BMC Veterinary Research

RESEARCH

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Hazardous effects of heavy metal pollution on Nile tilapia in the aquatic ecosystem of the Eastern Delta in Egypt

Walaa M. Shaalan[1,2*]

Abstract

Introduction Heavy metal pollution threatens the biodiversity and ecological equilibrium of the Nile River. This study investigates the impact of heavy metal pollution on aquatic animals such as Nile tilapia (*Oreochromis niloticus*) in the Damietta branch of the River Nile and El-Rayah El-Tawfeeky canal in Benha City in Egypt.

Methods Fish and water samples were collected from the Damietta branch and El-Rayah El-Tawfeeky during the fall of 2022. The concentrations of heavy metals in fish muscle tissues were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-OES). Histopathological examinations were performed on gill, liver, spleen, and muscle tissues, following standard histological procedures, to assess tissue damage and morphological alterations. Additionally, gene expression analysis was conducted using real-time polymerase chain reaction (RT-qPCR) to evaluate the expression levels of muscle growth (MyoD, IGF-1) and immune response (TNFa, IL6) genes.

Results Histopathological examinations revealed noteworthy alterations in tilapia gill, liver, spleen, and muscle, suggesting potential health risks. Gene expression analysis using Real-time polymerase chain reaction (RT-qPCR) indicated significant changes in genes related to muscle growth (MyoD, IGF-1) and immune response (TNFa, IL6) in fish from the Damietta branch relative to fish of El-Rayah El-Tawfeeky.

Conclusion The findings raise concerns about bioaccumulation of heavy, some of which surpass international safety limits, posing potential health risks to consumers. The study underscores the significance of continuous monitoring, utilizing chemical, histopathological, and molecular tools as bioindicators for environmental protection measures against aquatic pollution.

Keywords Heavy metals, Gene expression, River Nile, *Oreochromis niloticus*, Eastern Delta

Introduction

The increased heavy metal pollution is alarming and threatens the worldwide aquatic ecosystems [10, 30]. Heavy metals can enter into water from a variety of sources including idol immersion, hospital wastes, emptying of sewers, recreational activities, etc. However, the natural sources of heavy metals are through ore-bearing rocks, forest fires, vegetation, and windblown dust [10]. The River Nile is the main resource of water along Egypt. After Cairo, the Nile tracks the westnorth direction and then bifurcates into two main branches at El-kanater El-khayriya. The two branches are the Damietta branch and Rossetta branch that enclose the Delta in between. There is another canal called El-Rayah El-Twfeeky that was constructed in 1889 and starts from the Damietta branch at El-kanater El-khayrya as described in

*Correspondence:
Walaa M. Shaalan
walaa.shalan@fsc.bu.edu.eg
[1] Department of Zoology, Faculty of Science, Benha University, Benha 13518, Egypt
[2] Faculty of Biology and Biotechnology and Centre for Protein Diagnosis, Bioinformatics Group, Ruhr University Bochum, Bochum 44801, Germany

Fig. (1). The Nile River and its branches face serious ecological challenges due to water pollution [23].

Numerous discharges from point and non-point sources have contaminated the Nile River [70]. According to [7] The biodiversity of c fish can be adversely affected by metal pollution in the Nile River and its branches, which can alter the natural equilibrium of the river environment. Although the heavy metals Cu, Co, Fe, and Zn are essential micronutrients for living things, excessive concentrations can be harmful. Cr, Pb, Hg, As, and Cd heavy metals are carcinogenic microelements and do not have any beneficial biological impact on living animals [56]. Even at lower concentrations, cadmium, lead, tin, and chromium show significant toxicity [32]. Heavy metals are known to be non-biodegradable and to be deposited, integrated, and bioaccumulated in aquatic habitats, which in turn affects aquatic creatures [11]. The bioaccumulation level of heavy metals in fish tissues is affected by biotic and abiotic factors, such as water temperature, pH, dissolved oxygen concentration, fish biological habitat, body mass, and physiological conditions [12]. Fish health and physiological processes are negatively affected by the bioaccumulation of heavy metals in fish [44]. The type of fish, the concentration of the metals, and the length of exposure all have a substantial impact on the degree of metal toxicity that may be carcinogenic, teratogenic, and/or mutagenic [47]. Heavy metals have adverse effects on the nervous system of fish [33]. Fish exposed to heavy metal pollution have decreased gonadosomatic index (GSI), fecundities, fertilization rates, and hatching rates, which could have an adverse effect on fish growth and reproductive activity [28]. Therefore, heavy metals may have a negative impact on several metabolic processes in developing embryos, which may lead to morphological and functional abnormalities, developmental retardation, or even death in the most vulnerable cases. Additionally, heavy metals activate energy-demanding detoxification processes, reducing the energy available for growth in affected fish [59].

The heavy metals in the aquatic ecosystem can enter the food chain beginning from the fish gills, digestive system, and skin. Then most of them will be distributed into the fish body through the bloodstream until reach the fillet that can be consumed by human [33]. The nutritional value of fish to humans is its high-quality protein and inclusion of 2 types of Omega-3 unsaturated fatty acid [57]. Omega-3 can protect from different heart diseases, thrombosis, reduction of blood clotting [54]. However, the presence of heavy metals in fish fillets can adverse the benefits of omega-3 in fish and its beneficial effect on heart health [20]. In addition, heavy metals can accumulate in the food chain and have a major negative impact on human health, including cancer, by increasing their biomagnification over time [46].

The native Egyptian species, the Nile tilapia (*Oreochromis niloticus*), has expanded worldwide, mostly due to its value as it is easy to raise and reproduce in a variety of fish culture methods [6]. *O. niloticus* is a widespread species of freshwater fish utilized in toxicological investigations, primary field research, and laboratory research [2]. To ensure that a sustainable ecosystem continues to function well into the future, it is crucial to monitor the concentration of heavy metals and evaluate the degrees of contamination in the aquatic system. Monitoring heavy metal pollution in aquatic environments using fish tissues facilitates evaluating aquatic ecosystem's quality. Fish tissue contamination can serve as a crucial early warning indicator of sediment pollution and/or related water quality issues [8]. Fish tissue contamination can be evaluated for pollution's effects on fish, metal concentrations in fish that are dangerous for human consumption can be found, and appropriate action can be taken for the preservation of the environment, public health, and socioeconomic reasons [49].

