Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(1): 1329 – 361 (2025)
www.ejabf.journals.ekb.eg

Exploring the Potential Toxicity Induced by Heavy Metals in the Nile Tilapia Fish (*Oreochromis niloticus*) of Various Spatiotemporal Monitoring Patterns in the Water of the Damietta Branch

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ARTICLE INFO

Article History:
Received: Jan. 2, 2025
Accepted: Jan. 30, 2025
Online: Feb. 4, 2025

Keywords:
Heavy metals,
Damietta Branch,
Oreochromis niloticus,
Oxidative stress,
Electrophoresis

ABSTRACT

Heavy metals are not biodegradable and are deposited, incorporated, and bioaccumulated in aquatic organisms. The Nile tilapia, *Oreochromis niloticus*, is regarded as one of the most significant biomonitors in the aquatic ecosystem for detecting heavy metal contamination. During the current study, concentrations of heavy metals, markers of oxidative stress and inflammatory responses were assessed in the liver, kidney, and muscle tissues of *O. niloticus* fish collected from five key points in the Damietta Branch (El-kanater El-Khayriya, Benha, Zefta, Talkha, and El-Serw) in addition to undergoing their histopathological examination. Furthermore, native electrophoretic proteins and the relative gene expressions of antioxidant enzymes and inflammatory markers in the target tissues were assayed. The tilapia fish obtained from El-Serw had the highest quantities of heavy metals in their liver and muscle, followed by Talkha, Zefta, and Benha. When comparing the liver and muscle of tilapia fish collected from El-Serw, Talkha, Zefta, and Benha to those collected from E. EL-Khayria, measurements of the antioxidant system (both enzymatic and non-enzymatic) significantly decreased ($P \leq 0.05$) in tandem with rising levels of inflammatory markers. All of the tilapia fish's tissues that were taken from El-Serw had significant histopathological changes. Furthermore, the tissues of fish taken from El-Serw exhibited the lowest similarity percentages when compared to those from E. EL-Khayria, according to the electrophoretic protein patterns. According to the molecular assays, *O. niloticus* fish collected from El-Serw, Talkha, Zefta, and Benha had significantly lower relative gene expressions of antioxidant enzymes ($P \leq 0.05$) in conjunction with higher levels of inflammatory markers than fish collected from E. El-Khayria. The study revealed that severe histopathological, physiological, and molecular alterations were noticed in the target tissues of *O. niloticus* fish gathered from El-Serw with those gathered from other areas.

INTRODUCTION

Recreational activities, medical waste disposal, idol immersion, and sewer discharge are some ways through which heavy metals can be found in the water. But ore-bearing rocks, forest fires, flora, and wind-blown dust are all natural sources of heavy

metals **(Hama Aziz *et al.*, 2023)**. Egypt's main supply of water comes from the Nile River. The Nile travels west-north beyond Cairo until dividing into two major branches at El-kanater El-Khayriya. These branches are the Damietta branch and the Rosetta branch, which enclose the Delta in between. Water contamination poses serious ecological problems for the Nile River and its branches **(El-Saadani *et al.*, 2022)**. Aquatic animals are impacted by heavy metals since they are known to be non-biodegradable and deposited, integrated, and bioaccumulated in aquatic environments. The global aquatic ecosystem is at danger due to the concerning rise in heavy metal pollution **(Briffa *et al.*, 2020)**. Despite that certain heavy metals are necessary micronutrients for life, high levels of these metals can cause cancer and have no positive biological effects on living creatures. Even at lesser levels, several heavy metals exhibit notable toxicity **(Morshdy *et al.*, 2021)**. The gills, digestive tract, and skin of fish are the first points of entry for heavy metals into the food chain in the aquatic ecosystem. The majority of them are then transported by the bloodstream throughout the fish's body until they arrive with the human-consumed fillet **(Jamil Emon *et al.*, 2023)**.

In the aquatic ecosystem, fish are regarded as one of the most significant biomonitors for determining the level of heavy metal pollution. Additionally, because they are at the top of the food chain, they can absorb metals from water through their digestive tract, gills, and skin, or they can directly consume it. Eating fish can then expose people to these metals, which may result in acute or long-term illnesses **(Al-Yousuf *et al.*, 2000; Abou El-Gheit *et al.*, 2012)**. The degree of metal toxicity that may be carcinogenic, teratogenic, and/or mutagenic depends significantly on the type of fish, the concentration of the metals, and the duration of exposure **(El-Batrawy *et al.*, 2018)**.

To assess the impact of pollution on fish, detect harmful metal concentrations in fish tissues that may pose risks to human consumption, and take appropriate actions for environmental, public health, and socioeconomic reasons, evaluating fish tissue contamination is essential **(Panda *et al.*, 2023)**. Pollutant buildup alters the physiology of fish tissues. The microscopic analysis of target tissues using histopathological characteristics is the final step in determining the danger of environmental contaminants **(Fatima *et al.*, 2015)**. Histopathological alterations can serve as a gauge of the general health of the aquatic ecosystem as well as an indicator of the effects of different man-made toxins on organisms **(Saad *et al.*, 2011; Mahmoud & Abd El Rahman, 2017)**. Pollutants can have detrimental effects on fish tissues before they cause noticeable alterations in the fish's behavior and outward appearance **(Mahboob *et al.*, 2020)**.

Because they absorb toxins through their skin and gills, fish are the species most impacted by pollution **(Alm-Eldeen *et al.*, 2018)**. This causes disturbances in physiological and biochemical processes **(Afzal *et al.*, 2022)**. Even at low concentrations, prolonged exposure to water pollutants can change the tissues' shape, biochemistry, and histology **(Haredi *et al.*, 2020)**. Increased lipid peroxidation and the depletion of antioxidant enzymes are two examples of bioindicators of oxidative stress that fish

exposed to different pollutants may display **(Karami *et al*., 2016)**. Fish are employed to monitor contamination in the aquatic ecosystem because they are important members of the trophic chain and have the ability to absorb hazardous compounds, even in minute amounts **(Milla *et al*., 2011)**.

One of the most commercially significant fish species is the Nile tilapia, *Oreochromis niloticus*, which exhibits low disease susceptibility and can withstand a wide range of harsh climatic conditions **(Tayel *et al*., 2018)**. It is a useful aquatic species for toxicological study and is regarded as the most commercially cultivated species in many parts of the world, including Egypt **(Al-Awadhi *et al*., 2024)**. The purpose of this study was to measure the levels of heavy metals in *O. niloticus* muscles, liver, and kidney that were collected from several sites in the Damietta Branch. The study also sought to evaluate the negative consequences at the histopathological, physiological, and molecular levels that were caused in these target tissues of fish.

MATERIALS AND METHODS

1. Collection of tilapia fish (*Oreochromis niloticus*)

The fish were collected from five key points in the Damietta Branch (El-kanater El-Khayriya, Benha, Zefta, Talkha, and El-Serw) based on the Global Positioning System (GPS), which was used to record the geographical location of samples. The selected sites were known for their high production rates, as well as their possible contamination with high concentrations of harmful substances, as a result of manufacture using agricultural drainage water.

2. Heavy metals analysis

The method demonstrated by **Jiang *et al*. (2018)** was used to extract the metals from fish samples, including the liver, kidney, and muscles. Using an instrument called Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES), the concentrations of cadmium (Cd), lead (Pb), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and chromium (Cr) were measured in all digested samples. Using the ICP-OES, quality control and detection limits were established with a good recovery rate for the estimation of hazardous metals.

3. Biochemical assays

3.1. Preparation of the tissue homogenates

The liver, kidney, and muscles of fish were excised, washed with cold phosphate-buffered saline, quickly frozen with liquid nitrogen, ground, and then homogenized in 0.05 M Tris-HCl buffer (pH 7.4) following the method demonstrated by **Mohamed *et al*. (2020)**. The homogenates were left in the refrigerator overnight, shaken with a vortex for 15 seconds, and then centrifuged at 10,000 rpm at 4˚C for 15 minutes. The supernatants, including water-soluble proteins, were collected.

3.2. Markers of the oxidative stress

The clear supernatants of the investigated fish tissue homogenates were used to test these indicators. Reduced glutathione (GSH) **(Beutler *et al*., 1963)** and total antioxidant capacity (TAC) **(Koracevic *et al*., 2001)** were measured as µmol/g and mg/g tissue, respectively. Units per gram of tissue were used to quantify the activities of glutathione peroxidase (GPx) **(Paglia 7 Valentine, 1967)**, superoxide dismutase (SOD) **(Nishikimi *et al*., 1972)** and catalase (CAT) **(Aebi, 1984)**. Lipid peroxidation product (LPO) **(Ohkawa *et al*., 1979)** and total protein carbonyl content (TPC) **(Levine *et al*., 1994)** concentrations were measured as nmol/g wet tissue and nmol of reactive carbonyl compounds per mg protein of tissue, respectively.

3.3. Inflammatory markers

In accordance with the guidelines proposed by **March *et al*. (1985)** and **Engelmann *et al*. (1990)**, respectively, markers of inflammatory reactions, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), were measured in the tissue homogenates as Pg/g tissue using the quantitative sandwich enzyme immunoassay technique. A modified Ellman's approach was used to measure the AChE's activity **(Ellman *et al*., 1961)**.

4. Histopathological examination

Each fish's liver, kidney, and muscle tissues were autopsied, and the specimens were promptly stored in 70% ethanol and fixed in 10% buffered formalin. After being cleaned, the tissue samples were embedded in blocks of paraffin wax, clarified in xylene, and dehydrated in serial alcohol solutions. Hematoxylin and eosin staining was performed after the tissue sections, which had a thickness of 4 microns, were collected on glass slides and deparaffinized **(Bancroft & Gamble, 2008)**. In order to assess the histological alterations, the stained sections were lastly captured on camera with an Olympus microscope and a digital camera (14 MP USB).

5. Electrophoretic assays

Fish tissues weighing 0.2 grams were quickly frozen in liquid nitrogen and then homogenized in 1 milliliter of water-soluble extraction buffer. After centrifuging the homogenates for five minutes at 10,000 rpm, the clear supernatants were separated and put into different tubes. Each group's separate supernatants were combined into equal amounts and used as a single sample. To guarantee that the concentration of the protein loaded in each well is the same throughout all electrophoretic tests, the total protein concentration was determined in each pooled sample using the method proposed by **Bradford (1976)**. In accordance with the procedure described by **Laemmli (1970)** and most recently refined by **Darwesh *et al*. (2015)**, native proteins were electrophoretically separated using Polyacrylamide Gel Electrophoresis (PAGE) and then stained with Commassie Brilliant Blue.

Quantity One software (Version 4.6.2) was used to examine the PAGE plates that were imaged. This software was used to calculate the electrophoretically separated bands' relative mobility (Rf), band percent (B%), and relative band quantity (Qty%).

6. Molecular assay

Total RNA was extracted from fish tissues (about 20mg) by Trizol reagent (Invitrogen, USA). Concentration of the extracted RNA was detected by a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Solidity of the RNA was tested by 1% agarose gel electrophoresis according to the integrity of 18S and 28S rRNA bands. Then, total RNA samples were separately treated with RNase-free DNase I (Promega, Madison, WI, USA) before starting to create the complementary DNA (cDNA) with cDNA Kit (Invitrogen, Waltham,MA, USA). Expression of mRNA levels of antioxidant enzymes (SOD, CAT and GPX) and inflammatory markers (IL-6 and IL-1b) were assayed. The amplification process was carried out by qPCR using primers with sequences specific to the tested genes (Table 1) and its protocol was 10min at 95 °C, 15s at 95°C, and 60s at 60°C for 40 cycles. Levels of the relative mRNA were assessed based on the cycle threshold procedure and normalized to geometric means of β-actin as an internal reference gene (house-keeping gene) **(Qin *et al.*, 2013)**. The $2^{-\Delta\Delta Ct}$ equation was applied to calculate level of the relative mRNA **(Livak & Schmittgen, 2001)**.

7. Statistical analysis

The mean ± standard error (SE) was used to express the data compiled in Tables and illustrated Figs. The Statistical Package for Social Sciences (SPSS for Windows, version 11.0) was used to perform a one-way analysis of variance test (one-way ANOVA), which was followed by a post-hoc Bonferroni test. A "*P*" value of less than 0.05 indicated that the group differences were statistically significant.

