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Effect of bioaccumulation and biosedimentation of some heavy metals on histological features in the cichlid fish, *Tilapia zillii* inhabiting Lake Qarun, Egypt.

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ABSTRACT

The present study was aimed to investigate the bioaccumulation and biosedimentation factors of some heavy metals in *Tilapia zillii* inhabiting Lake Qarun, Egypt during the year, 2015- 2016. Water, sediments and fish samples were collected during the period of study. The present study exhibited that, the higher levels of bioaccumulation and biosedimentation factors were recorded in liver samples except for Mn; gills showed their high accumulation while the lower levels of all studied metals were observed in muscle samples. The results indicated the differences of some heavy metals accumulation in different tissues of fish with an increasing rate in the liver during winter and spring except for Zn that attained the higher level in the liver during autumn for bioaccumulation factor and spring for biosedimentation one. The liver of *T. zillii* accumulated higher levels of Cd, Cu, Fe and Zn than other organs. The results showed that the bioaccumulation factor of Cd, Fe and Mn in fish organs was greater than biosedimentation factor, and this implies that the fish bioaccumulated these metals from the water. While, biosedimentation factor of copper and zinc in the fishes was higher than bioaccumulation one, and this refers to the fact that the fishes bioaccumulated these metals from the sediment. Histopathologically results showed congestion in the interstitial blood vessel and multifocal necrotic areas in renal tubules. While, vacuolated hepatocytes, dilated central vein, compressed blood sinusoids, and congestion were seen in fish liver. Furthermore, intermuscular edematous areas between the muscle bundles were obvious. The increased rate of bioaccumulation, biosedimentation factors, and histopathological changes in the target organs indicated the high risk to fish health and human consumption. Thus, the current study showed that precautionary measures should be taken to prevent heavy metal pollution in the future.

INTRODUCTION

Lake Qarun constitutes one of the very important sectors in the Egyptian fisheries. It is a closed elongated marine lake with irregular shape (Figure 1). Lake Qarun contains different species of fish and is considered one of the most important wintering and nesting sites for many species of the migratory birds. It is approximately 55000 feddans (22000 hectares) or approximately 107 square kilometers **(Ghanem, 2011; Sabae & Mohamed, 2015; Ragab, 2017; Ali & Aboyadak, 2018; Bakry *et al.*, 2018; Mohamed, 2019** and **Elwasify *et al*., 2021).** It acts as a reservoir for agricultural and sewage drainage waters for El-Faiyoum Province. The drainage water discharged into the lake is highly concentrated with solids, nutrients, pesticides, heavy metals and organic matters. It resembles about 450 million cubic meters of agricultural drainage water and domestic waste water that decreases annually to attain balance by evaporation **(Mohamed, 2009; Ghanem, 2011; Mohamed, 2019; Elwasify *et al*., 2021).**

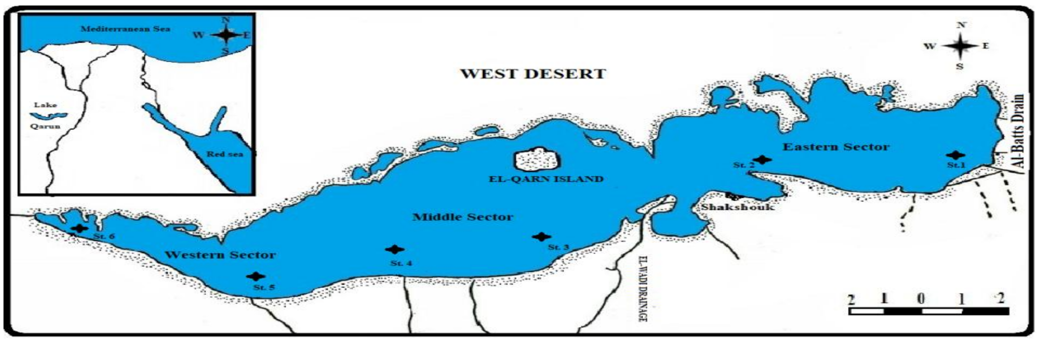

Fig. 1: A map of Lake Qarun showing the study area.

