

Effect of heavy metals from three types of water on biochemicals and antioxidants parameters in Nile tilapia as bio- indicators of pollution in fish farms

Abstract

Background: This experiment was conducted to study the effect of water sources on farmed Nile tilapia (*Oreochromis niloticus*). The physicochemical properties of three selected water sources were investigated. The effects of water quality changes on biochemical indicators and antioxidant levels in the fish living in these three water sources were also examined.

Materials and methods: The first group of experimental ponds was supplied with Nile River water from the Ismailia Canal, the second group with groundwater from a reservoir on the farm, and the third group with a mixture of groundwater and Ismailia Canal water. The experiment lasted for two consecutive seasons in 2025, spring and summer.

Results: The results showed significant changes in water quality in the three studied sources, but to varying degrees. Fish collected from the three areas also exhibited changes in biochemical indicators and antioxidant levels.

Conclusions: The results showed differences in water quality. All three water sources are suitable for fish farming, but to varying degrees.

Keywords: Nile tilapia, biochemical, antioxidants, Nile River, types of water.

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Introduction

Water serves as the foundation of life for all aquatic organisms, playing a crucial role in their survival, growth, and reproduction. The physical and chemical characteristics of water directly influence the health and biological functions of fish and other marine species.¹ Factors such as temperature, pH levels, dissolved oxygen, and the presence of pollutants determine whether an aquatic environment can support thriving marine life or pose challenges to their survival. Fish, like all aquatic creatures, rely entirely on their surrounding water for essential physiological functions. These include respiration, waste excretion, nutrient absorption, osmoregulation (maintaining salt and water balance), and reproduction. Since fish are fully immersed in their environment, any fluctuation in water quality can have immediate and significant effects on their overall well-being.² As a result, the success of any aquaculture system is largely dictated by the quality of the water in which fish are raised. When water quality deteriorates, it disrupts fish growth and negatively impacts production rates.³ Contaminants such as excessive ammonia, heavy metals, pesticides, and organic waste can impair fish metabolism, stunt their growth, interfere with reproductive cycles, or, in severe cases, lead to mass mortality events within aquaculture systems. Given these risks, fish farmers must actively manage and regulate water quality to ensure an environment that minimizes stress and supports healthy development. By maintaining optimal physical, chemical, and biological conditions, fish farmers can promote stronger immune responses in fish, improve their growth rates, and enhance overall productivity.⁴ Water quality in aquaculture is a dynamic factor that undergoes continuous changes due to both biological and environmental influences. The aquatic environment in fish farms, rivers, and ponds is affected daily by the metabolic activities of fish, the decomposition of organic matter, and the chemical and physical interactions occurring within the water system.⁵ These fluctuations make water quality management a complex challenge, requiring constant monitoring to ensure optimal conditions

for fish growth and survival. One of the most pressing concerns in aquaculture today is the contamination of aquatic environments with heavy metals. This issue has gained significant global attention due to the ability of fish to bioaccumulate toxic metals in their tissues, which can pose risks to both fish health and human consumers.⁶

While certain minerals, such as zinc and iron, are essential micronutrients required in trace amounts for normal physiological functions, such as cadmium, lead, and mercury can be highly toxic even in minute concentrations. These harmful metals can enter water sources through industrial discharge, agricultural runoff, and natural geological processes, ultimately disrupting aquatic ecosystems and affecting fish populations. There is an ongoing debate among researchers regarding the extent to which long-term exposure to water pollutants contributes to pathological changes in fish. Some studies suggest a direct correlation between heavy metal contamination and severe morphological, histological, and biochemical alterations in fish tissues, potentially leading to compromised immune systems, developmental abnormalities, and reduced reproductive success.⁷ Such changes not only impact the health and productivity of aquaculture species but also raise concerns about food safety and the potential transmission of toxic substances to humans through fish consumption. Seasonal fluctuations of biological modifications include biological factors such as fish proliferation and metabolism, environmental norms such as access to food, dissolved oxygen, water temperature, salinity and photoperiod, so organisms show differential antioxidant responses against environmental stresses.⁸ The direct relationship between circulation and surrounding environmental fluctuations and the availability of blood in fish are important biomarkers.⁹ Fish's response to environmental stresses can be monitored through a set of oxidative stress biomarkers.¹⁰ The current study aims to assess the impact of seasonal changes on biochemical parameters and antioxidants in Nile tilapia (*Oreochromis niloticus*) in three different water environments in Egypt.

Materials and methods

Study area

This study was conducted on three fish ponds with different water sources, which were supplied with three types of water: fresh water, groundwater, and mixed water in a fish farm in Al-Abbasa Al-Sharqiya. Water and fish samples were collected from the three waters (freshwater, groundwater, and mixed water) in the period from March 2025 to August 2025 during the spring and summer of 2025. Figure 1 shows the map of the farm located in Sharkia Governorate.



Figure 1 Map showing the location of the fish farm and showing the sampling locations of the study area in Al-Abbassa.

Determination of water quality parameters

To assess water quality, a systematic approach was employed to measure various physical and chemical parameters. These measurements were conducted using precise scientific instruments and standardized methods to ensure accuracy and reliability.

Regular monitoring of key water quality parameters

Water temperature, dissolved oxygen (DO), and pH levels were monitored on a weekly basis at two specific times: **6 a.m. and 12 noons**. These parameters were measured using specialized instruments, including:

- **Thermometer** for recording temperature variations.
- **Dissolved Oxygen Meter (YSI Model 57)** for assessing oxygen levels in the water.
- **pH Meter (Corning Model 345)** to determine the acidity or alkalinity of the water.

These three fundamental water quality indicators are essential in aquaculture, as they influence fish metabolism, growth rates, and overall health. Fluctuations in temperature can stress aquatic organisms, while inadequate oxygen levels may lead to suffocation, and extreme pH levels can disrupt biological functions.

Collection and preservation of water samples

To ensure a comprehensive analysis, additional water quality parameters were assessed biweekly. Water samples were collected from different water basins at a **depth of 1.75 meters** using a **one-liter PVC bottle**. These samples were then transferred into **one-liter polyethylene bottles** and immediately placed in an **ice box** to maintain their integrity until laboratory analysis. Proper storage was crucial in preventing any alterations in chemical composition before testing.

Laboratory analysis of chemical parameters

Several key chemical properties were analyzed in the laboratory following standardized procedures established by.¹¹ These included:

- Total Water Hardness:** Determined using the **EDTA titration method** with **Eriochrome Black T** as the indicator. Water hardness, primarily caused by dissolved calcium and magnesium, affects fish osmoregulation and overall water chemistry.
- Total Alkalinity:** Measured through **titration with 0.02 N hydrochloric acid (HCl)** in the presence of a **phenolphthalein indicator**. Alkalinity plays a crucial role in buffering pH fluctuations, ensuring a stable environment for aquatic organisms.
- Total Ammonia (NH₃):** Quantified using AOAC-approved techniques. Ammonia levels are a critical factor in aquaculture, as excessive accumulation can be toxic to fish, leading to stress, reduced growth, and increased mortality.