The goal of the current study is to assess the levels of heavy metals in the muscle, liver, spleen, and gill tissues of Nile tilapia (*Oreochromis niloticus*) as well as in water samples taken from the same Damitta branch study locations in Benha City. determining the level of pollution, evaluating the use of tilapia fish as a bioindicator for heavy metal pollution, and creating a model for environmental safety in locations with comparable pollution.

Materials and methods

Field study and sample collection

Fish (*Oreochromis niloticus*) and water samples were collected from the Damietta branch of the River Nile and the El-Rayah El-Twfeeky, in Benha city as shown in Fig. 1. Samples of water were taken between 10 and 20 cm below the water surface and were saved in glass containers. All containers and sampling equipment were pre-cleaned using acid-washing to prevent contamination. Using a dragnet and assistance from a local fisherman, 120 (60/site) adult fish with average body lengths of 18.19 ± 0.46, 16.51 ± 0.35 cm and average body weights of 68.85 ± 3.26, 62.54 ± 2.45 g were caught from the two study sites. The animals were collected and handled according to the guidelines of the Animal Ethics Committee of the Faculty of Science, Benha University, Egypt with approval number: BUFS-REC-2024-114Zoo. An overdose of MS222 (Syndel, Ferndale, WA, USA) was used to euthanize fish samples. Samples of muscle were taken in liquid nitrogen and immediately kept at −80 ^{0}C.

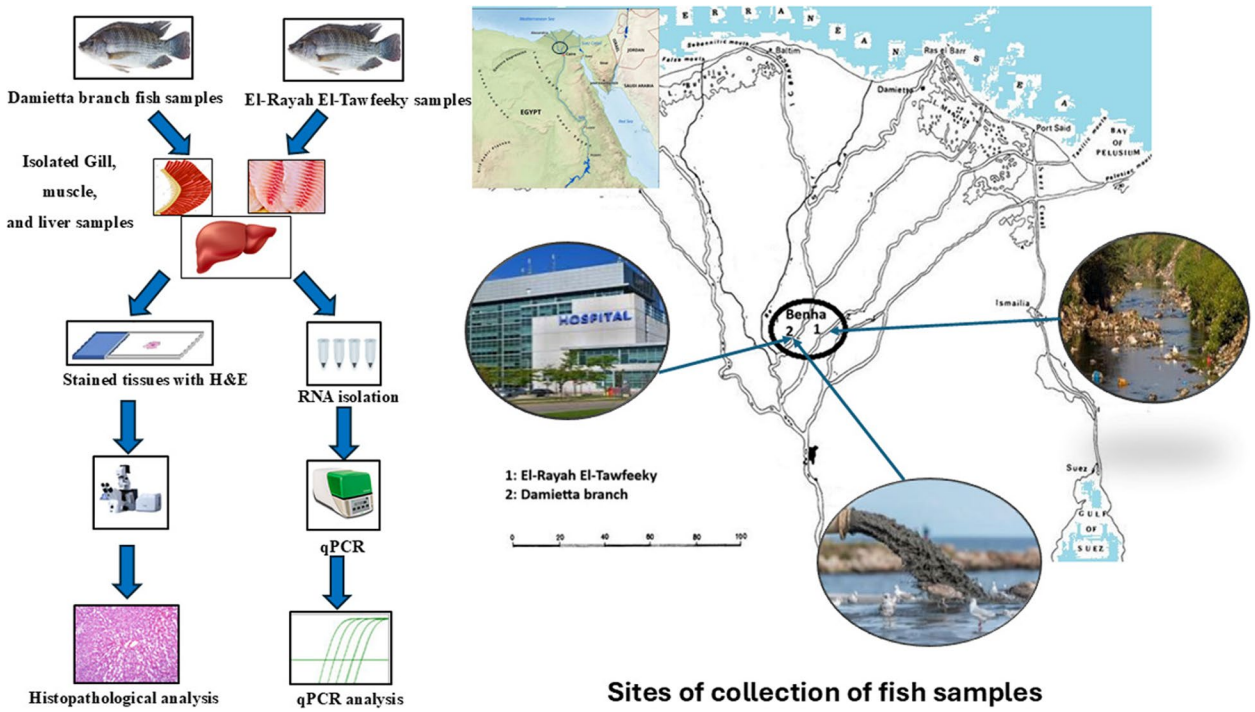

Fig. 1 Schematic illustration of the experimental set-up. The map shows the River Nile in Egypt and the Delta branches. A and B are the sites of sample collection from the Damietta branch and El-Rayah El-Tawfeeky canal

Measuring heavy metals in water and muscle samples

Calibrated Inductively coupled plasma optical emission spectrometry (ICP-OES; Perkin Elmer, Optima 4100DV, Germany) was used [19] to analyze the concentration of heavy metals in the muscle tissue and water samples after they had been treated with nitric acid (Merck, Darmstadt, Germany) and filtered through a glass filter (0.45 ml pore diameter, Sartorius, Göttingen, Germany) following the procedure outlined by [5]. Instrument calibration was performed using certified reference standards. For the muscle samples, they were prepared for measuring heavy metals according to [27]. Using deionized water, serial dilutions of 5, 10, 15, 20, 25, and 30 mg/L were prepared from a stock solution of 1000 mg/L of Mg, Cd, Hg, Cr, Cu, Ni, Pb, Zn. To create the Standard curve, these standards were measured using ICP. Subsequently, WINLAB32 software (PerkinElmer, Germany) measured the samples' concentration using this curve. The results were expressed in terms of micrograms per kilogram dry weight. The limit of detection (LOD) and limit of quantification (LOQ) were determined, and they were Mg: 0.5; 1.8, Cd and Zn: 0.003; 0.01, Hg: 0.020; 0.080, Cr: 0.0033; 0.011, Cu and Ni: 0.006; 0.02, and Pb: 0.0012; 0.004 mg/L, respectively. The recovery percent of the studied heavy metals 94–103%.