Tilapia zillii is one of the dominant fish species recorded from the lake waters **(Ghanem, 2011; Khalaf- Allah, 2001; Ragab, 2017; Mohamed, 2019)**. Fish living in polluted waters tend to accumulate different pollutants in their body. This accumulation related to species and concentration of the chemicals, the pollution degree of the environment, time of exposure, pathway of metal uptake, environmental conditions, fish age and its feeding habits and the function of metabolite organs **(Ghanem, 2006; Ghanem *et al*., 2015; Elwasify *et al*., 2021).**

The heavy metals contribute into the bodies of aquatic life including many sources; agricultural activities and fish feed transferred to the fish in two main ways, either directly through consumption of water and food through the digestive system, or indirectly through permeable membranes **(Garcia *et al*., 2016; Das *et al*., 2017** & **Hadi & Al-hamadawi, 2021)**. Notably, through feeding on polluted food, harmful substances bio-accumulate in the food chain **(Kim *et al*., 2016).** Fish are used as a good biomarker for the degree of metal pollution in aquatic ecosystems which is a higher level of food chain (**Al-SayeghPetkovšek *et al*., 2012**; **Kouamenan *et al*., 2020**) as fish may concentrate large amounts of some metals dissolved in water. Following the food chain, those chemicals can be transferred into human metabolism through consuming contaminated fish, and thus, humanbeings are reliable to serious biomagnifications and deterioration in health status (**Daviglus *et al*., 2002; Alinnor & Obiji, 2010; El-Batrawy *et al*., 2018**).

The changes in histological features of the target organs may be attributed to direct toxic effects of pollutants on edible organs **(Bernard, 2008; Mohamed & Sabae, 2015; Ibemenuga *et al*., 2019; Mohamed, 2019**; **Hadi & Al-hamadawi, 2021)**. Consequently, histological studies should determine the degree of pollution being a valuable tool to marker chronic effects of water pollution **(Pirbeigi *et al*. 2016; Hussain *et al*., 2019, 2021; Dai *et al*., 2020; Shahid *et al*., 2021).**

Many studies have demonstrated anthropogenic pollution of water and sediments by heavy metals, but the data on the bioaccumulation and biosedimentation of heavy metals in the different tissues of the studied fish was found limited **(Sarker *et al*., 2016; Adeyemi & Ugah, 2017; Maurya *et al*., 2018; Junejo *et al*., 2019; Abiyu *et al*., 2020; Sanou *et al.*, 2021)**.

Therefore, the present study was organized to determine the bioaccumulation and biosedimentation factors in some organs of the *Tilapia zillii*, as well as their effects on histological features of the kidney, liver and muscles as a bioindicator of contamination levels.

MATERIALS AND METHODS

All experiments of the present work were conducted at the laboratories of Zoology Department, Fuculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt, during the period of this study.

1. Samples collection:

Water, sediments and fish samples were collected from the lake during the period from October, 2015 to September, 2016.

Water and sediments sampling:

Ruttner bottle water sampler was used to collect surface water samples with its capacity of one/two litters. For measurement of heavy metals, samples were collected in polyethylene bottles. While, Van Veen type grab was used in the sediment sampling. After collection, the sediment samples were transferred to the laboratory in plastic bags.

Fish sampling:

A total number of 80 fish (about 20 fish/season) of the *Tilapia zillii* (Fig. 2) was seasonally collected for freshly examined or preserved for later examination. In the laboratory, fishes were identified; the total and standard lengths of each fish were measured and recorded to the nearest centimeter (cm). While, the body weight was determined to the nearest gram (g.). After dissection, a known weight of the muscles was kept under freezing condition at 4°C until the latter examinations.

Fig. 2: Lateral view of the cichlid fish, *Tilapia zillii,* collected from Lake Qarun.

2. Heavy metals analysis:

Concentrations of heavy metals in water were determined after digestion by nitric acid according to the method of **Eaton and Franson (2005)**. Concentrations of heavy metals in the sediment were determined by using the method suggested by **Oregioni and Aston (1984).** However, concentrations of heavy metals in the target organs were measured according to **APHA (1992)** to determine the correlation between the different metals during the different seasons. Concentrations of heavy metals in the water, sediment and target organs were detected after digestion for determination of the bio-accumulation and bio-sedimentation factors**.**

3. Bioaccumulation and biosedimentation factors:

In fish from the aquatic ecosystem, which includes water and sediments, the bioaccumulation factor **(BAF)** and biosedimentation factor **(BSF)** are calculated according to the following equations:

BAF= M_{tissue} (mg/kg) / M_{water} (mg/l) (Vassiliki & Konstantina, 1984)

BSF =M_{tissue} (mg/kg) / $M_{sediment}$ (mg/kg) (Evans & Engel, 1994)

Where: M_{tissue}: is the metal concentration in tissued of fish.

M_{water}: is the metal concentration in water.