Fish samples

As for Nile tilapia (*Oreochromis-Niloticus*), fish of approximately similar size were collected from the water every two weeks during the experiment period with an average weight of 50-80 grams from three different waters to filter out biochemical defense factors and oxidation.

Diagnostic kits

AST, ALT, urea, uric acid, glucose, serum creatinine and total protein, as well as GSH, SOD, CAT, and GST were measured by commercial diagnostic kits from Giza Egypt Company using a spectrophotometer.

Concentration heavy metals of water

To assess the presence and concentration of heavy metals in the aquatic environment, water samples were carefully collected, preserved, and analyzed using advanced laboratory techniques. These analyses are crucial in understanding the potential impact of heavy metal contamination on aquatic life and ensuring that water quality meets environmental safety standards.

Collection and transportation of samples

Water samples designated for heavy metal analysis were stored in **one-liter polyethylene bottles** and promptly transported to the laboratory under controlled conditions. To ensure the accuracy and reliability of the results, additional water samples were collected in **three separate glass containers** to measure specific heavy metals, namely:

- Iron (Fe)**
- Zinc (Zn)**
- Copper (Cu)**
- Lead (Pb)**
- Cadmium (Cd)**

These heavy metals were selected due to their potential to accumulate in aquatic ecosystems, posing risks to both fish and human health. Some metals, such as **iron and zinc**, are essential in trace amounts for biological functions, whereas **lead, cadmium, and excessive copper** can be highly toxic even at low concentrations.

Digestion and analytical procedure

To prepare the samples for analysis, the water underwent a **digestion process using nitric acid (HNO₃)**, following the standard methods outlined by.¹² The acid digestion step was necessary to break down organic and inorganic compounds, allowing for precise quantification of heavy metal concentrations.

Instrumental analysis using atomic absorption spectrophotometry

The concentrations of heavy metals in the water samples were determined using an **atomic absorption spectrophotometer (AAS), model PerkinElmer 3150**. This highly sensitive instrument enables the detection of trace metal elements by measuring their absorption of light at specific wavelengths. The results were expressed in **micrograms per liter (µg/L), based on dry weight**.

Concentration heavy metals in organs (muscles and gills)

To assess the accumulation of heavy metals in fish, a systematic approach was taken to collect, process, and analyze fish tissue samples. This evaluation is essential for understanding how pollutants from the aquatic environment affect fish health and the potential risks associated with human consumption.

Fish sample collection and preparation

Seven fish were **randomly selected from each pond** for heavy metal analysis. Once collected, the fish were thoroughly **washed with distilled water** to remove any external contaminants that could interfere with the accuracy of the results. To analyze the concentration of heavy metals within their tissues, **approximately 5 grams of wet tissue** was extracted from two key organs: Muscle tissue (representing edible parts commonly consumed by humans). Gills (which serve as primary contact points with waterborne contaminants).

Sample drying and digestion process

The collected tissue samples were subjected to a **drying process** to eliminate excess moisture. Once dried, the samples were **ignited** to remove organic matter before undergoing an **acid digestion process** using a combination of **concentrated nitric acid (HNO₃) and hydrochloric acid (HCl)**. This digestion method effectively breaks down tissue components, allowing for accurate measurement of heavy metal concentrations.

Heavy metal analysis using atomic absorption spectrophotometry

The concentrations of five heavy metals **iron (Fe), zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd)** were measured in both muscle and gill tissues using an **atomic absorption spectrophotometer (AAS)**. This highly precise instrument detects and quantifies trace metal elements by measuring their absorption of light at specific wavelengths.

Blood sampling and biochemical analysis

Blood sampling

At the end of the experiment, a blood sample was taken from the fish from the tail vein using a sterile heparin syringe. It was left without anticoagulant in the centrifuge tube to separate the serum by centrifuging at 3000 rpm for 7 minutes and stored at -15°C until analysis.

Biochemical analysis

To evaluate the physiological and metabolic health of fish, various biochemical parameters were analyzed using well-established laboratory methods. These analyses provide crucial insights into metabolic function, protein synthesis, kidney performance, and liver health, all of which are essential for assessing the overall well-being of aquatic organisms in aquaculture systems.

Serum glucose measurement

Blood glucose levels were assessed following the.¹³ Glucose is a key energy source, and its concentration in fish serum serves as an important indicator of metabolic activity and stress levels. Elevated or reduced glucose levels may reflect environmental stress, dietary imbalances, or disease conditions.

Total protein determination

Total protein concentration in serum was measured using the **Biuret test**; a widely accepted method established by.¹⁴ Protein levels are essential for evaluating liver function and overall nutritional status. Abnormal protein levels may indicate malnutrition, infections, or impaired liver function in fish.

Kidney function assessment

To assess renal function and nitrogenous waste metabolism, three key markers were measured using **colorimetric techniques**:

- I. Serum creatinine** was determined based on the method described by.¹⁵ Creatinine is a byproduct of muscle metabolism and serves as an indicator of kidney filtration efficiency.
- II. Serum urea** levels were measured following the method of.¹⁶ Urea is the primary waste product of protein metabolism, and elevated levels may suggest impaired kidney function or excessive protein breakdown.
- III. Serum uric acid** concentration was analyzed according to the technique outlined by.¹⁷ Uric acid accumulations can indicate metabolic disturbances, stress, or impaired excretion.

Liver enzyme analysis

Liver function was assessed by measuring the activity of two key enzymes:

- a. Aspartate aminotransferase (AST)**
- b. Alanine aminotransferase (ALT)**

These enzymes were evaluated using the method described by.¹⁸ AST and ALT play crucial roles in amino acid metabolism and are commonly used as biomarkers of liver damage. Elevated levels of these enzymes may signal hepatotoxicity, environmental stress, or exposure to harmful contaminants.

Oxidative stress biomarkers

Oxidative stress and antioxidant enzyme analysis in liver tissue

To assess the oxidative stress response and antioxidant defense mechanisms in fish, liver tissue samples were subjected to biochemical analysis. Various enzymatic and non-enzymatic antioxidant parameters were measured to evaluate cellular protection against oxidative damage. These biomarkers help determine the impact of environmental stressors, pollutants, and overall physiological health in aquaculture species.

Liver tissue sample preparation

Liver tissues were carefully homogenized using a **Potter–Elvehjem glass/Teflon homogenizer**, ensuring thorough cell disruption and extraction of intracellular components. The homogenized samples were then subjected to **centrifugation**, allowing for the separation of solid debris. The **supernatant** was carefully collected for biochemical estimation of key oxidative stress markers and antioxidant enzyme activity.

