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Heavy Metal Bioaccumulation and Related Histopathological Changes in Gills, Liver and Muscle of *Solea aegyptiaca* Fish Inhabiting Lake Qarun, Egypt.

Asmaa M. El-Agri[1*], Manal A. Emam[2], Hanan S. Gaber[1], Ekrami A. Hassan[2], Soha M. Hamdy[3]

1. Pollution Department, National Institute of Oceanography and Fisheries, Cairo, Egypt.
2. Biochemistry Department, Ain Shams University, Cairo, Egypt.
3.Chemistry Department, Fayoum University, Cairo, Egypt.

*Corresponding author: asmaamohamed_8817@yahoo.com

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ABSTRACT

Pollution of Lake Qarun with heavy metals has become a more serious concern during recent years as it affects the physicochemical characteristics of the water, sediments and biological components and may damage aquatic organisms as well. Lake Qarun receives the agricultural and sewage wastewater loaded with heavy metals through two main drains; El-Batts and El-Wadi. Therefore, an annual increase in the accumulation of pollutants and heavy metals has been detected in all its components (e.g. water, sediment and fish). The present study aimed to evaluate the seasonal variation of heavy metals' contents (Fe, Cu, Zn, Cd, Pb and Ni) in water and the bioaccumulation factor of these heavy metals in *Solea aegyptiaca* fish samples, collected from Lake Qarun. Thus, information was provided regarding histological changes in gills, liver and muscle organs. This study was carried out along Lake Qarun at four sites (Eastern, Southern, Northern and Western) during the winter and summer seasons, 2019. The results revealed that summer was higher in heavy metal concentration in water than winter. Additionally, the eastern and southern sectors showed increased water concentration of heavy metals. Concerning fish samples, the present study exhibited that, the maximum values of bioaccumulation factor were recorded in the gills and liver, whereas the minimum values were observed in the muscles. The gills of *Solea aegyptiaca* accumulate higher levels of Zn, pb and Ni, while, Fe, Cu and Cd accumulated mainly in the liver. Results showed obvious histopathological alternations and clear damage in gills, liver and muscles of *Solea aegyptiaca* collected from the eastern and southern sectors of Lake Qarun.

INTRODUCTION

Lake Qarun, located in El-Fayoum province, is one of the most important Egyptian water bodies. It is potentially at risk due to pollution and environmental degradation. This lake suffers from several environmental problems **(Mohamed & Gad, 2008)**. Lake Qarun has witnessed several drastic changes affecting its role as economic potential site for living natural resources **(Dardir & Wali, 2009)**. It receives agricultural, domestic and sewage drainage water from El-Fayoum province loaded with high levels of pollutants through a system of two main drains (El-Bats and El-Wadi drains) and some small drains that pass about 450 million cubic meters of wastewater annually to the Lake Qarun **(Goher, 2002; Fathi & Flower, 2005)**. The drainage waters discharged into the lake are high in solids, nutrients, pesticides, heavy metals and organics **(Gupta & Abd El-Hamid, 2003; Ali & Fishar, 2005; Mohamed & Gad, 2008; Mohamed, 2009; Ghanem, 2011; Mohamed, 2019)**.

Among the various toxic pollutants, heavy metal pollution of aquatic environment has recently become a great concern because being very harmful due to their non-biodegradable nature, long biological half-life and their potential to accumulate in different body parts of organisms **(Zahran *et al.*, 2015)**. They can also be concentrated along the food chain, producing their toxic effect at points lond after being removed from the source of pollution. 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)**.

Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals such as lead, cadmium, chromium, copper, mercury, zinc and iron. These metals accumulate differently in fish organs, and thus cause serious health hazards to humans **(Mansour & Sidky, 2002)**. For this reason, fish is a good bio-indicator for heavy metals and other organic pollutants **(Ahmed & Shubami-Othman, 2010)**. *Solea aegyptiaca* fish was successfully transplanted into the Lake Qarun from the Mediterranean Sea to improve the yield of its original strain in the lake **(Zaghloul *et al.*, 2011)**. Benthic fish, such as sole, into direct contact with sediments, feed on benthic invertebrates that accumulate metals, increasing the toxicity of these compounds through bioaccumulation/biomagnification process **(Drake *et al.*, 1984; Dinis, 1992)**. Thus, the presence of significant levels of toxic metals in these species can not only affect their physiological performance but also negatively affect consumer's health **(Castro-González & Méndez-Armenta, 2008)**. Consequently, *Solea aegyptiaca* was chosen for this study due to its great economic and commercial interest.

Accumulation of pollutants disrupts the physiology of fish tissues. The endpoint in assessing the risk of pollutants in the environment is the microscopic examination of target tissues through histopathological parameters **(Fatima *et al.*, 2015)**. Histopathological changes can be used as indicators of the impact of various anthropogenic pollutants on organisms and as a measure of the overall health of the entire aquatic ecosystem **(Saad *et al.*, 2011)**. Harmful effects of pollutants can be manifested in fish tissues before consequential changes in the external appearance and behavior of fish **(Mahboob *et al.*, 2020)**. The exposure of fish living in Lake Qarun to different types of wastes (industrial, agricultural and sewage) has led to several pathological changes in different fish organs **(Ibrahim *et al.*, 2009; Zaghloul *et al.*, 2011; Abou El-Gheit *et al.*, 2012; Tayel *et al.*, 2013)**.

The present study was organized to determine the seasonal variations of water heavy metals and the bioaccumulation factor in gills, liver and muscle of *Solea aegyptiaca* fish collected from different sites along the Lake Qarun and to evaluate the related histological changes in these organs.

MATERIALS AND METHODS

The present work was conducted at Shakshouk Fish Research Station, El-Fayoum Governate, National Institute of Oceanography and Fisheries, Egypt, during the period of study.

1. Samples collection

Water samples were collected from the studied sites during winter (from January to March) and summer (from July to September) for the year 2019. Fish samples were collected from the same sites where the water samples were taken as follows (Fig. 1):

Site (1): western sector of the lake; a relatively unpolluted area where no drainage water was recognized.

Site (2): northern sector of the lake, and lies northern of El-Qarn Island far from drainage water.

Site (3): midpoint of the southern sector, and lies near the mouth of El-Wadi drainage channel.

Site (4): eastern sector of the lake, and lies near the mouth of El-Bats drainage channel.

