for significant differences for metals between different sites and species using one-way ANOVA, and $p < 0.05$ was considered statistically significant. In addition, the relationships between the analyzed metals were determined by calculating the Pearson correlation index.

3. Results and discussion

Salts of heavy metals constitute a very serious form of pollution. They can bioaccumulate in fish tissues and the extent of bioaccumulation depends on species, age and trophic transfer (Islam et al. 2017). It was difficult to compare the contamination with trace metals in all fish species at different sites. Therefore, tilapia species were selected as indicators to account for the degree of pollution by heavy metals in the area of Qahr El-Bahr in Lake Al-Manzalah, where they were successfully obtained from all sampling sites. The concentrations of metals (Fe, Zn, Cu, Mn, Pb, Cd and Ni) in different fish species are listed in Table 3.

There were no differences in the order of heavy metal levels in the muscles and gills of the three fish species. Fe accumulated the most in the muscles and gills of the three tilapia species, and was followed by Zn, Ni, Mn, Cu, Pb, and Cd. The levels of essential elements (Fe, Zn, Ni, Mn, Cu) were higher than those of the non-essential ones (Pb, and Cd). The overall concentrations of the studied metals in the muscles and gills of the fish species were in the following descending order: *Tilapia zillii* > *Oreochromis aureus* > *Oreochromis niloticus*, and the metal concentrations showed distinct differences between different species (Elnabris et al. 2013; Islam et al. 2017). The present results indicate significant differences between the distribution of Fe, Zn, Pb, Cu, and Cd in different species ($p < 0.05$). However, there was no clear spatial variation in the contamination with heavy metals ($p > 0.05$) for the three species. In addition to species differences, differences in metal concentrations depend on the types of tissues analyzed (Abarshi et al. 2017), with gills containing a higher level of the studied metals compared to the muscles in all tilapia species (Table 3). The tissue of gills in all fish has the ability to accumulate significant levels of metals compared to other tissues and their surface has a negative charge and therefore provides a possible site for positively charged elements (Shovon et al. 2017).

Iron is the most important metal for biological life. It plays a greater biological role than any other heavy metal. Its toxicity causes diarrhea, hemorrhagic gastroenteritis, liver necrosis and leads to death by hepatic coma (Clarke et al. 1981). According to WHO (1989), the permissible limit of Fe concentrations in fish species is about 100 mg kg^{-1}. The concentrations of Fe in tissues of tilapia species are still below this limit and the fish can be considered as uncontaminated (< 100; Table 3).

Zn and Cu are essential micronutrients for all organisms. They are required at considerable levels as constituents of various enzymes in organisms to maintain certain biological functions. Zn was found in high concentrations in samples of some fish species, exceeding the limit (40 mg kg^{-1}) specified by FAO (1983). Zn levels were higher than the permissible limit in 14.2%, 28.4%, and 71.1% of the muscle samples of *Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zillii*, respectively. The concentration of metals, especially zinc, was more elevated in the gills than in the muscles because the gills are the main entrance for metals into the fish. They are taken up by fish directly from water, especially through mucus and gills (Skidmore 1964). The high concentration of zinc may be due to domestic, sewage and agricultural wastes discharged into the lake through different drains and affecting the entire area of the lake (Abdel-Satar

Table 3

Metal concentration (mg kg^{-1}) in the muscles and gills of fish species collected from Qahr El-Bahr

Fish species (muscle)		Fe	Zn	Cu	Mn	Pb	Cd	Ni
O. niloticus	Range Mean ± SD	36.4–83.9 53.3 ± 21.0	28.5–41.5 35.7 ± 4.8	2.0–4.2 2.6 ± 0.8	2.9–6.9 4.4 ± 1.7	1.5–3.3 2.2 ± 0.7	0.4–1.1 0.7 ± 0.3	4.39–6.01 5.51 ± 0.5
O. aureus	Range Mean ± SD	37.9–84.3 52.1 ± 15.5	30.7–45.6 37.3 ± 5.4	2.3–6.4 3.7 ± 1.4	2.9–7.5 4.9 ± 1.5	1.6–4.8 3.1 ± 1.2	0.4–1.4 1.0 ± 0.3	4.68–6.65 5.54 ± 0.6
T. zillii	Range Mean ± SD	60.9–98.3 78.1 ± 11.9	39.8–50.1 45.5 ± 3.7	3.3–4.8 4.1 ± 0.5	4.7–8.1 6.1 ± 1.1	2.2–4.9 3.8 ± 1.1	1.0–1.4 1.2 ± 0.2	5.52–6.73 6.05 ± 0.5
Fish species (gills)		Fe	Zn	Cu	Mn	Pb	Cd	Ni
O. niloticus	Range Mean ± SD	119.3–220.0 163.5 ± 41.7	55.0–76.6 65.4 ± 10.2	4.8–7.7 6.1 ± 1.2	21.3–54.2 32.3 ± 15.1	3.6–6.0 4.8 ± 1.1	1.0–1.4 1.3 ± 0.2	11.6–13.8 12.5 ± 1.0
O. aureus	Range Mean ± SD	131.5–221.5 176.5 ± 36.3	52.6–105.2 73.4 ± 18.5	5.9–10.1 7.4 ± 1.6	30.7–61.3 46.8 ± 13.3	5.5–7.2 6.3 ± 0.6	1.4–2.1 1.8 ± 0.3	11.4–14.0 12.5 ± 1.0
T. zillii	Range Mean ± SD	189.5–291.2 220.4 ± 42.7	66.9–105.2 87.7 ± 15.9	4.7–8.2 6.0 ± 1.4	35.9–68.9 49.2 ± 12.5	4.3–7.2 6.0 ± 1.1	1.4–2.3 1.8 ± 0.4	12.5–15.9 14.0 ± 1.2

2008; Abdel-Satar & Geneid 2009; El-Naggar et al. 2016; Elmorsi et al. 2019). FAO (1983) and WHO (1989) proposed a permissible limit of 30 mg kg^{-1} for Cu concentration. Cu concentrations in all fish muscle samples harvested from the study area were below the recommended level and did not appear to pose a contamination hazard.

Mn deficiency causes reproductive and skeletal abnormalities. Daily intake of small amounts of Mn is recommended for growth and good health of children. However, excess consumption of Mn can lead to neurologic and psychological disorders (Ahmed et al. 2016). Mn concentrations in the muscles of the studied fish were higher than the permissible concentration (1 mg kg^{-1}) recommended by WHO (1989). The increase in Mn levels in fish gills and muscles is related to a large amount of agricultural drainage water entering Lake Al-Manzalah. Our results are consistent with those of Mahmoud & Abd El Rahman (2017) for the fish species *Clarias gariepinus* and *Mugil capito* from the same lake. Agrochemicals, such as pesticides and herbicides, release Mn and contribute to its accumulation in fish (Ibrahim & Mahmoud 2005). Fish samples showed a similar accumulation of heavy metals to that observed in another study conducted in Lake Al-Manzalah by Elmorsi et al. (2019), where Fe, Mn, Ni and Zn were the most accumulated metals in fish. This may indicate that the enrichment of biota with heavy metals was affected by their concentrations in water, sediment and plants.

Pb and Cd play no role in biological processes of living organisms and are highly toxic non-essential elements even at low concentrations (Dimari et al. 2008). They are also potent mutagenic and carcinogenic agents (Markmanuel & Horsfall 2016). Pb inhibits impulse conductivity by inhibiting the activity of acetylcholine esterase and monoamine oxidase, leading to pathological changes in organs and tissues (Rubio et al. 1991). It also impairs the larval and embryonic growth of fish species (Dave & Xiu 1991).