Assessment of antioxidant and detoxification enzymes

To evaluate the antioxidant defense capacity of fish liver, the following parameters were analyzed using established biochemical methods:

- a. **Reduced Glutathione (GSH):** The levels of GSH, a crucial intracellular antioxidant that protects cells from oxidative stress and detoxifies harmful compounds, were measured according to the method described by.¹⁹ GSH plays a pivotal role in maintaining cellular redox balance and neutralizing free radicals.
- b. **Superoxide Dismutase (SOD):** The activity of SOD, an essential enzyme that catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, was determined calorimetrically based on the protocol established.²⁰ Elevated SOD activity suggests an adaptive response to oxidative stress, while decreased levels may indicate impaired antioxidant defense.
- c. **Catalase (CAT) Enzyme Activity:** CAT is a vital enzyme responsible for decomposing hydrogen peroxide (H₂O₂) into water and oxygen, preventing cellular damage caused by oxidative stress. Its activity was measured following the method outlined by.¹¹ The proper function of CAT is crucial for maintaining cellular homeostasis and protecting tissues from oxidative injury.
- d. **Glutathione-S-Transferase (GST):** The activity of GST, an enzyme involved in detoxification and the conjugation of harmful xenobiotics with glutathione, was assessed using the method described by.²² GST is a key component of cellular defense mechanisms against toxic substances, including pollutants and heavy metals in aquatic environments.

Statistical analysis

Results are expressed as the mean ± SE. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to assess the significant differences between the concentrations of various water quality parameters and mineral levels in the tissues of the studied fish in different environments. Differences were considered significant at a significance level of (P < 0.05).

Results

Physico-Chemical parameters of water

In fish ponds, the physical and chemical properties of water vary depending on the specific conditions prevailing in these ponds. The results obtained in this study: Water temperature, total hardness, ammonia and pH were decreased significantly (P < 0.05) in spring compared to summer in water samples collected from all water types. Conversely, transparency was higher in spring compared to summer. Dissolved oxygen decreased significantly (P < 0.05) in water during the summer in ground and mixed water, and increased significantly (P < 0.05) in fresh water, and was more apparent in groundwater in both

seasons. Total alkalinity during the summer in fresh and groundwater and increased significantly (P < 0.05) in mixed water. These results are recorded in (Table 1). The differences between the two seasons were recorded for the chemical treatments shown in the Figures 2-8. These figures showed the differences between the three water types for the treatments in the spring and summer seasons. The difference was observed between the two seasons for each chemical parameter in the study.

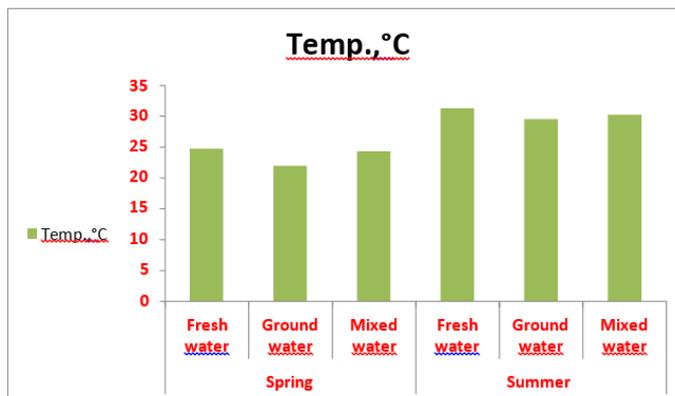


Figure 2 Seasonally variation of Temp., °C in the spring and summer seasons in the three types of water.

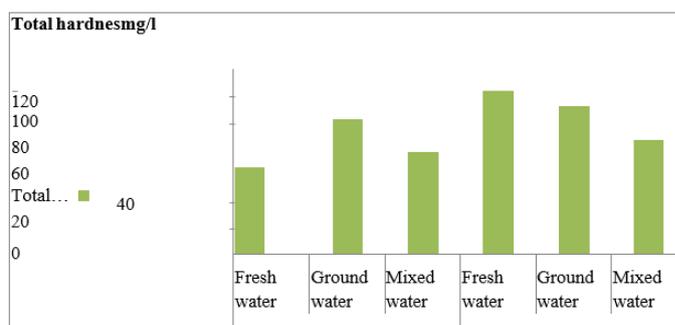


Figure 3 Seasonally variation of Total hardness in the spring and summer seasons in the three types of water.

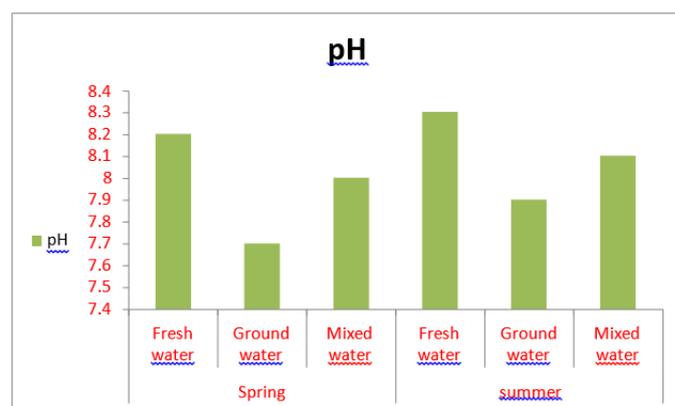


Figure 4 Seasonally variation of pH in the spring and summer seasons in the three types of water.

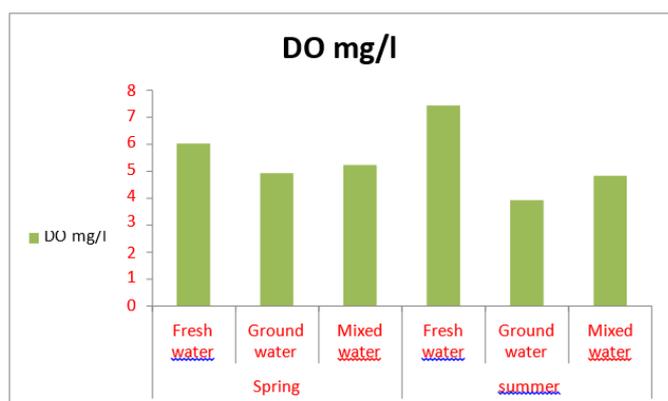


Figure 5 Seasonally variation of DO mg/l in the spring and summer seasons in the three types of water.

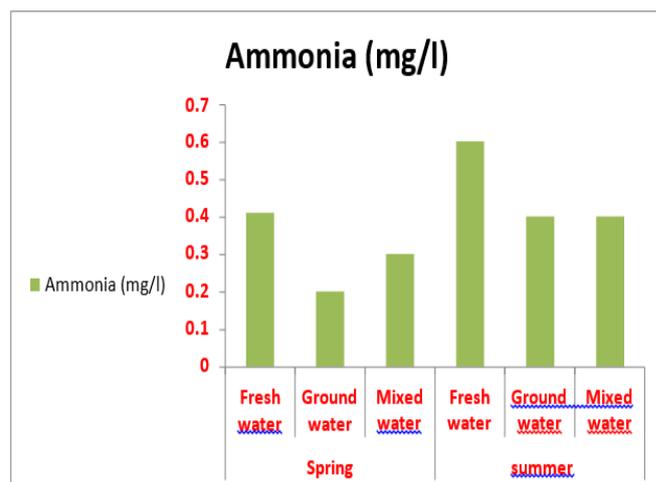


Figure 7 Seasonally variation of total Ammonia (mg/l) in the spring and summer seasons in the three types of water.