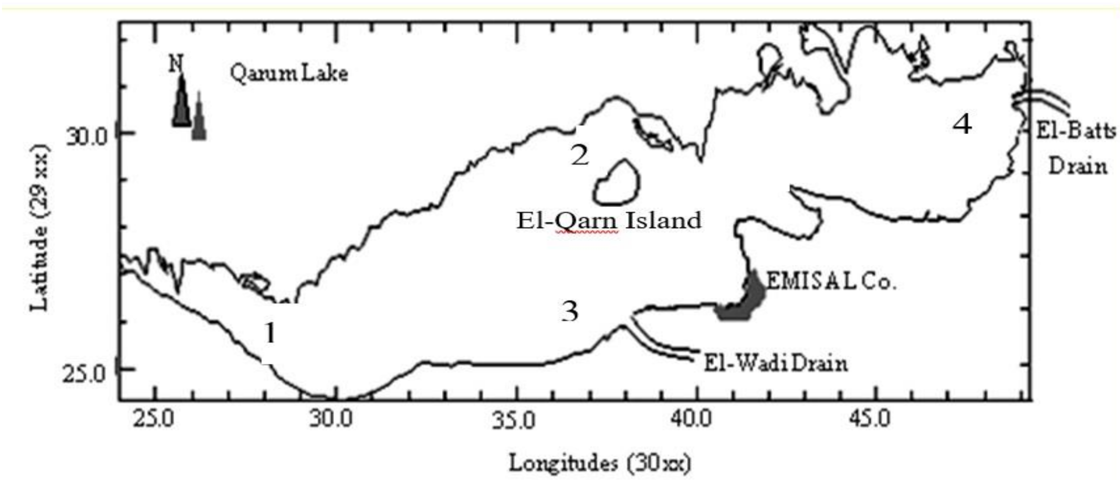

Fig. 1. A map of Lake Qarun showing the studied sites.

Water sampling

Surface water samples were collected from the sampling sites (n=3/ site) along the Lake Qarun, approximately 50 cm below the surface water, in polyethylene bottles (washed with detergent, then with deionized water, 2 M nitric acid (Merch), then deionized water again and finally surface water). Samples were filtered in the field and acidified with 10% HNO3 for preservation, placed in an ice bath and brought to the laboratory. The samples were filtered through a 0.45 µm micropore membrane filter and kept at -20ºC until analysis.

Fish sampling

Specimens of *Solea agyptiaca* were collected during winter and summer seasons from the four studied sites along Lake Qarun at El-Fayoum region; the samples were caught by fishermen's nets, and then transported to the laboratory. Then, each sample collected was dissected for its gill, liver and muscle tissues; muscle tissue was removed from the left side of the fish between the pelvic and dorsal fins. Tissue samples were dried in an oven at 105°C (about 5 g of fresh fish tissue) for 48 hours and then grounded to a fine powder for residual heavy metal determination. Additionally, sections of the gill, liver and muscle of each group were immediately excised and fixed in 10% formalin for histopathological examination.

2. Heavy metal analysis

Heavy metal concentrations in water (iron, copper, zinc, cadmium, lead and nickel) were determined by using Inductively Coupled Plasma Emission Spectrometer (ICP) (ICAP- 6300 Duo), according to **APHA (2012)**. The results were expressed in mg/1.

The dried samples (gills, liver and muscle) were digested according to the method of **Ghazally (1988)**, then the concentrations of Fe, Zn, Cu, cd, Pb and Ni in gills, liver and muscle were measured following the same methods used for water analysis. The results were expressed in mg/kg dry weight.

Bioaccumulation factor (BAF) is the ratio of the contaminant in an organism to the concentration in the ambient environment at a steady state **(U.S.EPA, 2010)**. Bioaccumulation of heavy metals was calculated in the different vital tissues (gills, liver and muscle) of *Solea agyptiaca* according to **Arnot and Gobas (2006)** using the following equation:

BAF= Metal concentration in the organ (mg/kg)/ Metal concentration in water (mg/1).

3. Histological examination

The fixed tissues (gills, liver and muscle) were dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sagittal sections were cut at 4 to 6 microns and at least 10 slides from each region were prepared. These were stained with Harri's haematoxylin and subsequently countered stain with eosin. Finally, the slides were microscopically examined to identify the histo-pathological features, then photographed using camera mounted on light microscope, and hence described **(Delafield, 1984)**.

Statistical analysis

The data were analyzed by general linear model using SPSS Statistical Package Program **(SPSS, 2015)** version 23. Means were compared by Duncan multiple range test when the differences were significant **(Duncan, 1955)**. Level of significance in all tests was $P \leq 0.05$. The results were expressed as means ± standard error (SE).

RESULTS

1. Heavy metals in water

The results of heavy metals concentration in water samples in the selected sites during winter and summer and their permissible limits were determined according to **WHO (1993)** and are presented in Table (1). The present study revealed that, the values of Fe ranged between 0.260 and 0.015 mg/l. They were higher in the eastern sector during summer and lower in the northern sector during winter. The results of the Cu concentration showed the highest value in the southern sector during summer (0.050

mg/l) with the lowest values in the northern and western sectors during winter and summer seasons. Regarding the Zn concentration; the results showed that the highest value (0.066 mg/l) was recorded during summer in the eastern sector, while the lowest value was detected during winter in the northern (0.003 mg/l) sector. Concerning Cd concentration; results indicated that the highest value was recorded in the eastern sector during winter (0.005 mg/l), while the lowest values were recorded in the western sector during summer (0.0001 mg/l). The values of Pb ranged between (0.114 and 0.014 mg/l) with the higher level in the eastern sector during summer and the lower in the western sector during winter. Finally, results showed that the maximal value of nickel concentration was recorded in the southern sector during winter (0.130 mg/l) and the minimal value was obtained during summer in the western sector (0.031 mg/l).

The present results revealed that the average concentration levels of Fe, Cu, Zn and Cd in the lake water were below the permissible limits of **WHO (1993)**, except for Pb during summer and Ni during winter and summer seasons.

Table 1. Concentration of heavy metals in water samples (mg/l) collected from Lake Qarun during winter and summer seasons, 2019. Each value is mean of replicates ± standard error (S.E) of means.