Pb and Cd concentrations in the muscles and gills of the three fish species were higher than the permissible limits recommended by FAO (1983) as criteria for human health protection, indicating that Pb and Cd may pose a risk to humans through the consumption of these contaminated fish species. Cd concentrations in fish muscles of the collected species were higher than its permissible limit (0.5 mg kg^{-1}), while Pb concentrations were 3 to 10 times higher than the limit of 0.5 mg kg^{-1} recommended by FAO (1983) in the muscles of all species.

Ni is a well-known essential metal necessary for enzymes and other cell components with critical functions for living organisms, yet very high intakes can lead to serious health problems (Elnabris et al. 2013), therefore IARC (2012) classified Ni as a human carcinogen. Ni concentrations in the muscles (4.39–6.73 mg kg^{-1}) and gills (11.4–15.6 mg kg^{-1}) of tilapia species were found to be lower than the limit (70–80 mg kg^{-1}) recommended by USFDA (1993).

Correlation coefficients were calculated to clarify the relationships between the analyzed metals. Matrix analysis showed significant positive correlations (n = 7; $p < 0.05$) for each pair of metals contained in the muscles: Zn/Cu (r = 0.68), Zn/Pb (r = 0.81), Zn/Cd (r = 0.64), Cu/Pb (r = 0.89), Cu/Cd (r = 0.77) and Pb/Cd (r = 0.83) for *Oreochromis niloticus*, Fe/Zn (r = 0.79), Fe/Cu (r = 0.91), Fe/Ni (r = 0.66) and Zn/Cu (r = 0.91) for *Oreochromis aureus* and finally between Fe/Ni (r = 0.55) and Pb/Cu (r = 0.56) for *Tilapia zillii*. Whereas positive correlations (n = 7; $p < 0.05$) were found for the following pairs of metals contained in the gills: Fe/Zn (r = 0.75), Fe/Pb (r = 0.90), Fe/Cd (r = 0.82), Fe/Ni (r = 0.85), Zn/Ni (r = 0.82), Pb/Ni (r = 0.91) and Pb/Zn (r = 0.90), Pb/Cd (r = 0.83) and Mn/Cu (r = 0.84) for *Oreochromis niloticus,* Fe/Ni (r = 0.88), Fe/Pb (r = 0.66) and Pb/Ni (r = 0.82) for *Oreochromis aureus* and Fe/Zn (r = 0.74), Fe/Ni (r = 0.90), Pb/Mn (r = 0.86), Pb/Zn (r = 0.76), Pb/Cu (r = 0.79), Pb/Cd (r = 0.90), Mn/Zn (r = 0.73), Mn/Cu (r = 0.93), Mn/Cd (r = 0.90), Cu/Zn (r = 0.87), Cu/Cd (r = 0.78) for *Tilapia zillii.* Significant positive correlations between metals indicate their release from the same sources (drainage water) and mutual dependence (Ali et al. 2019).

3.1. Heath risk assessment

As fish consumption is a possible source of heavy metal accumulation in humans, it is important to consider the daily intake of metals through fish consumption (Elnabris et al. 2013). The EDI was estimated by considering that a 70 kg person consumes 57 g fish per day. The EDI of heavy metals through the consumption of three tilapia fish species by humans is presented in Table 4. The results revealed that Mn, Ni, Cu, Pb, and Cd constituted the lowest daily intake, while Fe and Zn – the highest daily intake. The EDI values of Fe, Pb, and Cd in the selected fish species were higher than the maximum tolerable daily intake (MTDI) values recommended by FAO\WHO (2011) for a 70 kg person (MTDI-70), indicating a high human health risk associated with the consumption of the examined fish. Whereas the estimated daily intake of Zn, Cu, Mn and Ni in the muscles of tilapia species was below the corresponding permissible tolerable daily intake (Table 4).

The health risk assessment was carried out to determine potential risks resulting from the

Table 4

Estimated daily intakes of heavy metals for consumable fish collected from Qahr El-Bahr, the oral reference dose (RfD) and the maximum tolerable daily intake (MTDI)

Species		Estimated Daily Intakes (EDIs; mg kg^{-1}) Fe	Zn	Cu	Mn	Pb	Cd	Ni
O. niloticus	Range	29.7–68.3	23.2–33.8	1.7–3.4	2.3–5.6	1.2–2.7	0.3–0.9	3.6–4.9
	Mean ± SD	41.7 ± 17.1	29.1 ± 3.9	2.2 ± 0.6	3.6 ± 1.3	1.8 ± 0.5	0.5 ± 0.2	4.5 ± 0.4
O. aureus	Range	30.9–68.6	25.9–37.1	1.9–5.2	2.4–6.1	1.3-3.9	0.3–1.1	3.8–5.4
	Mean ± SD	42.4 ± 12.6	30.3 ± 4.4	3.0 ± 1.1	4.0 ± 1.2	2.5 ± 1.0	0.8 ± 0.3	4.5 ± 0.5
T. zillii	Range	49.6–80.0	32.4–40.8	2.7–3.9	3.8–6.6	1.8–4.0	0.8–1.2	4.5–5.5
	Mean ± SD	63.6 ± 9.7	37.0 ± 3.0	3.4 ± 0.4	5.0 ± 0.9	3.1 ± 0.9	1.0 ± 0.1	4.9 ± 0.4
RfD*		0.7	0.3	0.037	0.14	0.0035	0.001	0.02
MTDI**		0.8	1	0.5	1	0.003	0.0008	5
MTDI-70		56	70	35	70	0.21	0.056	350

* WHO (2018)
**FAO/WHO (2011)
MTDI-70 – maximum tolerable daily intake for a 70 kg person (mg day−1) = MTDI × 70 kg

consumption of the three fish species collected from the selected section of Lake Al-Manzalah. The results of the health risk assessment using the hazard quotient index are shown in Table 5. The HQ values for Fe, Zn, Mn, Cu and Ni were below 1 for all tilapia species from all sampling locations, indicating that there is no health risk associated with the exposure to these individual heavy metals. However, the HQ for Pb was above 1 in 14.3% of *Oreochromis aureus* samples and 42.8% of *Tilapia Zillii* samples. Whereas the HQ value for Cd was greater than 1 in 28.5% of *Oreochromis aureus* samples and 42.8% of *Tilapia Zillii* samples collected from Qahr El-Bahr, indicating that a potential risk may occur upon consumption of fish belonging to these species.

The health risk assessment of metal exposure from the consumption of tilapia fish species from Lake Al-Manzalah should allow for the combined effects of various heavy metals studied. Therefore, the HI value is quite necessary to assess the health risk associated with fish consumption (Zhu et al. 2016). The HI was above 1 for the three fish species. The minimum HI was observed for *Oreochromis niloticus* (1.21–2.10), while the highest HI was recorded in *Tilapia zillii* samples (2.08–2.84). Of all the studied metals, Cd and Pb were the major contributors to the HI values (34.3–40.6% and 33.7–35.5%, respectively), followed by Ni (9.6–15.7%) for the three tilapia species.

