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Impact of Bioaccumulation of Some Heavy Metals on Histopathological Biomarkers of *Mugil cephalus* Fish Samples in Relation to Water Quality and Sediment of Lake Qaroun, Egypt

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ABSTRACT

Lake Qaroun, one of Egypt's largest lakes, is a remnant of a much larger one; it is considered as a closed basin used as a general reservoir for agricultural wastewater drainage in El-Fayoum Province. Furthermore, the lake receives agricultural and partially treated sewage water from the El-Fayoum Governorate as well as fish farm drainage. The current study assessed the changes in physicochemical characteristics and heavy metal levels (Zn, Pb, Cd, and Cu) in water and sediment, as well as the bioaccumulation of these heavy metals in *Mugilcephalus* fish samples obtained from Lake Qaroun. Thus, information on histopathological changes in the gills, liver, kidney, end of the alimentary canal, and muscles was provided. This research was conducted in three different locations along Lake Qaroun (Eastern, southeastern, and western). This study confirms an increased heavy metal pollution in the eastern and southeastern sectors of Lake Qaroun, facing the drain outlet, as a result of the discharge of drainage water effluents without prior treatment, which directly affects water quality and fish health, and subsequently human health. The concentrations of Pb and Cd in the water exceeded the allowable limits. The gills are the target organ for Cd in *Mugil cephalus*, but Pb and Zn are preferentially collected in the kidneys, with the liver containing the highest concentrations of Cu ions. Histopathological changes with an evident damage were visible in the gills, liver, and kidneys of *Mugil cephalus* taken from the lake's eastern and southeastern areas. In addition, blood parameters of *Mugil cephalus* collected from various study sites throughout Lake Qaroun were included in this study. A highly significant decrease was detected in red blood cell counts, hemoglobin content, and hematocrit values of fish collected from the lake's eastern and southeastern parts in comparison to fish collected from the western sector, indicating more or less normal values.

INTRODUCTION

Environmental pollution has become a major global issue that is receiving a lot of attention since it poses a direct threat to human life. From a scientific standpoint, pollution is defined as any impairment of the suitability of the air, water, and soil for any of their beneficial uses, actual or potential, caused by man-made changes in the quality of these ecosystem components **(Zhao *et al*., 2015)**. Lake Qaroun is an important fishery habitat in Fayoum Governorate. Lake Qaroun, one of Egypt's great lakes, is a relic of a much larger one, "the historic Lake Moreis," and was originally a fresh water lake. It is a closed basin that serves as a general reservoir for agricultural wastewater drainage in the province of El-Fayoum **(Ghanem, 2011).** It collects polluted agricultural, household, and industrial water from El-Fayoum province through a system of two large drains (El-Bats and El-Wadi drains) and other small drains that transport yearly around 450 million cubic meters of wastewater to Lake Qaroun **(Fathi & Flower, 2005)**.

Lake Qaroun is important for the environment, being a wetland of an international importance for water birds in addition to supporting a large fishery **(Elwasify *et al*., 2021).** Throughout the twentieth century, the lake became increasingly environmentally unstable, and various publications have reported the lake's changing ecosystem **(Sabae & Mohamed, 2015)**.

Researchers have reported a gradual increase in Lake Qaroun's salinity over the last century as a result of its closed nature and extensive evaporation **(Fathi & Flower, 2005)**. Therefore, heavy metals, and pesticides carried by agricultural drainage water accumulate in lake components. Furthermore, toxic metals in water originating accidental spillage of chemical wastes, periodic precipitation contaminated with air-borne pollutants, discharge of industrial or sewage effluents, agricultural drainage, domestic wastewater, and petrol from fishery boots, all of which result in fish pollution

Heavy metal contamination of aquatic environments has recently become a major worry because it is extremely dangerous owing to its non-biodegradable nature, lengthy biological half-life, and ability to collect in numerous body organs of aquatic organisms **(Eldeeb *et al*., 2020, 2021; Alprol *et al.*, 2022; Hamad *et al*., 2024)**. They can also be concentrated along the food chain, producing their toxic effect. Thus, compared to the other types of aquatic pollution, heavy metals pollution is less visible but its effects on the ecosystem and humans can be intensive and very extensive **(Edem *et al.*, 2008).**

Fishes are frequently at the top of the aquatic food chain and may contain high concentrations of metals such as lead, cadmium, chromium, copper, mercury, zinc, and iron. These metals accumulate in different ways in fish organs, posing substantial health risks to human **(Mansour & Sidky, 2002)**. Subsequently, fish are an excellent bio-indicator of heavy metals and other organic contaminants **(Ahmad & Shuhaimi-Othman, 2010).**

Fishes represent an important element of the human diet and one of the primary protein sources for human, so it is not surprising that several research on metal pollution in various edible fish species have been conducted **(Türkmen *et al*., 2009)**. Based on their distinct life strategies, the monitored fish species (flathead mullet, *Mugilcephalus*) is a member of Family: Mugilidae, Order: Perciformes.

Members of the Family Mugilidae are euryhaline and tolerant to a wide range of salinities. Mullet (*Mugilcephalus*) is a perciform species that feeds primarily on zooplankton, benthic organisms, algae, diatoms, and plant detritus (**Cardona, 2000, 2006**).

Pollutant accumulation impairs the functioning of fish tissues. The microscopic evaluation of target tissues using histopathological characteristics is the endpoint in determining the risk of contaminants in the environment **(Fatima *et al.*, 2015)**. Histopathological alterations can be utilized as markers of the influence of various anthropogenic contaminants on organisms and as a measure of the overall health of the aquatic ecosystem **(Ahmed *et al.*, 2011)**. Pollutants' harmful effects can be seen in fish tissues before they cause changes in the fish's outward look and behavior. The exposure of fish lives in Lake Qaroun to different types of wastes (industrial, agricultural and sewage) has led to several pathological changes in different fish organs **(Tayel *et al.*, 2013)**. The following steps could be taken to fulfil the primary purpose of the current investigation:

1. Compare the water quality and amounts of several heavy metals (copper, zinc, lead, and cadmium) in water and sediment samples taken from the analyzed collection sites.
2. Determine the residual heavy metals (Cu, Zn, Pb, and Cd) in chosen important organs (gills, liver, kidney, end of alimentary canal, and muscles) of researched fish species taken from the three studied sites along Lake Qaroun.

3. Monitor the physiological status of the examined fish species and corroborate these findings with histological sections of *Mugilcephalus* gills, liver, and kidney collected from the three study sites along Lake Qaroun.
4. Cast light on the role that the competent authorities should play in regard to the situation of Lake Qaroun.

MATERIALS AND METHODS

1. Description of the studied ecosystem (Lake Qaroun)

Lake Qaroun is in Egypt's western desert, approximately 85 km south- west of Cairo. It is one of Egypt's most important and largest lakes. It is situated about 44 meters below the sea level in the lowest pit in El-Fayoum depression in the western desert, between 300° 34ˋ and 300° 49ˋ E longitudes and 290° 25ˋ and 290° 34ˋ N latitudes. The lake has an area of around226 km^2 (about 55000 feddans), with an average depth of 4m, with the deepest section being 8m to the northwest of El-Qarn Island. It is a closed basin that serves as a general reservoir for El-Fayoum Province's agricultural drainage water **(Ragab, 2017)**.

The lake receives yearly about one million cubic meter of drainage water through 12 drains, the majority of which are "El-Bats " and "El-Wadi "drains. Lake Qaroun is an economically significant lake due to its abundant fisheries resources. However, the action of agricultural and waste municipal drainage water released straight into the lake without prior treatment has caused significant changes in the lake's water quality **(Elghobashy *et al.*, 2001)**.