Human risk assessment

The evaluation of fish exposed to the reported doses of heavy metals was done by calculating the average daily human consumption dose (ADD, mg/kg/day) [39] of specific each metal according to the following equation: $ADD = \frac{C \times IR \times EF \times ED}{BW \times AT}$

where C is the concentration of heavy metals in fish muscle (mg/kg), IR is the intake rate (0.1424 kg/day for habitual fish consumers, 0.0312 kg/day for the general population), EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years), BW is body weight (70 kg), and AT is the averaging time (70 years × 365 days/year). To ensure the accuracy of the risk assessment, the hazard index (HI) was calculated according to standardized protocols and compared against international oral reference dose (RfD) values [45, 48] The RfD (the heavy metals' oral reference dose, expressed in mg/kg per day) is the upper limit of human metal consumption with average body weight of 70 kg. The upper limits for the studied heavy metals Mg, Cd, Hg, Cr, Cu, Ni, Pb, Zn are 0.06, 0.001, 0.006, 2.5, 0.14, 0.012, 0.025, and 0.214 according to [72].

$$\text{Hazard index (HI)} = \text{ADD/oral RfD}$$

It was reported as risk effects to humans when the HI is ≥ 1.0 [48].

Histological analysis

Samples of small muscle, gill, liver, and spleen tissue (10 fish/site) were transferred to 70% ethanol after being fixed for a full day in Davidson's fixative [13]. After that, they were gradually processed via ethyl alcohol and then embedded in paraffin wax. Lastly, 5µm-thick slices of paraffin blocks were cut, and Hematoxylin and eosin satin (H&E;; Merck, Darmstadt, Germany) [24]. After staining, the slides were examined using an Olympus light microscope (Olympus Corporation, Tokyo, Japan).

RNA extraction and investigation of gene expression

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to isolate total RNA from each fish (eight fish per location, randomly chosen) and reverse transcribed following the manufacturer's instructions of the reverse transcriptase kit (Applied Biosystems, Foster City, CA, USA) [62]. RNA integrity was assessed using gel electrophoresis, and all PCR assays were conducted in triplicate to ensure reliability of the results. Real-time quantitative PCR (RT-PCR) was performed using SYBR green (Thermo Scientific, Hudson, NH, USA) on Bio-Rad iCycler PCR detection system, and the fold change of the target genes between the two studied sites was determined by using the 2^-ΔΔCT method with B-actin acting as the internal control [61]. The primers were designed using primer3 software as listed in Table 1.

Statistical analysis

The data was presented as mean ± standard deviation (SD). Microsoft Excel was used to compute and report the fold change that represents the gene expression and the significance difference between the groups was calculated using a t test [53].

Results

Heavy metals level in water and tissue samples

The concentration of Mg, Cd, Hg, Cr, Cu, Ni, Pb, and Zn in muscle tissue were significantly increased in El-Ryah El-Tawfeek River samples compared to the River Nile samples ($p < 0.05$) as presented in Table 2. The concentrations of the measured metals, listed from highest to lowest, are as follows: Zn, Mg, Cr, Cu, Pb, Ni, Hg, and Cd. The concentrations of heavy metals in the Damietta branch fish were significantly increased compared to El Rayah El Tawfeeky fish. The habitual fish eater from the Damietta branch showed prediction for risk effects from Ni, Mg, and Pb metals. While the normal fish eater from the same site does not show any risk effects. On the other hand, habitual fish eaters were at risk from all the tested metals except Cu and Cr measured in fish muscle collected from El-Rayah El-Tawfeeky canal. Normal fish eaters are at risk from exposure to Cd and Ni metals. All water samples collected at the two studied sites showed a significant increase. However, there was a significant

Table 1 Primers of Real time PCR (RT-PCR)

Gene name	Forward primer	Reverse primer	Accession number
MyoD	ACGCATGTACTCCAGTCCAA	CAGTGAACACAGCAGTCGTC	XM_025907525.1
IGF-1	CACCCTCTCACTACTGCTGT	CACAGTACATCTCAAGGCGC	NM_001279503.1
TNFa	CATCTTTCCGGGTTGACTGC	CTGCAGAGTACCAGTTCCCA	XM_025902124.1
IL6	TTTCTTGCTACATGGCCACG	CCTCATGATCTCAAACCGCG	XM_005448195.3
B-actin	TCTCGGCTGTGGTGGTGAA	GACCCACACAGTGCCCATCT	XM_003455949

Table 2 Heavy metal concentrations in the muscle tissue of *Oreochromis niloticus* in mg/kg muscle weight collected from El Rayah ElTawfeeky canal and Damietta branch, Egypt

	El Rayah El Tawfeeky canal			Damietta River Nile		
Metal	Concentration (mg/kg)	HI (H.)	HI (N.)	Concentration (mg/kg)	HI (H.)	HI (N.)
Mg	74.533 ± 0.075*a	2.527	0.553	59.146 ± 0.980*	2.005	0.439
Cd	2.286 ± 0.115*a	4.651	1.019	0.270 ± 0.053*	0.549	0.120
Hg	10.360 ± 0.075*a	3.512	0.7696	1.500 ± 0.030*	0.508	0.111
Cr	63.020 ± 0.346*a	0.051	0.011	38.853 ± 0.449*	0.031	0.006
Cu	54.406 ± 0.116*a	0.790	0.173	38.986 ± 0.991*	0.566	0.124
Ni	23.560 ± 0.019*a	3.993	0.875	20.823 ± 0.106*	3.530	0.773
Pb	33.403 ± 2.068*a	2.718	0.595	14.673 ± 0.723*	1.193	0.261
Zn	212.086 ± 4.328*a	2.016	0.441	99.930 ± 0.370*	0.949	0.208

Tilapia collected from two sites, with Hazard Indices (HI) calculated for high (H.) and normal (N.) fish consumers. Asterisks (*) indicate statistically significant differences between the two sites ($p < 0.05$). and 'a' indicates a significant increase compared to the maximum allowable concentration [71]

increase ($p < 0.05$) in Mg, Cr, Cu, and Zn metals Table 3 for water samples collected from El-Rayah El-Tawfeeky canal compared to those collected from The Damietta branch.