Although the HI for all three species falls under the medium non-cancer risk category (1 > HI < 4), the consumption of fish from Qahr El-Bahr was found unsafe when consumed for an extended period of time. Of the three species studied, *Oreochromis niloticus* is relatively safe for consumption as posing the lowest health hazard, while *Tilapia zillii* is more predisposed to accumulate heavy metals in its tissues.

According to USEPA (2012), Fe, Mn, Zn, and Cu do not cause any carcinogenic effects as their CPS_o has not yet been established. The average TCR factors for Pb over a lifetime of exposure through the consumption of contaminated *Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zillii* were 1.5×10^{-5}, 2.1×10^{-5} and 2.6×10^{-5}, respectively. Since USEPA sets the value of 10^{-5} as an acceptable lifetime carcinogenic

Table 5

Carcinogenic and non-carcinogenic risk of metals due to fish consumption based on fish samples collected from Qahr El-Bahr

Species		HQ Fe	Zn	Cu	Mn	Pb	Cd	Ni	HI	TCR Pb	Cd	Ni
O. niloticus	Range	0.04–0.10	0.08–0.11	0.05–0.09	0.02–0.04	0.34–0.77	0.33–0.90	0.18–0.25	1.21–2.10	1×10^{-5}–2.3×10^{-5}	1×10^{-4}–3×10^{-4}	6×10^{-3}–8×10^{-3}
	Mean ± SD	0.06 ± 0.02	0.10 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.51 ± 0.16	0.53 ± 0.23	0.22 ± 0.01	1.51 ± 0.40	$2 \times 10^{-5} \pm 5 \times 10^{-6}$	$2 \times 10^{-4} \pm 9 \times 10^{-5}$	$8 \times 10^{-3} \pm 7 \times 10^{-4}$
O. aureus	Range	0.04–0.10	0.08–0.12	0.05–0.14	0.02–0.04	0.37–1.11	0.33–1.10	0.19–0.27	1.43–2.69	1×10^{-5}–3×10^{-5}	1×10^{-4}–4×10^{-4}	6×10^{-3}–9×10^{-3}
	Mean ± SD	0.06 ± 0.02	0.10 ± 0.01	0.08 ± 0.03	0.03 ± 0.01	0.71 ± 0.28	0.84 ± 0.27	0.23 ± 0.03	2.05 ± 0.50	$2 \times 10^{-5} \pm 8 \times 10^{-6}$	$3 \times 10^{-4} \pm 1 \times 10^{-4}$	$8 \times 10^{-3} \pm 9 \times 10^{-4}$
T. zillii	Range	0.07–0.11	0.11–0.14	0.07–0.11	0.03–0.05	0.51–1.14	0.84–1.16	0.23–0.27	2.08–2.84	2×10^{-5}–3×10^{-5}	3×10^{-4}–4×10^{-4}	8×10^{-3}–9×10^{-3}
	Mean ± SD	0.09 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.04 ± 0.01	0.89 ± 0.26	0.99 ± 0.12	0.25 ± 0.02	2.46 ± 0.27	$3 \times 10^{-5} \pm 8 \times 10^{-6}$	$4 \times 10^{-4} \pm 5 \times 10^{-5}$	$8 \times 10^{-3} \pm 7 \times 10^{-4}$

risk, the TCR of Pb appears to be negligible. On the other hand, all three fish species pose a significant health risk from the intake of Cd and Ni, with TCR ranging from 6×10^{-3} to 4×10^{-4}.

Cd poses the highest relative risk (RR) for the three tilapia species, followed by Pb and Ni, while Mn poses the lowest risk. The contribution of Pb and Cd to the overall relative risk index ranged from 34% to 41% (Figure 2). Thus, the consumption of tilapia species should be limited to avoid a potentially harmful exposure to these metals, especially Cd and Pb. Many coastal cities in Egypt rely primarily on fish as a source of protein in their meals, hence fish consumption is relatively high, so their exposure to heavy metal toxicity increases.

muscle fibers and the hypodermal layer, edema, hemorrhage in muscle fibers and balloon necrosis in hypodermal layers (Fig. 3a–f). These results are consistent with those obtained by El- Serafy et al. (2005), Yacoub et al. (2008) and Saad et al. (2011). These changes in the muscles may be attributed to the accumulation of heavy metals and/or inorganic fertilizers that are discharged from different drains into the lake with a large amount of wastes (Mahmoud & El-Naggar 2007; Yacoub et al. 2008; Tayel et al. 2018) and to parasitic infections (Saad et al. 2011; Abou El-Gheit et al. 2012).

The gills are the most delicate structure of the teleost body, having an external location. They are exposed to damage and pathological abnormalities

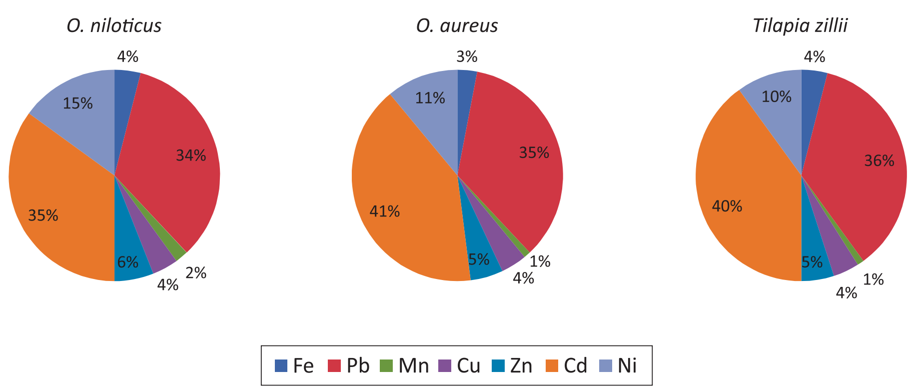

Figure 2

Relative risk of heavy metals for tilapia species

3.2. Histopathological observations

For field assessment, histopathology is a rapid and cost-effective method of detecting adverse, acute and chronic effects of exposure in various tissues and organs. *Oreochromis niloticus* is one of the most commercially important fish that can tolerate a wide range of severe environmental conditions and shows little susceptibility to diseases (Tayel et al. 2008).

The muscular system constitutes the largest part of the fish body. Its overall functions include locomotion, pumping of blood, synchronized movement of skeletal components, peristaltic constriction of visceral organs and their related structures (Kadry et al. 2015). Muscles which are mainly composed of segmental myomeres are covered with skin. Each myomere is regarded as a muscle and its fibers are parallel along the body axis (Bayomy & Tayel 2007; Yacoub et al. 2008).