2. Description of the studied sites of collection

Water, sediment, and fish samples were taken from the following three sites along Lake Qaroun where drainage is discharged straight without any prior treatment (Fig.1).

Site (1): It is located in the lake's eastern quarter, at the mouth outflow of the El-Bats drainage canal.

Site (2): It is located in the southeastern section near the mouth discharge of the El-Wadi drainage canal.

Site (3): Western sector, it is an unpolluted area west of the lake, with no identified drainage water.

3. Sampling

Water, sediment, and fish samples were obtained from the researched collection locations for the following study.

3.1.Water sampling and analysis

Surface water samples were collected in plastic bottles at the sampling sites along Lake Qaroun, roughly 50cm below the surface water (washed with detergent, followed by deionized water, 2M nitric acid (Merck), then deionized water again, and lastly surface water). In the field, samples were filtered and acidified with 10% HNO_3 for preservation before being deposited in an ice bath and transported to the laboratory. The materials were filtered using a 0.45µm micropore membrane filter and stored at -20°C until analysis.

3.2. Physicochemical analysis of water

The water samples collected from different locations in the studied ecosystems were subjected to a number of physicochemical analyses as mentioned later.

- For on-site sampling, the pH was measured by means of a pocket-pH meter (Micro Checkit ® pH^+, Lovibond, England).
- Dissolved oxygen (mg/l) concentration was determined at the sampling site by means of an oxygen meter (model, YSI 58).

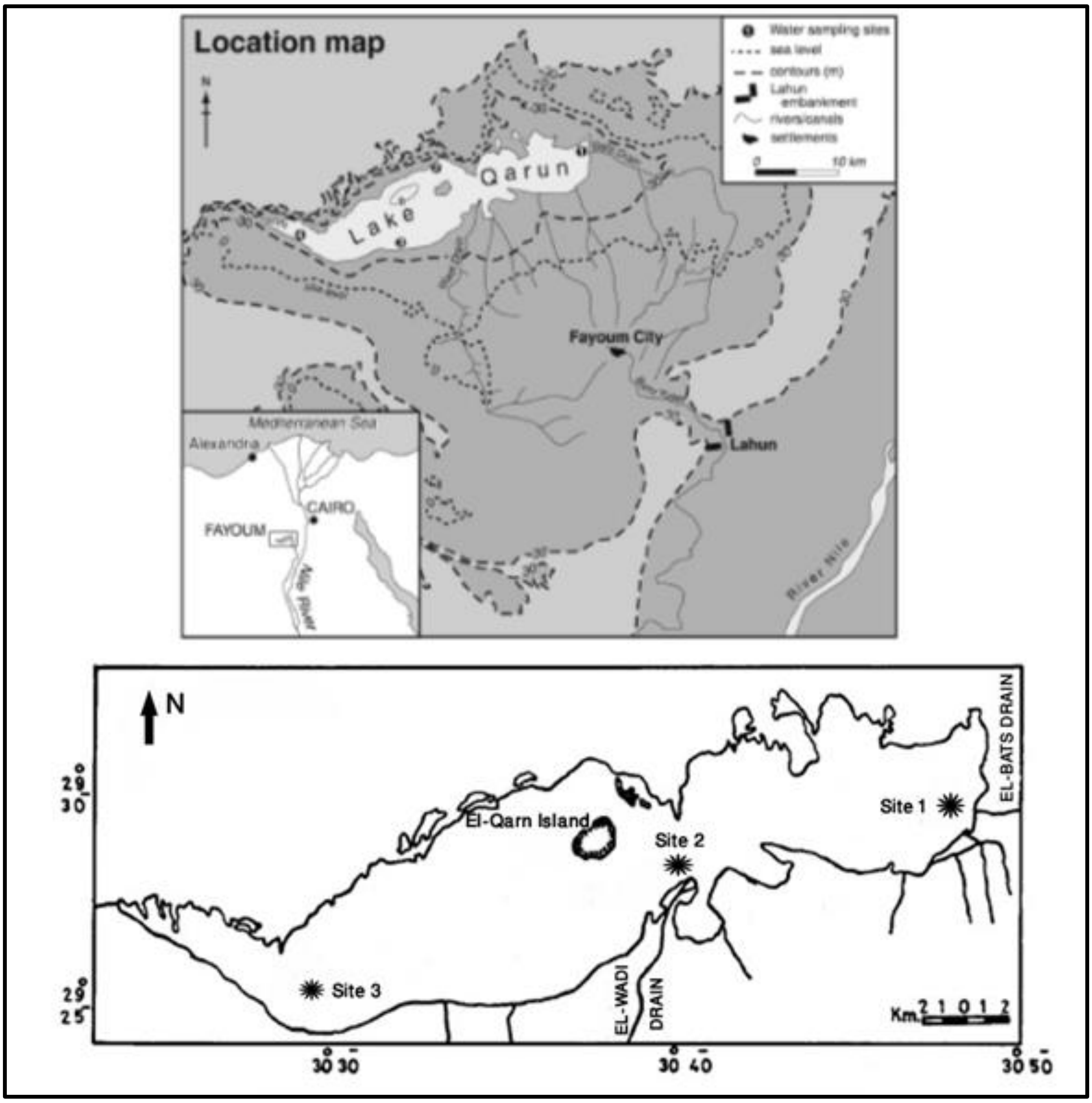

Fig. 1. The three studied sites of collection along Lake Qaroun

- Salinity was measured by using a salinity-conductivity meter (model, YSI 58).
- Total hardness and total alkalinity were measured by titration method according to the American Public Health Association standard methods **(Salihu & Bakar, 2018)**.
- Ammonia and nitrite were evaluated according to the method described by the American Public Health Association standard methods **(Salihu & Bakar, 2018)**.
- Heavy metal concentrations in water were determined by an atomic absorption spectrophotometer (Model, Perkin Elmer-2280). Heavy metals examined in this study were copper, zinc, lead, and cadmium **(Vaidya & Gadhia, 2012)**.
- For each analyzed water sample, the reported amounts of different ions were used to estimate the expected salts hypothetically and the residual amount of sulfate ions. The latter items were used to calculate the concentration of sodium. The total dissolved salts (TDS) were determined gravimetrically at 105 –110°C (Method 160.3).

3.3. Sediment sampling and analysis for heavy metals

Surface sediment samples from each of the three studied sites were collected and placed in a cooler and transported to the laboratory. Sediment samples were dried at 105^{o}C till reaching a constant weight then ashed at 420^{o}C for 24hrs. The ash was dissolved in concentrated nitric acid, and the impacted heavy metals (Cu , Zn , Pb and Cd) were detected according to **Haritonidis and Malea (1995),** using an atomic absorption spectrophotometer; (Perkin Elmer, 2280).

3.4. Fish sampling and analysis

3.4.1. Residual heavy metals in some selected organs of the studied fishes

Mugilcephalus specimens were collected from three study sites along Lake Qaroun in El-Fayoum using fishermen's nets and then brought to a laboratory for investigation. The muscle tissue was extracted from the left side of the fish sample between the pelvic and dorsal fins. Moreover, the gills, liver, kidney, and alimentary canal tissues were isolated. All tissues were cleaned with deionized water and placed in acid washed labelled vials and frozen (-20°C) until they attained a consistent weight.