Histopathological analysis of gill, liver, spleen, and muscle in Damietta branch of River Nile

Some histopathological changes were recognized in tilapia fish collected from the Damietta branch of River Nile. The microscopic examination of the gills of Nile tilapia revealed mild histopathological changes where the secondary lamellae of these gills were covered by a thin layer of single epithelium. The gill filaments were covered by stratified epithelium with aggregation of few mononuclear cells in some of these filaments (Fig. 2A). Partial fusion of some secondary lamellae and aggregation of some inflammatory cells in gill filaments, mainly lymphocytes were also seen (Fig. 2B). Focal areas of hepatocellular degenerative changes in the form of hydropic and vacuolar degeneration of hepatocytes with focal aggregation of few lymphocytes in-between hepatocytes and activation of kupffer cells were the main detectable microscopic changes in the examined Tilapia liver (Fig. 2C). However, aggregation of some melanomacrophages within the hepatopancreas was also recorded (Fig. 2D). The microscopic examination of the spleen showed normal cyto-architecture of the spleen. The examined spleen was covered by a thin capsule and contained red and white pulps with ellipsoids and melanomacrophage centres which are formed from the aggregation of macrophages and melanin pigments scattered throughout the splenic parenchyma (Fig. 2F). The examined muscles tissue of tilapia showed normal histological structure of these muscles where unbranched and elongated muscle fibres with peripheral flattened nuclei and loose collagenous tissue in-between these muscle fibres were found (Fig. 2E).

Table 3 The heavy metals concentrations in water samples from the selected sites in mg/L

Metal	Max allowable conc. (mg/L; [72]	El-Rayah El-Tawfeeky Water (mg/L)	Damietta River Nile Water (mg/L)
Mg	0.4	$0.757 \pm 0.004^{*a}$	$0.589 \pm 0.006^{*}$
Cd	0.00001	$0.238 \pm 0.008^{*}$	$0.241 \pm 0.011^{*}$
Hg	0.002	$0.153 \pm 0.006^{*}$	$0.135 \pm 0.008^{*}$
Cr	0.05	$0.437 \pm 0.004^{*a}$	$0.381 \pm 0.015^{*}$
Cu	1.5	$0.545 \pm 0.012^{*a}$	$0.359 \pm 0.025^{*}$
Ni	0.07	$0.244 \pm 0.004^{*}$	$0.222 \pm 0.023^{*}$
Pb	0.1	$0.152 \pm 0.002^{*}$	$0.146 \pm 0.006^{*}$
Zn	0.01	$1.044 \pm 0.015^{*a}$	$0.975 \pm 0.022^{*}$

The concentrations of heavy metals in water samples collected from the two studied sites, compared to the maximum allowable concentrations set by [71]. Statistically significant differences ($p < 0.05$) between the two locations are marked with an asterisk (*), and 'a' indicates a significant increase compared to the maximum allowable concentration

Histopathological analysis of gill, liver, spleen, and muscle in El-Rayah El-Tawfeeky canal

The examined gill of tilapia collected from El-Rayah El-Tawfeeky revealed severe histopathological changes. Mononuclear inflammatory cellular aggregation in the gill filaments with activation of mucous cells were prevalent lesions, particularly at the tip of these filaments (Fig. 3A). Fusion of secondary lamellae with extensive inflammatory cellular infiltration of gill filament was also prominent (Fig. 3B). In addition, focal areas of desquamation and necrosis of gill lamellar epithelium were observed (Fig. 3C). Moreover, congestion with aggregation of mononuclear cells and eosinophilic granule cells (EGCs) in the gill arch with the proliferation of mucous cells were also recognized (Fig. 3D). Multiple areas of necrosis and degeneration of the hepatocytes with perivascular lymphocytic cellular aggregation were scattered throughout the examined liver of tilapia (Fig. 3E). Mononuclear infiltration of the hepatopancreas and hyperplasia of the bile ductal epithelium were detected (Fig. 3F& G). Furthermore, vacuolation of the pancreatic acini was also noticed in the hepatopancreas (Fig. 3H). The examined spleen revealed thickening of the splenic capsule due to fibrous connective tissue proliferation with severe depletion of the splenic hematopoietic tissue and necrosis of the ellipsoidal sheaths (Fig. 3J). In addition, destruction of melanomacrophage centres was prevalent in the examined spleen (Fig. 3K). Perivascular oedema and aggregation of melanomacrophage cells around splenic blood vessels were also recorded (Fig. 3L). Vacuolation and even cavitation of the muscles were the main microscopic changes recorded in the skeletal muscles of tilapia (Fig. 3I).

Relative gene expression of MyoD, IGF-1, TNFa, and IL6

The results of gene expression of MyoD in muscle tissue showed a significant decrease in its expression in the samples of El- Rayah El-Tawfeky canal relative to that of the Damietta branch of River Nile samples ($p \leq 0.05$). In addition to reporting a significant decrease in the IGF-1 gene in muscle tissue of the fish from El-Rayah El-Tawfeky relative to the River Nile fish as in (Fig. 4), on studying the gene expression of TNFa and IL6 genes in spleen tissue, It was observed that there was a significant increase in the TNFa gene by 25.69-fold in fish from El-Rayah El-Tawfeeky relative to that from Damietta branch of River Nile as shown in (Fig. 5). The IL6 gene revealed a significant increase by 5.22 folds in fish samples collected from El-Rayah El-Tawffeky compared to that from Damietta branch ($p \leq 0.05$).