Table 1. Sequences of the primers specific for the target genes

Gene Description	Accession No.	Sequences (5'- 3')
Superoxide dismutase	AY491056.1	**F:** CATGCCTTCGGAGACAACAC
		R: ACCTTCTCGTGGATCACCAT
Glutathione peroxidase	NM_001279711.1	**F:** CGCCGAAGGTCTCGTTATTT
		R: TCCCTGGACGGACATACTT
Catalase	JF801726.1	**F:** CCCAGCTCTTCATCCAGAAAC
		R: GCCTCCGCATTGTACTTCTT
Interleukin-1β	-	**F**: AAGATGAATTGTGGAGCTGTGTT
		R: AAAAGCATCGACAGTATGTGAAAT
Interleukin-6	XM_019360734.2	**F**: GTCGCCTCCAGTGGTTACAA
		R: GAAGTCCAGCACCTCTTGCT
β-actin	XM_003455949	**F:** TGGTGGGTATGGGTCAGAAAG
		R: CTGTTGGCTTTGGGGTTCA

RESULTS AND DISCUSSION

1. Heavy metals analysis

Heavy metals are a class of naturally occurring metallic elements with high atomic weights and densities. They are released into the environment through mining, industrial processes, and agricultural practices. The measurement of heavy metals is crucial for assessing their presence, concentration, and potential effects on organisms, particularly fish due to their toxicity and persistence. Depending on the metal's kind, concentration, length of exposure, and sensitivity of the species, they can have a variety of harmful consequences on fish. Therefore, understanding the effects of heavy metals on fish is crucial for assessing the health of aquatic ecosystems and ensuring the safety of fish as a food source for humans **(Ezzat *et al.*, 2024)**.

Comparing the levels of heavy metal pollution in several fish species at various locations was challenging. Thus, tilapia species were chosen as markers to evaluate the level of heavy metal contamination in the Damietta Branch's water. From every sampling location, they were effectively collected. Heavy metal bioaccumulation in *O. niloticus's* liver, kidney, and muscle is shown by the results presented in Table (2), showing differences in the concentrations of the metals under study depending on where the fish were taken. This agrees with the study proposed by **Islam *et al.* (2017)**. It was observed that the metals consistently followed the sequence of iron > manganese > chromium > copper > nickel > lead > cadmium in all the studied tissues. Iron exhibited the highest level, while cadmium showed the lowest values compared to the other measured metals. This is consistent with the hypothesis of **Elnabris *et al.* (2013)** that the essential elements (Fe, Ni, Mn, and Cu) were present in greater quantities than the non-essential ones (Pb and Cd).

It was discovered throughout the current investigation that the types of tissues examined affected the metal concentrations. Compared to the other tissues under study, the muscles have larger concentrations of the metals under investigation. This result agrees with those of **Abarshi *et al.* (2017)** and **Shovon *et al.* (2017)**, who highlighted that these tissues can accumulate higher concentrations of metals than other tissues because of the negative charge on their surface, which creates a potential location for positively charged elements.

In the liver and muscle of *O. niloticus*, it was found that concentrations of all determined heavy metals increased significantly ($P \leq 0.05$) in tilapia fish collected from El-Serw, followed by Talkha, and Zefta, then Benha compared to those collected from El-Kanater EL-Khayria. Regarding the kidney tissues, no significant differences were noticed among tilapia fish collected from Benha and Zefta compared to those collected from El-Kanater EL-Khayria. Whereas, in the kidney of fish collected from El-Serw, concentration of the heavy metals increased significantly ($P \leq 0.05$), followed by those

collected from Talkha. This might be related to the higher concentrations of heavy metals in the water matrix from which tilapia fish were collected, as reported by **Eldourghamy *et al*. (2024)**. They demonstrated that concentrations of heavy metals were higher during all four seasons at water collection sites of both Talkha and El-Serw.

2. Markers of the oxidative stress

Reactive oxygen species (ROS) and oxidative stress are caused by environmental stress in fish **(Gu *et al*., 2020)**. Promoting the antioxidative capacity is a viable method to enhance fish health and maintain a balanced physiological status **(Yang *et al*., 2023)**. These enzymes' activity serves as a crucial gauge for the cellular antioxidant defense system's activation and defense against oxidative stress, which is the imbalance between the generation and breakdown of ROS, including lipid peroxides, hydrogen peroxide, and superoxide anion **(Valavanidis *et al*., 2006)**. Major antioxidant enzymes in the body, such as SOD, GPx, and CAT, can neutralize undesirable O^{2-} and H_2O_2, as well as ROOH produced by free radicals **(Abdel-Tawwab *et al*., 2017)**. The SOD, CAT, and GPx enzymes are principally responsible for the enzymatic inactivation of ROS in muscle tissue **(Terevinto *et al*., 2010)**. Changes in transcription and gene expression may be linked to the decline in enzyme function **(Delles *et al*., 2014)**. Elevating the oxidative load lowers SOD, CAT, and GPx levels, which is linked to an increase in peroxidation products (LPO and TPC), according to **Hoseinifar *et al*. (2023)**.

Measurements of the antioxidant system (both enzymatic and non-enzymatic) in *O. niloticus's* liver and muscle showed a significant ($P \leq 0.05$) decline with the level of pollution. From this perspective, these measurements decreased markedly in tilapia fish collected from El-Serw, followed by Talkha, Zefta, and then Benha compared to those collected from El-Kanater El-Khayria (Table 3 & Fig. 1).

Regarding the kidney tissues, the variables of the antioxidant system displayed non-significant differences among tilapia fish collected from Benha and Zefta compared to the normal values of those collected from El-Kanater El-Khayria. However, in the kidneys of fish collected from El-Serw, these measurements decreased significantly ($P \leq 0.05$), followed by those collected from Talkha. The decrease in the antioxidative enzymes (SOD, CAT, and GPx) is responsible for elevating the levels of ROS, thereby increasing the LPO and TPC in the hepatic, renal, and muscular tissue of the Nile tilapia **(Xu *et al*., 2022)**. **Reyad *et al*. (2021)** found in their investigations that the antioxidant defenses and activity of antioxidant enzymes could be valuable biomarkers of water contamination. Even at low concentrations of heavy metals in water, they may be altered by changing water quality characteristics, which could result in eventual heavy metal bioaccumulation and large concentrations of metals in fish tissues.

3. Inflammatory markers

The biomolecular reaction of tilapia fish exposed to heavy metals showed that TNF-α protein expression, activation, and concentration rose in proportion to the tissue's

heavy metal content **(Rumahlatu *et al.*, 2019)**. One of the fundamental molecular processes behind the toxicity of heavy metals is oxidative stress **(Chen *et al.*, 2018)**. Certain cellular inflammatory factors such as IL-6 and immunological factors are drastically modified when the immune system is inhibited **(Jantawongsri *et al.*, 2021)**. In the liver and muscle of *O. niloticus*, it was found that the levels of inflammatory markers, including the cytokines TNF-α and IL-6, increased significantly ($P \leq 0.05$) in tilapia fish collected from El-Serw, followed by Talkha, Zefta, and then Benha compared to those collected from El-Kanater El-Khayria (Table 4). This matches the finding of **Hossain *et al.* (2021)**, who postulated that there is a relationship between increasing inflammatory markers and the depletion of antioxidant enzymes, which occurred as a result of the bioaccumulation of heavy metals.

Regarding the kidney tissues, the levels of these cytokines displayed non-significant variation among tilapia fish collected from Benha and Zefta compared to the normal values of those collected from E. El-Khayria. However, in the kidneys of fish collected from El-Serw, these measurements increased significantly ($P \leq 0.05$), followed by those collected from Talkha. Based on the measurements, tissues exposed to pollution had a markedly elevated level of IL-6 **(Liu *et al.*, 2022)**. This suggests that heavy metals may specifically target the tissues' cytokines TNF-α and IL-6 genes to have negative effects. Our results imply that in addition to tissue damage, heavy metal exposure results in immune system dysfunction and muscular atrophy. Oxidative stress, inflammation, apoptosis, tissue damage, and immunosuppression from heavy metal exposure are all intricately linked.

4. Histopathological examination

Histopathology is categorized as a speedy and cost-effective tool for detecting unfavorable effects (acute and chronic) caused in numerous tissues and organs as a result of exposure to heavy metals **(Tayel *et al.*, 2014)**. Fish detoxification is mostly carried out by the liver, which rids the body of toxins that enter from the intestine **(El-Naggar *et al.*, 2009)**. Fish liver damage can cause a variety of physiological problems, which can ultimately lead to the fish's demise **(Mahboob *et al.*, 2020)**. In the present study, the liver sections of *O. niloticus* collected from E. El-Khayria (Fig. 2a) showed thickness of blood vessels (Th), hypoxia (Hy), and pyknosis of nuclei (H & E, 400X). In the liver section of fish collected from Benha (Fig. 2b), hemorrhage (Hr), hemolysis (Hs), and hemosiderin (Hn) between hepatocyte cells were detected. Dilatation (Di), hemosiderin (Hs) in blood vessels, and fatty degeneration (ft) were noticed in hepatocytes in fish collected from Zefta (Fig. 2c). In the liver section of fish collected from Talkha (Fig. 2d), degeneration (D), necrosis (N), fibrosis (Fb) in hepatocytes, congestion (Cn) in blood sinusoids, and pyknosis of nuclei (Pk) were identified. The liver section of fish collected from El-Serw (Fig. 2e) showed degeneration (D) in the wall of the hepatic portal vein, hemosiderin (Hs) around the wall of the hepatic portal vein, and pyknosis of nuclei (Pk). This is congruent with the findings of **Hashem *et al.* (2020)**, who claimed that the liver is engaged in the

detoxification of heavy metals, which accumulate in the tissue leading to degeneration and necrosis of hepatocytes. The fertilizers, salts, and sewage discharged into the hemosiderin **(Authman & Abbas, 2007)** and water matrix **(Mahmoud & Abd El Rahman, 2017)**, which accumulate in liver cells, may contribute to the rapid and continual death of red blood cells. Furthermore, degradation of hepatocytes might be triggered by oxygen shortage due to intravascular hemolysis and vascular dilatation **(Saad *et al.* 2011)**. According to **El-Naggar *et al.* (2009)**, fatty degeneration may result from an imbalance between the production and consumption of fat or from an accelerated rate of energy reserve usage.

Fish kidneys are crucial organs for excretion, osmoregulation, and hematopoiesis **(Faheem *et al.*, 2016)**. Toxicants enter the kidney through the bloodstream, and several studies have confirmed that kidneys are the primary sites affected by toxicants **(Smorodinskaya *et al.*, 2023)**. During the current study, it was observed that the kidney sections of *O. niloticus* collected from E. El-Khayria (Fig. 3a) showed dilatation of blood vessels (Di), congestion of blood vessels (Cn), necrosis in hematopoietic tissue (N), and degeneration in kidney tubules (D) (H & E, 400X). Dilatation of blood vessels (Di), stagnant blood in hematopoietic tissue (St), and fibrosis (Fb) were observed in the renal tissue of fish collected from Benha (Fig. 3b). In the renal tissue of fish collected from Zefta (Fig. 3c), necrosis (N) and shrinkage (Sk) of glomeruli, hemorrhage (Hr) in hematopoietic tissue, and focal necrosis (FN) were detected. Degeneration in kidney tubules (D), hemosiderin pigment (Hs), and fibrosis (Fb) in renal tissue, and hemolysis (Hs) in hematopoietic tissue were identified in fish collected from Talkha (Fig. 3d). The kidney section of fish collected from El-Serw (Fig. 3e) showed degeneration (D) and necrosis (N) in glomeruli, hemorrhage in hematopoietic tissue (Hr), and dilatation in the lumen of kidney tubule (Di). According to current findings, the most deleterious abrasions were observed in the kidneys compared to other studied tissues, resulting in renal failure **(Waris *et al.*, 2023)**. Additionally, **Pal and Reddy (2018)** demonstrated that the toxicants caused degeneration and necrosis of renal tubules, glomerulus expansion or atrophy, aggregation of lymphocytes, and hemorrhage within the hematopoietic tissues. These findings revealed various abnormalities detected histopathologically, ultimately leading to renal failure.