$M_{sediment}$: is the metal concentration in sediment.

4. Histopathological study:

After the dissection of the fish, samples were taken from kidney, liver and muscle and immediately fixed in alcoholic Bouin`s solution for at least 24 hours, for the purpose of preparing tissue to identify pathological tissue changes by using heamatoxylin and eosin to be examined by light microscope **(Pearse, 1968).**

RESULTS AND DISCUSSION

1. Bioaccumulation and biosedimentation factors:

1.1.Bioaccumulation factor (BAF):

The presented data in Table (1) reveal that, the bioaccumulation factor for **cadmium** was at its peak during spring in the liver and declined in the muscles during autumn (0.459 and 0.072, respectively). The maximal rate of bioaccumulation factor for **copper** was detected in the liver during winter and the minimal one was determined in the muscles during autumn; being 0.313 in the former and 0.047 in the latter. It was peaked for **iron** during winter in the liver (1.282) and gills (1.193) and decreased gradually to reach its minimal value (0.211) during autumn in the muscles.

The results show that, bioaccumulation factor for **manganese** ranged between 0.011 during autumn in the muscles and 0.123 during winter in the gills. The increasing level of bioaccumulation factor for **zinc** was measured during autumn in the liver and the decreasing one was recorded during winter in the muscles; being 0.407 in the first and 0.075 in the second (Table 1).

Concentration of heavy metals in an aquatic organism depends on species, invasion pathways and chemical composition of material, metabolic characters of organs and the surrounding condition **(Ozmen *et al*., 2008; Younis *et al*., 2015; Mohamed, 2019; Elwasify *et al*., 2021)**. Experimental specimens showed several changes in gills, kidney, liver and muscle. The first barrier against toxins is gills, it decreases both the uptake of toxins and their magnification in other organs. Due to regular contact with water, gills are directly exposed to pollution (**Shahid *et al*., 2021)**.

The current work showed that, the sediments contain very high levels of some heavy metals compared to water. This difference could be explained by the tendency of metals to accumulate in sediments and biomagnify along aquatic food chains **(Yi *et al*., 2011; Kouakou *et al*., 2016; Sanou *et al*., 2021).**

The present study matches with **Ahmed *et al*. (2019)** and **Elwasify *et al*. (2021)** who found that, the bioaccumulation factors (BAFs) of some metals showed the lower level in the muscles. Level of bioaccumulation factor refer to that, metals concentrations in organisms exceeded the concentrations of metals found in the environment **(Ghanem, 2019**; **Sanou *et al*., 2021).**

The present data indicates that, the difference in the levels of bioaccumulation in different organs of fish may be due to the physiological role of edible organ with its regulatory ability and feeding behavior of the organism which play a significant role in the difference of bioaccumulation in those organs **(Ghanem, 2014** & **2019).**

Table 1: Bio-accumulation and bio-sedimentation factors of some heavy metals in the different organs of *T. zillii* collected from Lake Qarun during the period from autumn, 2015 to summer, 2016.

Metals	Seasons / Organs	Bio-accumulation factor				Bio-sedimentation factor			
		Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Cd	Gills	0.253	0.171	0.345	0.139	0.204	0.134	0.291	0.096
	Kidney	0.157	0.132	0.345	0.129	0.126	0.103	0.291	0.089
	Liver	0.121	0.145	0.459 ▲	0.161	0.097	0.114	0.388 ▲	0.111
	Muscles	0.072 ↓	0.118	0.299	0.118	0.058 ↓	0.093	0.252	0.082
Cu	Gills	0.071	0.093	0.099	0.111	0.337	0.484	0.410	0.333
	Kidney	0.087	0.123	0.123	0.101	0.414	0.639	0.514	0.299
	Liver	0.127	0.313 ▲	0.179	0.204	0.601	1.626 ▲	0.747	0.609
	Muscles	0.047 ↓	0.059	0.066	0.068	0.221	0.304	0.276	0.203 ↓
Fe	Gills	0.708	1.193	0.988	1.161	0.113	0.134	0.939 ▲	0.096
	Kidney	0.321	0.577	0.645	0.561	0.051	0.065	0.613	0.046
	Liver	0.379	1.282 ▲	0.815	1.001	0.061	0.144	0.774	0.083
	Muscles	0.211 ↓	0.245	0.451	0.431	0.034	0.028 ↓	0.429	0.036
Mn	Gills	0.066	0.123 ▲	0.075	0.046	0.042	0.098 ▲	0.045	0.044
	Kidney	0.024	0.024	0.035	0.024	0.015	0.019	0.021	0.023
	Liver	0.069	0.067	0.061	0.049	0.044	0.053	0.036	0.046
	Muscles	0.011 ↓	0.013	0.017	0.009	0.007 ↓	0.009	0.009	0.009
Zn	Gills	0.236	0.109	0.354	0.206	1.161	0.686	1.556	1.429
	Kidney	0.167	0.149	0.293	0.201	0.823	0.944	1.286	1.399
	Liver	0.407 ▲	0.322	0.472	0.294	2.005	2.031	2.075 ▲	2.046 ▲
	Muscles	0.173	0.075 ↓	0.204	0.128	0.851	0.473 ↓	0.898	0.891