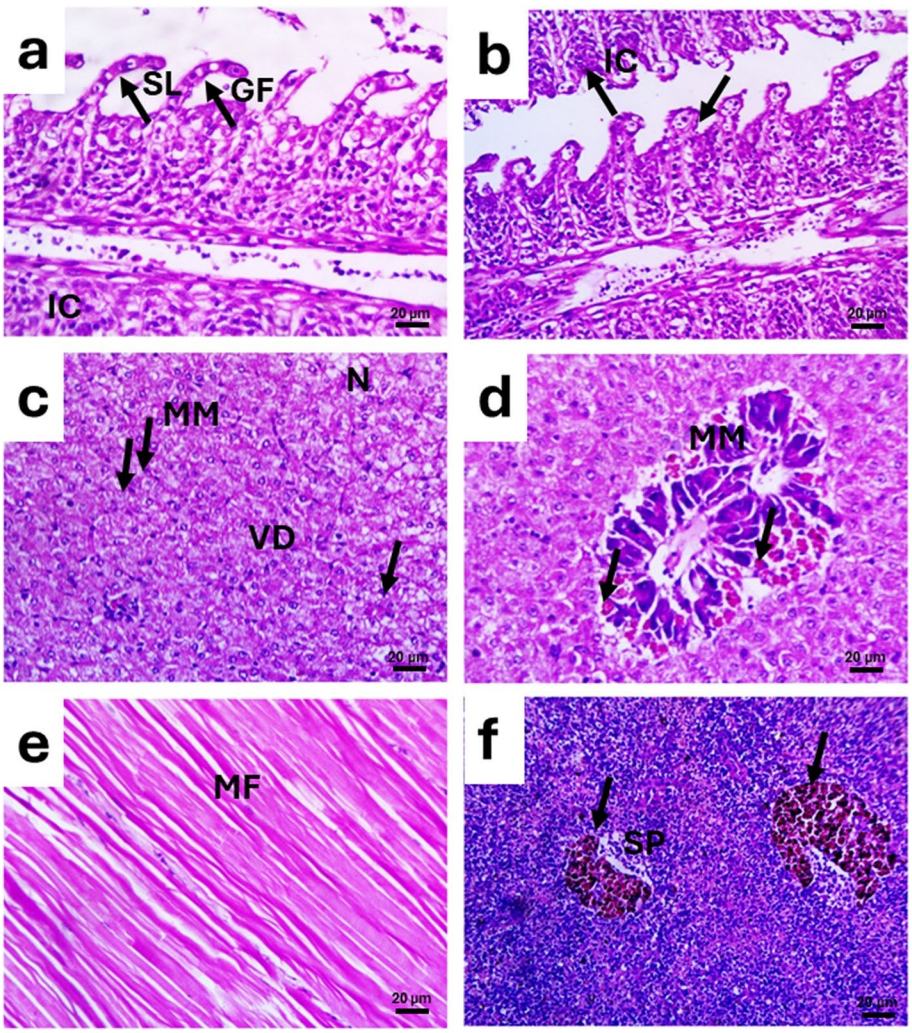

Fig. 2 Photomicrograph of gills (**A & B**), Liver (**C & D**) Skeletal muscles (**E**) spleen (**F**) of tilapia collected from Damietta branch of River Nile. H&E stain X 200. **A** Secondary lamellae covered by thin layer of single epithelium (SL) oriented perpendicular to the gill filament (GF) covered by a stratified epithelium with aggregation of a few mononuclear cells. **B** Partial fusion of some secondary lamellae (arrows) with aggregation of a few inflammatory cells in the gill filament. **C** Hydropic and vacuolar degeneration of some hepatocytes with activation of kupffer cells (arrows). **D** Aggregation of melanomacrophages within the hepatopancreas (arrows). **E** Unbranched and elongated muscle fibers with peripheral flattened nuclei and loose collagenous tissue in-between the muscle fibers. **F** Splenic red and white pulps with two melanomacrophage centers (arrows)

Discussion

Heavy metals can enter the aquatic ecosystem from various sources, such as the Earth's crust, sewage spills, agricultural practices, and oil extraction. Once introduced into sediments, these metals may not fully dissolve, remaining accessible to the ecosystem [74]. Dissolved metals are bioavailable and can accumulate in aquatic organisms. Fish living in these ecosystems may absorb heavy metals through their gills, digestive system, or the food chain [52] The present study reported different concentration of Mg, Cd, Hg, Cr, Cu, Ni, Pb, and Zn metals in water samples collected from El-Rayah El-Tawfeeky canal and the Damietta branch. Pollution sources in these water systems include the disposal of dead animals and sewage discharge [1, 22]. Previous findings were summarized in Table 4. Al-Afify and Abdel-Satar [4] showed that river sites, particularly during low-flow seasons, tend to have higher concentrations of metals like Mn, Ni, and Cd. In this study, metal concentrations in fish muscles from the El-Rayah El-Tawfeeky canal were significantly higher than those from the Damietta Nile River branch. Such elevated levels are likely due to sediment accumulation, which can act as a reservoir for metals, entering the aquatic food chain through biomagnification [18].