According to **Yacoub *et al.* (2008)**, the muscular system, which makes up the majority of the fish's body and is covered in skin, is mostly made up of segmental myomeres that are thought of as muscle. Its fibers run parallel to the body axis. According to **Kadry *et al.* (2015)**, it is in charge of peristaltic constriction of visceral organs, blood pumping, coordinated skeletal component movement, and locomotion. During the present study, the muscle section of *O. niloticus* collected from E. El-Khayria (Fig. 4a) showed separation of the epidermal and dermal layers (S), degeneration in the dermal and muscle layers (D), and edema (E) in the muscle layer (H & E, 400X). Hemorrhage (Hr), edema (E), and degeneration (D) in the muscle layer were noticed in

the muscle tissue of fish collected from Benha (Fig. 4b). In the muscle tissue of fish collected from Zefta (Fig. 4c), degeneration (D) in the epidermal layer, necrosis (N) in the dermal layer, and focal necrosis (FN) and hemorrhage in the muscle layer were detected. Degeneration (D), necrosis (N), hemosiderin (Hn) in the dermal layer, and hemorrhage (Hr) were identified in the muscle layer in fish collected from Talkha (Fig. 4d). The muscle section of fish collected from El-Serw (Fig. 4e) showed parasite form (P), hemorrhage (Hr), edema (E), and Kupffer cell (K) in the muscle layer (Pk). This is in line with the theory set by **Fernandes *et al.* (2008)** that osmoregulatory and circulatory damage is caused by heavy metal exposure. Deterioration of the epithelial layer, separation of the epidermis from the dermal layer, and degeneration of irritants floating or dissolved in water decrease their surface area and impair respiratory function according to **Tayel *et al.* (2014)**. Additionally, wastewater discharge into natural water bodies with high organic matter content raises organic phosphorus levels, which can lower fish gaseous exchange capacity **(Dalzochio *et al.*, 2018)**.

5. Electrophoretic assays

One common method for separating, identifying, and measuring the numerous proteins expressed in different tissues is electrophoresis. According to **Aboulthana *et al.* (2016)**, it was regarded as the most widely used instrument for determining the stoichiometry of a particular subunit of a protein complex. The similarity % number solely correlates with qualitative changes and gives an indicator of the tissue's physiological state. Compared to the control group, a lower similarity percentage suggests differences in the quantity and arrangement of electrophoretically separated bands. If not, changes can be made quantitatively by keeping their identification data's bands normal, but changes can also happen in their quantities **(Deabes *et al.*, 2021)**. The most significant macromolecules that perform crucial roles in all living cells are proteins. Depending on the proportion of oxidation-sensitive amino acid residues, they are oxidizable. Thus, electrophoretic separation, identification, and characterisation were used to investigate the chemical makeup and physiological role of particular proteins **(El-Shamarka *et al.*, 2024)**. During the present study, the electrophoretic technique was used to assess the physiological state of various tissues (liver, kidney, and muscle) in fish. This approach is in line with **Mohamed *et al.* (2020)**, who showed that an electrophoretic method can differentiate between fish slices based on their protein composition. While there were slight differences for fish from different sources, consistent and repeatable electrophoretic patterns were seen for samples of the same species that varied in size and provenance.

As shown in Fig. (5), it was noticed that the native protein pattern was represented electrophoretically in liver tissue of *O. niloticus* collected from El-Kanater El-Khayria by 7 bands identified at Rfs 0.05, 0.12, 0.30, 0.43, 0.58, 0.73, and 0.92 (Int. 162.83, 170.28, 159.83, 174.75, 169.96, 172.91, and 172.83; B% 13.72, 13.16, 14.14, 17.92, 13.37, 17.00, and 10.68; Qty 5.24, 5.02, 5.40, 6.84, 5.10, 6.49, and 4.08, respectively). In liver tissue of

fish collected from Benha, the native protein pattern was represented by 9 bands identified at Rfs 0.05, 0.13, 0.19, 0.30, 0.43, 0.50, 0.64, 0.73, and 0.92 (Int. 159.74, 160.52, 155.88, 164.47, 182.99, 149.74, 154.99, 160.68, and 218.88; B% 11.14, 13.39, 10.02, 12.59, 8.51, 9.83, 10.60, 21.53, and 2.39; Qty 4.38, 5.27, 3.94, 4.95, 3.35, 3.87, 4.17, 8.47, and 0.94, respectively). The data from the phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 62.20%. In liver tissue of fish collected from Zefta, the native protein pattern was represented by 8 bands identified at Rfs 0.04, 0.12, 0.18, 0.30, 0.43, 0.49, 0.64, and 0.73 (Int. 160.87, 164.17, 157.87, 159.79, 175.15, 156.83, 156.74, and 170.51; B% 12.15, 13.78, 11.04, 13.86, 17.15, 11.84, 11.84, and 8.35; Qty 4.78, 5.42, 4.35, 5.45, 6.75, 4.66, 4.66, and 3.29, respectively). The phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 65.20%.

In liver tissue of fish collected from Talkha, the electrophoretic protein pattern was represented by 9 bands identified at Rfs 0.05, 0.12, 0.18, 0.31, 0.44, 0.50, 0.65, 0.73, and 0.83 (Int. 159.70, 163.04, 160.22, 162.17, 175.54, 154.22, 158.14, 173.12, and 150.13; B% 10.36, 12.82, 8.80, 6.68, 16.64, 9.43, 10.26, 14.90, and 10.11; Qty 4.55, 5.62, 3.86, 2.93, 7.30, 4.14, 4.50, 6.54, and 4.44, respectively). The phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 63.00%. In liver tissue of fish collected from El-Serw, the electrophoretic protein pattern was represented by 7 bands identified at Rfs 0.12, 0.18, 0.31, 0.44, 0.65, 0.74, and 0.83 (Int. 165.49, 153.15, 164.74, 175.41, 157.79, 177.44, and 150.76; B% 16.81, 11.95, 13.82, 16.78, 12.77, 15.67, and 12.20; Qty 6.31, 4.49, 5.19, 6.30, 4.80, 5.89, and 4.59, respectively). The phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 59.90% indicating higher physiological changes in the number and arrangement of protein bands among the liver tissue of fish collected from Benha and Zefta compared to the tissue of fish collected from E. El-Khayria, which is considered control tissue.

Four common bands were identified at Rfs 0.12, 0.30, 0.43, and 0.73. No characteristic bands were identified.

Regarding the renal tissue of *O. niloticus* collected from E. El-Khayria (Fig. 6), it was noticed that the native protein pattern was represented electrophoretically in by 6 bands identified at Rfs 0.21, 0.38, 0.51, 0.60, 0.74, and 0.97 (Int. 113.44, 131.84, 168.54, 115.49, 128.22, and 158.07; B% 13.81, 16.05, 23.33, 12.68, 13.77, and 20.37; Qty 5.38, 6.25, 9.09, 4.94, 5.37, and 7.94, respectively). In renal tissue of fish collected from Benha, the native protein pattern was represented by 6 bands identified at Rfs 0.21, 0.39, 0.52, 0.60, 0.75, and 0.97 (Int. 118.58, 121.92, 158.83, 113.11, 123.01, and 152.61; B% 10.04, 15.49, 32.66, 11.86, 13.64, and 16.31; Qty 4.66, 7.19, 15.16, 5.51, 6.33, and 7.57, respectively). The data from the phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 83.3%.

In renal tissue of fish collected from Zefta, the native protein pattern was represented by 6 bands identified at Rfs 0.21, 0.39, 0.52, 0.60, 0.75, and 0.97 (Int. 117.83, 130.53, 160.98, 109.86, 128.15, and 150.34; B% 11.28, 13.88, 35.95, 14.49, 6.81, and 17.59; Qty 4.95, 6.10, 15.79, 6.36, 2.99, and 7.72, respectively). The phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 78.70%. In renal tissue of fish collected from Talkha, the electrophoretic protein pattern was represented by 4 bands identified at Rfs 0.21, 0.52, 0.75, and 0.97 (Int. 117.91, 152.10, 122.81, and 150.09; B% 16.55, 38.32, 18.80, and 26.33; Qty 5.20, 12.04, 5.91, and 8.27, respectively). The phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 75.70%.

In renal tissue of fish collected from El-Serw, the electrophoretic protein pattern was represented by 5 bands identified at Rfs 0.10, 0.31, 0.52, 0.75, and 0.97 (Int. 116.43, 122.11, 133.39, 129.35, and 150.40; B% 11.35, 15.31, 28.33, 16.21, and 28.80; Qty 3.29, 4.43, 8.20, 4.70, and 8.34, respectively). The phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 62.40% indicating higher physiological changes in the number and arrangement of protein bands among the renal tissue of fish collected from Benha and Zefta compared to the tissue of fish collected from E. El-Khayria.

Three common bands were identified at Rfs 0.51, 0.74, and 0.97. Two characteristic (unique) bands were identified at Rfs 0.10 and 0.31 (Int. 116.43 and 122.11; B% 11.35 and 15.31; Qty 3.29 and 4.43, respectively) in renal tissue of fish collected from El-Serw.

As shown in Fig. (7), it was noticed that the native protein pattern was represented electrophoretically in muscle tissue of *O. niloticus* collected from E. El-Khayria by 5 bands identified at Rfs 0.14, 0.34, 0.62, 0.72, and 0.91 (Int. 144.34, 158.40, 158.21, 163.04, and 145.60; B% 15.03, 19.79, 17.88, 25.22, and 22.09; Qty 4.84, 6.37, 5.76, 8.12, and 7.11, respectively). In renal tissue of fish collected from Benha, the native protein pattern was represented by 6 bands identified at Rfs 0.15, 0.34, 0.47, 0.62, 0.72, and 0.91 (Int. 148.10, 156.86, 165.11, 153.84, 173.14, and 149.59; B% 12.41, 17.16, 14.99, 19.33, 19.75, and 16.36; Qty 4.95, 6.85, 5.98, 7.72, 7.88, and 6.53, respectively). The data from the phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 86.80%.

In muscle tissue of fish collected from Zefta, the native protein pattern was represented by 5 bands identified at Rfs 0.15, 0.34, 0.47, 0.62, and 0.72 (Int. 148.99, 157.10, 161.97, 161.31, and 176.54; B% 15.83, 21.11, 19.74, 20.16, and 23.17; Qty 4.83, 6.44, 6.02, 6.15, and 7.07, respectively). The phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 74.60%.

In muscle tissue of fish collected from Talkha, the electrophoretic protein pattern was represented by 5 bands identified at Rfs 0.25, 0.34, 0.48, 0.62, and 0.73 (Int. 149.13,

156.65, 160.75, 157.00, and 168.43; B% 16.15, 19.87, 21.38, 16.03, and 26.57; Qty 4.99, 6.14, 6.61, 4.95, and 8.21, respectively). The phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 60.80%.

In muscle tissue of fish collected from El-Serw, the electrophoretic protein pattern was represented by 5 bands identified at Rfs 0.25, 0.34, 0.49, 0.73, and 0.81; (Int. 149.17, 155.93, 147.71, 173.12, and 151.79; B% 14.86, 18.83, 24.97, 23.00, and 18.33; Qty 4.77, 6.05, 8.02, 7.39, and 5.89, respectively). The phylogenetic tree showed that this protein pattern is physiologically similar to the tissue in fish collected from E. El-Khayria by 42.00%, indicating higher physiological differences in the number and arrangement of protein bands among the muscle tissue of fish collected from Benha and Zefta compared to the tissue of fish collected from E. El-Khayria.

Two common bands were identified at Rfs 0.34 and 0.72. Only one characteristic band was identified at Rf 0.81 (Int. 151.79; B% 18.33; Qty 5.89) in muscle tissue of fish collected from El-Serw.

The impact of heavy metals, particularly Pb and Cd, which can render proteins and peptides vulnerable to structural changes in subcellular compartments and tissues, may be indicated by the electrophoretic changes in the native protein patterns in the target tissues **(Hechtenberg & Beyersmann, 1991)**. Furthermore, depending on the kind, concentration, and duration of exposure, the metals can alter the activity of liver enzymes, resulting in tissue alterations **(Paris-Palacios *et al.*, 2000)**. In addition, since Cd can change cellular adhesion and antioxidant defense mechanisms, it may be the cause of changes in the electrophoretic patterns **(Yano & Marcondes, 2005)**.