Seasons	Sites	Iron (mg/l)	Copper (mg/l)	Zinc (mg/l)	Cadmium (mg/l)	Lead (mg/l)	Nickel (mg/l)
Winter	Western sector	0.019±0.004 e	0.009±0.001 d	0.005±0.0004 d	0.0005±0.0002 de	0.014±0.001 f	0.058±0.007 D
	Northern sector	0.015±0.005 e	0.015±0.002 d	0.003±0.0004 d	0.0006±0.0002 de	0.018±0.003 f	0.078±0.005 C
	Southern sector	0.065±0.002 d	0.026±0.002 c	0.017±0.001 c	0.001±0.0004 d	0.037±0.002 e	0.130±0.011 A
	Eastern sector	0.088±0.004 cd	0.034±0.006 b	0.015±0.001 c	0.005±0.0004 a	0.038±0.003 e	0.115±0.005 Ab
Summer	Western sector	0.095±0.003 cd	0.010±.0.001 d	0.051±0.0004 b	0.0001±0.00001 e	0.055±0.002 d	0.031±0.002 E
	Northern sector	0.111±0.006 c	0.011±0.0004 d	0.054±0.002 b	0.0004±0.0004 de	0.071±0.002 c	0.033±0.002 E
	Southern sector	0.201±0.023 b	0.050±0.001 a	0.064±0.002 a	0.002±0.0002 c	0.105±0.003 b	0.083±0.003 C
	Eastern sector	0.260±0.029 a	0.041±0.002 b	0.066±0.003 a	0.004±0.0003 b	0.114±0.003 a	0.100±0.006
	P value	** 0.000	** 0.000	** 0.000	** 0.000	** 0.000	** 0.000
	P.L. WHO, (1993)	---	2	5	0.01	0.05	0.02

Values with different letters in column have a statistically significant difference at P ≤0.05. P.L. = Permissible level.

2. Bioaccumulation factor (BAF)

The results of the bioaccumulation factor (BAF) of the studied heavy metals (Fe, Cu, Zn, Pb, Cd and Ni) in gills, liver and muscle of *Solea aegyptiaca* fish collected from different studied sectors along the Lake Qarun during winter and summer are presented in Tables (2, 3 and 4), respectively. The concentration of the analyzed metals in the gills, liver and muscle of the analyzed fish were higher than their concentrations in water and followed a descending order of Zn> Fe> Cu> Cd> Ni> Pb. The current results indicated that the calculated BAFs of Zn, pb and Ni metals had favorable bioaccumulation pattern in gill tissues (6656.063, 24.002 and 67.366, respectively), while Fe, Cu and Cd were mainly higher in liver being 8576.385, 2622.037 and 433.708, respectively. Meanwhile, the lowest bioaccumulation values of all studied metals were recorded in muscle tissues.

Bioaccumulation factor of heavy metals in gills

Table 2. Bioaccumulation factor (BAF) of heavy metals (Fe, Cu, Zn, Cd, Pb and Ni) in gills of *Solea aegyptiaca* from the studied sites in winter and summer, 2019.

Seasons	Sites	Iron	Copper	Zinc	Cadmium	Lead	Nickel
Winter	**Western sector**	7200.000	560.000	12517.400	80.000	28.571	52.586
	Northern sector	10252.867	318.200	17615.667	105.000	30.000	40.769
	Southern sector	4487.800	400.385	6601.941	263.000	36.568	41.308
	Eastern ector	3935.602	320.794	8332.467	69.400	39.289	42.087
Summer	**Western sector**	2578.105	207.400	1927.647	440.000	5.509	105.806
	Northern sector	2496.667	271.091	2015.185	310.000	10.324	117.606
	Southern sector	1726.950	289.460	2095.578	216.500	20.143	73.807
	Eastern sector	1632.380	266.585	2142.621	115.000	21.614	64.960
Mean		**4288.796**	**329.239**	**6656.063**	**199.863**	**24.002**	**67.366**

Bioaccumulation factor of heavy metals in liver

Table 3. Bioaccumulation factor (BAF) of heavy metals (Fe, Cu, Zn, Cd, Pb and Ni) in liver of *Solea aegyptiaca* from the studied sites in winter and summer, 2019.

Seasons	Sites	Iron	Copper	Zinc	Cadmium	Lead	Nickel
Winter	**Western sector**	18617.526	2604.444	6197.400	266.000	20.000	51.948
	Northern sector	22793.333	1710.200	15052.333	271.667	25.944	49.449
	Southern sector	9508.462	1754.500	4290.176	270.000	22.892	38.023
	Eastern sector	5728.784	1725.000	6324.000	76.000	27.816	52.869

Summer	**Western sector**	3672.000	4997.000	1117.314	1650.000	5.709	29.839
	Northern sector	3234.504	4927.273	1164.444	577.500	9.155	104.909
	Southern sector	2468.308	1603.660	1323.281	212.500	12.029	64.939
	Eastern sector	2588.165	1654.219	1579.242	146.000	12.368	59.100
Mean		**8576.385**	**2622.037**	**4631.024**	**433.708**	**16.989**	**56.384**

Bioaccumulation factor of heavy metals in muscle

Table 4. Bioaccumulation factor (BAF) of heavy metals (Fe, Cu, Zn, Cd, Pb and Ni) in muscle of *Solea aegyptiaca* from the studied sites in winter and summer, 2019.

Seasons	Sites	Iron	Copper	Zinc	Cadmium	Lead	Nickel
Winter	**Western sector**	1773.316	360.778	4042.600	134.000	17.143	13.328
	Northern sector	3150.200	348.467	8040.000	155.000	31.111	15.295
	Southern sector	2102.569	412.308	4025.882	160.000	21.622	21.638
	Eastern sector	1263.784	250.588	4858.667	42.600	22.447	27.713
Summer	**Western sector**	487.053	262.100	851.176	910.000	2.600	40.323
	Northern sector	493.694	253.909	900.370	290.000	4.268	44.545
	Southern sector	596.269	224.460	1059.688	97.000	9.486	36.446
	Eastern sector	584.165	197.634	1504.394	73.750	9.518	34.300
Mean		**1306.381**	**288.781**	**3160.347**	**232.794**	**14.774**	**29.199**