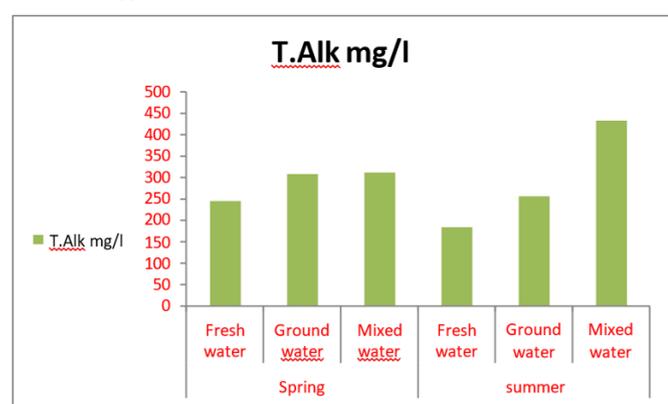


Figure 6 Seasonally variation of total alkalinity in the spring and summer seasons in the three types of water.

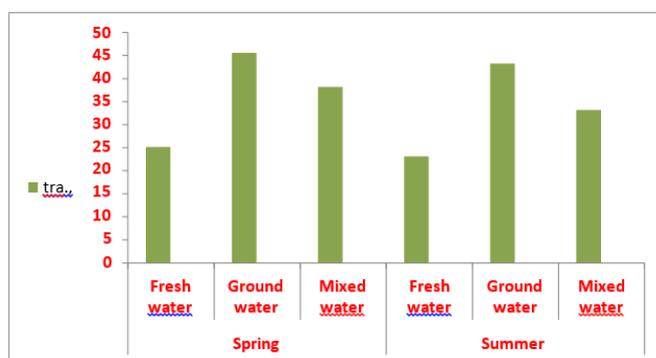


Figure 8 Seasonally variation of Transparency (cm) in the spring and summer seasons in the three types of water.

Table 1 Seasonal variation in samples of different water ponds (physical and chemical parameters) during the spring and summer seasons of 2025(mean ± SE)

Seasons parameters	Spring			Summer		
	Fresh water	Ground water	Mixed water	Fresh water	Ground water	Mixed water
Temp., °C	24.63±0.19	21.86±0.15 *	24.21±0.14	31.19±0.97	29.454±0.32	30.11±0.43
Total hardness mg/l	75.0±6.17 *	111.4±14.58	86.8 ± 7.22	133.0±4.17	121.4±8.58	95.8 ± 6.22
pH	8.2± 0.042	7.7 ± 0.052 *	8.0 ± 0.05	8.3± 0.032	7.9 ± 0.072	8.1 ± 0.08
DO mg/l	6.0 ± 0.260	4.9 ± 0.08	5.2 ± 0.26	7.4 ± 0.320	3.9 ± 0.08 *	4.8 ± 0.36
T.Alk mg/l	245.2 ± 3.1	308.2 ± 6.66	311.9 ± 3.46	184.2 ± 1.139*	256.2 ± 4.66	432.7 ± 3.16
Ammonia (mg/l)	0.41±0.004	0.2±0.004	0.3±0.014	0.6±0.004	0.4±0.004	0.4±0.004
Transparency	25.05±0.76	45.48±0.76 *	38.0 ± 3.72	23.04±0.76	43.18±0.66 *	33.1 ± 2.72

Values are presented as the mean ± standard error of samples. Values marked "Different" differ significantly (P<0.05). (P<0.05).

Heavy metals concentrations in water

The present study evaluated the concentration of five heavy metals (Fe, Zn, Cu, Cd and Pb) in the three selected waters. The results obtained showed that heavy metal concentrations in water are closely related to water quality and are detected in the following order: freshwater, groundwater and mixed water, the results showed decrease significantly (P< 0.05) iron, copper and lead in spring

compared to summer in water samples collected from all water types. Conversely, zinc was higher increase significantly (P < 0.05) in groundwater and mixed water in spring compared to summer, but it was higher increase significantly (P < 0.05) in fresh water in summer. As for cadmium, there were differences in the results in both seasons, as shown in (Table 2).

Table 2 Seasonal variation of heavy metal concentrations in samples of different water ponds during the spring and summer seasons of 2025 (mean ± standard error)

parameters	Spring			Summer		
	Fresh water	Ground water	Mixed water	Fresh water	Ground water	Mixed water
Fe	207.2±9.5*	571.0±23.8	465.0±13.5	255.64±53.0	751.7±45.87 *	641.7±36.3
Zn	72.42±6.7	124.0±4.8 *	106.4±2.9*	94.36±3.8*	77.4±2.72	68.4.4±3.9
Cu	23.86±3.3*	39.4±3.1	26.75±1.47	41.6±3.4	65.62±4.4	34.5±2.47
Cd	1.07±0.3	3.01±0.18	1.3.±0.06	2.2 ±0.07	3.6±0.21 *	2.83.±1.4
Pb	17.5±0.65 *	28.5±4.43	24.4±2.3	19.8 ±2.9	49.4±2.8	37.4±2.6

Values are presented as the mean ± standard error of samples. Values marked "Different" differ significantly (P<0.05). (P<0.05).

Averages and Averages value of heavy metals in types of water

The average seasonal concentrations and average values of iron and lead are higher significantly (P < 0.05) than the normal rate in the three types of water, while the other metals are below the permissible limit. Cadmium is the metal with the decrease significantly (P <

0.05) average concentration in water, while iron has the increase significantly (P < 0.05) concentration. The order of accumulation of heavy metals in water for the three types is: Fe > Zn > Cu > Pb > Cd (Table 3). The concentration of Fe in the water samples exceeded the permissible limits in ground and mixed water but less in fresh water. As for lead, it was in excess of the permissible limit for the three types prescribed.

Table 3 Averages and Averages value of heavy metals three different of water fish ponds during spring and summer seasons 2025

Average	Fe(µg/l)	Zn(µg/l)	Cu(µg/l)	Cd(µg/l)	Pb (µg/l)
Average spr.,	414.4	100.94	30	1.79*	23.4
Average sum.,	549.68 *	73.3*	47.2*	2.87	35.5*
Average value	482.04	87.12	38.6	2.3	29.45
EOS (1993)	300	5000	1000	10	10

Values are presented as the mean ± standard error of samples. Values marked "Different" differ significantly (P<0.05). (P<0.05).

Heavy metals in Fish muscle and gills

It showed the concentration of heavy metals (µg/g) in the muscles and gills collected from different types of water. There are significant differences (P < 0.05) in concentration of heavy metals in both

muscles and gills. The results for heavy metal concentrations in the muscles and gills of fish are given in Table 4. The concentration of heavy metals measured in fish organs is generally lower than the levels issued.