Table 2. Data on the heavy metals in the most susceptible tissues to pollutant accumulation in tilapia fish (*Oreochromis niloticus*) gathered from various points in the water of the Damietta Branch

			E. EL-Khayria	Benha	Zefta	Talkha	El-Serw
Liver	**Cd**	**mg/ Kg**	0.01±0.00	0.02±0.00^{a}	0.05±0.00^{ab}	0.11±0.00^{abc}	0.13±0.00^{abcd}
	Pb		0.04±0.00	0.10±0.00^{a}	0.20±0.00^{ab}	0.44±0.01^{abc}	0.55±0.01^{abcd}
	Cu		0.76±0.00	1.63±0.00^{a}	3.51±0.01^{ab}	7.54±0.02^{abc}	9.42±0.03^{abcd}
	Fe		12.58±0.02	27.05±0.04^{a}	58.16±0.09^{ab}	125.05±0.20^{abc}	156.31±0.25^{abcd}
	Mn		3.26±0.01	7.00±0.02^{a}	15.05±0.03^{ab}	32.35±0.07^{abc}	40.44±0.09^{abcd}
	Ni		0.28±0.00	0.60±0.01^{a}	1.29±0.02^{ab}	2.77±0.04^{abc}	3.47±0.05^{abcd}
	Cr		0.84±0.01	1.80±0.01^{a}	3.88±0.03^{ab}	8.34±0.05^{abc}	10.43±0.07^{abcd}
Kidney	**Cd**		0.01±0.00	0.01±0.00	0.02±0.00	0.07±0.00^{abc}	0.09±0.00^{abcd}
	Pb		0.03±0.00	0.03±0.00	0.03±0.00	0.31±0.01^{abc}	0.39±0.01^{abcd}
	Cu		0.58±0.00	0.52±0.00	0.55±0.00	5.80±0.02^{abc}	7.25±0.03^{abcd}
	Fe		5.08±0.02	5.12±0.03	5.17±0.02	50.51±0.20^{abc}	63.14±0.25^{abcd}
	Mn		2.68±0.01	2.71±0.02	2.67±0.02	26.64±0.07^{abc}	33.30±0.09^{abcd}
	Ni		0.18±0.00	0.21±0.01	0.19±0.01	1.77±0.04^{abc}	2.22±0.05^{abcd}
	Cr		0.46±0.01	0.44±0.01	0.47±0.02	4.61±0.05^{abc}	5.77±0.07^{abcd}
Muscle	**Cd**		0.01±0.00	0.03±0.00^{a}	0.06±0.00^{ab}	0.13±0.00^{abc}	0.17 ± 0.00^{abcd}
	Pb		0.06±0.00	0.12±0.00^{a}	0.26±0.00^{ab}	0.55±0.01^{abc}	0.69±0.01^{abcd}
	Cu		0.95±0.00	2.04±0.01^{a}	4.38±0.01^{ab}	9.42±0.03^{abc}	11.78±0.04^{abcd}
	Fe		15.73±0.03	33.82±0.05^{a}	72.70±0.12^{ab}	156.31±0.25^{abc}	195.39±0.31^{abcd}
	Mn		4.07±0.01	8.75±0.02^{a}	18.81±0.04^{ab}	40.44±0.09^{abc}	50.55±0.11^{abcd}
	Ni		0.35±0.01	0.75±0.01^{a}	1.61±0.02^{ab}	3.47±0.05^{abc}	4.34±0.06^{abcd}
	Cr		1.05±0.01	2.26±0.01^{a}	4.85±0.03^{ab}	10.43±0.07^{abc}	13.03±0.08^{abcd}

Data were expressed as mean ± SE calculated from five replicates. In comparison with tilapia fish collected from El-Kanater EL-Khayria, "**a**" indicates a significant difference ($P \leq 0.05$); "**b**" indicates a significant difference ($P \leq 0.05$) from fish collected from Benha; "**c**" indicates a significant difference ($P \leq 0.05$) from fish collected from Zefta; and "d" indicates a significant difference ($P \leq 0.05$) from fish collected from Talkha.

Table 3. Data of the enzymatic and non-enzymatic antioxidants in the most susceptible tissues to pollutant accumulation in tilapia fish (*Oreochromis niloticus*) gathered from various points in the water of the Damietta Branch

		E. EL-Khayria	Benha	Zefta	Talkha	El-Serw
Liver	**TAC (**µmol/g**)**	5.99±0.05	4.34±0.04^{a}	3.01±0.04^{ab}	2.16± 0.03^{abc}	1.25 ± 0.03^{abcd}
	GSH (mg/g tissue**)**	83.37±0.54	72.49±0.47^{a}	60.38±0.45^{ab}	48.20± 0.40^{abc}	35.42 ± 0.38^{abcd}
	SOD (IU/g tissue**)**	35.78±0.36	22.42±0.31^{a}	17.76±0.30^{ab}	12.92± 0.26^{abc}	8.37 ± 0.25^{abcd}
	CAT (IU/g tissue**)**	54.05±0.52	38.30±0.45^{a}	25.19±0.44^{ab}	12.34± 0.38^{abc}	7.40 ± 0.37^{abcd}
	GPx (IU/g tissue**)**	34.45±0.54	29.96±0.47^{a}	29.08±0.46^{ab}	25.29± 0.40^{abc}	24.55 ± 0.38^{abcd}
Kidney	**TAC (**µmol/g**)**	2.98±0.08	3.00±0.07	2.71±0.06	2.18± 0.06^{abc}	1.12 ± 0.05^{abcd}
	GSH (mg/g tissue**)**	48.92±0.48	46.54±0.42	48.30±0.41	31.92± 0.35^{abc}	24.87 ± 0.34^{abcd}
	SOD (IU/g tissue**)**	15.06±0.26	14.09±0.23	15.71±0.22	10.05± 0.19^{abc}	8.73 ± 0.18^{abcd}
	CAT (IU/g tissue**)**	25.90±0.35	26.03±0.30	24.87±0.29	17.02± 0.26^{abc}	10.46 ± 0.25^{abcd}
	GPx (IU/g tissue**)**	20.54±0.31	19.86±0.27	20.34±0.26	12.08± 0.23^{abc}	7.64 ± 0.22^{abcd}
Muscle	**TAC (**µmol/g**)**	4.60±0.04	3.39±0.03^{a}	2.25±0.03^{ab}	1.41±0.03^{abc}	0.78±0.03^{abcd}
	GSH (mg/g tissue**)**	56.75±0.36	38.26±0.32^{a}	25.59±0.31^{ab}	19.64±0.27^{abc}	10.07±0.26^{abcd}
	SOD (IU/g tissue**)**	8.80±0.17	7.16±0.14^{a}	5.43±0.14^{ab}	3.46±0.12^{abc}	1.28±0.12^{abcd}
	CAT (IU/g tissue**)**	18.24±0.31	15.25±0.27^{a}	12.87±0.26^{ab}	10.19±0.22^{abc}	7.86±0.22^{abcd}
	GPx (IU/g tissue**)**	15.87±0.28	12.32±0.24^{a}	10.02±0.24^{ab}	8.71±0.21^{abc}	5.76±0.20^{abcd}

Data were expressed as mean ± SE calculated from five replicates. In comparison with tilapia fish collected from El-Kanater EL-Khayria, "**a**" indicates a significant difference ($P \leq 0.05$); "**b**" indicates a significant difference ($P \leq 0.05$) from fish collected from Benha; "**c**" indicates a significant difference ($P \leq 0.05$) from fish collected from Zefta; and "d" indicates a significant difference ($P \leq 0.05$) from fish collected from Talkha.

Table 4. Data of the markers of inflammatory reactions in the most susceptible tissues to pollutant accumulation in tilapia fish (*Oreochromis niloticus*) gathered from various points in the water of the Damietta Branch

		E. EL-Khayria	Benha	Zefta	Talkha	El-Serw
Liver	**TNF-α (**Pg/g tissue**)**	127.91±0.25	151.59±0.28^{a}	187.04±0.29^{ab}	215.10±0.33^{abc}	239.55±0.34^{abcd}
	IL-6 (Pg/g tissue**)**	151.70±0.39	197.46±0.45^{a}	227.07±0.46^{ab}	258.13±0.53^{abc}	278.97±0.55^{abcd}
	AChE (ng/g tissue**)**	1.49±0.03	1.81±0.04^{a}	2.77±0.04^{ab}	3.03±0.04^{abc}	3.25±0.05^{abcd}
Kidney	**TNF-α (**Pg/g tissue**)**	65.51±0.41	67.34±0.47	64.60±0.48	81.24±0.55^{abc}	98.91±0.57^{abcd}
	IL-6 (Pg/g tissue**)**	78.81±0.15	80.03±0.17	78.35±0.18	107.35±0.21^{abc}	122.58±0.21^{abcd}
	AChE (ng/g tissue**)**	1.15±0.03	1.07±0.04	1.11±0.04	2.13±0.04^{abc}	2.79±0.05^{abcd}
Muscle	**TNF-α (**Pg/g tissue**)**	23.82±0.15	29.40±0.17^{a}	37.22±0.18^{ab}	46.45±0.20^{abc}	54.42±0.21^{abcd}
	IL-6 (Pg/g tissue**)**	28.66±0.06	38.96±0.06^{a}	47.95±0.07^{ab}	54.04±0.08^{abc}	67.21±0.08^{abcd}
	AChE (ng/g tissue**)**	0.84±0.01	1.27±0.01^{a}	1.64±0.01^{ab}	1.94±0.02^{abc}	2.76±0.02^{abcd}

Data were expressed as mean ± SE calculated from five replicates. In comparison with tilapia fish collected from El-Kanater EL-Khayria, "**a**" indicates a significant difference ($P \leq 0.05$); "**b**" indicates a significant difference ($P \leq 0.05$) from fish collected from Benha; "**c**" indicates a significant difference ($P \leq 0.05$) from fish collected from Zefta; and "d" indicates a significant difference ($P \leq 0.05$) from fish collected from Talkha.

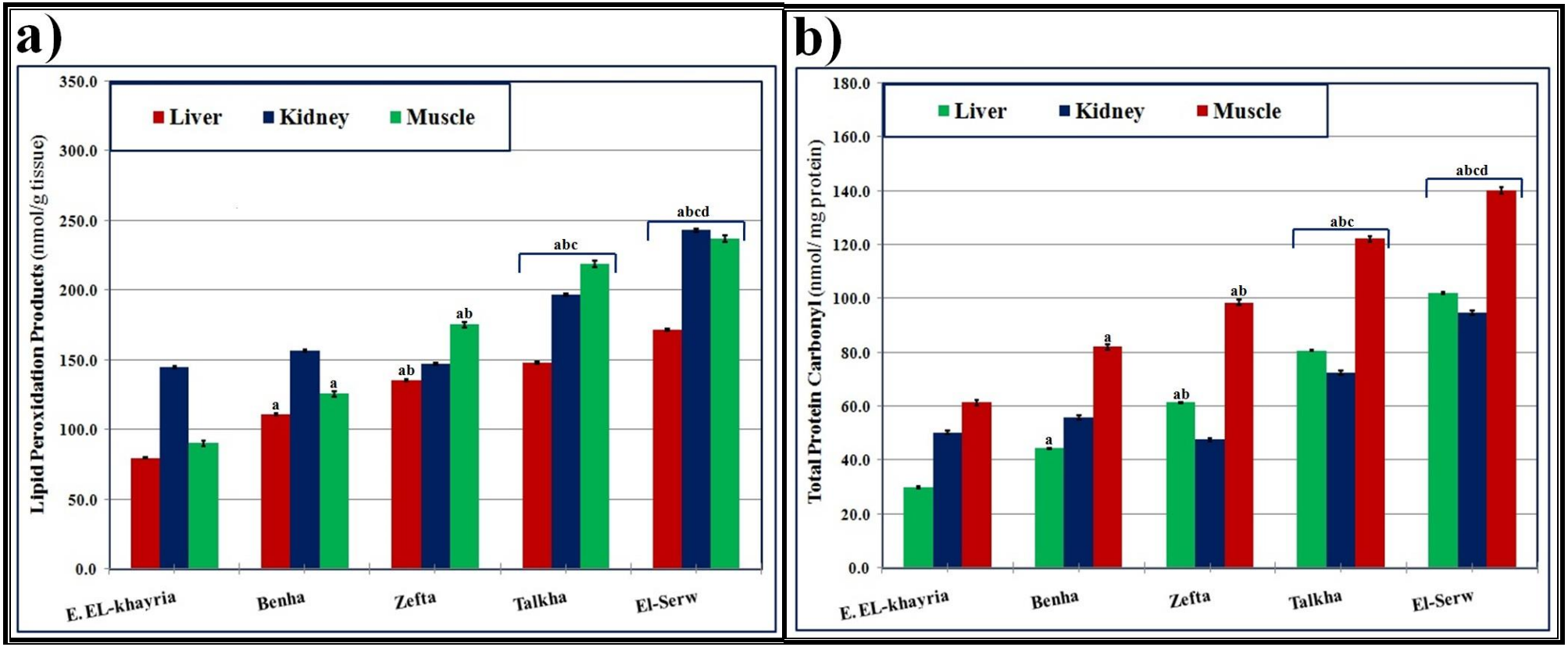

Fig. 1. Data showing the differences in **a)** lipid peroxidation product, and **b)** total protein carbonyl content in the most susceptible tissues to pollutant accumulation in tilapia fish (*Oreochromis niloticus*) gathered from various points in the water of the Damietta Branch. Data were expressed as mean ± SE calculated from five replicates. " In comparison to tilapia fish collected from El-Kanater EL-Khayria, "**a**" indicates a significant difference ($P \leq 0.05$), "**b**" indicates a significant difference ($P \leq 0.05$) from fish collected from Benha, "**c**" indicates a significant difference ($P \leq 0.05$) from fish collected from Zefta, and "d" indicates a significant difference ($P \leq 0.05$) from fish collected from Talkha