3. Histological observations

Histopathological observations of gills: gill sections of fish collected from **the western** sector of the lake (Fig. 2) **(A)** during winter and **(B)** during summer, showed nearly normal structure of primary (PL) and secondary lamellae (SL) and wide water channel (WC). While, the gill sections of fish collected from **the northern** sector of the lake (Fig. 2) **(C)** during winter and **(D)** during summer, revealed moderate case of hyperplasia (HP), moderate fusion of secondary lamellae (F) and bending or curling of some secondary lamellae (Cr). Moreover, the gill sections of fish collected from **the southern** sector of the lake exposed to effluents discharged directly from El-Wadi drainage channel (Fig. 2) **(E)** during winter and **(F)** during summer, showed clear histopathological changes including severe hyperplasia of epithelial cells (HP), complete fusion of secondary lamellae (F), deposition of hemosiderin granules (Hn) and rupture of pillar cells and capillaries that led to ballooned like lamellae packed with erythrocytes or lamellar telangiectasis (LT) at the tips of secondary lamellae. Furthermore, the gill sections of fish collected from **the eastern** sector of the lake exposed to effluents discharged directly from El -Bats drainage channel (Fig. 2) **(G)** during winter and **(H)** during summer, exhibited clear histopathological changes which included severe hyperplasia of epithelial cells (HP), vasodilation with blood congestion (BC) and complete degeneration or destruction of primary lamellae and secondary lamellae (D) with focal necrosis (N) in most areas of gill filament resulting in disorganization of the normal gill structure.

Histopathological observations of liver: sections of fish liver from **the western** sector of the lake (Fig. 3) **(A)** during winter and **(B)** during summer, showed normal structure of central vein (CV) and hepatic cells (HC). Regarding liver sections of fish collected from **the northern** sector of the lake (Fig. 3) **((C)** during winter and **(D)** during summer, there was moderate histopathological changes which include congested blood vessels (CBV), edema between liver cells (E) and appearance of vacuolar structures (V) in hepatic cells. On the other hand, liver samples obtained from fish inhabiting **the southern** sector of the lake exposed to effluents discharged directly from El-Wadi drainage channel (Fig. 3) **(E)** during winter and **(F)** during, revealed marked histopathological changes which included cytoplasmic vacuolization of the hepatocytes or oil droplets appeared (OD), thick-walled blood vessel (BV), highly infiltration of lymphocytes around degenerated blood vessel and hemosiderin deposition (Hn), congested blood vessels in central vein (CBV) and some necrotic hepatocytes (N) also recorded. Furthermore, liver sections of fish collected from **the eastern** sector of the lake that exposed to effluents discharged directly from El -Bats drainage channel (Fig. 3) **(G)** during winter and **(H)** during summer, showed severe damage represented as necrotic areas encapsulated by fibrous tissue in central vein and leukocytes forming neoplasia cyst (NC), multivacuolation (V) with disoriented hepatic tissue, necrotic areas (N), severe hemorrhage in central vein (H), blood hemolysis, widened blood sinusoid (WS) and distortion of both pancreatic acini and blood vessels were also detected.

Histopathological observations of the muscles: the muscle of fish collected from **the western** sector of the lake (Fig. 4) **(A)** during winter and **(B)** during summer, show normal muscle structure with mild edema between muscle fibers (E). Muscle sections of fish collected from **the northern** sector of the lake (Fig. 4) **(C)** during winter and **(D)** during summer, exhibited histopathological changes which included vacuolar degeneration (VD) and necrosis (N) of muscle cells. Concerning the muscle of fish inhabiting **the southern** sector of the lake exposed to effluents discharged directly from El-Wadi drainage channel (Fig. 4) **(E)** during winter and **(F)** during summer, there was marked histopathological changes which included severe edema between the muscle fibers (E), atrophy and disruption of muscle fibers (DMF). Moreover, sections of fish muscle from **the eastern** sector of the lake exposed to effluents discharged directly from El -Bats drainage channel (Fig. 4) **(G)** during winter and **(H)** during summer, detected severe damage represented as severe deposition of hemosiderin granules between the muscle fibers (Hn) and disorganization of the normal muscle structure.

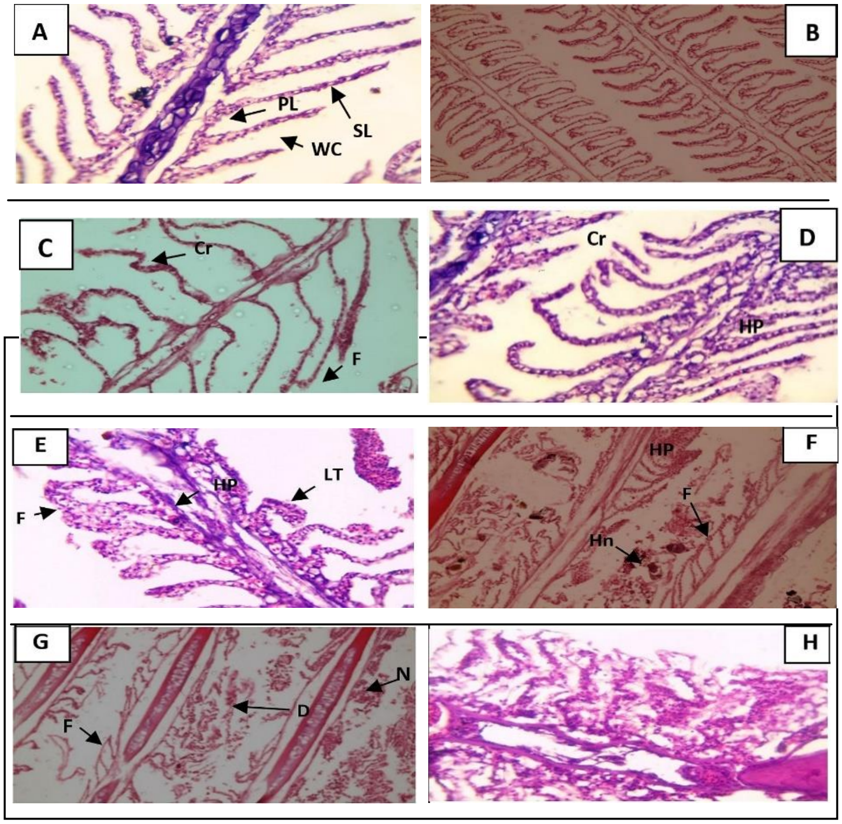

Fig. 2. Photomicrograph sections in gills of *Solea aegyptiaca* fish collected from different sites along Lake Qarun. **Western** sector of the lake during winter **(A)** and summer **(B)**; **Northern** sector of the lake during winter **(C)** and summer **(D)**; **Southern** sector of the lake (exposed to El-Wadi drain) during winter **(E)** and summer **(F)**; **Eastern** sector of the lake (exposed to El-Bats drain) during winter **(G)** and summer **(H)**.