Table 4 values of heavy metals (µg/g) in muscles and gills of Nile tilapia reared in different water types. During spring and summer 2025 (mean ± standard error)

parameters	muscles			Gills			E.O.S.Q. C2005) (µg/g)
	Fresh water	Ground water	Mixed water	Fresh water	Ground water	Mixed water	
Fe	89.7±7.67	154.6±11.4*	76.32±7.45	164.8±22.7	347.7±34.8*	179.4±28.7	3000
Zn	64.8±3.5	87.1±4.6*	59.45±3.4	116.8±8.5	212.7±12.8*	165.1±9.8	4000
Cu	13.7± 1.6*	19.5± 1.4	16.75± 1.4	32.4± 6.2	37.8± 7.9	26.7± 5.6*	2000
Cd	1.18±0.12*	2.6±0.18	2.12±0.09	1.14±0.04	1.28±0.06	1.17±0.06	50
Pb	32.7±14.4*	52.3±17.7	47.4±15.4	94.7±22.4	128.4±27.7*	82.6±18.5	500

Values are presented as the mean ± standard error of samples. Values marked "Different" differ significantly (P<0.05).

Serum biochemical analysis

The results provided key information on the effect of heavy metals present in different water types on blood parameters in Nile tilapia. Differences were observed between the different water types in all

biochemical values. Levels of aminotransferase enzymes (AST and ALT), urea, uric acid, and blood glucose were significantly higher (P < 0.05) during the summer compared to the spring. In contrast, total protein and creatinine levels were not significant across the seasons and were recorded in all three water types (Table 5).

Table 5 Biochemical profile of Nile tilapia collected from three types of water fish farm (fresh, ground and mixed water) during spring and summer seasons 2025

Seasons parameters	Spring			Summer		
	Fresh water	Ground water	Mixed water	Fresh water	Ground water	Mixed water
AST(U/l)	64.4±3.24	112.4 ±2.31	73.6±3.33	119.33±7.21	127.2±4.52*	118.67±5.5
ALT (U/l)	241.5 ±2.45	227.00±1.00	236.67±2.60	248.1±6.58	251.37±4.8*	248.2 ±4.03
Creatinine(mg/dl)	0.43±0.01	0.75±0.04	0.69±0.08	0.38±0.01	0.69±0.07	0.68±0.02
Urea (mg/dl)	8.2±0.51	14.6±1.02	13.4±0.68	11.6±0.16*	21.9±1.63	20.4±0.38
Uric acid (mg/dl)	0.86±1.06	2.41±3.33	1.54±1.07	1.95±2.06*	2.78±0.17	2.86±0.06
Glucose (mg/dl)	56.9±3.4	132.6±7.33	144.3±4.03	73.9±5.58	176.7±8.18*	149.6±7.73
Total protein (g/dl)	5.6±2.53	4.4±0.11	3.8±1.13	5.1±0.13	3.8±1.05	2.4±1.14

Values are presented as the mean ± standard error of samples. Values marked "Different" differ significantly (P<0.05).

Oxidative stress biomarkers

In the current study, the effect of different types of water on biomarkers of oxidative stress was studied, which showed that the

levels of GSH, CAT, SOD, and GST in the liver were higher significant (P < 0.05) in three types of water during the summer compared to those collected during the spring, which are recorded in Table 6.

Table 6 Oxidative stress biomarkers in the liver tissue of Nile tilapia collected from three types of water fish farm (fresh, ground and mixed water) during spring and summer seasons 2025

Seasons parameters	Spring			Summer		
	Fresh water	Ground water	Mixed water	Fresh water	Ground water	Mixed water
GSH (nmol/g tissue)	14.20±0.4	19.13±1.5	15.21±1.10	16.39±0.72	24.3±1.42*	17.30±1.70
CAT (µg/mg tissue)	1.32±0.05	1.64±0.02	1.62±0.02	1.44±0.01	1.83±0.01	1.68±0.01
SOD (µg/mg tissue)	415.00±37.4	512.33±18.3	523.27±9.7	432.42±22.8*	536.32±14.3	516.60±13.46
GST (µg/mg tissue)	0.32±0.03	0.45±0.04	0.39±0.02	0.40±0.02	0.63±0.02*	0.53±0.07

Values are shown as mean ± standard error of triplicates.

Within the column values with different letters are significantly different (P<0.05).

Discussion

Water quality is routinely analyzed in fish farms to determine its suitability for biological processes. It is defined by various chemical and physical characteristics, along with the presence of organic and inorganic substances, all of which influence aquatic life.²³ Ensuring proper water quality is essential for sustaining fish health, growth, and productivity. Water temperature is one of the most critical factors in aquaculture, as it directly affects physiological processes such as growth rate, metabolic activity, reproduction, and disease resistance.²⁴ Similarly, the amount of dissolved oxygen plays a crucial role in fish survival, influencing respiration and overall biological functions. For most aquatic species, 30–35°C represents the upper tolerance limit.²⁵ Variations in water temperature were observed across different seasons and water sources. Spring samples showed lower temperatures, whereas freshwater samples exhibited higher temperatures compared to other aquatic environments (Table 1). The fluctuation in water temperature is influenced by multiple environmental factors, including seasonal changes, time of sampling, duration of sunlight exposure, turbidity levels, wind speed, moisture content, and vegetation density.²⁶ These factors interact to create varying thermal conditions in aquatic ecosystems, which in turn impact fish health, feeding behavior, and reproductive cycles. The concentration of hydrogen ions (pH) significantly influences fish biodiversity and overall aquatic ecosystem stability. The optimal pH range for fish production falls between 6.5 and 9.0, ensuring a balanced environment for physiological functions. However, extreme pH values can be detrimental. A pH of 4.0 is considered lethal, while values between 4.0 and 5.0 inhibit reproduction, and 4.0–6.5 results in slow growth rates. Similarly, a pH of 11 is regarded as the alkaline death threshold for aquatic organisms.²⁷ Seasonal variations affect pH levels, with

freshwater samples recording a pH of 8.3 in summer and groundwater showing a pH of 7.7 in spring. The higher pH values in freshwater during summer are attributed to increased photosynthetic activity, which consumes carbon dioxide (CO₂) and results in a more alkaline environment.²⁸ Water alkalinity refers to its capacity to neutralize acids and is primarily determined by the concentration of carbonates, bicarbonates, and hydroxides present in the water. It serves as a critical indicator of water stability, buffering against drastic pH changes. In this study, water alkalinity showed a significant increase during the summer across all three water samples, with the most pronounced rise observed in mixed water. The observed increase in alkalinity is attributed to the higher concentration of bicarbonate ions (HCO₃⁻), which result from the microbial decomposition of organic matter. As bacteria break down organic compounds, bicarbonate is formed as a byproduct, leading to increased alkalinity. Similar to alkalinity, water hardness—which measures the concentration of dissolved calcium and magnesium ions—also increased during the summer across all three water sources. This seasonal rise was most noticeable in mixed water. Since both alkalinity and hardness are closely linked to carbonate and bicarbonate content, their simultaneous increase suggests a direct relationship between microbial decomposition processes and seasonal water chemistry changes. Among the most widely used indicators in water chemistry, pH significantly influences the solubility and availability of essential nutrients for aquatic organisms. It plays a pivotal role in determining the biological productivity of water bodies, as certain pH ranges optimize the absorption of minerals and nutrients by fish and plants.²⁹ In this study, pH levels increased during both spring and summer across all three water types. Notably, all recorded values remained on the alkaline side, suggesting a water environment conducive to biological activity. The rise in pH is largely