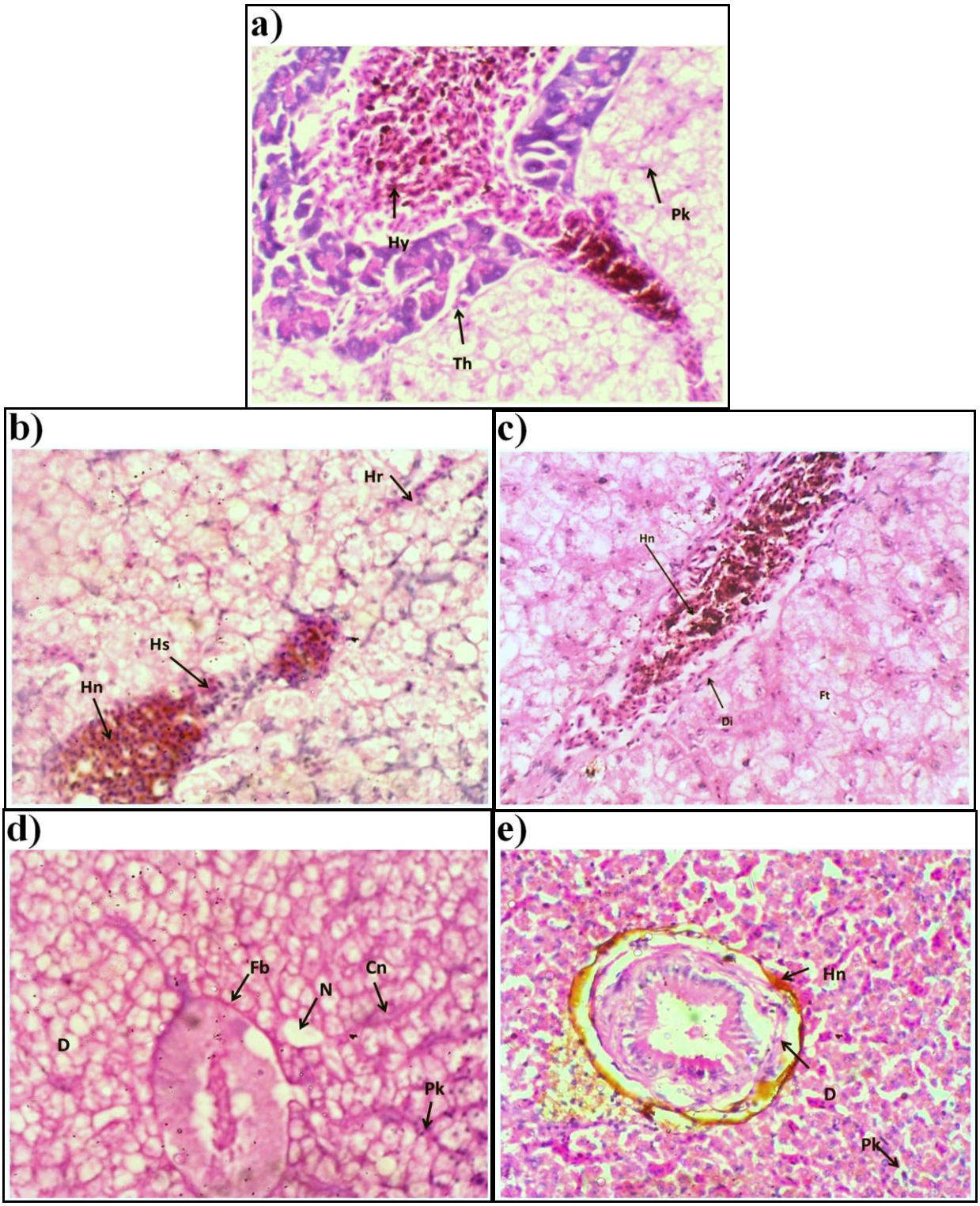

Fig. 2. Representative photomicrographs of H&E-stained liver sections of rats (Scale bar 50µm) showing the histopathological differences in tilapia fish (*Oreochromis niloticus*) gathered from **a)** E. EL-Khayria, **b)** Benha, **c)** Zefta, **d)** Talkha, and **e)** El-Serw

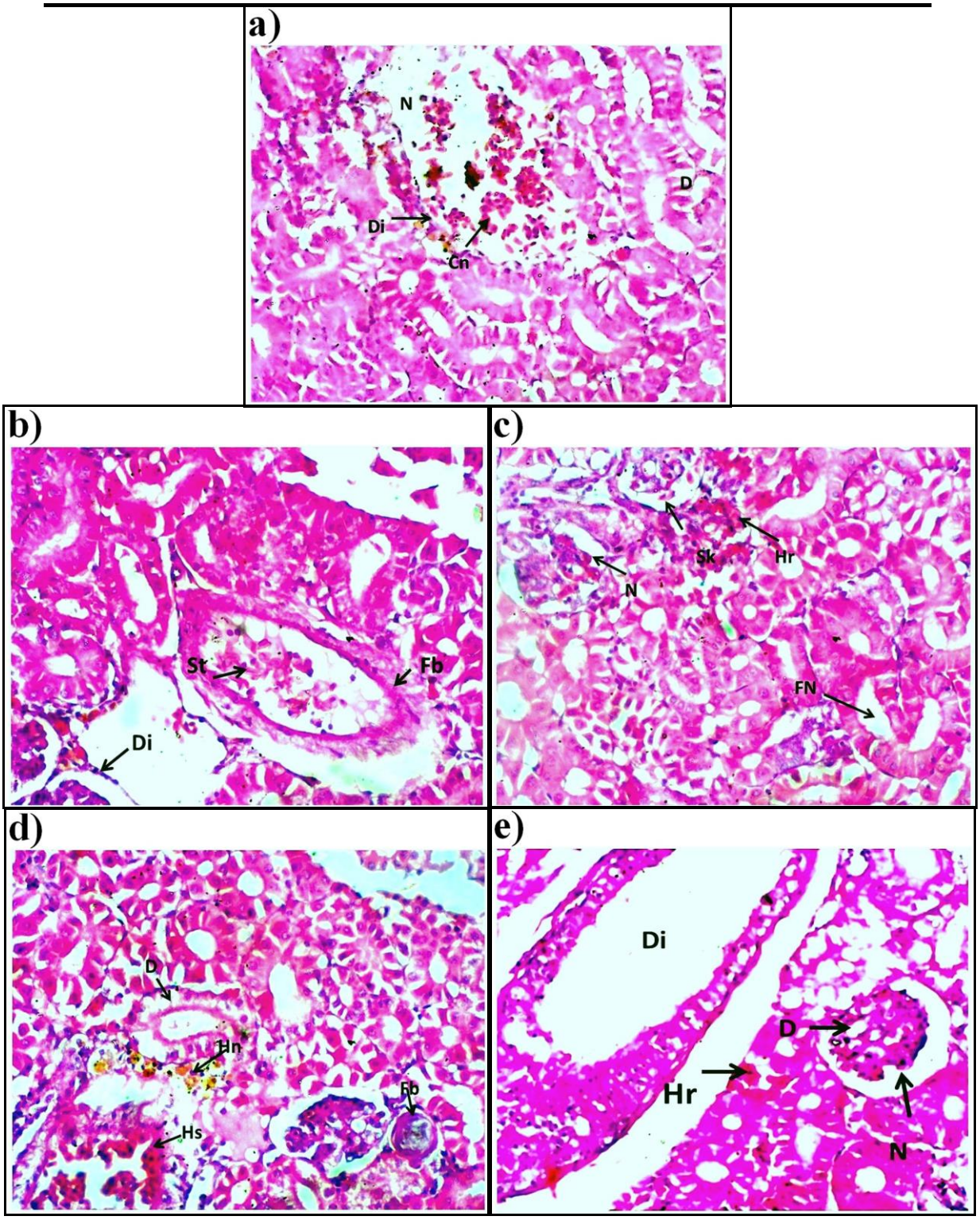

Fig. 3. Representative photomicrographs of H&E-stained kidney sections of rats (Scale bar 50µm) showing the histopathological differences in tilapia fish (*Oreochromis niloticus*) gathered from **a)** E. EL-Khayria, **b)** Benha, **c)** Zefta, **d)** Talkha, and **e)** El-Serw

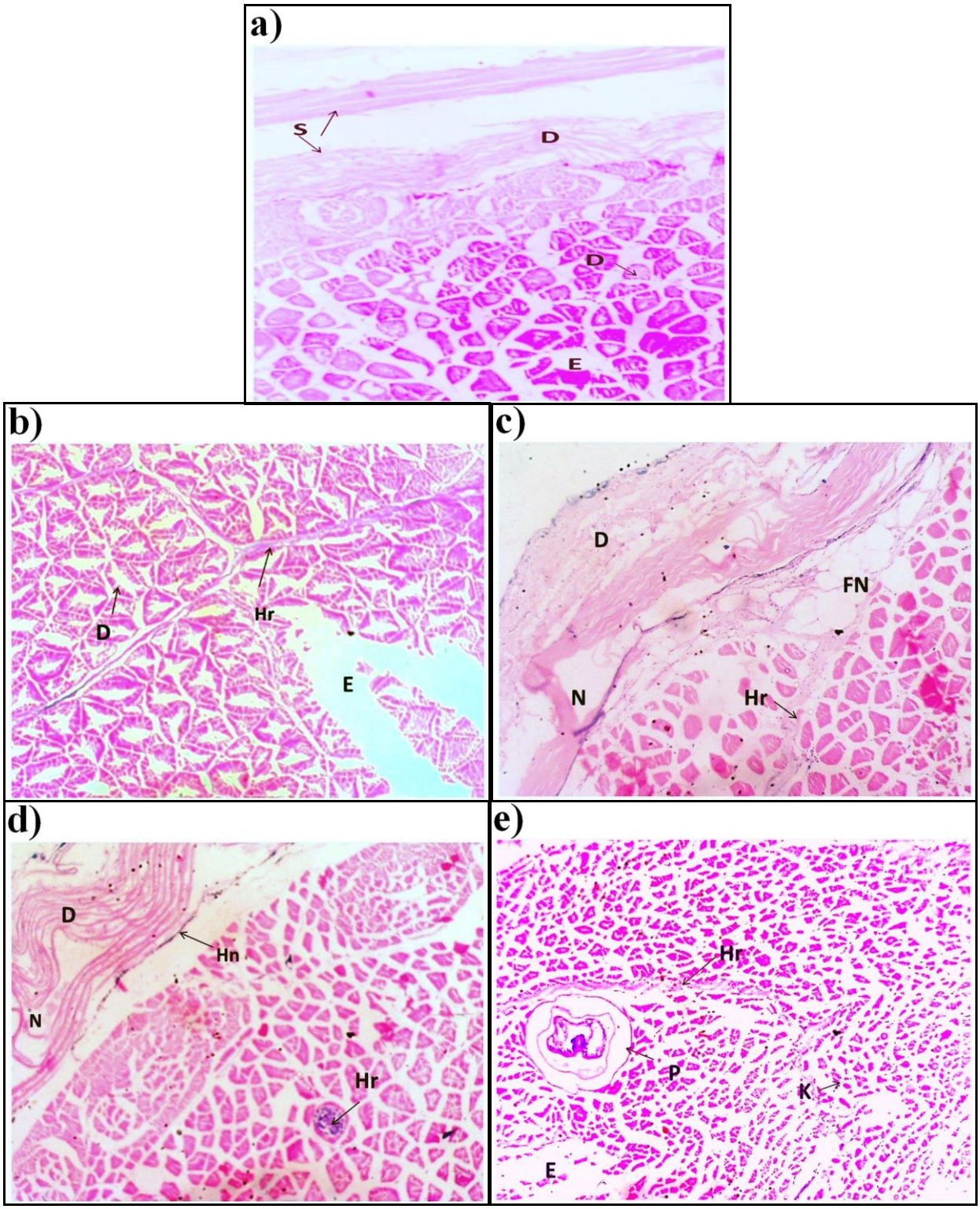

Fig. 4. Representative photomicrographs of H&E-stained muscle sections of rats (Scale bar 50µm) showing the histopathological differences in tilapia fish (*Oreochromis niloticus*) gathered from **a)** E. EL-Khayria, **b)** Benha, **c)** Zefta, **d)** Talkha, and **e)** El-Serw