The results of histopathological examination of skin and muscles of *Oreochromis niloticus* indicate degeneration of the epithelial layer, separation of epidermis from the dermal layer, degeneration of

by irritants that are suspended or dissolved in water, thereby reducing their surface area and retarding the respiratory function (Tayel et al. 2014). The importance of gills as a target organ for contaminants results from their large surface area and the fact that they are in constant and direct contact with irritants. Exposure to heavy metals results in respiratory, osmoregulatory and circulatory impairment (Fernandes et al. 2008). In the present study, histopathological changes were observed in the gills of *Oreochromis niloticus*, including degeneration, necrosis, hemorrhage progressing to hemolysis in the secondary lamellae, edema, curling, lamellar aneurysm as well as hyperplasia progressing to complete fusion of the secondary lamellae (Fig. 4a–f). According to Mallat (1985), the edema of the gill epithelium is one of the major structural changes caused by exposure to heavy metals. The current results confirm that this lesion is due to heavy metal exposure. Alvarado et al. (2006) reported that a significant increase in the number of chloride cells in the gills induces epithelial thickening of the filament, which enhances the migration of chloride cells to

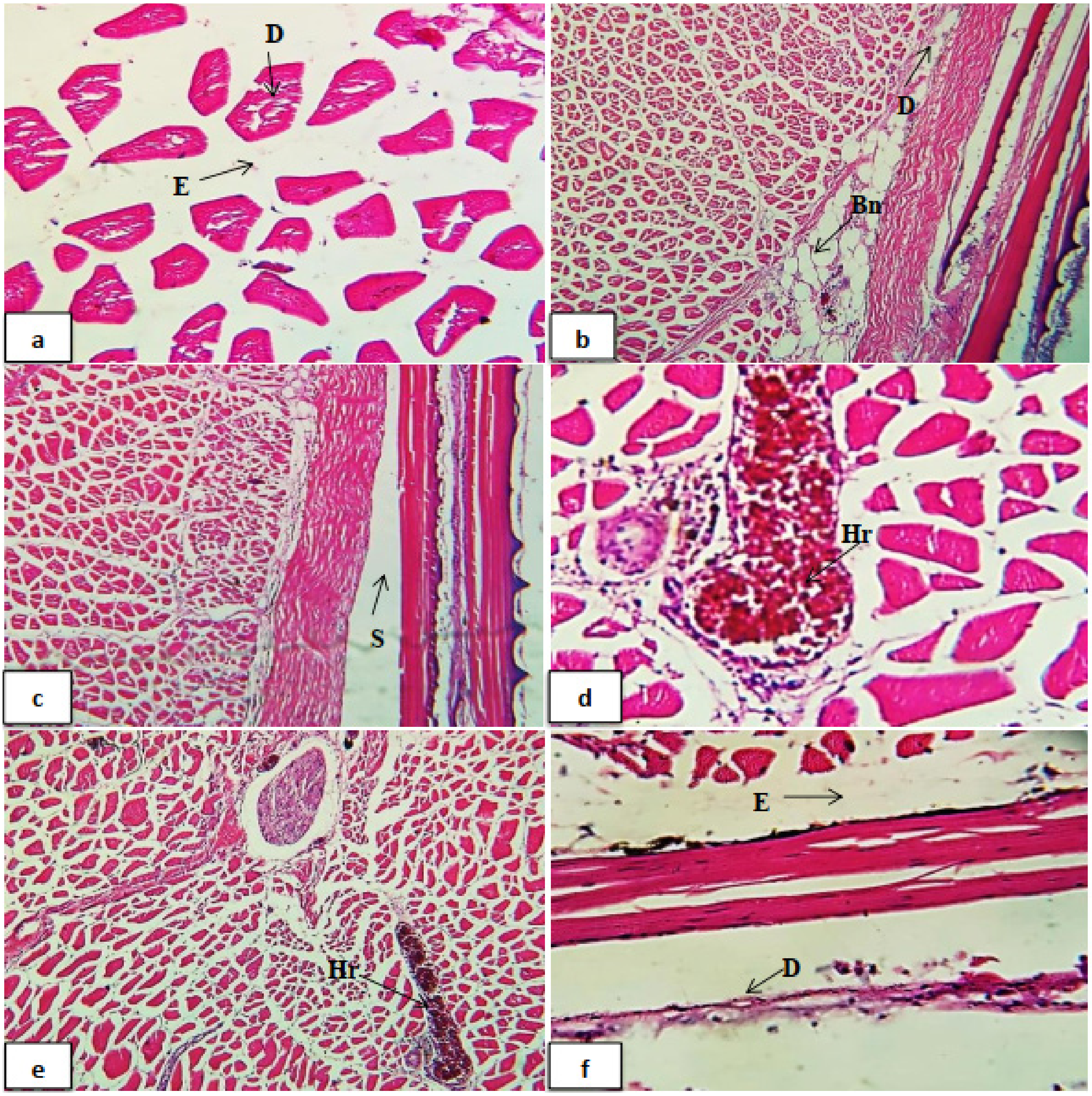

Figure 3

a–f. Vertical section of skin and muscles of *O. niloticus* obtained from Qahr El-Bahr, Lake Al-Manzalah, stained with H&E, showing: degeneration (D) of epithelial and hypodermal layers, muscle fibers; edema (E) & hemorrhage (Hr) between myomeres; separation (S) of epidermis from the dermal layer and balloon necrosis (Bn) in the hypodermal layer (a, d, f × 400) & (b, c, e × 100)

the edges of the secondary lamellae and causes hypertrophy and fusion of the secondary lamellae. This may be viewed as a non-specific biomarker response to the exposure to heavy metals and disruption of fish health.

The intralamellar hyperplasia is a consequence of excess mucus production. Penetration of contaminants activates the secondary lamellae epithelium to increase the number of mucus cells. Hyperplasia with excess mucus lamellae causes fusion, which reduces