[PL, Primary lamella; SL, Secondary lamella; WC, Wide water channel; HP, Hyperplasia; BC, Blood congestion; D, Destruction of primary lamellae and secondary lamellae; N, Necrosis; F, Fusion of secondary lamellae; Hn, hemosiderin granules; Cr, Curling of secondary lamellae and LT, Lamellar telangiectasis].

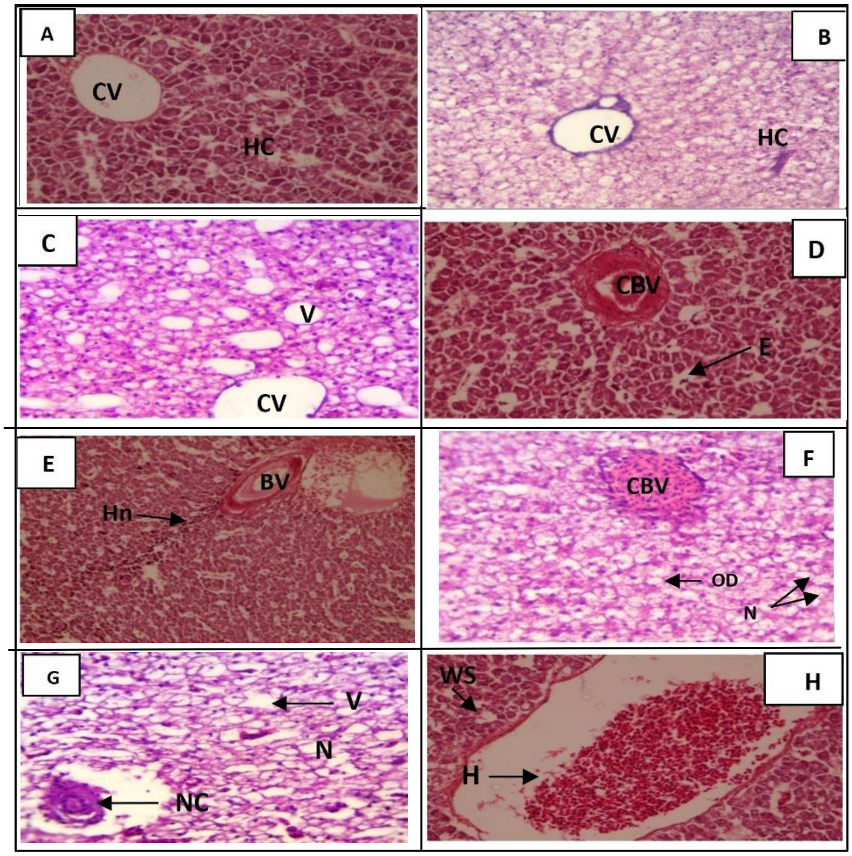

Fig. 3. Photomicrograph sections in livers of *Solea aegyptiaca* fish collected from different sites along Lake Qarun. **Western** sector of the lake during winter **(A)** and summer **(B)**; **Northern** sector of the lake during winter **(C)** and summer **(D)**; **Southern** sector of the lake (exposed to El-Wadi drain) during winter **(E)** and summer **(F)**; **Eastern** sector of the lake (exposed to El-Bats drain) during winter **(G)** and summer **(H)**.

[CV, central vein; HC, Hepatic cells; N, necrotic area; OD, oil droplet; V, vacuoles; NC, neoplasia cyst; H, Hemorrhage, WS, Widened blood sinusoid; CBV, congested blood vessel; Hn, hemosiderin deposition; E, Edema and V, Vacuoles].

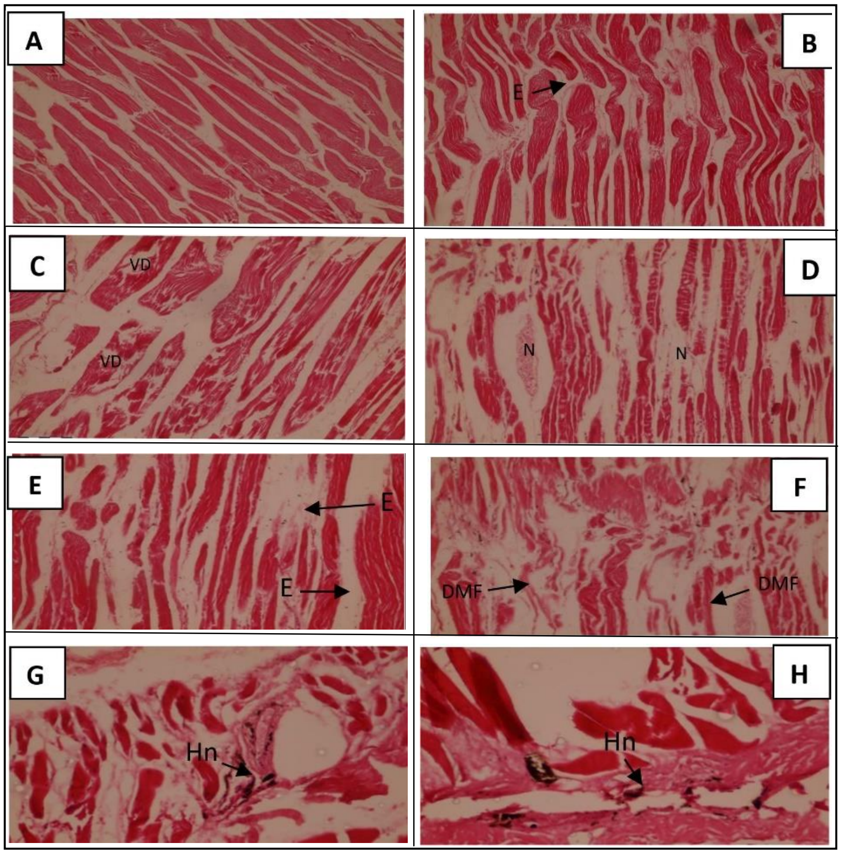

Fig. 4. Photomicrograph sections in muscles of *Solea aegyptiaca* fish collected from different sites along Lake Qarun. **Western** sector of the lake during winter **(A)** and summer **(B)**; **Northern** sector of the lake during winter **(C)** and summer **(D)**; **Southern** sector of the lake (exposed to El-Wadi drain) during winter **(E)** and summer **(F)**; **Eastern** sector of the lake (exposed to El-Bats drain) during winter **(G)** and summer **(H)**.
[E, Edema; DMF, Disruption of muscle fibers; VD, Vacuolar degeneration; N, Necrosis and Hn, Hemosiderin deposits].