attributed to increased vegetation density and phytoplankton growth, which enhance photosynthetic activity and drive carbon dioxide (CO₂) consumption.³⁰ Since CO₂ naturally forms carbonic acid in water, its depletion during photosynthesis leads to a shift toward alkalinity. Dissolved oxygen (DO) is one of the most critical factors for sustaining aquatic life. It supports the breakdown of organic materials, fuels biochemical processes, and facilitates respiration in fish and other aquatic organisms. Furthermore, DO levels influence the oxidation of organic matter in both water and sediment, impacting nutrient cycling and overall water quality.³¹ In this study, dissolved oxygen concentrations decreased during spring and summer in groundwater samples compared to fresh and mixed water. The lowest oxygen levels were recorded in groundwater during the summer, likely due to elevated water temperatures and increased oxidation of organic matter. Warmer water holds less dissolved oxygen, and higher temperatures accelerate organic decomposition, leading to further oxygen depletion. Ammonia (NH₃) is the most common nitrogenous waste product found in aquatic ecosystems. It originates from fish excretion, decaying organic matter, and microbial activity. Ammonia levels are heavily influenced by temperature and oxygen availability, with higher temperatures and lower oxygen levels exacerbating its accumulation. The current findings indicate that ammonia concentrations increased during spring and summer, particularly in freshwater samples compared to other water types. The highest ammonia levels were recorded in freshwater during the summer, a trend that can be attributed to organic matter accumulation, increased microbial decomposition, and subsequent oxygen depletion. As large quantities of organic material decompose, oxygen consumption rises, leading to the formation of higher ammonia concentrations.³² Heavy metal contaminations in aquatic ecosystems originates from both natural and anthropogenic sources. Naturally, heavy metals enter water bodies through atmospheric deposition, geological weathering, and sediment erosion. However, human activities such as agricultural runoff, industrial discharge, urban waste disposal, and wastewater treatment plants significantly contribute to metal accumulation in water systems. In this study, cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) concentrations increased during summer across all water samples, with the highest levels observed in groundwater (Table 2). This seasonal rise in heavy metal content aligns with findings from previous studies conducted in Abbassa.³³ And Abbassa.³⁴ All of which reported similar trends.

To assess bioaccumulation levels, concentrations of Fe, Zn, Cu, Cd, and Pb were measured in fish muscles and gills. The results indicated that iron (Fe) exhibited the highest concentration, followed by zinc (Zn), lead (Pb), copper (Cu), and cadmium (Cd). Notably, iron was detected in significantly higher concentrations in the gills compared to fish collected from all water sources. Despite elevated zinc and copper levels in fish tissues, their concentrations remained lower than those detected in the water samples, suggesting selective bioaccumulation mechanisms. Fish from groundwater-fed ponds exhibited the highest heavy metal concentrations, whereas fish from freshwater ponds showed the lowest levels. This pattern highlights the impact of water source variability on metal accumulation in aquatic organisms. The gills play a fundamental role in the absorption of metals from water due to their direct exposure to the aquatic environment. As the primary site for respiration and ion exchange, gills contain thin epithelial tissues, making them highly susceptible to metal accumulation.³⁵ These findings support previous research indicating that gills act as a primary target for metal deposition conversely; muscle tissues exhibited the lowest metal concentrations, consistent with previous studies demonstrating the limited ability of fish muscles to accumulate heavy metals.³⁶ Since muscles primarily function in

locomotion rather than filtration or metabolic detoxification, they serve as a relatively poor reservoir for heavy metal retention. Despite seasonal fluctuations, heavy metal concentrations in both fish gills and muscle tissues remained below permissible limits established by,³⁷ indicating that the fish were still within safe consumption thresholds. Biochemical analysis serves as an essential tool for assessing the physiological impact of pollution on aquatic organisms. Such evaluations help determine target organs affected by toxic exposure and provide early warning signals of environmental stress.³⁸ In the current study, serum glucose levels increased in fish from groundwater sources, particularly during spring and summer (Table 3). The rise was most evident in summer, suggesting a stress-induced metabolic response to environmental pollutants. Elevated glucose levels likely resulted from enhanced glycogen breakdown (glycogenolysis), increased glucose synthesis (gluconeogenesis), and inhibition of glucose metabolism. These physiological adjustments reflect an energy-demanding response to counteract environmental stressors.³⁹ Total protein levels are frequently used in disease diagnosis and liver function assessment, given that most plasma proteins are synthesized in the liver.⁴⁰ In this study, total blood protein levels were lower in fish from mixed water samples during summer and spring (Table 5). The most significant reduction occurred in summer, aligning with previous findings by.⁴¹ The observed decrease in protein levels may indicate hepatic dysfunction, possibly due to disruptions in protein metabolism and synthesis. Such disturbances can be triggered by chronic exposure to heavy metals, pollutants, or impaired nutrient absorption. The kidneys play a pivotal role in maintaining fluid balance, regulating electrolytes, and ensuring homeostasis in fish. Any physiological disruption caused by heavy metal exposure or environmental stress can impair renal function, leading to metabolic imbalances,⁴² In this study, biochemical parameters pointed toward potential alterations in kidney activity, emphasizing the need for continuous monitoring of aquatic pollution and its physiological effects on fish health. The physiological state of fish can be assessed by analyzing key biochemical markers, particularly those related to kidney and liver function. These biomarkers provide insight into the impact of environmental stressors, such as heavy metal contamination and oxidative damage, on aquatic organisms. Serum urea and uric acid concentrations were found to be higher during spring and summer in fish from mixed and groundwater sources, with the highest levels observed in summer. Similarly, creatinine levels were elevated in groundwater during spring, indicating potential renal dysfunction. These findings are consistent with previous studies by.⁴³ Elevated creatinine and urea levels in blood are widely recognized as indicators of kidney impairment, often caused by excessive production of reactive oxygen species (ROS) and oxidative damage to renal.⁴⁴ Kidney dysfunction in fish is commonly linked to pollutant exposure, which disrupts normal filtration processes and leads to the accumulation of metabolic waste in the bloodstream. Transaminases, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are key enzymes involved in protein metabolism and act as intermediaries between carbohydrate and amino acid metabolism. These enzymes facilitate the mobilization of L-amino acids for gluconeogenesis, making them essential under both physiological and pathological conditions.⁴⁵ In this study, AST and ALT levels increased during summer across all water types, with the highest elevations recorded in groundwater samples. These results align with findings from.⁴⁶ Increased AST and ALT activity in serum is a common biomarker of liver dysfunction, as damaged liver cells release these enzymes into circulation. The rise in transaminase levels suggests hepatic insufficiency, which may be caused by toxic exposure, oxidative damage, or cellular degradation.⁴⁷ One of the major toxicological mechanisms affecting aquatic organisms is oxidative stress, which