3.4.2. Blood and tissue sampling

To lessen capture stress, the fish for blood analysis were transported to the laboratory alive in an aerated tank with a sandy bottom. Blood was extracted from the caudal artery. The needle (heparinized glass pipette) was inserted rather deeply in a dorso-cranial direction via the middle line right behind the anal fin. Furthermore, serum was obtained by centrifuging another blood sample for 12 minutes at 5000rpm and stored at -20^{o}C for further investigation. After the fish was decapitated, a bit of white epi-axial muscle and liver was removed for further examination.

3.4.3. Hematological analysis:

As mentioned before, blood samples were withdrawn from the caudal artery. The blood samples are examined as follow:

I) Red and white blood cells count. The total number of erythrocytes (RBCs) was carried according to the procedure described by **Haritonidis and Malea (1995)** using diluted solution. Leucocytes (WBCs) count was carried out according to the procedure described by **Lucas (1906)**.

II) Hemoglobin content: hemoglobin was estimated by using Cyanmethmoglobin method described by **Van Kampen and Zijlstra (1961)**.

III) Hematocrit value: The packed cell volume (PCV) was performed in a simple hematocrit graduated tube, upon hematocrit centrifuge at 3000rpm for about 10 minutes.

IV) Blood indices: Blood indices were calculated as follows **(Wiernik, 2003)**:

a) Mean corpuscular volume (μm^3/cell)

$$\mathbf{MCV = P.C.V.\ (\%)\ /\ RBCs\ 10^6/mm^3 *10}$$

b) Mean corpuscular hemoglobin (pg / cell)

$$\mathbf{MCH = Hb_{(g/100ml.\ blood)}/RBCs_{\ 10^6/mm^3} *10}$$

C) Mean corpuscular hemoglobin concentration (g/dl):

$$\mathbf{MCHC = Hb_{(g/100ml.\ blood)}/\ P.C.V.\ (\%) *100}$$

3.4.4. Biochemical analysis

Serum analysis: frozen serum samples were analyzed for the following analysis: The concentration of serum glucose was measured by using BIOme-rieux kit **(Trinder, 1969).** Serum total protein content was determined by Biuret test **(King & Wooton, 1964)**.Serum total lipid concentration was determined colorimetrically by sulphovanillin reaction **(Gofman *et al.*, 1950)**.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated colorimetrically as described by **Reitmans and Frankel (1957)**. Serum creatinine was measured by the colorimetric method **(Westgard *et al.*, 1981)**. The serum uric acid was measured by using enzymatic determination method **(Praetorius & Poulsen, 1953)**.

3.4.5. Histological analyses

The digestive tract samples were submerged in phosphate buffered formalin (8%, pH 7.2) for histological examination. Following this step, the samples were dried and embedded in paraffin according to conventional histological methods. Sections were stained with hematoxylin and eosin and viewed under a light microscope. Organisms from toxicity studies were examined for histopathological damage in various target tissues (liver, kidney, and gills). During 5-10 minutes, fish were anaesthetized with 0.1% 2-phenoxyethanol 99%. Dissection was used to obtain the fish's liver, kidney, and gill tissues, which were then fixed in Bouin's fixative for 24 hours. The materials were embedded in paraffin wax after being dehydrated in varying dilutions of ethanol. Hematoxylin and Eosin were used to stain histological sections of 4μ thickness. Light microscopy (LeitzLaborlux S) and photography (Sony DKC-CM30) were used to examine the sections.

3.4.6. Muscle analysis (Meat quality)

Muscle samples were transferred directly after decapitation to weighing bottles and accurately weighed. The bottles were then placed in a drying oven, which was thermostatically regulated at 105°C for 72hrs. The loss in weight was taken as equivalent to the weight of the water of the sample.

- Muscle total protein was determined using the semi-microkjeldahl method as reported by **Joslyn (2012)**.
- Muscle total lipids were determined by the standard method reported in **AOAC (1970)**. The process of extraction was carried out in Soxhlet apparatus using petroleum ether.
- Muscle ash was determined by burning the samples in the muffle furnace for16hrs at 550°C **(Anderson *et al.*, 1993)**.

5. Statistical analysis:

Statistical analysis system, version 6.2 (SAS, 2000) was used to analyze the data using the variance (F-test) and Duncan's multiple range test to find differences in means (among the three researched sites of collection for fish species). Principal component analysis (PCA) and correlation matrix was showed to compare physico-chemical factors and heavy metals in surface water. It was realized by IBM SPM statistics program version 22.

RESULTS AND DISCUSSION

1. Water quality

Water quality in the aquatic ecosystem is thought to be the most important element affecting the health and illness status of both cultivated and wild fish **(Fathi & Flower, 2005)**. The results in Table (1) depict the quality indicators of water collected from the three separate examined collection sites of Lake Qaroun (Eastern, southeastern, and western sectors). The analysis of variance (F-test) revealed significant differences in pH, dissolved oxygen, total hardness, total alkalinity, salinity, ammonia, and nitrite levels among the various study locations (F-values = 15.3, 55, 3.5, 6.2, 5.2, 34, and 108, respectively). The highest value of dissolved oxygen was evidently found in water samples from the lake's western sector (site III), followed by the southeastern sector (site II) and the eastern sector (site I). This could be attributed to agricultural discharges of excessive levels of organic chemicals, inorganic salts, and heavy metals, which cause hypoxia and fish mortality. Furthermore, the present data revealed high levels of water ammonia and nitrite in water samples collected from the lake's eastern and southeastern sectors, with the lowest levels found in the western sector.

2. Heavy metals in surface water

Due to the industrial expansion and the widespread use of pesticides in agriculture, heavy metals are widely disseminated in aquatic systems. They can cause an increase or decrease in the growth rate of some fishes and, within limits, are necessary for food efficiency and growth rate **(Chebotareva *et al.*, 2009)**. Higher concentrations of heavy metals over fish tolerance limits, on the other hand, have an effect on fish populations, lowering growth, reproduction, and/or survival, and may even kill fish **(Zaghloul *et al*., 2007)**. The results showed that the amounts of copper, cadmium, and lead in water obtained from the lake's eastern and southeastern locations were higher than in water samples collected from the lake's western site (Fig. 2), which revealed no source of pollution. The high copper, lead, and cadmium concentrations in water collected from the lake's eastern and southeastern sectors could be attributed to the quality of the industrial and agricultural drainage water, which is rich in fertilizers and chemicals and feeds the lake.

Furthermore, the findings revealed that water samples collected from the lake's western side had lower quantities of all heavy metals examined, except zinc. There were clearly significant differences (F-test) in all heavy metal concentrations among the different examined collection sites (F-values = 191, 319, 13, and 21 respectively), as shown in Table (1). The current findings demonstrated that the average concentration levels of Cu, Zn, Pb, and Cd in lake water were below the permitted limits, with the exception of Cd in the eastern and southeastern sectors and Pb metal in the eastern sector **(FAO, 1989)**. The majority of the parameters showed statistically significant correlation with one another, based on the correlation matrix among the different parameters, showing a tight link between these parameters, as shown in Table (1). Additionally, principal component analysis was used to classify the physical and chemical parameters chosen in Lake Qaroun based on how similar their water quality is, as shown in Figs. (3, 4). The similarity coefficients of all the stations under investigation are listed in Table (1). PCA exhibited that the maximum positive relationship was observed amongst Cd and between pH (0.979), total hardness (0.983), and total alkalinity (0.949) while Cd correlate with DO and salinity that showed a negative correlation being -0.976 and -0.988, respectively. These multivariate data are derived from the analysis of the water quality parameters of the water samples of all stations in Lake Qaroun.