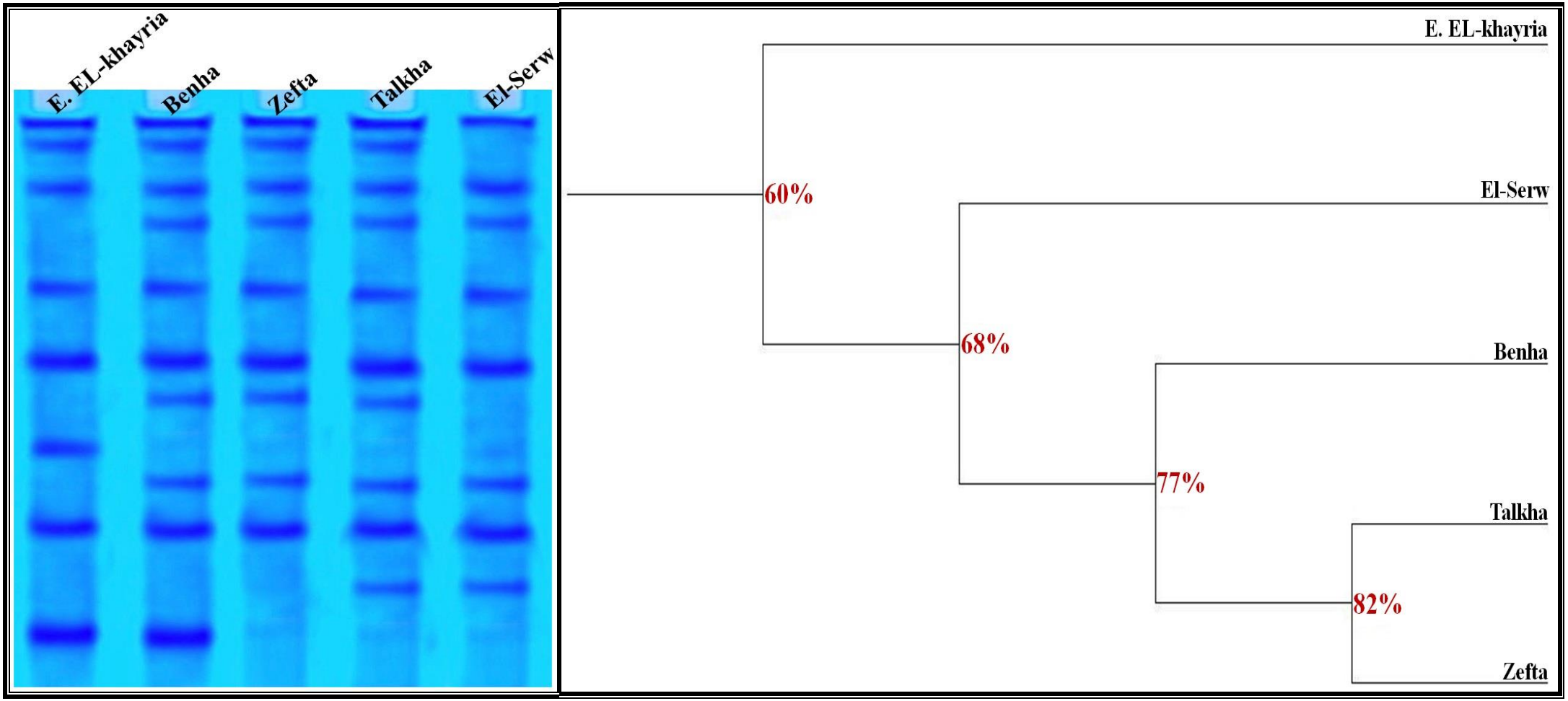

Fig. 5. The native electrophoretic protein pattern showing the physiological differences in liver of tilapia fish (*Oreochromis niloticus*) gathered from various points in the water of the Damietta Branch

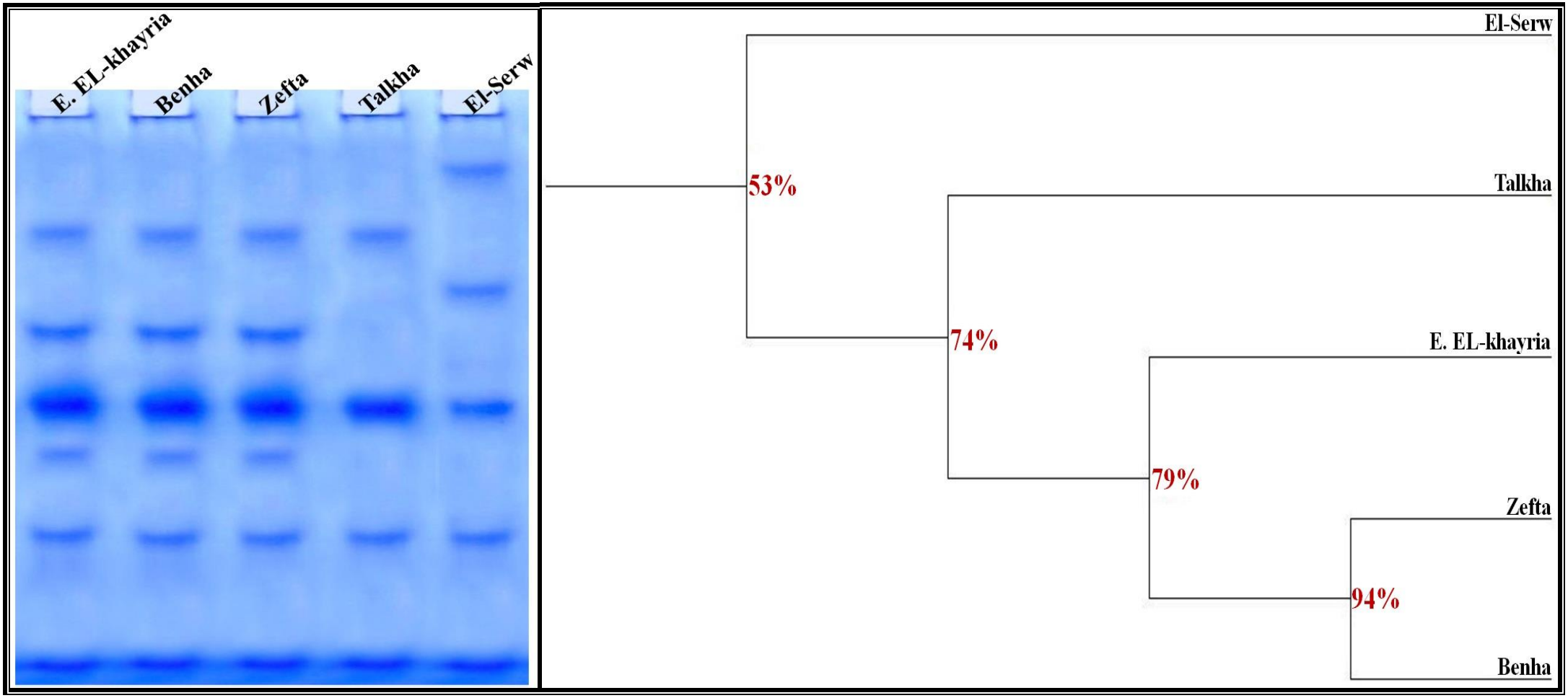

Fig. 6. The native electrophoretic protein pattern showing the physiological differences in kidney of tilapia fish (*Oreochromis niloticus*) gathered from various points in the water of the Damietta Branch.

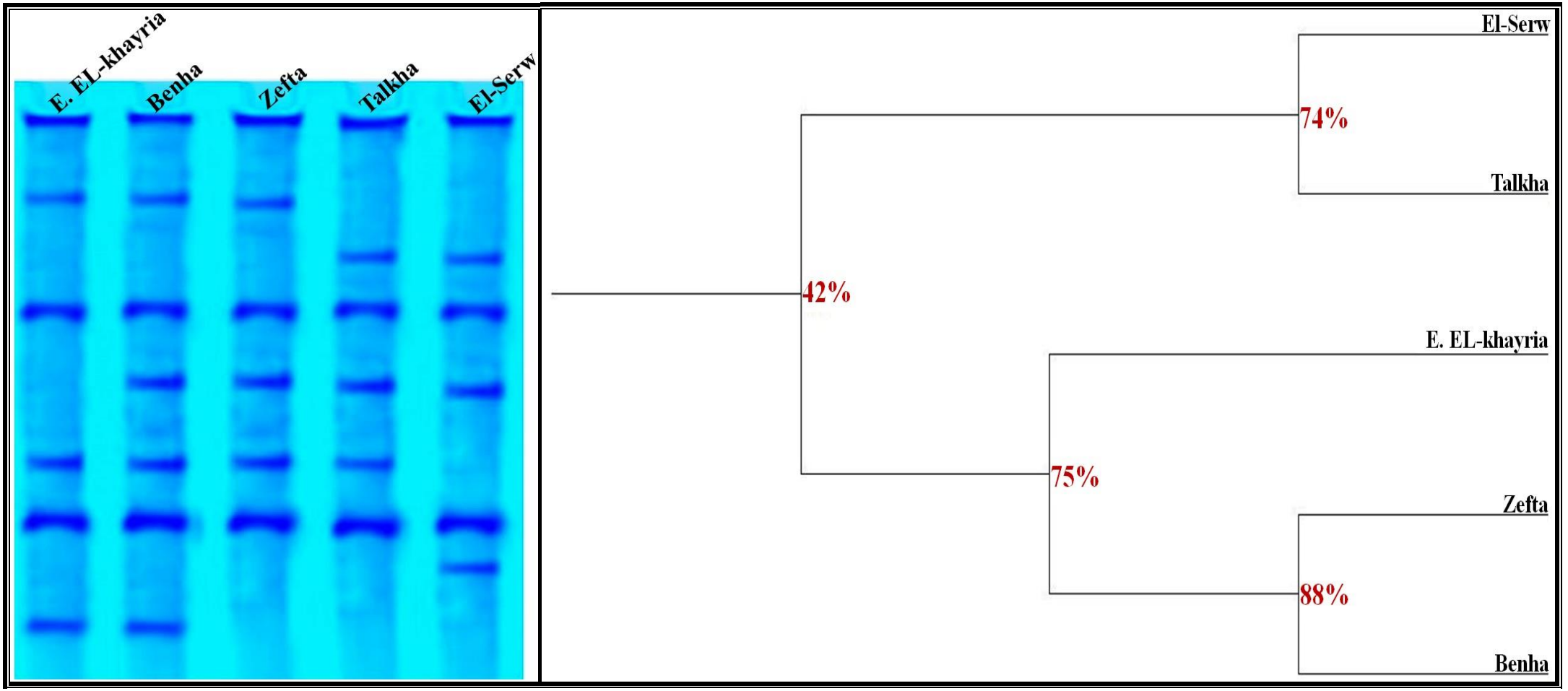

Fig. 7. The native electrophoretic protein pattern showing the physiological differences in muscle of tilapia fish (*Oreochromis niloticus*) gathered from various points in the water of the Damietta Branch

6. Molecular assay

The level of ROS increases when fish are subjected to stressful conditions, resulting in cell impairment due to lipid peroxidation **(Mahmoud *et al.*, 2018)**. The secretion of antioxidative enzymes, such as SOD, CAT, and GPx, which are thought to be significant markers of the activation of the cellular antioxidant defense system and protection against oxidative stress, activates a number of antioxidative responses to deal with the effects of ROS on the animals' cells **(Gobi *et al.*, 2018)**.

During the present study, it was found that the relative gene expression of the antioxidant enzymes (SOD, CAT, and GPx) decreased significantly ($P \leq 0.05$) in the liver and muscle of *O. niloticus* fish collected from El-Serw, followed by Talkha, Zefta, and then Benha compared to those collected from E. El-Khayria (Table 5). In terms of kidney tissues, the relative gene expression showed non-significant differences among tilapia fish collected from Benha and Zefta compared to the normal values of those collected from El-Khayria. However, in the kidneys of fish collected from El-Serw, these measurements decreased significantly ($P \leq 0.05$), followed by those collected from Talkha. Lowering the activity of antioxidant enzymes may be related to alterations or reductions in gene expression and transcription **(Delles *et al.*, 2014)**. Because of increased proteolysis and protein oxidation brought on by oxidative stress, antioxidant enzyme activity may decline **(Carvalho *et al.*, 2017)**. This may have produced more delicate fillets due to the high density, which can result in lower expression of the CAT and GPx enzymes, protein fragmentation or aggregation, and decreased protein solubility **(Guillemin *et al.*, 2012)**.