DISCUSSION

The presence of trace metals in the Lake Qarun is mainly of allochthonous origin due to either agricultural influx, wastes of fish farms or sewage via the surrounding cultivated lands **(Ali & Fishar, 2005)**. The distribution of heavy metals showed irregular patterns in the lake as a result of interference between several factors such as surrounding environment, closed basin and climatic effects **(Abdel-satar *et al.*, 2010)**. The present results revealed that metal concentrations followed a descending order of Fe > Pb ≅ Ni > Zn > Cu > Cd. The generally higher concentration of Fe is related to the abundance of Fe-bearing minerals **(Moalla *et al.*, 1998)** from the Tertiary basalt sheets that surrounds the Lake Qarun and the lacustrine deposits **(Metwaly *et al.*, 2010; Abdel Wahed *et al.*, 2015; El-Sayed *et al.*, 2015)**.

A relative increase was recorded in the concentrations of all the studied metals (Fe, Cu, Zn, Pb, Cd and Ni) in the eastern and southern sectors facing the outlet of the drains.

These results are coincided with those of many investigators **(Ali & Fishar, 2005; Hussein *et al.*, 2008; Abdel-satar *et al.*, 2010; Abou El-Gheit *et al.*, 2012; Abdel Wahed *et al.*, 2015; Goher *et al.*, 2018; Mohamed *et al.*, 2019)**. **Elghobashy *et al.*, (2001)** attributed the increase of heavy metals in drainage water to the decomposition of the organic matter and/or the use of fertilizers and other chemicals in agriculture. Thus, anthropogenic influences, rather than natural enrichment of water by metals may be the main reasons **(Wasim Aktar *et al.*, 2010)**.

In the Qarun Lake water, all metals exhibited their highest values during summer season. This may be due to the metal liberation from the bottom sediment to the water column by the degradation of organic matter under the effect of the high temperature and the microbial activity **(Goher *et al.*, 2014)**. In addition, the drainage water inflow to the lake during winter is higher than the water lost by evaporation, thus possibly increasing the lake water level and decreasing the heavy metal concentrations **(Abdel Wahed *et al.*, 2014)**. These results are in accordance with thaose obtained by **Redwan and Elhaddad (2016)**, **Goher *et al.* (2018)** and **Mohamed *et al.* (2019)**.

The bioaccumulation factor is evaluated in relation to the concentration of the aqueous metal at which the studied fish inhabits. Accumulation of metals in aquatic biota includes complicated relation between exogenous and endogenous factors, such as bioavailability of metal, physicochemical characteristics of surrounding water, species, age and physiological status **(Moiseenko & Kudryavtseva, 2001)**. In the present study, the nonessential metals (Pb, Cd and Ni) are bioaccumulated with less efficiency compared to the high bioaccumulation efficiency of essential metals (Fe, Cu and Zn) in the various tissues. The relatively higher BAF of essential elements may be due to their role as an activator of numerous enzymes present in fish **(Uluturhan & Kucuksezgin, 2007)**. Gills and liver tissues were witnessed to be active bioaccumulators for most metals since these tissues have a considerable mass in which the accumulated metals may be detoxified, regulated or excreted **(Reinfelder *et al.*, 1998)**. These results concur with those of **Jayaprakash *et al.* (2015)** and **Abdel-Khalek *et al.* (2016)** as they showed that the highest BAF values of Ni, Pb, Mn, Co, Cr, Fe, Cu and Zn were observed in the gills and liver tissues.

Gills could be important as a site of direct metal uptake from water **(Storelli *et al.*, 2006)**. High metal concentrations in gills can point out water as the main source of contamination **(Bervoets & Blust, 2003)**. The present findings affirmed that gill tissues bioaccumulate higher amounts of Zn, pb and Ni in comparison to other studied organs. This may be due to the MTs binding proteins produced as a result of metals' interaction in gill uptake sites and involved in the process of biotransformation and defense pathways. This process plays an important role in the protection of tissues from damage by heavy metal toxicants **(Di Giulio & Hinton, 2008; Saeed & Shaker, 2008)**. **Reid and Mcdonald (1991)** reported that, the gill surface is negatively charged and thus provides a potential site for gill-metal interaction for positively charged metals.

Meanwhile, the MTs are able to form stable chelates with Pb **(Sharif *et al.*, 1993)**. The detected highest Pb bioaccumulation in gill tissues are in line with the findings of **Ureña *et al.* (2007)** and **Zaghloul *et al.* (2011)**. Additionally, **Palermo *et al.* (2015)** observed increased MT levels in the gills after exposure to Ni, implying that the presence of Ni in these tissues could induce MT synthesis. Moreover, **Hogstrand *et al.* (2011)** reported that the main target of waterborne Zn toxicity are the gills.

The liver is the main metabolic organ, the amount of pollutants in fish liver is directly proportional to the degree of pollution in the aquatic environment **(Yılmaz, 2009; Tapia *et al.*, 2012)**. Many experimental and field studies showed that liver was the target organ for the accumulation of many metals and was highly active in the uptake and storage of metals because of its role in the storage, redistribution, detoxification, transformation of contaminants and the high induction rate of metallothioneins (MTs) in the liver **(Dural *et al.*, 2007; Yılmaz *et al.*, 2010)**. In this context, liver has been recommended by many authors as the best environmental indicator of both the water pollution and chronic exposure to heavy metals **(Agah *et al.*, 2009; Messaoudi *et al.*, 2009)**. The haemopoietic functions of fish liver with abundant blood supply explain its higher accumulation of metals **(Mohamed, 2008)**.