Table 1. Quality of water collected from the different studied sites along Lake Qaroun **(**Data are represented as means of eight samples ± SE**)**

Parameter	Eastern sector (Site I)	Southeastern sector (Site II)	Western sector (Site III)	F-value
pH	8.45 ± 0.09 **A**	8.20 ± 0.08 **B**	7.85 ± 0.06 **C**	**15.3****
Dissolved oxygen mg/l	6.05 ± 0.09 ***C***	6.65 ± 0.13 **B**	7.65 ± 0.09 **A**	**55****
Total hardness as $CaCO_3$ mg/l	815 ± 9.4 **A**	803± 8.5 **A/B**	785 ± 5.67 **B**	**3.5***
Total alkalinity as $CaCO_3$ mg/I	383 ±5.1 **A**	368 ± 6.6 **A/B**	354 ± 6.05 **B**	**6.2****
Salinity g/l	37 ±0.76 **B**	38.5 ± 0.57 A/**B**	40.5 ± 0.94 **A**	**5.2****
NH_3 mg/l	0.49 ±0.04 **A**	0.36 ± 0.02 **B**	0.15 ± 0.02 **C**	**34****
NO_2 mg/l	0.60 ±0.04 **A**	0.35 ± 0.19 **B**	0.09 ± 0.004 **C**	**108****
Cu^{+2} P.l. = 1.0 mg/l	0.465 ± 0.013 **A**	0.40 ± 0.02 **B**	0.10 ± 0.003 **C**	**191****
Zn^{+2} P.l. = 5.0 mg/l	0.02 ± 0.004 **B**	0.035 ± 0.006 **B**	0.36 ± 0.02 **A**	**319****
Cd^{+2} P.l. = 0.01 mg/l	0.30 ± 0.04 **A**	0.24 ± 0.05 **A**	0.04 ± 0.004 **B**	**13****
Pb^{+2} P.l. = 0.05 mg/l	0.140 ± 0.02 **A**	0.045 ± 0.009 **B**	0.015 ± 0.002 **B**	**21****

P.l. = Permissible level in water according to **WHO (1993)**;
Means with the different letters for each parameter are significantly different;
* Significant difference (*P*<0.05); ** Highly Significant difference (*P*< 0.01).

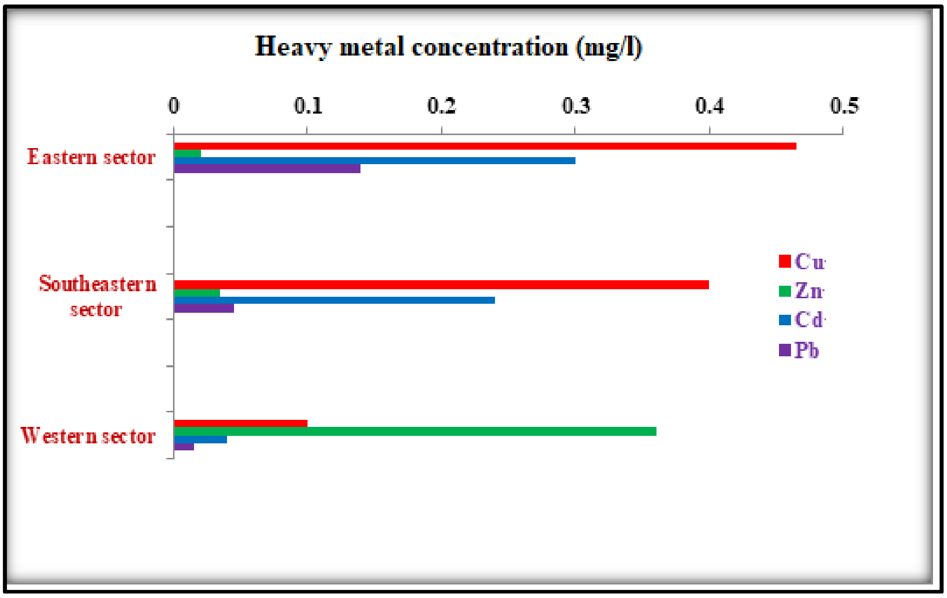

Fig. 2. Heavy metal concentrations (mg/l) in water samples collected from different studied sites along Lake Qaroun

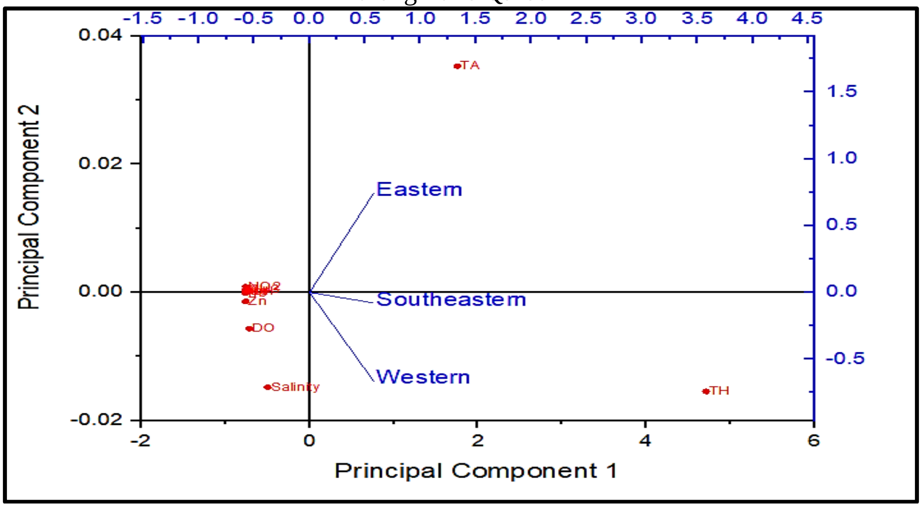

Fig. 3. Principal component analysis for the water quality parameters in Lake Qaroun

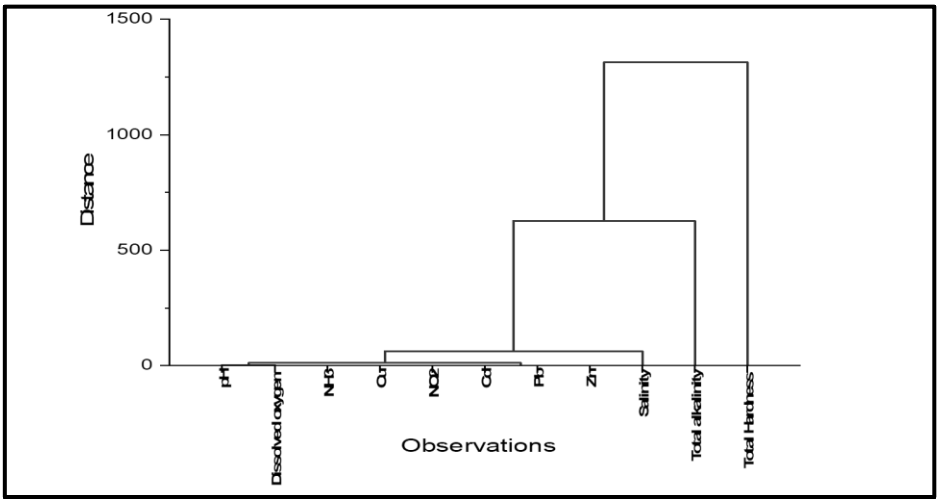

Fig. 4. Dendrogram of the relationship amongst physicochemical parameters in Lake Qaroun

3. Sediment heavy metals

The function of sediments in the cycling of chemical elements has often been underestimated in aquatic ecosystems research, and the interchange of elements (particularly nutrients) between sediments, and water remains a critical topic **(Arain *et al*., 2008)**.