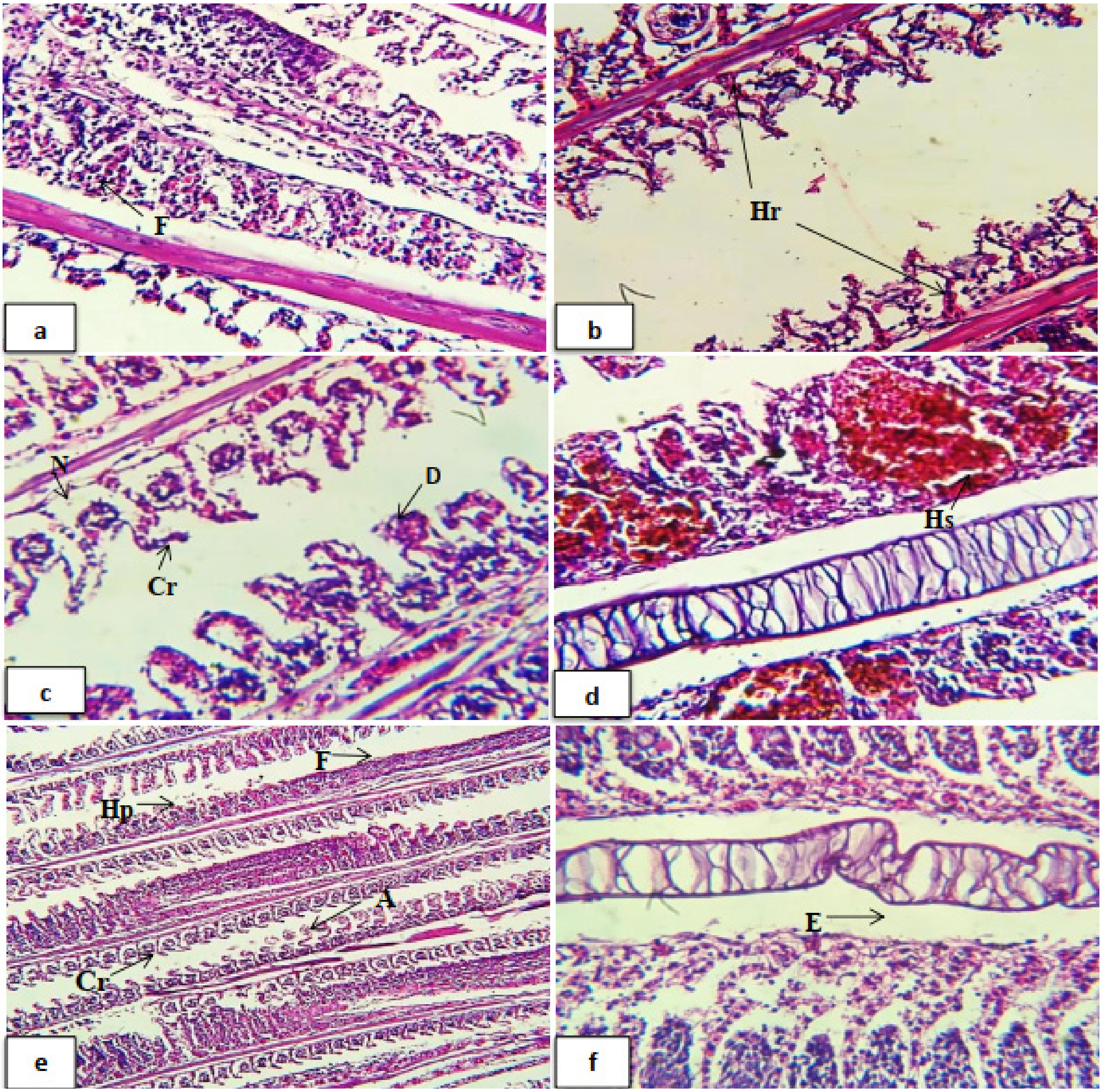

Figure 4

a–f. Longitudinal section of gills in *O. niloticus* obtained from Qahr El-Bahr, Lake Al-Manzalah, stained with H&E, showing: degeneration (D), necrosis (N), hemorrhage (Hr), hemolysis (Hs), edema (E), curling (Cr), aneurysm (A), hyperplasia (Hp) progressing to complete fusion (F) of primary and secondary lamellae (a, b, c, d, & f × 400; e × 100)

the surface area of the gills and thus affects the ability of the gas exchange (Kumari et al. 2012). The discharge of wastewater into natural water bodies containing a large amount of organic matter results in high levels of organic phosphorus (Marcogliese et al. 2015), which can reduce the gaseous exchange capacity in fish (Dalzochio et al. 2018).

The liver is the main organ of detoxification in fish, with one of the main functions being to cleanse the body of any poisons coming from the intestine (El-Naggar et al. 2009). Any damage to the fish liver ultimately leads to multiple physiological disorders and subsequent fish death (Mahboob et al. 2020). The liver of *Oreochromis niloticus* caught from Lake Al-Manzalah in Qahr El-Bahr showed many histopathological changes, including degeneration, necrosis, fatty degeneration, hemorrhage and accumulation of hemosiderin in hepatocytes. Hemorrhage, hemosiderin and degeneration in blood vessels were observed in addition to congestion in blood sinusoids (Fig. 5a–f).

These changes may be due to fertilizers, salts and sewage discharged into Lake Al-Manzalah. Tayel et al. (2014) and Mahmoud & Abd El Rahman (2017) found similar histopathological changes in the liver of *Mugil* species and *Clarias gariepinus* caught in the same lake. Degeneration and necrosis of hepatocytes may be due to the accumulation of heavy metals. This is consistent

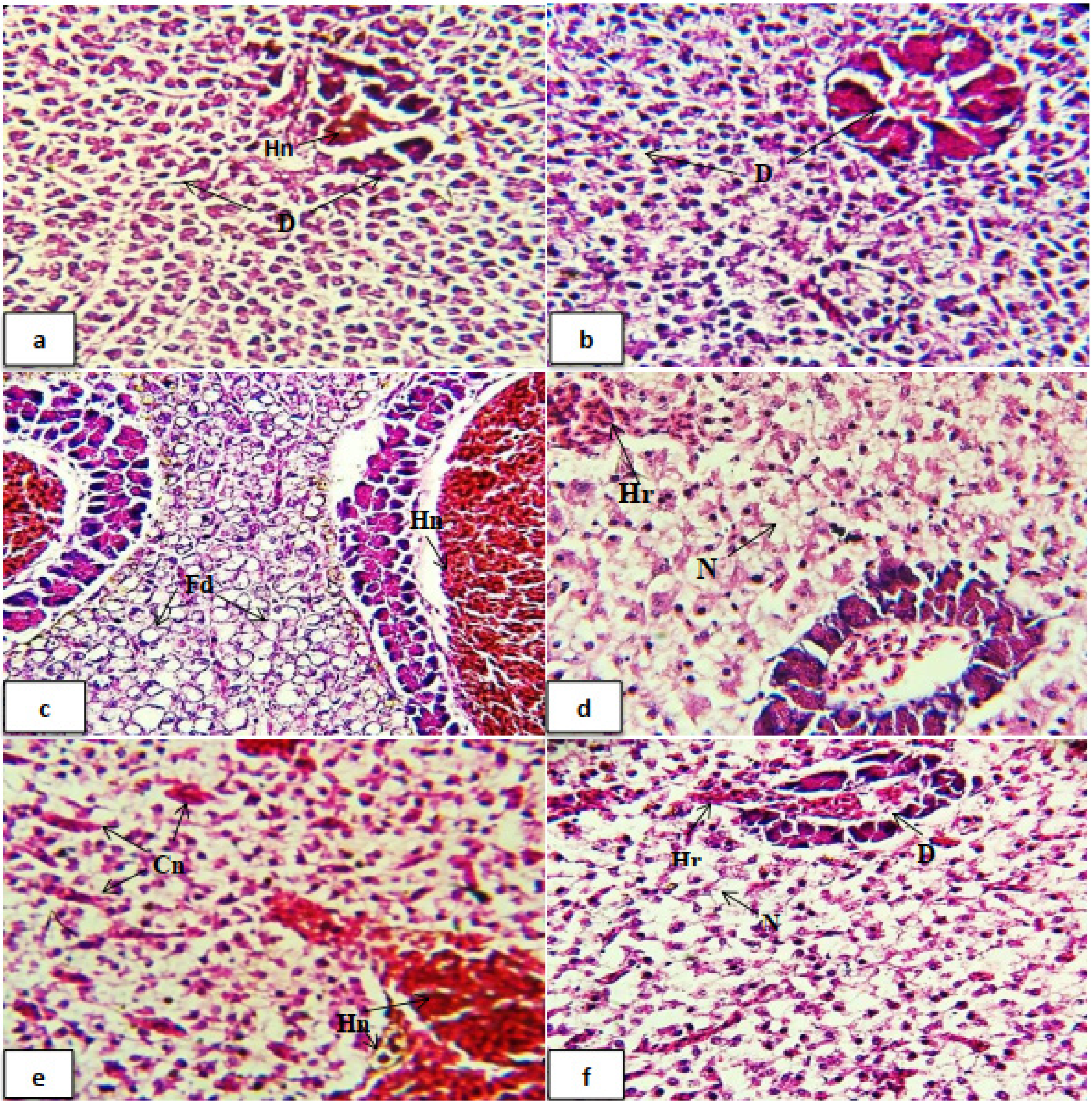

Figure 5

a–f. Liver sections of *O. niloticus* obtained from Qahr El-Bahr, Lake Al-Manzalah, stained with H&E, showing: degeneration (D), fatty degeneration (Fd), necrosis (N), hemorrhage (Hr) and hemosiderin (Hn) in hepatocytes, hemorrhage (Hr), hemosiderin (Hn) degeneration (D) in blood vessels, congestion (Cn) in blood sinusoids (× 400) with the findings of Authman & Abbas (2007) who stated that the liver is involved in a detoxification of toxins such as heavy metals. Accumulation of hemosiderin in liver cells may contribute to the rapid and continuous destruction of red blood cells (Hashem et al. 2020; Tayel et al. 2018; Ibrahim & Mahmoud 2005). Degeneration of hepatocytes can be caused by oxygen deficiency due to intravascular hemolysis and vascular dilation (Gaber & Gaber 2006). Toxins secreted by microorganisms in sewage water may cause necrosis and hemorrhage (Saad et al. 2011). Fatty degeneration can be caused by an increased rate of utilization of energy reserves or an induced imbalance between fat utilization and production (El-Naggar et al. 2009). The liver is an organ that excretes and binds proteins such as metallothionein. Metal-binding proteins, which are present in the nuclei of hepatocytes, increase the cell damage (Mela et al. 2007).