occurs when there is an imbalance between ROS production and the antioxidant defense system. When the body's natural antioxidants are overwhelmed, excessive ROS accumulation leads to cell damage, lipid peroxidation, and disruption of physiological functions.⁴⁸ A primary consequence of oxidative stress is lipid oxidation, where free radicals interact with lipids, resulting in cellular injury. This process is a hallmark of free radical-induced toxicity and is particularly concerning in aquatic environments contaminated with heavy metals.⁴⁹ Superoxide dismutase (SOD) and catalase (CAT) serve as the first line of defense against oxidative damage. These enzymes neutralize reactive oxygen species, preventing oxidative stress-related cellular damage. However, their activity varies based on the extent of metal exposure and the antioxidant system's response.⁵⁰ In this study, SOD and CAT enzyme activity increased during summer in freshwater and groundwater samples, while higher activity was recorded in mixed water samples during spring (Table 6). This suggests that fish exposed to groundwater pollutants—particularly heavy metals—activate their antioxidant defenses to counteract oxidative stress.⁵¹ Glutathione-S-transferase (GST) plays a crucial role in detoxification, facilitating the elimination of harmful xenobiotic compounds. This enzyme catalyzes the conjugation of xenobiotics with glutathione (GSH), enhancing their water solubility and excretion.⁵² In the present study, hepatic GST levels increased significantly during summer across all aquatic environments. This elevation may indicate an adaptive response to environmental pollutants, particularly heavy metal exposure. High levels of GSH in fish tissues serve as a protective mechanism, enabling fish to counteract oxidative stress and enhance detoxification. These findings are consistent with prior⁵³ which identified a direct correlation between GSH elevation and antioxidant defense activation in fish exposed to metal contamination.

Conclusion

The present study investigated the effect of three types of water on fish farms by examining some chemical and physical properties and the concentration of various heavy metals in water and fish muscles collected from three fish farms with different water sources. Nile tilapia was used as a bioindicator of water pollution by detecting its accumulation in muscles. This work also studies the effect of different types of water on fish biochemistry by detecting glucose, liver and kidney functions, and the activity of some blood enzymes. All fish samples were used as bioindicators of water pollution. The results confirm that the three types are suitable for fish farming, but to varying degrees.

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Conflicts of interest

We declare that there is no conflict of interest of any kind.

References

1. Boyd CE. Guidelines for aquaculture effluent management at the farm-level. *Aquaculture*. 2003;226:101–112.
2. Alam A, Al-Hafedh YS. Diurnal dynamics of water quality parameters in an aquaculture system based on recirculating green water technology. *J Appl Sci Environ Manage*. 2006;10:19–21.
3. Iwama GK, Vijayan MM, Morgan JD. *The stress response in fish. Ichthyology, recent research advances*. Oxford and IBH Publishing Co, Pvt. Ltd, New Delhi. 2000.
4. Isyagi NA, Veverica KL, Asiimwe R, et al. *Manual for commercial pond production of the African catfish in Uganda, Kampala*. 2009;222.
5. Keppeler EC, Valenti PMCM, Pereira LV. Temporal variation in the water quality of ponds and effluent of grow-out ponds of Amazon River prawn *Macrobrachium amazonicum*. *Rev Peru Biol*. 2012;19:299–306.
6. Mansour SA, Sidky MM. Ecotoxicological studies: 3. Heavy metals contaminating water and fish from Fayoum Gov, Egypt. *Food Chemistry*. 2002;78:15–22.
7. Khattaby AA, Fayza E Abbas, Soltan M A, et al. Effect of using different water sources on the growth performance of mono sexed Nile tilapia (*Oreochromis niloticus*) reared in earthen ponds. *Abbassa Int. J. Aqua. Special Issue (The Third Scientific Conference Al Azhar University, Cairo*. 2010;129–142.
8. Buet A, Banas D, Vollaire Y, et al. Biomarker Responses in European Eel (*Anguilla anguilla*) Exposed to Persistent Organic Pollutants. A Field Study in the Vaccarès Lagoon (Camargue, France). *Chemosphere*. 2006;65(10):1846–1858.
9. Gaber HS, El-Kasheif MA, Ibrahim SA, et al. Effect of water pollution in El-Rahawy Drainage Canal on hematology and organs of freshwater fish *Clarias gariepinus*. *World Applied Sciences Journal*. 2003;21(3):329–341.
10. Doherty VF, Ogunkuade OO, Kanife UC. Biomarkers of oxidative stress as indicators of Environmental pollution in some selected fishes in Lagos, Nigeria. American– Eurasian. *Journal of Agriculture and Environmental Sciences*. 2010;7(3):359–365.
11. AOAC. *Official Methods of Analysis of the Association of Official, Analytical Chemists*. 15th ed. Association of Official Analytical Chemists, Arlington, VA. 1990.
12. APHA, AD Eaton. *American Public Health Association Mary Ann. H. Franson, American Water work Association (Ed.), Standard methods for the examination of water and wastewater*. 2005.
13. Trinder P. Determination of blood glucose using 4- aminophenazone. *Journal of Clinical Pathology*. 1959;22:246.
14. Gornal AG, Bardawil CJ, Dais MM. *The Journal of Biological Chemistry*. 1949;177:751.
15. Fabiny DL, Eriinghausen G. Automated reaction-rate method for determination of serum creatinine with the CentrifChem. *The Chem Clinic*. 1971;17:696.
16. Henry JB, Todd JC, Sanford AH, et al. *Clinical Diagnosis and Measurement by laboratory Methods*. 16th Ed, W.B. Saunders and Co, Phila-Delphia. 1974;260.
17. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analytst*. 1972;97:142–145.
18. Reitman S, Frankel S. A calorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*. 1957;28:56–63.
19. Beutler E, Duron O, Kelly MB. Improved method for the determination of blood GSH. *Journal of Laboratory and Clinical Medicine*. 1963;61:883–887.
20. Nishikimi M, Roa NA, Yogi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*. 1972;46:849–884.
21. Aebi H. Catalase in vitro. *Methods in Enzymology*. 1984;105:121–126.
22. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*. 1974;249:7130–7139.
23. Mapfumo E, Willms W, Chanasyk D. Water Quality of Surface Runoff from Grazed Fescue Grassland Watershed in Alberta. *Water Quality Research Journal Canada*. 2002;37:543–562.