Table (2) shows the mean amounts of copper, zinc, cadmium, and lead in sediment samples taken from the three distinct examined areas around Lake Qaroun. There were clearly significant differences (F-test) in all heavy metal concentrations among the different examined collection sites (F-values = 69.7, 4.1, 52, and 33, respectively). The results showed that the quantities of copper, cadmium, and lead in sediment samples collected from the lake's eastern and southeastern sections were higher than in the lake's unpolluted western sector. Furthermore, the results revealed that sand samples obtained from the lake's western sector had much higher zinc contents than those gathered from the other analyzed collection sites. This finding agrees with that of several authors **(Camargo & Martinez, 2007; Elwasify *et al*., 2021)**.While, it differs with the outcome of **De Mora *et al*. (2004)**, who stated that the seabed composition has mostly greater nickel sulfide content, which is the natural source of nickel in coastal locations, rather than anthropogenic activity.

Table 2. Heavy metals concentration in sediment samples (mg/kg dry weight) collected from the different studied sites along Lake Qaroun (Data are represented as means of eight samples ± SE).

Studied site of collection	Cu^{+2}	Zn^{+2}	Cd^{+2}	Pb^{+2}
Eastern sector	2.30 ± 0.15 **A**	24.5 ± 1.32 **B**	0.30 ± 0.04 **A**	3.05 ± 0.32 **A**
Southeastern sector	1.80 ± 0.11 **B**	26.0 ± 1.13 **A/B**	0.31 ± 0.04 **B**	2.1 ± 0.15 **B**
Western sector	0.50 ± 0.04 **C**	29.5 ± 1.32 **A**	0.07 ± 0.007 **C**	0.60 ± 0.11 **C**
F-values	**69.7****	**4.1***	**52****	**33****

Means with the different letters for each parameter are significantly different;
* Significant difference (P<0.05); ** Highly Significant difference (P< 0.01).

4. Residual heavy metals

Table (3) displays the concentrations of the studied heavy metals (copper, cadmium, lead, and zinc) in some vital organs (gills, liver, kidneys, end of alimentary canal, and muscles) of *Mugilcephalus* collected from different studied sectors along Lake Qaroun.

4.1. Copper concentrations

Table (3) shows that the copper concentrations in numerous key organs of *Mugilcephalus* samples obtained from the eastern sector had the greatest values, followed by fish gathered from the southeastern area, while the lowest values were found in the unpolluted western sector. Furthermore, the investigated *M. cephalus* species obtained from the polluted eastern and southeastern sections of the lake indicated substantial differences, with the exception of the gills and kidneys of fish collected from the polluted southeastern sector of the lake, which demonstrated a non-significant difference at P> 0.05 (Table 3). According to the current findings, copper accumulated in the following order in the examined organs of fish species: liver > kidneys > gills > end of alimentary canal > muscles.

4.2. Cadmium concentrations

The amounts of cadmium in certain essential organs of *M.cephalus* investigated fish species are shown in Table (3). The greatest values were found in fish gathered from the lake's eastern sector, followed by fish collected from the lake's southeastern section, while the lowest values were found in fish collected from the lake's unpolluted western sector. According to the current findings, cadmium bioaccumulation occurred in fish species in the following order: kidney > gills > end of alimentary canal > liver > muscles.

4.3. Lead concentrations

Table (3) exhibits the lead concentrations in the gills, liver, kidneys, end of the alimentary canal, and muscles of *M.cephalus*. Based on the current findings, lead accumulated in the following order in the *M.cephalus* organs: kidneys > end of alimentary canal > liver > gills > muscles.

4.4. Zinc concentrations

Table (3) shows zinc concentrations in several selected important organs of examined fish species. The greatest levels were found in the western sector of Lake Qaroun, followed by fish caught in the eastern and southeastern sections of the lake, where main drain effluent was discharged. According to the current findings, zinc accumulated in the following order in *Mugilcephalus* organs: kidneys > liver > gills > end of alimentary canal > muscles. Heavy metals are primarily stored in metabolic organs such as the liver, which detoxicates metals by generating metallothioneins **(Kargm& Erdem, 1991)**.

Kidney, gills, and liver tissues were found to be active bioaccumulators for most metals due to their large bulk, which allows the collected metals to be detoxified, controlled, or expelled **(Reinfelder, *et al*., 1998)**. These findings are consistent with those of **Jayaprakash *et al.* (2015)** and **Abdel-Khalek *et al.* (2016)**, who found the highest levels of Ni, Pb, Mn, Co, Cr, Fe, Cu, and Zn in the gills and liver tissues.

Heavy metal levels in fish gills were generally higher than in muscle tissue. The element combined with the mucus, which is impossible to be entirely removed from the lamellae before tissue was prepared for analysis, could explain the metal concentration in the gill. Adsorption of metals onto the gill surface, being the primary target for contaminants in water, could also have a significant impact on gill total metal levels **(Heath, 2017)**. However, as previously documented by **Shenouda *et al*. (1992)** and **Mohamed and Gad (2008)**, the low levels of metals in the muscles may be ascribed to the lack of blood flow to the muscular tissues.

In the current study, nonessential metals (Pb and Cd) are bioaccumulated with reduced efficiency in the various tissues compared to essential metals (Cu and Zn), which have a high bioaccumulation efficiency. Furthermore, as mentioned by **Authman and El-Sehamy (2007)** and **Mohamed and Gad (2008)**, differences in the pattern of metals distribution in the studied *Mugilcephalus* could be due to differences in countless factors, such as feeding habitats, ecological needs, metabolism, and physiology.

Table 3. Residual metal concentrations in some selected organs (mg/kg dry weight) of *Mugilcephalus* collected from the different studied sites along Lake Qaroun

Residual copper concentrations					
Studied site of collection	**Gills**	**Liver**	**Kidney**	**End of alimentary canal**	**Muscles P.l. = 20 ppm**
Eastern sector	7.70 ± 0.30 **A**	23.0 ± 1.51 **A**	20.0 ± 1.50 **A**	4.7 ± 0.22 **A**	1.85 ± 0.13 **A**
Southeastern sector	5.35 ± 0.10 **B**	15.0 ± 1.13 **B**	11.5 ± 0.94 **B**	3.4 ± 0.26 **B**	1.6 ± 0.11 **A**
Western sector	3.15 ± 0.25 **C**	9.5 ± 0.90 **C**	6.0 ± 0.76 **C**	2.05 ± 0.21 **C**	1.0 ± 0.08 **B**
F-values	**55****	**31****	**40****	**32****	**16****
Residual cadmium concentrations					
Studied site of collection	**Gills**	**Liver**	**Kidney**	**End of alimentary canal**	**Muscles P.l. = 0.5 ppm**
Eastern sector	3.55 ± 0.09 **A**	1.2 ± 0.11 **A**	4.45 ± 0.21 **A**	2.9 ± 0.42 **A**	0.77 ± 0.09 **A**
Southeastern sector	2.15 ± 0.06 **B**	0.74 ± 0.05 **B**	2.85 ± 0.17 **B**	1.75 ± 0.24 **B**	0.47 ± 0.05 **B**
Western sector	0.71 ± 0.01 **C**	0.25 ± 0.02 **C**	0.95 ± 0.06 **C**	0.55 ± 0.09 **C**	0.16 ± 0.017 **C**
Residual lead concentrations					
Studied sites of collection	**Gills**	**Liver**	**Kidney**	**End of alimentary canal**	**Muscles P.l. = 0.6 ppm**
Eastern sector	2.65 ± 0.13 **A**	6.75 ± 0.28 **A**	17 ± 0.76 **A**	13 ± 0.38 **A**	1.0 ± 0.03 **A**
Southeastern sector	2.45 ± 0.13**B**	5.65 ±0.25**B**	15 ± 0.76**B**	10.5 ± 0.57**B**	0.8 ± 0.038**B**
Western sector	0.7 ± 0.10**C**	1.10 ± 0.07**C**	2.35 ± 0.09**C**	1.90 ± 0.04**C**	0.45 ± 0.07**C**
F-values	**85****	**184****	**164****	**218****	**38****