4. Conclusions

The study showed that tilapia fish species caught in the area of Qahr El-Bahr in Lake Al-Manzalah contained varying concentrations of metals and the degree of their accumulation varied among different tilapia species. The average level of heavy metals ranged as follows: *Tilapia zillii* > *Oreochromis aureus* > *Oreochromis niloticus*. The accumulation rate of heavy metals in the muscles and gills of tilapia fishes were in the following order; Fe > Zn > Ni > Mn > Cu > Pb > Cd. The studied metals do not pose carcinogenic health hazards individually but their combined effects are potentially hazardous to the health of consumers (HP > 1). The muscles of *Oreochromis niloticus* occurring in the Qahr El-Bahr area of Lake Al-Manzalah are almost safe for human consumption. Thus, *Oreochromis niloticus* can withstand the risk of heavy metals in the study area. In general, a moderate intake of tilapia fish from Lake Al-Manzalah is strongly recommended for consumers to avoid serious health problems, including cancer and kidney malfunctions. Histopathological changes, including degeneration, fatty degeneration, necrosis, edema, hemorrhage, hemolysis, hemosiderin, curling and hyperplasia were found in the muscles, gills and liver of *Oreochromis niloticus* due to the accumulation of heavy metals in fish organs.

References

Abarshi, M.M., Dantala, E.O. & Mada, S.B. (2017). Bioaccumulation of heavy metals in some tissues of croaker fish from oil spilled rivers of Niger Delta region, Nigeria. *Asian Pac. J. Trop. Biomed.* 7(6): 563–568.

Abdel-Satar, A.M. & Goher, M.E. (2009). Nutrient status and phosphorus speciation of Manzalah Lake sediment, Egypt. *Thalassia Salentina* 32: 3–16.

Abdel-Satar, A.M. (2001). Environmental studies on the impact of the drains effluent upon the southern sector of Lake Manzala, Egypt. *Egypt. J. Aquat. Biol. & Fish.* 5(3): 17–30.

Abdel-Satar, A.M. (2008). Chemistry of major ions, nutrient salts and heavy metals in the Manzalah Lake system, Egypt. *Egypt. J. Aqua. Res.* 34(2): 130–148.

Abdel-Satar, A.M., Geneid, Y.A. (2009). Evaluation of heavy metal status in ecosystem of Lake Manzalah, Egypt. *Global. J. Environ. Res.* 3(3): 194–204.

Abdel-Satar, A.M.; Ali, M.H.H & Goher, M.E. (2017). Distribution and speciation of Fe, Mn, Zn, Cu, Pb and P in surface sediments of Mariut Lake, Egypt. *Oceanological and Hydrobiological Studies* 46(2): 154–167.

Abou El-Gheit, E.N., Abdo, M.H. & Mohmoud, S.A. (2012). Impact of blooming phenomenon on water quality and fishes in Qarun Lake, Egypt. *International Journal of Environmental Science and Engineering (IJESE)* 3: 11–23.

Abu Khatita, A.M., Shaker, I.M. & Shetaia, S.A. (2015). Urabanization and human activities around Manzala Lake, Egypt: studies on heavy metals distribution and environmental impacts. *Sedimentology of Egypt* 22: 69–83.

Ahmed, M., Donia, N. & Fahmy, M. (2006). Eutrophication assessment of Lake Manzala, Egypt using geographical information systems (GIS) techniques. *Journal of Hydroinformatics* 8(2): 101–109.

Ahmed, Md.K., Abdul Baki, M., Kundu, G.K., Islam, Md.S., Islam, Md.M. et al. (2016). Human health risks from heavy metals in fish of Buriganga River, Bangladesh. *SpringerPlus* 5: 1697.

Ali, M.M., Ali, M.L., Proshad, R., Islam, S., Rahman, Z. et al. (2019). Heavy metal concentrations in commercially valuable fishes with health hazard inference from Karnaphuli river, Bangladesh. *Human and Ecological Risk Assessment: An International Journal* DOI: 10.1080/10807039.2019.1676635.

Ali, M.M.H. (2008). Assessment of some water quality characteristics and determination of some heavy metals in Lake Manzala, Egypt. *Egypt. J. Aquat. Biol. & Fish.* 12(2): 133–154.

Alvarado, N.E., Quesada, K., Hylland, I., Marigómez, L. & Soto, M. (2006). Quantitative changes in metallothionein expression in target cell-types in the gills of turbot (*Scophthalmus maximus*) exposed to Cd, Cu, Zn and after a depuration treatment. *Aquatic Toxicology* 77(1): 64–77.

Al-Yousuf, M.H., El-Shahawi, M.S. & Al-Ghais, S.M. (2000). Trace metals in liver, skin and muscle of Lethrimus lentjan fish species in relation to body length and sex. *The Science of the Total Environment* 256(2–3): 87–94.

Atique Ullah, A.K.M., Maksud, M.A., Khan, S.R., Lutfa, L.N. & Quraishi, S.B. (2017). Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh. *Toxicology Reports* 4: 574–579.

Authman, M. & Abbas, H. (2007). Accumulation and distribution of copper and zinc in both water and some vital tissues of two fish species (*Tilapia zillii* and *Mugil cephalus*) of Lake Qarun, Fayoum Province, Egypt. *Pak. J. Biol. Sci.* 10(13): 2106–2122.

Bayomy, M.F.F. & Tayel, S.I. (2007). Effect of industrial wastes on the bony fish *Clarias gariepinus* inhabiting the River Nile (Egypt). *J. Egypt. Ger. Soc. Zool.* 54C: 239–255.

Clarke, M.L., Harvey, D.G. & Humphreys, D.J. (1981). *Veterinary Toxicology*. London: Bailliere Tindall

Dalzochio, T., Rodrigues, G., Simões, L.A., de Souza, M., Petry, I.E. et al. (2018). In situ monitoring of the Sinos River, southern Brazil: water quality parameters, biomarkers, and metal bioaccumulation in fish. *Environ. Sci. Pollut. Res. Int.* 25: 9485–9500.

Dave, G & Xiu, R (1991). Toxicity of mercury, copper, nickel, lead and cobalt to embryos and larval of Zebra fish *Brachydanio rerio*. *Archive of Environmental Contamination and Toxicology* 21: 126–134.

Dimari, G.A., Abdulrahman, J.C. & Garba, S.T. (2008). Metals concentrations in tissues of *Tilapia gallili*, *Clarias lazera* and *Osteo glossidae* caught from Alau Dam, Maiduguri, Borno State, Nigeria. *American Journal of Environmental Sciences* 4(4): 373–379.