24. Moustafa MM, Ali MHH, Abdel-Satar AM, et al. Water quality assessment of Rosetta and Damietta branches, River Nile, Egypt. *African J Biol Sci.* 2010;6(2):127–142.
25. Abdo MH, Sabae SZ, Haroon BM, et al. Physico-chemical characteristics, microbial assessment and antibiotic susceptibility of pathogenic bacteria of Ismailia Canal water, River Nile, Egypt. *J of Amer Sci.* 2010;6(5):234–250.
26. Hala Elshahat Ghannam, Mohamed YM Aly, et al. Biochemical and Histopathological Alterations Induced by Sublethal Concentrations of Silver Nanoparticles and Silver Nitrate in the Nile tilapia (*Oreochromis niloticus*). Egypt. *J Aquat Biol & Fish.* 2025;29(3):1475–1494.
27. Ayaz Khan M, Yousafzai AM, Afshan N, et al. Physicochemical Parameters of water collected from River Panjkora, Khyber Pakhtunkhwa, Pakistan. *World J Fish and Marine Sci.* 2015;7(6):462–471.
28. Abdel-Satar AM. Water quality assessment of river Nile from Idfo to Cairo. *Egyptian J of Aquatic Research.* 2005;31:200–223.
29. Osman AGM, Kloas W. Water quality and heavy metal monitoring in water, sediment and tissues of the African catfish *Clarias gariepinus* (Burchell, 1822) from the river Nile, Egypt. *Journal of Environmental Protection.* 2010;1:389–400.
30. Sabae SZ, Mohamed FAS. Effect of environmental pollution on the health of Tilapia spp. from Lake Qarun. *Global Veterinaria.* 2015;14:304–328.
31. Tayel SI, Yacoub AM, Mahmoud SA. Histopathological and haematological responses to freshwater pollution in the Nile catfish *Clarias gariepinus*. *J Egypt Acad Soc Environ Develop.* 2008;9(4):43–60.
32. Saad SMM, El-Deeb AE, Tayel SI, et al. Haematological and histopathological studies on *Clarias gariepinus* in relation to water quality along Rossetta branch, River Nile, Egypt. *Journal of Experimental Biology (Zoology).* 2011;7(2):223–233.
33. Aly MYM. Comparison of heavy metals levels in muscles, liver and gills of three fish species collected from agricultural drainage water at El-Abbassa fish farm. Egypt. *J Aquat Biol & Fish.* 2016;20(3):103–112.
34. Ghannam EH, Aly YM. Influence of different water resources on physical, chemical and heavy metals levels of El-Abbassa Fish Farms, El-Sharqia Governorate, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries.* 2018;22(5):165–174.
35. Kotze PJ, HH du Preez, JHJ van Vuren. Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa. 12. Department of Zoology, Rand Afrikaans University, PO Box 524, Auckland Park 2006, South Africa. 2006.
36. Hatem H Abd-elrahman a, Mohamed YM Aly a, Mahmoud Rasly El-desouky b, et al. Protective Effects of Vitamin C Nanoparticles Supplemented Diet against Toxicity of Fe²⁺ and Mn²⁺ Mixture on Nile Tilapia; *Oreochromis niloticus*. *Journal of Water and Environment Technology.* 2024;22(5):232–240.
37. EOSQC. (Egyptian Organization for Standardization and Quality Control), *The permissible limits for fish.* 2005;1–889.
38. David M, Ramesh H, Patil V, et al. Sodium cyanide-induced modulations in the activities of some oxidative enzymes and metabolites in the fingerlings of *Cyprinus carpio* (L). *Toxicological and Environmental Chemistry.* 2010;92:1841–1849.
39. Yekeen TA, Fawole O. Toxic effects of endosulfan on haematological and biochemical indices of *Clarias gariepinus*. *African Journal of Biotechnology.* 2011;10:14090–14096.
40. Yang JL, Chen HC. Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry and erythrocyte morphology. *Chemosphere.* 2011;53:877–882.
41. El-Gaml SA, Khalil R, Hashish EA, et al. Protective effects of selenium and alpha-tocopherol against lead-induced hepatic and renal toxicity in *Oreochromis niloticus*. *Journal of Aquaculture Research and Development.* 2015;6:1–5.
42. Palaniappan PLRM, Krishnakumar N, Vadivelu M. Bioaccumulation of lead and the influence of chelating agents in *Catla catla* fingerlings. *Environmental Chemistry Letters.* 2009;7:51–54.
43. Sayed AH, Hamed HS. Induction of apoptosis and DNA damage by 4-nonylphenol in African catfish (*Clarias gariepinus*) and the antioxidant role of *Cydonia oblonga*. *Ecotoxicology and Environmental Safety.* 2017;139:97–101.
44. Upasani CD, Balaraman R. Protective effect of spirulina on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. *Phototherapy Research.* 2003;17:330–334.
45. Manjunatha B, Tirado J, Selvanayagam M. Sub lethal toxicity of potassium cyanide on Nile tilapia (*Oreochromis niloticus*): biochemical response. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2015;7(3):379–382.
46. Canli EG, Canli M. Low water conductivity increases the effects of copper on the serum parameters in fish *Oreochromis niloticus*. *Environmental Toxicology and Pharmacology.* 2015;39(2):606–613.
47. Mohamed FAS, Gad NS. Bioaccumulation, some blood biochemical changes and histological alterations in selected tissues of *Oreochromis niloticus* exposed to zinc and/ or cadmium. *American-European Journal of Agricultural and Environmental Sciences.* 2009;5:441–455.
48. Nishida Y. The chemical process of oxidative stress by copper (II) and iron (III) ions in several neurodegenerative disorders. *Monatshfte für Chemie – Chemical Monthly.* 2011;142:375–384.
49. El-Batrawy OA, El-Gammal MI, Mohamadein LI, et al. Impact assessment of some heavy metals on tilapia fish, *Oreochromis niloticus*, in Burullus Lake, Egypt. *The Journal of Basic and Applied Zoology.* 2018;79(1):13.
50. Atli G, Canli M. Response of antioxidant system of freshwater fish *Oreochromis niloticus* to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. *Ecotoxicology and Environmental Safety.* 2010;73:1884–1889.
51. Hamed HS. Ameliorative effects of Spirulina platensis on deltamethrin-induced biochemical alterations and oxidative stress in the African catfish; *Clarias gariepinus*. *Open Journal of Marine Science.* 2016;6:1–10.
52. Luo Y, Su Y, Lin RZ, et al. 2-Chlorophenol induced ROS generation in fish *Carassius auratus* based on the EPR method. *Chemosphere.* 2006;65(6):1064–1073.
53. Saglam D, Atli G, Dogan Z, et al. Response of the Antioxidant System of Freshwater Fish (*Oreochromis niloticus*) Exposed to Metals (Cd, Cu) in Differing Hardness. *Turkish Journal of Fisheries and Aquatic Sciences.* 2014;14:43–52.