The current study revealed that the highest bioaccumulation of Fe, Cu and Cd were in liver tissues. These results are supported by **Javed and Usmani (2012)**, **Omar *et al.* (2014)** and **Elwasify *et al.* (2021)** who reported that, liver has Fe and Cu accumulation properties. Moreover, the present result is in agreement with that of **Kaoud and El-Dahshan (2010)** stating that, the detected high accumulation of cadmium in fish liver might be due to its strong binding with cystine residues of metallothionein. **Yılmaz *et al.* (2007)** reported that gills and liver tissues of the marine fish, *Leuciscus cephalus* and *Lepornis gibbosus* accumulated the highest amount of Cd, whereas the lowest level was recorded in the muscle tissues.

Muscles are often examined for metal content due to their use for animal and human consumption **(Alibabic *et al.*, 2007)**. The low heavy metals bioaccumulation in fish muscle may be related to the increase of metal deposition in the metabolically active organs such as liver, kidney and gill **(Pyle *et al.*, 2005)**. **Rauf *et al.* (2009)** showed that the low concentration of metals in fish muscle may reflect the low levels of the MTs binding proteins in the muscle.

Bioaccumulation of metals may lead to high mortality rate or cause many biochemical and histological alterations in the survived fish **(Rashed, 2001a, 2001b; Soltan *et al.*, 2005)**. Microscopic examination of target tissues is an important end point in the evaluation of toxic potential and risk assessment of chemicals in the environment **(Velma & Tchounwou, 2010)**. In addition, it is crucial for the discrimination between different polluted sites **(Dietze *et al.*, 2001)**. Sections of gills, liver and muscle of *Solea aegyptiaca* collected from the less polluted northern site of the lake during winter and summer showed much more improvement than samples collected from other sites. This may be attributed to the low heavy metal concentrations in water (as low agricultural discharge) also due to the better water quality. However, histopathological alternations and clear damage were obvious in gills, livers and muscle of fish collected from the eastern and southern sites exposed to heavy metal pollution from El -Bats and El-Wadi drainage channels at these sites which were generally in accordance with the results of the chemical analysis of water heavy metals. This suggests too slow defence mechanisms in these tissues to immobilize or eliminate heavy metals and shows the sensitivity of fish cells to metal exposure **(Mela *et al.*, 2007; Omar *et al.*, 2013)**.

Gills are directly affected by contaminants owing to their direct and continuous contact with the external medium and their functions in respiratory gas exchange, osmoregulation, excretion of nitrogenous waste and acid-base regulation **(Bhagwant & Elahee, 2002)**. The present study showed clear histopathological changes of gills of

Solea aegyptiaca collected from the Lake Qarun during winter and summer summarized in severe hyperplasia of epithelial cells, fusion of secondary lamellae, bending or curling of some secondary gill lamellae, vasodilation with blood congestion. Additionally, histopathological changes exhibited degeneration of primary and secondary lamellae with focal necrosis in most areas of gill filament, deposition of hemosiderin granules, and rupture of pillar cells and capillaries that led to ballooned like lamellae packed with erythrocytes or lamellar telangiectasis at the tips of secondary lamellae. These observations are similar to those obtained by **Ibrahim and Tayel (2005)**, **Yacoub *et al.* (2008)**, **Ibrahim *et al.* (2009)**, **Abou El-Gheit *et al.* (2012)** and **Tayel *et al.* (2013)**, who revealed these malformations in gills to increase of ammonia and heavy metals, pH change, oxygen depletion, occurrence of microorganisms and increase turbidity in water polluted by sewage and agricultural discharge in the Lake Qarun.

The intralamellar hyperplasia is a consequence of excess mucus production. Penetration of contaminants activates the secondary lamellae epithelium to increase the number of mucus cells. Hyperplasia with excess mucus lamellae causes fusion, which reduces the surface area of the gills and thus affects the ability of the gas exchange **(Kumari *et al.*, 2012)**. Decreased dissolved oxygen levels are associated with an increased Melanomacrophage centers number **(Fournie *et al.*, 2001)** with high haemosiderin content **(Agius & Roberts, 2003)** and resulting in deposition of hemosiderin in gills. On the other hand, toxic environmental conditions can result in several structural changes, which appear to be a compensatory mechanism to increase the epithelial thickness, thus preventing entry of toxic ions into the bloodstream or to compensate for osmotic imbalance **(Velcheva *et al.*, 2010)**. The observed lifting and swelling of the lamellar epithelium mainly develop with the purpose to increase the distance across which waterborne irritants can diffuse to reach the bloodstream **(Mallatt, 1985)**. Ballooning dilatation in gill filament is considered an ion trap that acts to concentrate trace metals and favor cell adhesion between neighboring secondary lamellae **(Bhagwant & Elahee, 2002)**.

The liver receives particular attention in toxicological investigations of different fish species due to its high metabolic activity **(Velcheva *et al.*, 2010)**, where one of the important functions of the liver is to clean any poison or pollutant from the blood coming from the intestine **(El-Naggar *et al.*, 2009)**. It is also one of the organs most affected by contaminants in the water **(Rodrigues & Fanta, 1998)**. Any damage to the fish liver ultimately leads to multiple physiological disorders and subsequent fish death **(Mahboob *et al.*, 2020)**. The present study revealed clear histopathological changes of liver of *Solea aegyptiaca* collected from the Lake Qarun including severe congested blood vessels, cytoplasmic vacuolization with disoriented hepatic tissue, blood hemolysis, widened blood sinusoid, lymphocytes infiltration, hemosiderin deposition and some necrotic hepatocytes. These results agree with those obtained by **Yacoub *et al.* (2008); El-Naggar *et al.* (2009); Zaghloul *et al.* (2011)** and **Abou El-Gheit *et al.* (2012)** who found the same observations with respect to different fish species collected from the Lake Qarun. **Tayel *et al.* (2013)** suggested a strong link between water quality changes and observed liver lesions in fish inhabiting the water of the Lake Qarun, they postulated that these lesions may be due to oxygen depletion, parasitic infection, elevation in ammonia and heavy metals.