El-Bokhty, E.E.B .(2010). Fisheries management of *Oreochromis niloticus* and *Oreochromis aureus* caught by Trammel Nets and Basket Traps in Lake Manzalah, Egypt. *World Journal of Fish and Marine Sciences* 2(1): 51–58.

Elewa, A.A., Saad, E.A., Shehata, M.B. & Ghallab, M.H. (2007). Studies on the effect of drain effluents on the water quality of Lake Manzala, Egypt. *Egypt. J. Aquat. Biol. & Fish.* 11(2): 65–78.

Elmorsi, R.R., Abou-El-Sherbini, K.S., Mostafa, G.A. & Hamed, M.A. (2019) Distribution of essential heavy metals in the aquatic ecosystem of Lake Manzala, Egypt. *Heliyon* 5(8): e02276.

Elnabris, K.J., Muzyed, S.K. & El-Ashgar, N.M. (2013). Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza Strip (Palestine). *Journal of the Association of Arab Universities for Basic and Applied Sciences* 13(1): 44–51.

El-Naggar, A.M., Mahmoud, S.A. & Tayel, S.I. (2009). Bioaccumulation of some heavy metals and histopathological alterations in liver of *Oreochromis niloticus* in relation to water quality at different localities along the River Nile, Egypt. *World J. Fish. & Marine Sci.* 1(2): 105–114.

El-Naggar, N., Rifaat, A.E. & Khalil, M.Kh. (2016) Numerical modeling on water flow in Manzala Lake, Nile Delta, Northern Egypt. International Journal of Contemporary Applied Sciences 3(4): 28–44.

El-Refaie, Gh. (2010) Temperature impact on operation and performance of Lake Manzala Engineered Wetland, Egypt. *Ain Shams Engineering Journal* 1(1): 1–9.

El-Serafy, S.S., Ibrahim, S.A. & Mahmoud, S.A. (2005). Biochemical and histopathological studies on the muscles of the Nile Tilapia (*Oreochromis niloticus*) in Egypt. *Egypt. J. Aquatic. Biol. & Fish.* 9(1): 81–96.

EL-Shafei, H.M. (2016). Assessment of some water quality characteristics as guidelines for the management of pond fish culture in Lake Manzala, Egypt. *International Journal of Fisheries and Aquatic Studies* 4(2): 416–420.

FAO (Food and Agricultural Organization). (1983). Compilation of legal limits for hazardous substances in fish and fishery products, FAO Fishery Circular No. 464, pp. 5–100.

FAO (Food and Agricultural Organization). (2014). Report of fisheries and aquaculture markets in the Middle East. (http://www.thefishsite.com/articles/1870/fao-report-fisheries-and-aquaculture markets-in-the-middle-east).

FAO/WHO (Food and Agricultural Organization/World Health Organization). (2011). Food Standards Programme Codex Committee on Contaminants In Foods Fifth Session. The Hague, The Netherlands, 21–25 March 2011.

Fatima, M., Usmani, N., Firdaus, F., Zafeer, M.F., Ahmad, S. et al. (2015). In vivo induction of antioxidant response and oxidative stress associated with genotoxicity and histopathological alteration in two commercial fish species due to heavy metals exposure in northern India (Kali) river. *Comparative Biochemistry and Physiology*, *Part C* 176–177: 17–30.

Fernandes, C., Fernandes, A.F., Ferreira, M. & Salgado, M. (2008). Oxidative stress response in gill and liver of *Liza saliens*, from the Esmoriz-Paramos coastal lagoon, Portugal. *Arch. Environ. Contam. Toxicol.* 55: 691–700.

Gaber, H.S. & Gaber, S.A. (2006). The effect of water quality of Lake Quarun on liver and gonads of *Oreochromis aureus* (L.) and *Tilapia zillii* (Gev.). *Egypt. J. Aquat. Res.* 32(2): 335–349.

Goher, M.E., Abdo, M.H., Bayoumy, W.A. & Mansour, El-Ashkar T.Y. (2017). Some heavy metal contents in surface water and sediment as a pollution index of El-Manzala Lake, Egypt. *Journal of Basic and Environmental Sciences* 4: 210–225.

Goldberg, E.D., Koide, M., Hodge, V., Flegal, A.R. & Martin, J. (1983). U.S. mussel watch: 1977–1978 results on trace metals and radionuclides. *Estuarine, Coastal and Shelf Science* 16(1): 69–93.

Hamed, Y.A., Abdelmoneim, T.S., ElKiki, M.H., Hassan, M.A. & Berndtsson, R. (2013). Assessment of heavy metals pollution and microbial contamination in water, sediments and fish of Lake Manzala, Egypt. *Life Sci. J.* 10(1): 86–99.

Hashem, M.H., Tayel, S.I., Sabra, E.A., Yacoub, A.M. & Heiba, A.A. (2020). Impact of the water quality of El-Rahawy Drain on some genetic and histopathological aspects of

Oreochromis niloticus. *Egypt J. Aquat. Biol. & Fish.* 24(2): 19–38.

Hegazy, W.H., Hamed, M.A., Toufeek, M.E.S. & Mabrouk, B.K.A. (2016). Determination of some heavy metals in water of the southern region of Lake Manzlah, Egypt. *Egypt. J. Aquat. Biol. & Fish.* 20(4): 69–81.

Hossen, H. & Negm, A. (2016). Performance of water bodies extraction techniques "embedded in ERDAS": Case Study Manzala Lake, Northeast of Nile Delta, Egypt. Nineteenth International Water Technology Conference, IWTC19 Sharm ElSheikh, 21–23 April 2016 50.

IARC (International Agency for Research on Cancer). (2012). Monographs on the evaluation of carcinogenic risks to humans: chemical agents and related occupations. A review of human carcinogens. Lyon, France: International Agency for Research on Cancer 100F: 224–248.

Ibrahim, S.A. & Mahmoud, S.A. (2005). Effect of heavy metals accumulation on enzyme activity and histology in liver of some Nile fish in Egypt. *Egypt. J. Aquat. Biol. & Fish.* 9(1): 203– 219.

Islam, Md.S., Ahmed, Md.K. & Habibullah-Al-Mamun, Md. (2017). Heavy metals in sediment and their accumulation in commonly consumed fish species in Bangladesh. *Archives of Environmental & Occupational Health* 72(1): 26–38.

Kadry, S.M., Tayel, S.I., Afify, M.F.H. & El-Sayed, R.A. (2015). Eco-histopathological studied on *Oreochromis niloticus* fish living in Damietta Branch in Egypt. *World Journal of Pharmaceutical Sciences* 3(5): 815–825.

Kumari, U., Mittal, S. & Mittal, A.K. (2012). Surface ultrastructure of the gill filaments and the secondary lamellae of the catfish, *Rita rita*, and the carp, *Cirrhinus mrigala*. *Microsc. Res. Tech.* 75: 433–440.

Mahboob, S., Al-Ghanim, K.A., Al-Balawi, H.F., Al-Misned, F. & Ahmed, Z. (2020). Toxicological effects of heavy metals on histological alterations in various organs in Nile tilapia (*Oreochromis niloticus*) from freshwater reservoir. *Journal of King Saud University- Science* 32(1): 970–973.

Mahmoud, S.A. & Abd El Rahman, A.A. (2017). Eco-Toxicological studies of water and their effect on fish in El Manzalah Lake. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 8: 2497–2511.