The highest concentration of Zn that was recorded in fish muscle is similar to those observed in fish collected

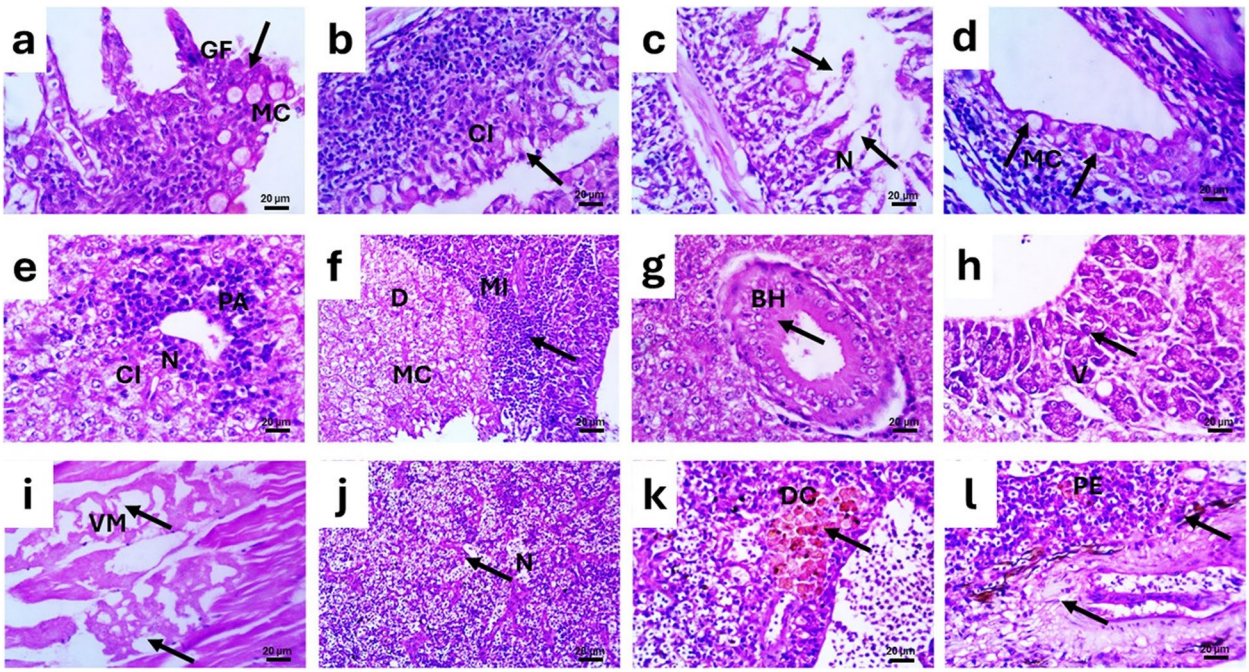

Fig. 3 Photomicrograph of gills (**A-D**), Liver (**E–H**) Skeletal muscles (**I**) spleen (**J-L**) of tilapia collected from El-Rayah El-Tawfeeky. H&E stain X 200. **A** Mononuclear cellular aggregation in the tip of the gill filament with activation of mucous cells. **B** Extensive inflammatory cellular infiltration of gill filament with fusion of secondary lamellae. **C** Necrosis of the gill lamellae (arrows). **D** Aggregation of mononuclear cells and eosinophilic cell in the gill arch with activation of mucous cells. E. necrosis and degeneration of the hepatocytes with perivascular lymphocytic cellular aggregation. F. extensive degeneration of hepatic cells with mononuclear infiltration of the hepatopancreas (arrows). **G** Bile ductal hyperplasia. **H** Vacuolation of the pancreatic acini. **I** Vacuolation of the skeletal muscles. **J** Severe depletion of the splenic hematopoietic tissue and necrosis of the ellipsoidal sheath. **K** Destruction of melanomacrophage center. **L** Perivascular edema with aggregation of melanomacrophage cells around splenic blood vessel

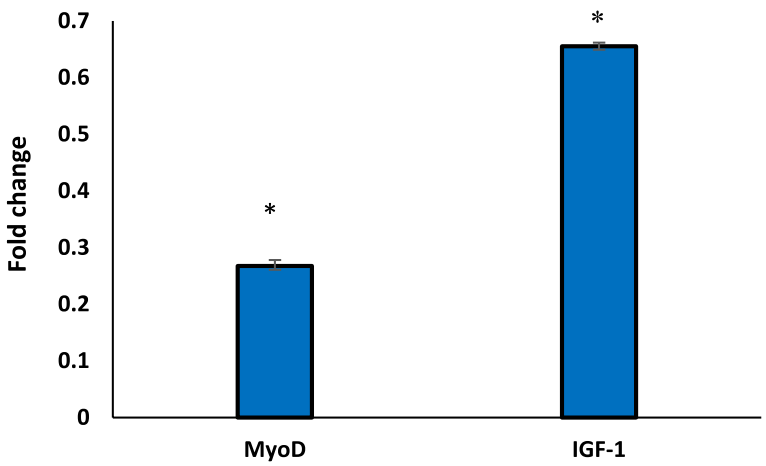

Fig. 4 Differential gene expression of MyoD and IGF-1 genes in tilapia muscle of El-Rayah El-Tawfeeky canal and Damietta River branch. The expression was represented by fold change between El-Rayah El-Tawfeeky relative to the River Nile samples ± standard deviation where $n = 8$ and $p \leq 0.05$

from the Southern Caspian Sea, Iran [64]. Fish from the Rabul River in Pakistan have been reported to contain chromium concentrations between 489–703 mg/kg [3], while fish from Calicut, India, contained 0.74 µg/g of chromium [58]. Additionally, marine fish from Kochi Waters were reported to have 0.5 mg/kg of Mn, whereas [40] found 2.9 µg/g of Mn in several fish species from Indian waters. Fish from the Kabul River in Pakistan contained 75–135 µg/g of Nickel [3], and those collected from Iskenderun Bay in Turkey had 0.11–12.9 µg/g [67].