Residual zinc concentrations					
Studied site of collection	**Gills**	**Liver**	**Kidney**	**End of alimentary canal**	**Muscles P.l. = 0.6 ppm**
Eastern sector	16 ±0.75**B**	19.5 ±0.94**B**	25.5 ±1.3**B**	4.2 ±0.11**B**	10 ±0.38**B**
Southeastern sector	17 ± 0.75**B**	21.5 ± 0.94**B**	27.5 ± 0.94**B**	4.4 ± 0.3**B**	9 ± 0.38**B**
Western sector	20 ±0.57**A**	25 ±0.76**A**	31.5 ±0.94**A**	6.0 ±0.538**A**	12 ±0.38**A**
F-values	**11.4****	**9.9****	**7.9****	**11.8****	**16.3****

Data are represented as means of eight samples ± S.E;
P.l. = Permissible level in fish tissues for human consumption according to **WHO (1993)**;
Means with different letters in the same column are statistically significantly varied;
* Significant difference (P<0.05); ** Highlysignificant difference (P<0.01).

5. Histological studies

5.1. Gills of species under study

Gill sections of fish species collected from eastern and southeastern sectors of the lake depicted in Fig. (5) show clear histopathological changes, which include the necrosis of epithelial cells,epithelial hyperplasia with ballooning degeneration and desquamation of the epithelium. The changes in gill epithelia of *Mugilcephalus* included edema with lifting of lamella repithelium, hyperplasia, cell degeneration, intense rupture and peeling of lamella repithelia.These observations are similar to those obtained by **Ibrahim *et al*. (2009)**, **Abou El-Gheit *et al*. (2012)** and **Tayel, *et al*. (2013)**, who related these malformations in gills to the increase of ammonia and heavy metals, pH change, oxygen depletion, occurrence of microorganisms and the increased turbidity in water polluted by sewage and agricultural discharge in the Lake Qaroun **(Rajeshkumar *et al.*, 2015)**.

5.2. Liver of fish samples

Fish liver was obtained from the lake's eastern and southeastern portions, where the water quality has experienced deterioration. Fig. (5) demonstrated significant histological alterations, including damage to parenchymal cells, disintegration and necrosis of hepatic cells, and intravascular hemolysis in the liver's blood vessel. Additionally, there are parenchymal hepatocyte shrinkage, fatty vacuolation, karyomegaly, pyknosis, and formation of increased lipofuscin granules, rough endoplasmic reticulum disorganization, and smooth endoplasmic reticulum proliferation **(Ziyaadini *et al*., 2017; Shahida *et al*., 2020)**.

From the above finding, it can be concluded that, the changes in histological features of the liver may be due to the toxic impact of different pollutants on the liver cells which is the site of detoxification of all types of harmful chemicals. The same diagnosis were detected by **Vasanthi *et al*. (2013)** in case of *Mugillcephalus* due to heavy metal pollution in Ennore Estuary. **Tayel*et al*. (2013)** suggested a strong link between water quality changes and observed liver lesions in fish inhabiting the water of the Lake Qaroun; they postulated that these lesions may be due to oxygen depletion, parasitic infection, elevation in ammonia and heavy metals.

5.3. Kidney samples

Kidneys of fish species obtained from contaminated eastern and southeastern sections of Lake Qaroun (Fig. 5) reveal a gradual destruction of kidney tubules accompanied with tubular necrosis, injury of the wall of renal blood vessels, and depletion of hemopoietic tissues **(Mohamed, 2009)**.

There were also indications of renal epithelial disintegration, nuclei relocation, glomeruli shrinkage, Bowman's capsule breakage, and significant inflammatory cell infiltration. In the current investigation, histopathological changes were linked to exposure to several heavy metals in the surrounding media. Heavy metal accumulation in fish kidneys may have a role in their damage.

However, accumulation patterns of heavy metals depend on both uptake and elimination rates **(Ghanem, 2019)**. Copper, zinc, and lead all altered the ionic composition of the aquatic environment. The most noticeable alterations in the kidney were the vacuolar degeneration of the tubular epithelium, blood vessel congestion, and the appearance of pigment granules between the renal tubules and the closest renal corpuscles. The presence of stressors and pollutants in contaminated water with higher levels of Cd, Pb, and Cu may explain the emergence of histopathological abnormalities in the kidneys of investigated fish **(Pirbeigi *et al*., 2016)**.

Sections of the gills, liver, and kidneys of the analyzed fish species obtained from the unpolluted western sector of the lake (Photomicrographs 1C, 2C, and 3C) were more or less identical and resembled those of the normal fish (it was chosen as a control group).