Mahmoud, S.A. & El-Naggar, A.M. (2007). Alterations in *Clarias gariepinus* caused by pollutants at El-Rahawy area, Rosetta branch, River Nile, Egypt. *J. Egypt. Acad. Environ. Develop.* 8(2): 61–70.

Mallatt, J. (1985). Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Aquatic Science* 42: 630–648.

Marcogliese, D.J., Blaise, C., Cyr, D., de Lafontaine, Y., Fournier, M. et al. (2015). Effects of a major municipal effluent on the St. Lawrence River: a case study. *AMBIO* 44: 257–274.

Markmanuel, D.P. & Horsfall, Jnr M. (2016). Evaluation of carcinogenic and non-carcinogenic risk of cadmium and nickel in land snails (*A. achatina* and *L. flammea*) and marine snails (*P. aurita* and *T. fuscatus*) commonly consumed in Nigeria. *Acta Chim. Pharm. Indica* 6(4): 123–134.

Mehanna, S.F., Shaker, I.M. & Farouk, AE-d. (2014). Impacts of excessive fishing effort and heavy metals pollution on the Tilapia production from Lake Manzalah. In: 4th Conference of Central Laboratory for Aquaculture Research (57–74): Egypt.

Mela, M.R., Ventura, F., Carvalho, D.F., Pelletier, C.E. & Ribeiro, C.A. (2007). Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. *Ecotoxicology and Environmental Safety* 68(3): 426–435.

Miri, M., Akbari, E., Amrane, A., Jafari, S.J. Eslami, H. et al. (2017). Health risk assessment of heavy metal intake due to fish consumption in the Sistan region, Iran. *Environ. Monit. Assess.* 189: 583.

Mohanta, V.L., Naz, A. & Mishra, B.K. (2020). Distribution of heavy metals in the water, sediments, and fishes from Damodar River basin at steel city, India: a probabilistic risk assessment. *Human and Ecological Risk Assessment: An International Journal* 26(2): 406–429.

OEHHA (The Office of Environmental Health Hazard Assessment). (2009). Air toxics hot spots program technical support document for cancer potencies. Appendix B. Chemical-specific summaries of the information used to derive unit risk and cancer potency values. Updated 2011.

Orabi, O.H. & Osman, M.F. (2015). Evaluation of some pollution at Manzala Lagoon: Special reference to medical importance of mollusca in Egypt. *J. Environ. Anal. Toxicol.* 5(5): 1–31.

Rubio, R., Tineo, P., Torreblance, A., Del-Romo, J. & Mayans, J.D. (1991). Histological and electron microscopical observations on the effects of lead on gills and midget gland of *Procamarus clarkii*. *Toxicology and Environmental Chemistry* 31(1): 347–352.

Saad, S.M.M., El-Deeb, A.E., Tayel, S.I. & Ahmed, N.A.M. (2011). Haematological and histopathological studies on *Clarias gariepinus* in relation to water quality along Rossetta Branch, River Nile, Egypt. *Egypt. J. Exp. Bio. (Zool.)* 7(2): 223–233.

Sallam, G.A.H. & Elsayed, E.A. (2018). Estimating relations between temperature, relative humidity as independed variables and selected water quality parameters in Lake Manzala, Egypt. *Ain Shams Engineering Journal* 9(1): 1–14.

Shaheen, N., Irfan, N.M., Khan, I.N., Islam, S., Islam, M.S. et al. (2016). Presence of heavy metals in fruits and vegetables: health risk implications in Bangladesh. *Chemosphere* 152: 431–438.

Shovon, M.N.H., Majumdar, B.C. & Rahman, Z. (2017). Heavy Metals (Lead, Cadmium and Nickel) Concentration in Different Organs of Three Commonly Consumed Fishes in Bangladesh. *Fish. Aqua. J.* 8: 207. DOI: 10.4172/2150-3508.1000207.

Skidmore, J. (1964). Toxicity of zinc compounds to aquatic animals with special references to fish. *Quart. Rev. Biol.* 39(3): 227–248.

Tayel, S.I., Ahmed, N.A.M. & EL-Hossiny, M.A. (2014). Impact of diffused pollution on histological and hematological properties of *Mugil cephalus* and *Mugil capito* collected from Lake Manzalah, Egypt. *International Journal of Environmental Science and Engineering* 5: 51–67.

Tayel, S.I., Mahmoud, S.A., Ahmed, N.A.M. & Abdel Rahman, A.S. (2018). Pathological impacts of environmental toxins on *Oreochromis niloticus* fish inhabiting the water of Damietta branch of the River Nile, Egypt. *Egypt. J. Aquat. Biol. & Fish.* 22(5): 309–321.

Tayel, S.I., Yacoub, A.M. & Mahmoud, S.A. (2008). Histopathological and haematological responses to freshwater pollution in the Nile catfish *Clarias gariepinus*. *J. Egypt. Acad. Soc. Environ. Develop.* 9(4): 43–60.

USEPA (United States Environmental Protection Agency). (2000). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, EPA 823 B-00-008, November 2000. Volume 2: Risk Assessment and Fish Consumption Limits. 3rd ed. Office of Water: Washington, DC.

USEPA (United States Environmental Protection Agency). (2011). USEPA Regional Screening Level (RSL) Summary Table: November 2011.

USEPA (United States Environmental Protection Agency). (2012). EPA Region III Risk-Based Concentration (RBC) Table 2008 Region III, 1650 Arch Street, Philadelphia, Pennsylvania 19103

USFDA (1993). "Food and Drug Administration, Guidance Document for Nickel in Shell Fish," DHHS/PHS/FDA/ CFSAN/ Office of Seafood, Washington DC.

WHO (World Health Organization). (1989). Heavy Metals – Environmental Aspects of Environment and Health Criteria. No.85. WHO, Geneva, Switzerland.

WHO (World Health Organization). (2018). Jecfa [WWW Document]. URL. http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1376, Accessed date: 27 September 2018

Yacoub, A.M., Mahmoud, S.A. & Tayel, S.I. (2008). Health status of *Oreochromis niloticus* in fish farm irrigated with drainage water in El-Fayoum Province, Egypt. *Egypt. J. Aquat. Res.* 34(1): 161–175.

Yacoub, A.M., Siliem, T.A., Kadry, S.M. & Mabrouk, D.B. (2005). Effect of drainage water on the chemical characteristics of Lake Manzalah waters, Egypt. *J. Egypt. Acad. Soc. Environ. Develop. (D- Environmental Studies)* 6(1): 133–152.

Yu, Y., Wang, X., Yang, D., Lei, B., Zhang, X. et al. (2014). Evaluation of human health risks posed by carcinogenic and non-carcinogenic multiple contaminants associated with consumption of fish from Taihu Lake, China. *Food and Chemical Toxicology* 69: 86–93.

Zahran, M.A., El-Amier, Y.A., Elnaggar, A.A., Abd El-Azim, H. & El-Alfy, M.A. (2015). Assessment and distribution of heavy metals pollutants in Manzala Lake, Egypt. *Journal of Geoscience and Environment Protection* 3: 107–122.

Zhu, H., Xu, Y., Yan, B., Guan, J., Zhou, Q. et al. (2016). Risk assessment of heavy metals contamination in sediment and aquatic animals in downstream waters affected by historical gold extraction in Northeast China. *Human and Ecological Risk Assessment: An International Journal* 22(3): 693–705.