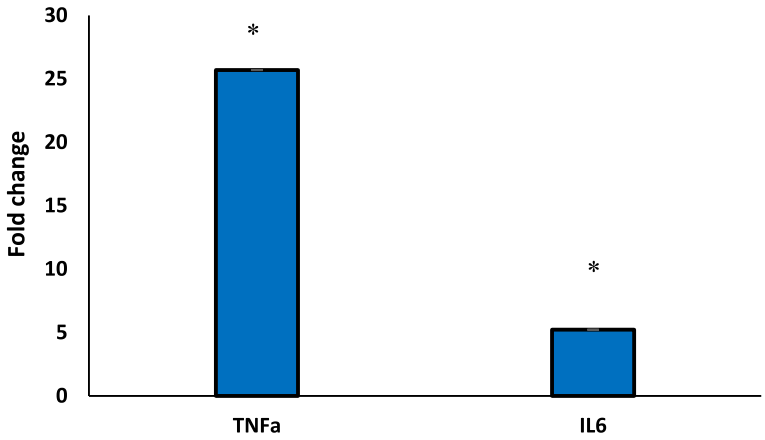

Fig. 5 Differential gene expression of TNFa and IL6 genes in tilapia spleen of El-Rayah El-Tawfeeky canal and Damietta River branch. The expression was represented by fold change between El-Rayah El-Tawfeeky relative to the River Nile samples ± standard deviation where $n = 8$ and $p \leq 0.05$

Table 4 Summary of previous studies on heavy metal concentrations in fish tissue

Fish species	Mg	cd	Hg	Cr	Cu	Ni	pb	Zn	Reference
11 Species (mg/kg)		0.02–0.06		0.20–0.65	0.79–2.26	0.44–9.75	0.03–8.62	15.2–29.5	[41]
Common carp (µg/kg)	235–275	0.9–2.4					18–110		[68]
Trout barb (µg/kg)	232–272	0.7–1.6					33–112		
Wallago attu (µg/g)		68		533.3	46.3	106.7	599.3	649	[3]
Aorichthys seenghala (µg/g)		60.7		565.3	132.7	94.7	350.7	1167.7	
Cyprinus carpio (µg/g)		53.3		489	303	74.7	226.3	826.3	
Labeo dyocheilus (µg/g)		66.7		647.3	191.7	117.7	528.7	883	
Ompok bimaculatus (µg/g)		71.7		703	241.3	135	407	902	
Corpus corpino (µg/g)		46.42- 95.90		24.37–50.43			78.43–168.9		[64]
Mugila auratus (µg/g)		19.27–34.62		43.90–130.29			53.67–88.52		
Rutilus frisikutum (µg/g)		20.89–58.71		31.07–82.15			99.15–138.80		
Saurida tumbil (µg/g)		–	0.0005	0.2	1.5	–		7.3	[58]
Megalaspsis cordyla (µg/g)		–	0.003	0.3	2.5	–		7.2	
Sphyraena jello (µg/g)		–	0.004	0.1	1.6	–		6.2	
Trichiurus savala (µg/g)		0.1	–	0.6	2.8	–		6	
Chiloscyllium spp. (µg/g)		–	–	0.5	2.6	–		8.3	
Johnieops dussumieri (µg/g)		–	0.005	1	2.4	0.1		7.4	
Perna viridis (µg/g)		0.1	–	0.9	6.5	0.8		15.7	
Pamphia malabaricus (µg/g)		0.1	–	0.8	4.4	–		10.3	
Sepia sp. (µg/g)		–	0.004	0.3	4.5	0.2		16.2	
Brownspotted grouper (mg/kg)		3		38.6	11	17.3	0.8		[73]
Squaretail coral grouper (mg/kg)		4.1		68.6	14.5	30.7	0.7		
Black pomfret (mg/kg)		5.1		96.3	18.9	53.5	0.8		
Goldbanded jobfish (mg/kg)		1.7		113.3	8.2	92.1	0.7		
Blueskin seabream (mg/kg)		2.2		49.4	7.7	35.8	0.8		

In the present study, the concentrations of copper in Tilapia muscle were 54.4 mg/kg in fish from El-Rayah El-Tawfeeky and 38.9 mg/kg in fish from the Damietta River branch. This copper concentration in El-Rayah El-Tawfeeky is higher than what was reported in fish from the Bangshi River in Bangladesh (8.33–43.18 mg/kg) [50]. In comparison, [41] reported cadmium levels of 0.02–0.06 µg/g in the Pearl River Delta, China, whereas [69] found 0.09–0.89 mg/kg in fish from the Bangshi River, Bangladesh. High mercury concentrations were found in some fish species from the Red Sea [73]. Lead concentrations ranged from 0.22–0.85 mg/kg in fish from the Middle Black Sea, Turkey [66], and similar levels were detected in different species from the Red Sea [73]. Excessive metal contamination in aquatic environments can severely damage commercial fisheries, lead to significant financial losses, and pose health risks to humans [76]. Fish collected from El-Rayah El-Tawfeeky canal showed potential health risks to humans, primarily through the consumption of these fish due to high levels of all measured metals. Researchers are increasingly concerned about heavy metal accumulation in aquatic environments due to their toxic effects on both aquatic organisms and humans through the food chain [65].

The predicted heath risk for fish eaters differs between the habitual fish eater and normal fish eater from both selected sites. Previous studies have been done in fish collected from Buriganga river in Bangladesh. They found that the values of hazardous index (HI) for Cu, Co, Zn, Fe, Ca, Mg, Se, and Ni in are less than 1 that indicate the non-potential health risk for human that may eat *Puntius ticto, Puntius sophore, Puntius chola, Labeo rohita or Glossogobius giuris* [36]. In previous studies on fish in Egypt, no health effects were reported for any metals at the average ingestion rate for a typical adult, as all HI values were less than 1. However, for habitual fish consumers, while HI values for Fe, Mn, Zn, and Cu remained less than 1, lead (Pb) showed exceptions across different seasons at the three sites, with particularly high HI values for lead in habitual fish eaters, indicating concerning levels [26]. Łuczyńska et al. [77] measured the heavy metals in crucian carp (Carassius carassius Linnaeus, 1758), flounder (Platichthys flesus Linnaeus, 1758), Gilthead seabream (Sparus aurata Linnaeus, 1758), mackerel (Scomber scombrus Linnaeus, 1758), Blue grenadier (Macruronus novaezelandiae Hector, 1871), rainbow trout (Oncorhynchus mykiss Walbaum, 1792), tench (Tinca tinca Linnaeus, 1758), tilapia (Oreochromis niloticus Linnaeus, 1758), Walleye pollock (Gadus chalcogrammus Pallas, 1814) and perch (Perca fluviatilis Linnaeus, 1758). They observed that all these fish are safe for consumers as their HI values are less than 1.