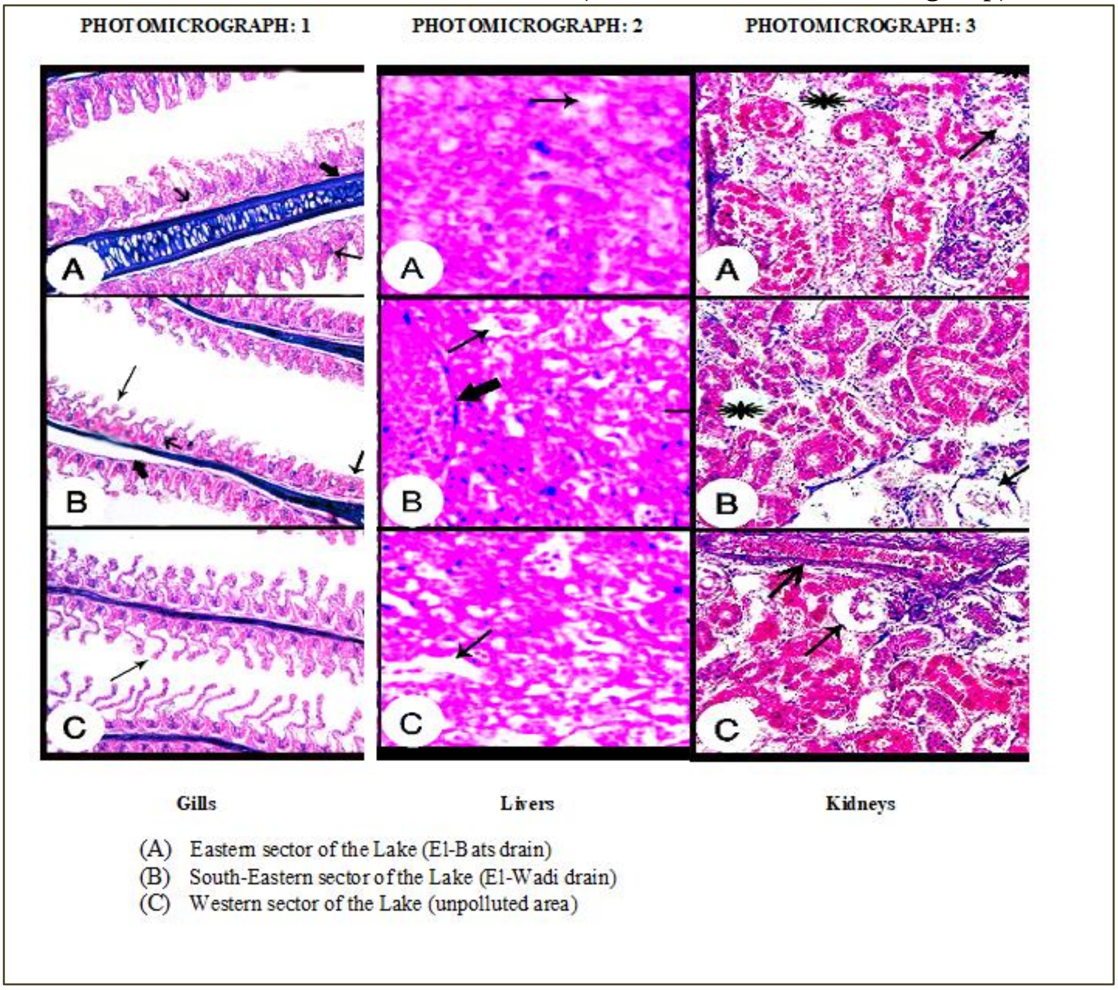

Fig. 5. Photomicrographs of Histological sections in livers of *Mugilcephalus* collected from different sites along Lake Qaroun

6. Bloodparameters

Hematological investigations have been developed to assess fish health problems. In fact, blood is the most convenient indicator of the general status of the animal body. As a result, hematological studies are potential methods for evaluating physiological changes produced by contaminants in the environment.

6.1. Red blood cell counts

The current findings (Table 4) demonstrated that RBCs of *Mugilcephalus* obtained from the several study sites along the lake differed significantly (F-values = 163). The eastern and southeastern sectors of the lake that were exposed to the discharge of El-Bats and El-Wadi drainage water showed a significant decrease in RBCs, while higher values were recorded in samples collected from the unpolluted western sector of the lake, where no source of pollution was identified **(Abdel-Khalek, 2015)**.

6.2. Hemoglobin content

Table (9) demonstrates that the hemoglobin concentration of *Mugilcephalus* obtained from the various study sites throughout Lake Qaroun differed significantly (F-values = 33). Furthermore, *Mugilcephalus* collected from the lake's eastern and southeastern sectors that were exposed to agricultural drainage water had a significant decrease in hemoglobin content, whereas higher values were recorded in samples collected from the lake's unpolluted western sector. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) increase in response to the reduction in blood Hb. This could be attributed to hemolytic activity, which caused fluid loss to tissues and a subsequent drop in plasma volume **(Swift, 1981)**. Lower Hb levels may be caused by the influx of water from farms, industries, and sewage containing heavy metals such as Cd and Pb, which affect the characteristics of hemoglobin by diminishing their affinity for oxygen binding capacity **(Adakole, 2012)**.

Based on the study of **Gaafar *et al.* (2010)**, a sustained decrease in hemoglobin concentration is detrimental to oxygen transport, and erythrocyte degradation could be caused by the pathological condition in fish.

6.3. Hematocrit values

In terms of *M. cephalus* hematocrit levels (Table 4), there were extremely significant differences among fish taken from the various study sites along Lake Qaroun (F-values = 63). In addition, it was noticed that, *M. cephalus* collected from the lake's eastern and southeastern sectors that were exposed to agricultural drainage water showed a significant decrease in hematocrit values, whereas higher values were recorded in samples collected from the lake's unpolluted western sector, where no source of pollution was identified. The same histological changes were observed in the current investigation. This is supported by visible damage in the gills of fish collected from the lake's eastern and southeastern parts.

6.4. White blood cell counts

The white blood cell counts of *M. cephalus* (Table 4) reveal that there were highly significant variances in WBCs of fish obtained from the several examined collection sites along Lake Qaroun (F-values =18.9). Furthermore, *M. cephalus* from the unpolluted western sector had a considerable drop in WBCs count. The rise of WBCs in polluted sites could be attributed to their defensive nature, as leukocytosis is directly proportional to the amount of damage and stress, resulting in the stimulation of immunological defense **(Javed & Usmani, 2012)**.

6.5. Blood parameters

For blood parameters, the highly significant decrease in RBCs, Hb, and hematocrit values of *Mugilcephalus* collected from the eastern and southeastern sectors of the lake in comparison to those of *Mugilcephalus* collected from the western sector may be due to a reduction in red blood corpuscles production in the hematopoietic organs under the action of high heavy metal concentrations recorded in water samples **(Zaghloul *et al.*, 2007)**. The comparison of *Mugilcephalus* white blood cell count revealed that there was a highly significant difference in WBCs count of fish obtained from the eastern and southeastern areas of the lake, compared to fish gathered from the western sector of the lake. The highly significant decrease in RBCs, Hb and Ht values of fish collected from the eastern and southeastern sectors of the lake in relation to those of the fish collected from western sector may be traced back to a reduction in red blood corpuscles production in the hematopoietic organs under the action of high heavy metal concentrations recorded in water samples **(Zaghloul *et al.*, 2007)**. This decrease could potentially be attributed to intrahepatic and intrasplenic hemorrhage caused by the effect of accumulating heavy metals.

Table 4. Blood parameters of *Mugilcephalus* collected from the different studied sites along lake Qaroun

Sites of collection	RBCs ($X10^6/mm^3$)	Hb (g/dl)	Ht (%)	MCV (femto liter)	MCH (pg/cell)	MCHC (g/dl)	WBCs ($X 10^3/mm^3$)
Eastern sector	1.25±0.06 **C**	5.45±0.36 **B**	16.5±0.94 **B**	132±1.7 A	43.5±0.94 A	33.0±0.38 A	41.0± 1.13 A
Southeastern sector	1.55±0.06 **B**	6.0±0.26 **B**	19±1.13 **B**	121±2.83 B	38.5±0.19 B	31.5±0.57 B	37.0±1.5 B
Wester sector	2.5±0.04 A	8.85±0.32 A	31.5±0.94 A	126±1.89 A/B	35±0.76 C	28.5±0.18 C	30.5±0.94 **C**
F-values	**163****	**33****	**63****	**5.2***	**37****	**31.5****	**18.9****

Data are represented as means of eight samples ± S.E;
Means with different letters in the same column are significantly different;
* Significant difference (P<0.05); ** Highly Significant difference (P<0.01).

7. Serum constituents

Serum component analyses have been shown to be helpful in the detection and diagnosis of metabolic disturbances and disease **(Zaghloul *et al.*, 2016)**.

Table (5) contains information on the variations in blood glucose, total protein, aspartate amino transferase (AST) and alanine amino transferase (ALT) activities, creatinine, and uric acid of *Mugilcephalus* collected from the several study sites along Lake Qaroun.

It was obvious that fish species collected from the polluted eastern and southeastern sectors of the lake had significantly higher serum glucose levels, whereas samples collected from the unpolluted western sector of the lake, where no effluents were discharged, had normal values in this parameter. The reported hyperglycemia in *Mugilcephalus* collected from the eastern and southeastern parts of Lake Qaroun may be due to increased glycogen breakdown in the liver, which may be affected by the bioaccumulation of the studied heavy metals and/or the increase in plasma concentrations of catecholamines and corticosteroids as a stress response of fish subjected to environmental alteration.

The serum total protein of *Mugilcephalus* collected from the various studied sites along Lake Qaroun (Table 5) reveal highly significant differences (F-values = 147), with the highest values in *Mugilcephalus* collected from the unpolluted western sector of the lake and the lowest values in fish collected from the polluted eastern and southeastern sectors of the lake that were exposed to El-Bats and El-Wadi drain effluents without prior treatments. Serum total proteins have a significant function in metabolism and water balance regulation **(Heath, 1995)**.