In addition to controlling inflammation, homeostasis, and cell proliferation, the pro-inflammatory cytokines TNF-α and IL-6 are linked to autologous immune metabolism **(Jiang & Li, 2022)**. The amounts of cellular inflammatory factors are markedly changed when the immune system is suppressed **(Jantawongsri *et al.*, 2021)**. The study revealed a significant ($P \leq 0.05$) increase in the relative gene expression of the inflammatory markers TNF-α and IL-6 in the liver and muscle of *O. niloticus* fish collected from El-Serw, followed by Talkha, Zefta, and then Benha, compared to those collected from E. El-Khayria (Table 6). Regarding the kidney tissues, the relative expression of these genes displayed non-significant differences among tilapia fish collected from Benha and Zefta compared to the normal values of those collected from E. El-Khayria. However, these values significantly ($P \leq 0.05$) increased in the kidneys of fish obtained from El-Serw, and then from Talkha.

The current study's results are in line with those reported by **Soliman *et al.* (2023)**, who highlighted that the disruption of homeostasis may be the cause of the rise in mRNA expression of these cytokines, resulting in a unique immunological response that includes inflammation and cellular damage. Additionally, these cytokines trigger rapid immunological responses against the stressor by disturbing cellular homeostasis. Excessive cytokine production can lead to pathological outcomes, including severe immune disorders and systemic inflammatory responses **(Jiang *et al.*, 2021)**. The substantial rise in TNF-α and IL-6 protein expression with increasing heavy metal concentrations corroborates the outcomes of **Rumahlatu *et al.***

(2019). Oxidative stress, inflammation, apoptosis, immune system dysfunction, and tissue damage and muscular atrophy, brought on by exposure to heavy metals, are all intricately linked **(Liu *et al*., 2022)**.

Table 5. Data of the relative expression of the antioxidant genes in the most susceptible tissues to pollutant accumulation in tilapia fish (*Oreochromis niloticus*) gathered from various points in the water of the Damietta Branch

		E. EL-khayria	Benha	Zefta	Talkha	El-Serw
Liver	SOD	2.87±0.02	2.21±0.01^{a}	1.48±0.01^{ab}	1.06±0.01^{abc}	0.81±0.01^{abcd}
	CAT	3.91±0.01	2.45±0.01^{a}	1.51±0.01^{ab}	1.13±0.01^{abc}	0.77±0.01^{abcd}
	GPx	1.88±0.02	1.32±0.02^{a}	1.15±0.02^{ab}	0.94±0.02^{abc}	0.67±0.01^{abcd}
Kidney	SOD	1.94±0.01	1.98±0.01	1.89±0.01	1.11±0.01^{abc}	0.85±0.01^{abcd}
	CAT	2.36±0.01	2.31±0.01	2.29±0.01	1.62±0.01^{abc}	1.11±0.01^{abcd}
	GPx	1.53±0.01	1.49±0.01	1.55±0.01	1.12±0.01^{abc}	0.68±0.01^{abcd}
Muscle	SOD	2.73±0.01	1.81±0.01^{a}	1.46±0.01^{ab}	1.17±0.01^{abc}	0.77±0.01^{abcd}
	CAT	2.16±0.01	1.48±0.01^{a}	1.13±0.01^{ab}	0.87±0.01^{abc}	0.66±0.01^{abcd}
	GPx	3.01±0.01	2.15±0.01^{a}	1.67±0.01^{ab}	1.26±0.01^{abc}	0.92±0.01^{abcd}

Data were expressed as mean ± SE calculated from five replicates. In comparison with tilapia fish collected from El-Kanater EL-khayria, "**a**" indicates a significant difference ($P \leq 0.05$); "**b**" indicates a significant difference ($P \leq 0.05$) from fish collected from Benha; "**c**" indicates a significant difference ($P \leq 0.05$) from fish collected from Zefta; and "**d**" indicates a significant difference ($P \leq 0.05$) from fish collected from Talkha.

Table 6. Data on the relative expression of the inflammatory genes in the most susceptible tissues to pollutant accumulation in tilapia fish (*Oreochromis niloticus*) gathered from various points in the water of the Damietta Branch

		E. EL-Khayria	Benha	Zefta	Talkha	El-Serw
Liver	IL-6	3.29±0.02	3.90±0.03^{a}	4.81±0.03^{ab}	5.67±0.03^{abc}	7.75±0.03^{abcd}
	IL-1β	4.12±0.02	5.74±0.03^{a}	6.88±0.03^{ab}	8.16±0.03^{abc}	9.87±0.03^{abcd}
Kidney	IL-6	3.03±0.02	3.11±0.03	2.97±0.03	5.73±0.03^{abc}	7.15±0.03^{abcd}
	IL-1β	3.78±0.02	3.67±0.03	3.74±0.03	5.15±0.03^{abc}	6.79±0.03^{abcd}
Muscle	IL-6	2.24±0.03	2.75±0.03^{a}	3.12±0.04^{ab}	3.75±0.04^{abc}	4.14±0.04^{abcd}
	IL-1β	3.11±0.02	3.55±0.02^{a}	4.05±0.02^{ab}	4.97±0.03^{abc}	6.09±0.03^{abcd}

Data were expressed as mean ± SE calculated from five replicates. In comparison with tilapia fish collected from El-Kanater EL-Khayria, "**a**" indicates a significant difference ($P \leq 0.05$); "**b**" indicates a significant difference ($P \leq 0.05$) from fish collected from Benha; "**c**" indicates a significant difference ($P \leq 0.05$) from fish collected from Zefta; and "**d**" indicates a significant difference ($P \leq 0.05$) from fish collected from Talkha.

CONCLUSION

The current study's results demonstrated that the liver and muscle of *O. niloticus* fish obtained from El-Serw had the greatest quantities of heavy metals. Increasing levels of inflammatory markers in the liver, kidney, and muscle of tilapia fish collected from El-Serw were linked to a substantial ($P \leq 0.05$) drop in antioxidant system measurements. Furthermore, the tissues of fish taken from El-Serw exhibited the lowest similarity percentages when compared to those from E. EL-Khayria, according to the electrophoretic protein patterns. Upon comparing the target tissues of *O. niloticus* fish obtained from El-Serw to those collected from other places, the molecular assays revealed a substantial ($P \leq 0.05$) change in the relative gene expressions of antioxidant enzymes and inflammatory markers.

REFERENCES

Abarshi, M.M.; Dantala, E.O. and 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-Tawwab, M.; Gamal, O. and Sherien, H. (2017). Effect of dietary active charcoal supplementation on growth performance, biochemical and antioxidant responses, and resistance of Nile tilapia, Oreochromis niloticus (L.) to environmental heavy metals exposure. Aquaculture, 479: 17-24.

Abou El-Gheit, E.N.; Abdo, M.H. and 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.

Aboulthana, W.M.; Ismael, M. and Farghaly, H.S. (2016). Assessment of mutagenicity induced by toxic factors affecting ovarian tissue in rats by electrophoresis and molecular dynamic modeling. Int. J. Curr. Pharm. Rev. Res., 7(6): 347-59.

Aebi, H. (1984). Catalase in vitro. Methods Enzymol., 105: 121-126. **Afzal, G.; Ahmad, H.I.; Hussain, R.; Jamal, A.; Kiran, S.; Hussain, T.; Saeed, S. and Nisa, M.U.** (2022). Bisphenol A induces histopathological, hematobiochemical alterations, oxidative stress, and genotoxicity in common carp (Cyprinus carpio L.). Oxid. Med. Cell. Longev., 2022(1): 5450421.

Al-Awadhi, R.; Abdelrazek, H.; Fouad, A.; El-Fahla, N. and Tawfik, N. (2024). Protective Role of Dietary Anthocyanidin Against Genotoxicity and Hepatotoxicity Influenced by Imidacloprid in the Nile Tilapia. Egypt. J. Aquatic Biol. Fish., 28(4): 2293-2322.

Alm-Eldeen, A.A.; Donia, T. and Alzahaby, S. (2018). Comparative study on the toxic effects of some heavy metals on the Nile Tilapia, Oreochromis niloticus, in the Middle Delta, Egypt. Environ. Sci. Pollut. Res., 25: 14636-14646.

Al-Yousuf, M.H.; El-Shahawi, M.S. and 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.

Authman, M. and 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.

Bancroft, J.D. and Gamble, M. (2008). Theory and practice of histological techniques, Elsevier health sciences.

Beutler, E.; Duron, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2): 248-254.

Briffa, J.; Sinagra, E. and Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon, 6(9): e04691.

Carvalho, R.H.; Ida, E.I.; Madruga, M.S.; Martı´nez, S.L.; Shimokomaki, M. and Este´vez, M. (2017). Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry meat. Food Chemistry, 215: 129-137.

Chen, P.; Bornhorst, J.; Diana Neely, M. and Avila, D.S. (2018). Mechanisms and Disease Pathogenesis Underlying Metal-Induced Oxidative Stress. Oxid. Med. Cell. Longev., 2018: 7612172.

Dalzochio, T.; Rodrigues, G.; Simões, L.A.; de Souza, M.; Petry, I.E.; Andriguetti, N.B.; Silva, G.J.H.; da Silva, L.B. and Gehlen, G. (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(10): 9485-9500.

Darwesh, O.M.; Moawad, H.; Barakat, O.S. and Abd El-Rahim, W.M., (2015). Bioremediation of textile reactive blue azo dye residues using nanobiotechnology approaches. Research Journal of Pharmaceutical Biological and Chemical Sciences, 6(1): 1202-1211.

Deabes, M.M.; Aboulthana, W.M.; Marzouk, E.M.A.; Mohamed, M.I. and Ahmed, K.A. (2021). Evaluation of Hepato- and Neuroprotective Effect of Chemical Constituents in Saussurea costus Extract against the Toxicity Induced by Chloropyrifos Ethyl in Rats. Egyptian Journal of Chemistry, 64(2): 631-647.

Delles, R.M.; Xiong, Y.L.; True, A.D.; Ao, T. and Dawson, K.A. (2014). Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity. Poultry Science, 93: 1561-1570.

Delles, R.M.; Xiong, Y.L.; True, A.D.; Ao, T. and Dawson, K.A. (2014). Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity. Poultry Science, 93: 1561-1570.

El-Batrawy, O.A.; El-Gammal, M.I.; Mohamadein, L.I.; Darwish, D.H. and El-Moselhy, K.M. (2018). Impact assessment of some heavy metals on tilapia fish,

Oreochromis niloticus, in Burullus Lake, Egypt. J. Basic Appl. Zool., 79(1): 1-12.

Eldourghamy, A.S.; Goher, M.E.; Hagag, Y.M. and Rizk, N.M.H. (2024). Assessment of the Water Quality in the Damietta Branch of the Nile River, Egypt. Egyptian Journal of Aquatic Biology & Fisheries, 28(3): 1129-1157.

Ellman, G.L. ; Courtney, K.D. ; Andres, V.J. and Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.

Elnabris, K.J.; Muzyed, S.K. and 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. and 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-Saadani, Z.; Mingqi, W.; He, Z.; Hamukwaya, S.L.; Abdel Wahed, M.S.M. and Abu Khatita, A. (2022). Environmental Geochemistry and Fractionation of Cadmium Metal in Surficial Bottom Sediments and Water of the Nile River, Egypt. Toxics, 10(5).

El-Shamarka, M.E.A.; Aboulthana, W.M.; Omar, N.I. and Mahfouz, M.M. (2024). Evaluation of the biological efficiency of Terminalia chebula fruit extract against neurochemical changes induced in brain of diabetic rats: an epigenetic study. Inflammopharmacology, 32: 1439-1460.

Engelmann, H. ; Novick, D. and Wallach, D. (1990). Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. J. Biol. Chem., 265: 1531-1563.

Ezzat, N.; Al-Hawary, I.; Elshafey, A.; Althobaiti, N. and Elbialy, Z. (2024). Effect of Seasonal Changes in Heavy Metals on the Histomorphology of the Liver and Gills of Nile Tilapia (*Oreochromis niloticus* L.) in Burullus Lake, Egypt. *Egyptian Journal of Veterinary Sciences*, 55(6): 1705-1716.

Faheem, M.; Jahan, N. and Lone, K. (2016). Histopathological effects of bisphenol-a on liver, kidneys and gills of Indian major carp, *Catla catla* (Hamilton, 1822). J. Anim. Plant Sci., 26: 514-522.

Fatima, M.; Usmani, N.; Firdaus, F.; Zafeer, M.F.; Ahmad, S.; Akhtar, K.; Dawar Husain, S.M.; Ahmad, M.H.; Anis, E. and Mobarak Hossain, M. (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. and 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.