Histopathological study

Gills are the first target for pollution, being in close contact with the surrounding environment and the primary site for heavy metal absorption. Therefore, the histopathological alterations of gills were generally attributed to the toxic effects of heavy metals. It was reported previously that fish exposed to metals have also been observed to exhibit similar gill changes [9]. It has been demonstrated that the histology of the gills reflects various environmental circumstances for fish and is susceptible to copper exposure [9]. In the present study, there was a fusion of secondary lamellae with extensive inflammatory cellular infiltration of the gill filament. Similar results were reported for grass carp exposed to heavy metals [63].

Melanomacrophages, hypertrophy, and biological disarray were noted in the fish treated with various aluminum doses [29]. A high concentration of pollutants that cause hepatocyte loss could be the cause of the necrosis seen in the centrilobular zone [51]. The examined spleen of tilapia fish collected from RTB revealed thickening of the splenic capsule, decrease in splenic haematopoietic tissue, necrosis, and oedema**.** The observed data align with previous research by [16], which demonstrated immune suppression in the splenic region of rainbow trout following exposure to various chemical toxicants. Changes to the spleen, under specific physiological and histological conditions, have been documented in studies by [25]. *Lates calcarifer* exposed to copper had thickening and separation of muscular bundles with significant oedema [43]. It was previously reported for tilapia fish exposed to heavy elements and microbes contaminants to have degeneration, necrosis, atrophy, and disintegration of muscular bundles in addition to enlargement of the muscle fiber [17].

Gene expression analysis

Understanding the extent of harm is an essential aspect of ecotoxicological studies. Tissue-specific gene expression, often used as a biomarker, helps indicate the type, degree, and condition of alterations in response to toxic exposure, as emphasized by [37]. Numerous studies have utilized tissue-specific expression levels to explore these responses. Different stressors were reported to affect muscle growth as was reported for tilapia [60]. After the liver and spleen, muscle exhibits a notable increase in gene expression due to the bio-accumulative nature of heavy metals. The present study revealed a significant decrease in MyoD and IGF-1 gene expression in the Tilapia muscle of RNB compared to that from DRN. The myogenic determining factor (MyoD) gene is one of the muscle-specific transcription factors

that control the development of the muscle, proliferation, and myofibril formation [75]. It was reported that the fish MyoD gene was negatively affected by different stressors. It has been reported that the MyoD gene in fish is negatively affected by various stressors. A study revealed low expression of the MyoD gene in gibel carp exposed to 168 h of acute thermal stress [31]. MyoD and Myf5 are two examples of muscle-specific genes whose transcription is modulated by Wnt signalling during myogenesis via PKA and the transcription factor CREB [14]. It was reported previously that Wnt signalling pathways are vulnerable to heavy metal exposure in the environment and are essential for regular cellular processes [38]. Previous study presented the effects of zinc and cobalt exposure on rainbow trout's IGF-I, IGF-II, and GH expression levels. It was reported that the exposure of rainbow trout for time to zinc and cobalt resulted in significantly decrease of the expressions of IGF-1 gene and that may be due to the interactions between these metals and metal binding proteins [21]. Our results showed a significant increase in TNFα and IL6 gene expression in the spleens of Tilapia from RNB compared to those from DRN. In the Asian carp head kidney, a positive correlation was found between the expression of mir155 and the mRNA levels of pro-inflammatory cytokines, such as TNF-α [35]. It was revealed that the biomolecular response to exposure of *D. setosum* to Cd heavy metals demonstrated that TNF-α protein expression, activation, and concentration increased as the concentration of Cd-containing heavy metals increased [55]. Oxidative stress is one of the basic chemical mechanisms that underlie toxicity caused by metals [15]. Certain cellular inflammatory factors such as IL-6 and immunological factors significantly changed when the immune system is repressed [34]. A significant increase in IL-6 was measured in the spleen of carp exposed to the pollutant difenoconazole [42]. This suggests that heavy metals may target the MyoD gene in muscle, as well as the TNF-α and IL-6 genes in the spleen, leading to harmful effects. Our findings suggest that exposure to heavy metals causes immunological system malfunction and muscle atrophy in addition to spleen tissue damage. Tissue damage, immunosuppression brought on by heavy metal exposure, oxidative stress, inflammation, and apoptosis are all deeply interrelated.

Conclusion and recommendations

In the present study, various biomarkers at different levels were used to identify the heavy metal pollution in Tilapia fish and the surrounding water. It gives indication about the health condition of the fish and the risk effect to the consumer. This approach provided an indication of the health condition of the fish and the potential risks to consumers. The data from this study highlights one of the most harmful forms of pollution that affects the environment and cannot be ignored. These toxins pose significant threats to both aquatic life and the human population. The levels of bioaccumulation of heavy metals in fish muscle were found to be close to exceeding safety thresholds at the studied sites, raising concerns for consumer health. Therefore, it is crucial to regularly monitor the metal levels in fish species from the Benha Damietta Branch and El-Rayah El-Tawfeeky canal. To protect these sites from further pollution, efforts should focus on preventing waste from entering the watershed, reducing environmental risks, and consistently monitoring the river ecosystem before metal concentrations reach harmful levels and endanger both aquatic life and humans. The findings of this study could provide valuable information to decision-makers on how to better safeguard the River Nile, its branches, and related canals from potential environmental hazards.

Acknowledgements
Author would like to thank anonymous referees for their valuable comments.

Authors' contributions
W. M. S. conducted the experiment, analyzed the data, wrote and revised the manuscript.

Funding
The authors did not receive support from any organization for the submitted work.

Availability of data and materials
All data supporting the findings of this study are available within the paper. No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The experiments were approved by the Ethical Committee of the Faculty of Science, Benha University, Egypt, with approval number: BUFS-REC-2024-114Zoo.

Competing interest
The author has no competing interests to declare that are relevant to the content of this article.
The authors declare no competing interests.

Received: 27 August 2024 Accepted: 5 November 2024
Published online: 28 December 2024

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