Results concerning serum aspartate amino transferase (AST) activity of *Mugilcephalus* collected from the various studied sectors along Lake Qaroun showed highly significant differences (F-values = 37.6), with the highest values in *Mugilcephalus* collected from the eastern and southeastern sectors of the lake; however, the lowest values were those recorded in fish collected from the unpolluted western sector of the lake.

The serum alanine amino transferase (ALT) of *Mugilcephalus* collected from the various studied collection sites along Lake Qaroun (Table 5) revealed highly significant differences (F-values = 52), with the highest values in the polluted eastern and southeastern sectors of the lake, and the lowest values in fish from the unpolluted western sector of the lake where no source of pollution was identified.

There were highly significant differences (F-values = 267) in serum creatinine of *Mugilcephalus* collected from the different studied sectors along Lake Qaroun, with the highest values in the eastern and southeastern sectors of the lake and the lowest values in fish collected from the unpolluted western sector of the lake.

Table 5. Serum constituents of *Mugilcephalus* collected from the different studied sites along Lake Qaroun

Studied site of collection	Glucose (mg/dl)	Total protein (g/dl)	AST (U/l)	ALT (U/l)	Creatinine (mg/dl)	Uric acid (mg/dl)
Eastern sector	101 ± 1.5 **A**	2.9± 0.08 **C**	26.0 ± 1.51 **A**	18.5 ± 0.94 **A**	3.70 ± 0.08 **A**	8.80± 0.42 **A**
Southeastern Sector	80.5 ± 3.21 **B**	3.45 ± 0.21 **B**	21.0 ± 0.80 **B**	17.5 ± 0.94 **B**	2.90 ± 0.08 **B**	6.15 ± 0.09 **B**
Western sector	46.5 ± 1.32 **C**	6.05 ± 0.09 **A**	13.0 ± 0.76 **C**	8.0 ± 0.38 **C**	1.45 ± 0.06 **C**	3.95 ± 0.09 **C**
F-values	**158****	**147****	**37.6****	**52****	**267****	**93****

Data are represented as means of eight samples ± S.E.
Means with different letters in the same column are significantly different;
* Significant difference (*P*< 0.05); ** Highly Significant difference (*P*< 0.01).

8. Muscle chemical composition (meat quality)

Table (6) displays data indicating the variations in muscle chemical composition of *Mugilcephalus* obtained from the several study sites along Lake Qaroun.

8.1. Muscle water content

Muscle composition varies by species, sex, maturity, and season. There were highly significant changes in the muscle water content of *Mugilcephalus* (Table 6) across fish taken from different study sites (F-values = 19). The most significant results for muscle water content were found in the lake's contaminated eastern and southeastern portions, which were exposed to the effluents of the El-Bats and El-Wadi drainage canals without prior treatment. The lowest muscle water content readings for *Mugilcephalus* were found in samples collected from the lake's unpolluted western side. Protein content reductions were seen in big muscles due to the decrease in protein used with the increasing age and dietary protein intake **(Abdel-Tawwab *et al*., 2010)**.

8.2. Muscle total protein

Highly significant changes (F-values =16) were recorded in the muscle total protein of *Mugilcephalus* collected from the various examined collection sites along Lake Qaroun (Table 6). The highest values were detected in fish collected from the polluted eastern and southeastern sectors of Lake Qaroun, where El-Bats and El-Wadi drainage canals discharge their effluents without prior treatment.

8.3. Muscle total lipids

There were extremely significant differences (F-values ≥ 101) in the muscle total lipids of *Mugilcephalus* obtained from the several study sites along Lake Qaroun (Table 6). The highest values were found in *M. cephalus* obtained from the lake's unpolluted western shore, whereas the lowest values were registered in fish gathered from the lake's polluted eastern and southeastern shores.

8.4. Muscle ash (%)

The muscle ash % of *Mugilcephalus* obtained at three distinct study sites along Lake Qaroun (Table 6) reveal extremely significant variances (F- values = 5.7).

The highest levels were found in *Mugilcephalus* samples collected from the lake's contaminated eastern and southeastern parts. The lowest *Mugilcephalus* levels were found in samples collected from the lake's unpolluted western portion, where no released effluents were found.

Fish exposed to agricultural effluents in the lake's eastern and southeastern sections showed deterioration in meat quality, with large increases in muscle water content and ash and significant decreases in muscle total protein and total lipids in the current field investigation **(Ghanem, 2019)**.

The substantial bioaccumulation of metals in gills may explain the decrease in muscle total protein and total lipids of fish exposed to agricultural drainage water. As previously observed in the study of **Reader *et al.* (1989)**, these metals induce damage to the gill structure and a decrease in the rate of oxygen consumption, resulting in a dramatic decrease in metabolic rate aligned with a slow fish growth. The decrease in total muscle protein and total lipids may possibly be ascribed to a decrease in insulin level, as identified by the present study's recognized hyperglycemia, which has a stronger effect on proteogenic and lipogenic pathways **(Ablett *et al.*, 1981)**.

Table 6. Muscle chemical composition of *Mugilcephalus* collected from different studied sites of lake Qaroun

Site of collection	Water content (%)	Total protein (% of wet weight)	Total lipids (% of wet weight)	Ash (%)
Eastern sector	79.5 ± 0.57 A	14.0 ± 0.38 C	2.45 ± 0.07 C	2.75 ± 0.09 A
Southeastern sector	77.0 ± 0.60 B	15.0 ± 0.37 B	3.15 ± 0.09 B	2.60 ± 0.11 A
Western sector	75.0 ± 0.40 C	17.0 ± 0.38 A	4.0 ± 0.08 A	2.30 ± 0.08 B
F-values	**19****	**16****	**101****	**5.7***

Data are represented as means of eight samples ± S.E.
Means with different letters in the same column are significantly different;
* Significant difference (P< 0.05); ** Highly Significant difference (P< 0.01).

CONCLUSION

The current field investigations included the analysis of water quality (pH, DO, total hardness, total alkalinity, salinity, NH_3 & NO_2) of samples obtained directly from different places around Lake Qaroun, as well as the study of some physiological and biochemical parameters of fish maintained in the various studied sites. Copper, zinc, cadmium, and lead were the heavy metals addressed in this study. The eastern sector of the lake had the highest values for copper, zinc, cadmium, and lead, followed by the southeastern section of the lake, and the western sector of the lake had the lowest values.

Fish exposed to agricultural effluents in the lake's eastern and southeastern sections exhibited deterioration in meat quality, with large increases in muscle water content and ash and significant decreases in muscle total protein and total lipids in the current field investigation. The accumulation of heavy metals identified in the target organs caused adverse consequences on various organ functions. The increase in bioaccumulation, biosedimentation factors, and histological changes in edible organs may be interpreted as a warning sign for fish and human health.

Furthermore, it is vital to be more cautious with regard to water resources in aquaculture in order to limit or prevent future contamination in a way that would give good fish production while preventing any harm that may endanger the health of the end-users. In general, fish caught from the western side of the lake demonstrated strong growth indices, high meat quality, and safe flesh for human consumption. However, effluents from drainage water dumped straight into the eastern and southeastern areas of Lake Qaroun without prior treatment limit fish development and impair meat quality, posing a threat to man. Furthermore, the findings of this study call attention to the necessity for prompt action by the responsible authorities to address the issue, as well as ongoing monitoring of changes in lake conditions to aid in the formulation of appropriate lake management policies for Lake Qaroun.

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