Gobi, N.; Vaseeharan, B.; Chen, J.-C.; Rekha, R.; Vijayakumar, S.; Anjugam, M. and Iswarya, A. (2018). Dietary supplementation of probiotic Bacillus licheniformis Dahb1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against *Aeromonas hydrophila* in tilapia *Oreochromis mossambicus*. Fish Shellfish Immunol., 74: 501-508.

Gu, Z.; Jia, R.; He, Q.; Cao, L.; Du, J.; Jeney, G.; Xu, P. and Yin, G. (2020). Oxidative stress, ion concentration change and immune response in gills of common carp (Cyprinus carpio) under long-term exposure to bisphenol A. *Comp. Biochem. Physiol. Part - C: Toxicol. Pharmacol.*, 230: 108711.

Guillemin, N.P.; Jurie, C.; Renand, G.; Hocquette, J.F.; Micol, D.; Lepetit, J. and Picard, B. (2012). Different phenotypic and proteomic markers explain variability of beef tenderness across muscles. International Journal of Biology, 4: 26-38.

Hama Aziz, K.H.; Mustafa, F.S.; Omer, K.M.; Hama, S.; Hamarawf, R.F. and Rahman, K.O. (2023). Heavy metal pollution in the aquatic environment: efficient and low-cost removal approaches to eliminate their toxicity: a review. RSC Adv., 13(26): 17595-17610.

Haredi, A.M.M.; Mourad, M.; Tanekhy, M.; Wassif, E. and Abdel-Tawab, H.S. (2020). Lake Edku pollutants induced biochemical and histopathological alterations in muscle tissues of Nile Tilapia (*Oreochromis niloticus*). Toxicol. Environ. Health Sci., 12: 247-255.

Hechtenberg, S. and Beyersmann, D. (1991). Inhibition of sarcoplasmic reticulum Ca+2-ATPase activity by cadmium, lead and mercury. Enzyme, 45: 109-115.

Hoseinifar, S.H.; Fazelan, Z.; El-Haroun, E.; Yousefi, M.; Yazici, M.; Van Doan, H. and Paolucci, M. (2023). The Effects of Grapevine (*Vitis vinifera* L.) Leaf Extract on Growth Performance, Antioxidant Status, and Immunity of Zebrafish (*Danio rerio*). *Fishes*, 8(6): 326.

Hossain, Z.; Hossain, M.S.; Ema, N.S. and Omri, A. (2021). Heavy metal toxicity in Buriganga river alters the immunology of Nile tilapia (*Oreochromis niloticus* L). Heliyon, 7(11): e08285.

Islam, Md.S.; Ahmed, Md.K. and 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.

Jamil Emon, F.; Rohani, M.F.; Sumaiya, N.; Tuj Jannat, M.F.; Akter, Y.; Shahjahan, M.; Abdul Kari, Z.; Tahiluddin, A.B. and Goh, K.W. (2023). Bioaccumulation and Bioremediation of Heavy Metals in Fishes-A Review. Toxics, 11(6):510.

Jantawongsri, K.; Nørregaard, R.D.; Bach, L.; Dietz, R.; Sonne, C.; Jørgensen, K.; Lierhagen, S.; Ciesielski, T.M.; Jenssen, B.M.; Haddy, J.; Eriksen, R. and Nowak, B. (2021). Histopathological effects of short-term aqueous exposure to environmentally relevant concentration oflead (Pb) in shorthorn sculpin (*Myoxocephalus scorpius*) under laboratory conditions. Environmental Science and Pollution Research, 28(43): 61423-61440.

Jantawongsri, K.; Nørregaard, R.D.; Bach, L.; Dietz, R.; Sonne, C.; Jørgensen, K.; Lierhagen, S.; Ciesielski, T.M.; Jenssen, B.M.; Haddy, J.; Eriksen, R. and Nowak, B. (2021). Histopathological effects of short-term aqueous exposure to environmentally relevant concentration of lead (Pb) in shorthorn sculpin (*Myoxocephalus scorpius*) under laboratory conditions. Environmental Science and Pollution Research, 28(43): 61423-61440.

Jiang, C.; Yang, X.; Xu, H.; Mao, M.; Chen, B.; Yang, Z.; Guan, Y. and Wang, H. (2021). Molecular characterization and expression profiles of two interleukin genes IL-8 and IL-10 in Pacific cod (*Gadus macrocephalus*). Aquaculture Reports, 21: 100788.

Jiang, L. and Li, J. (2022). lncRNA GMDS-AS1 upregulates IL-6, TNF-α and IL-1β, and induces apoptosis in human monocytic THP-1 cells via miR-96-5p/caspase 2 signaling. Mol. Med. Rep., 25(2): 1-9.

Jiang, Z.; Xu, N.; Liu, B.; Zhou, L.; Wang, J.; Wang, C.; Dai, B. and Xiong, W. (2018). Metal concentrations and risk assessment in water, sediment and economic fish species with various habitat preferences and trophic guilds from Lake Caizi, Southeast China. Ecotoxicol. Environ. Saf., 157: 1-8.

Kadry, S.M.; Tayel, S.I.; Afify, M.F.H. and 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.

Karami, A.; Romano, N.; Galloway, T. and Hamzah, H. (2016). Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*). Environ. Res., 151: 58-70.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature, 227(5259): 680-685.

Levine, R.L. ; Williams, J.A. ; Stadtman, E.R. and Shacter, E. (1994). Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol., 233: 346-357.

Liu, F.; Li, X.; Bello, B.K.; Zhang, T.; Yang, H.; Wang, K. and Dong, J. (2022). Difenoconazole causes spleen tissue damage and immune dysfunction of carp through oxidative stress and apoptosis. Ecotoxicology and Environmental Safety, 237: 113563.

Livak, K.J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods, 25: 402-408.

Mahboob, S.; Al-Ghanim, K.A.; Al-Balawi, H.F.; Al-Misned, F. and 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, M.M.A.; El-Lamie, M.M.M.; Kilany, O.E. and Dessouki, A.A. (2018). Spirulina (*Arthrospira platensis*) supplementation improves growth performance, feed utilization, immune response, and relieves oxidative stress in Nile tilapia (*Oreochromis niloticus*) challenged with Pseudomonas fluorescens. Fish Shellfish Immunol., 72: 291-300.

Mahmoud, S.A. and 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.

March, C.J. ; Mosley, B. ; Larsen, A. ; Cerretti, D.P. ; Braedt, G. ; Price, V. ; Gillis, S. ; Henney, C.S. ; Kronheim, S.R. ; Grabstein, K. ; Conlon, P.J. ; Hopp, T.P. and Cosman, D. (1985). Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. Nature, 315: 641-647.

Milla, S.; Depiereux, S. and Kestemont, P. (2011). The effects of estrogenic and androgenic endocrine disruptors on the immune system of fish: a review. Ecotoxicol., 20: 305-319.

Mohamed, M.F.; Ahmed, N.M.; Fathy, Y.M. and Abdelhamid, I.A. (2020). Impact of heavy metals on *Oreochromis niloticus* fish and using electrophoresis as bio-indicator for environmental pollution of rosetta branch, river Nile, Egypt. Eur. Chem. Bull., 9(2): 48-61.

Morshdy, A.E.M.A.; Darwish, W.S.; Hussein, M.A.M.; Mohamed, M.A.A. and Hussein, M.M.A. (2021). Lead and cadmium content in Nile tilapia (*Oreochromis niloticus*) from Egypt: A study for their molecular biomarkers. Sci. Afr., 12: e00794.

Nishikimi, M.; Rao, N.A. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem. Biophys. Res. Comm., 46: 849-864.

Ohkawa, H. , Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Paglia D.E. and Valentine, W.N. (1967). Studies on the Quantitive and Qualitative Charecterization of Erythrocyte Glutathione Peroxidase. The Journal of Laboratory and Clinical Medicine, 70(1): 158-163.

Pal, S. and Reddy, P. B. (2018). Bisphenol A (BPA) induced histopathological and biochemical alterations in the liver and kidney of stinging cat fish *Heteropneustes fossilis*. Trends Fish Res., 7(1): 67-73.

Panda, B.P.; Mohanta, Y.K.; Parida, S.P.; Pradhan, A.; Mohanta, T.K.; Patowary, K.; Wan Mahari, W.A.; Lam, S.S.; Ghfar, A.A.; Guerriero, G.; Verma, M. and Sarma, H. (2023). Metal pollution in freshwater fish: A key indicator of contamination and carcinogenic risk to public health. Environ. Pollut., 330: 121796.

Paris-Palacios, S.; Biagianti-Risbourg, S. and Vernet, G. (2000). Biochemical and (ultra) structural hepatic perturbation of Brachydaniorerio (Teleostei,

Cyprinidae) exposed to two sublethal concentrations of copper sulphate. Aquat. Toxicol., 50: 109-124.

Qin, T. ; Chen, J. ; Wang, D. ; Hu, Y. ; Zhang, J. ; Wang, M. ; Qiu, S. ; Gao, Z. ; Yu, Y. and Huang, Y. (2013). Selenylation modification can enhance immune-enhancing activity of Chinese angelica polysaccharide. Carbohydrate polymers, 95: 183-187.

Reyad, Y.A.; Elbahnaswy, S. and Zaki, V.H. (2021). Bioaccumulation of some heavy metals in water and tissues of naturally infected Oreochromis niloticus from two polluted sites in Egypt, with reference to related oxidative stress. Egyptian Journal of Aquatic Biology & Fisheries, 25(4): 139-157.

Rumahlatu, D.; Duran-Corebima, A.; Amin, M. and Rohman, F. (2019). Effect of cadmium on theconcentration and expression of TNF-α protein in sea urchin Diadema setosum (Leske, 1778). Hidrobiológica: [revista del Departamento de Hidrobiología], 29: 181-188.

Saad, S.M.M.; El-Deeb, A.E.; Tayel, S.I. and 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.

Shovon, M.N.H.; Majumdar, B.C. and 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.

Smorodinskaya, S.; Kochetkov, N.; Gavrilin, K.; Nikiforov-Nikishin, D.; Reznikova, D.; Vatlin, A.; Klimuk, A.; Odorskaya, M.; Nikiforov-Nikishin, A.; Ponomarev, A.; Marsova, M. and Danilenko, V. (2023). The Effects of Acute Bisphenol A Toxicity on the Hematological Parameters, Hematopoiesis, and Kidney Histology of Zebrafish (*Danio rerio*). Animals, 13(23): 3685.

Soliman, H.A.M.; Salaah, S.M.; Hamed, M. and Sayed, A.E.D. (2023). Toxicity of co-exposure of microplastics and lead in African catfish (*Clarias gariepinus*). Front. Vet. Sci., 10: 1279382.

Tayel, S.I.; Ahmed, N.A.M. and 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. and 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.

Terevinto, A.; Ramos, A.; Castroman, G.; Cabrera, M.C. and, Saadoun, A. (2010). Oxidative status, in vitro iron-induced lipid oxidation and superoxide dismutase, catalase and glutathione peroxidase activities in rhea meat. Meat science, 84: 706-710.

Valavanidis, A.; Vlahogianni, T.; Dassenakis, M. and Scoullos, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to

toxic environmental pollutants. Ecotoxicology and environmental safety, 64: 178-189.

Waris, S.; Hayat, S.; Mazhar, M. and Mahmood, S. (2023). Hematological and histopathological effects of xenoestrogen (Bisphenol A) on liver and kidney functions of *Mozambique tilapia* fish (*Oreochromis mossambicus*). Intl. J. Appl. Exp. Biol., 2: 79-87.

Xu, M.; Chen, X.; Huang, Z.; Chen, D.; Li, M.; He, J.; Chen, H.; Zheng, P.; Yu, J.; Luo, Y. and Yu, B. (2022). Effects of dietary grape seed proanthocyanidin extract supplementation on meat quality, muscle fiber characteristics and antioxidant capacity of finishing pigs. *Food Chem.*, 367: 130781.

Yacoub, A.M.; Mahmoud, S.A. and 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.

Yang, H.; Li, Y.; Wang, G.; Xie, J.; Kaneko, G. and Yu, E. (2023). Dietary grape seed proanthocyanidin extract improved the chemical composition, antioxidant capacity, myofiber growth and flesh quality of Nile tilapia muscle. *Aquac. Rep.*, 33: 101878.

Yano, C.L. and Marcondes, M.C.C.G. (2005). Cadmium chloride induced oxidative stress in skeletal muscle cells *in vitro*. Free Radicals Biol. Med., 39: 1378-1384.