Degeneration and necrosis of hepatocytes may be due to the accumulation of heavy metals. This is consistent with the findings of **Authman and Abbas (2007)** who stated that the liver is involved in a detoxification of toxins such as heavy metals. Accumulation of hemosiderin in liver cells may be contributed to the rapid and continuous destruction of the red blood cells **(Ibrahim & Mahmoud, 2005; Tayel *et al.*, 2018; Hashem *et al.*, 2020)**. Degeneration of hepatocytes can be caused by oxygen deficiency due to intravascular hemolysis and vascular dilation **(Gaber & Gaber, 2006)**. Toxins secreted by microorganisms in sewage water may cause necrosis and hemorrhage **(Saad *et al.*, 2011)**. Fatty degeneration can be caused by an increased rate of utilization of energy reserves or an induced imbalance between fat utilization and production **(El-Naggar *et al.*, 2009)**. It is worth noting that, the liver is an organ that excretes and binds proteins such as metallothionein. metal-binding proteins, which are present in the nuclei of hepatocytes and increase the cell damage **(Mela *et al.*, 2007)**. These histopathological changes suggest high metabolic activity in hepatocytes in response to the uptake of heavy metals **(Thophon *et al.*, 2003)**.

The muscular system constitutes the largest part of the fish body. Its overall functions include locomotion, pumping of blood, synchronized movement of skeletal components, peristaltic constriction of visceral organs and their related structures (**El-Serafy *et al.*, 2005; Tayel, 2007; Kadry *et al.*, 2015)**. Muscles which are mainly composed of segmental myomeres are covered with skin. Each myomere is regarded as a muscle and its fibers are parallel along the body axis **(Bayomy & Tayel, 2007; Yacoub *et al.*, 2008)**. The results of histopathological examination of *Solea aegyptiaca* muscles indicate hemosiderin deposition and edema between muscle fibers, atrophy and disruption of muscle fibers, vacuolar degeneration and necrosis of muscle bundles. These results are in agreement with those observed by many investigators who have studied the effects of different pollutants on fish muscles **(El-Serafy *et al.*, 2005; Sitohy *et al.*, 2006; Ahmed, 2007; Yacoub *et al.*, 2008; Saad *et al.*, 2012; Tayel *et al.*, 2013; Yacoub *et al.*, 2021)**. These alterations in muscles may be attributed to inorganic fertilizers **(Mahmoud, 2002)**, ammonia **(Tayel, 2003)**, heavy metals **(Sitohy *et al.*, 2006; Ibrahim, 2007; Yacoub *et al.*, 2008)**, parasitic infection **(Mahmoud & El-Naggar, 2007)** and changes in water quality **(Saad *et al.*, 2011; Abou El-Gheit *et al.*, 2012)**.

CONCLUSION

This study confirms previous reports on the increased heavy metal pollution in the eastern and southern sectors of the Lake Qarun, facing the outlet of the drains, as a result of discharging the drainage water effluents without prior treatment, which directly affect water quality and fish health and consequently affect human health. Pb and Ni concentrations in the water were higher than the permissible limits. Distribution of heavy metals in tissues varies with respect to different metals and organs even of the same organism. In *Solea aegyptiaca*, the gills act as the target organ for zinc, lead and nickel while iron, copper and cadmium prefer to accumulate specifically in the liver. Furthermore, the use of histological alternations as biomarkers of the deleterious effects of heavy metal bioaccumulation in *Solea aegyptiaca* organs are very realistic approaches. It is recommended to treat wastewater before entrance to the Lake Qarun or stopping the discharge of these pollutants into the lake to protect fish and human from the negative effects of pollution. Therefore, further monitoring programs should be conducted.

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ARABIC SUMMARY

التراكم الحيوي للمعادن الثقيلة والتغيرات النسيجيه المصاحبه في الخياشيم والكبد والعضلات من أسماك موسى القاطنه لبحيرة قارون، مصر.

أسماء محمد العجري[1], منال عاصم امام[2], حنان سيد جابر[1], اكرامى أحمد حسن[2] و سها محمد حمدى[3]

1 قسم التلوث، المعهد القومى لعلوم البحار والمصايد، مصر.

2 قسم الكيمياء الحيوية،جامعة عين شمس، مصر

3 قسم الكيمياء ،جامعة الفيوم، مصر.

أصبح تلوث بحيرة قارون بالمعادن الثقيلة مصدر قلق أكبر خلال السنوات الأخيرة لأنه يؤثر على الخصائص الفيزيائية- الكيميائية للمياه والرواسب والمكونات البيولوجية وقد يلحق الضرر بالكائنات المائية. تتلقى بحيرة قارون مياه الصرف الزراعي ومياه الصرف الصحي المحملة بالمعادن الثقيلة من خلال مصرفين رئيسيين هما مصرفا البطس والوادي، وبالتالي هناك زيادات سنوية في تراكم الملوثات والمعادن الثقيلة بجميع مكوناتها (مثل المياه والرواسب والأسماك). تهدف هذه الدراسة الى تقييم التباين الموسمى لمحتويات المعادن الثقيلة (الحديد، النحاس، الزنك، الكادميوم، الرصاص والنيكل) في الماء ومعامل التراكم الحيوى لهذه المعادن الثقيلة في عينات أسماك موسى، التي تم تجمعيعها من بحيرة قارون، وتوفير معلومات عن التغيرات النسيجية في الخياشيم والكبد و العضلات. وقد أجريت هذه الدراسة على طول بحيرة قارون في أربعة مواقع (الشرق والجنوب والشمال والغرب) خلال موسمي الشتاء والصيف لعام 2019م. وكشفت النتائج أن الصيف كان أعلى في تركيز المعادن الثقيلة في الماء من فصل الشتاء. وبالإضافة إلى ذلك، أظهر القطاعان الشرقي والجنوبي زيادة في تركيز المعادن الثقيلة في المياه. وفيما يتعلق بعينات الأسماك، أظهرت هذه الدراسة أن أعلي قيم لمعامل التراكم االحيوى تم تسجيلها في الخياشيم والكبد وأقل قيم في العضلات. سجلت خياشيم اسماك موسى نسب تراكم عاليه من الزنك والرصاص والنيكل، بينما تراكم الحديد والنحاس والكادميوم بشكل اساسى فى الكبد. وأظهرت النتائج تغيرات نسيجيه وتلف واضح في الخياشيم والكبد والعضلات لأسماك موسى التي تم تجميعها من القطاعين الشرقي والجنوبي من بحيرة قارون.