Data indicate that, the general increase in mean concentration of heavy metals in the samples may be due to metal bioaccumulation from water during the wet seasons. This finding disagrees with that of **Elwasify *et al*. (2021)** who recorded elevation in metal concentration arising from reduced water volume during the dry season.

The present study detected the differences of some heavy metals accumulation in different tissues of fish with decreasing rate in the muscles during autumn except zinc that increased in the liver during winter and spring and showed the higher level in the liver during autumn compared to other seasons.

Liver of *T. zillii* accumulated higher levels of Cd, Cu, Fe and Zn than other organs, while gills accumulated higher value of Mn than the sites of metabolite. Results concur with that of **Mohamed (2019)** and **Elwasify *et al*. (2021)** who found that Cu, Fe and Zn concentrations increased in fish liver. Remarkably, the higher accumulation in liver may be due to its detoxification function and may influence its physiological responses and histological structure **(Ferguson, 1989; Ghanem, 2019; Elwasify *et al*., 2021).**

<u>1.2. Bio-sedimentation factor (BSF):</u>

The present study as shown in Table (1) indicates that, the bio-sedimentation factor for **cadmium** attains its high rate during spring in the liver and the lower one during autumn in the muscles; being 0.388 in the first and 0.058 in the second.

However, bio-sedimentation factor for **copper** fluctuates between 0.203 during summer in the muscles and 1.626 during winter in the liver. The low level of bio-sedimentation factor for **iron** is recorded during winter in the muscles (0.028) and the higher one (0.939)is obtained during spring in the gills (Table 1).

Data reveals that, bio-sedimentation factor for **manganese** exhibits its maximum value during winter in the gills (0.098) and the minimum one (0.007) during autumn in the muscles. Moreover, bio-sedimentation factor for **zinc** shows the decreasing level in the muscles during winter and the increasing levels in the liver during spring and summer; being 0.473 in the first, 2.075 in the second and 2.046 in the third (Table 1).

The bio-sedimentation factor is defined as the ratio of heavy metal concentration in the body of organisms to that in the sediment. It makes it possible to evaluate the effectiveness of the bioaccumulation of heavy metals in the organism and give an idea of the speed of substance absorption and excretion by a living organism **(Liu *et al*., 2010; Zhao *et al*., 2012; Coulibaly, 2013).**

Heavy metals with high bioavailability could be absorbed by aquatic organisms depending on their absorption mechanisms, which are also affected by its availability in the aquatic environment (**Coulibaly, 2013**).

Results exhibited that, bio-sedimentation rate of heavy metals in the surrounding media of studied samples may be due to the increasing activity of sewage and anthropogenic pollution. This finding agrees with that of **Camargo and Martinez (2007)** and **Elwasify *et al*. (2021).** While, it disagrees with that of **De Mora *et al*. (2004)** who mentioned that the seabed composition has mostly more nickel sulphide content which is the natural source of nickel in the coastal areas, rather than the anthropogenic activities.

In the study area, pollution at Lake Qarun increased by the human activities such as fishing and waste water from sewage and agriculture drainage which reflect on its concentration in the target organs of organisms. Data referred to specification of target organs to specific metal. This observation coincides with that of **Abdel-Baki *et al*. (2011)**, **Ziyaadini *et al*. (2016)**, **Elwasify *et al*. (2021)** and **Sanou *et al*. (2021)** who declared that, enhancing of BSF for some metals can be used as specifically measurement of those metals as evidence for aquatic pollution.

The present study exhibited that, the higher concentration of some metals in gills may be attributed to its direct contact with the surrounding media; while liver and kidney showed that, the increasing levels may be due to their functional role as detoxification and excretion. The present finding agrees with that of **Ghanem (2006)**, **Asante *et al*. (2014)**, **Elwasify *et al.* (2021)** and **Hadi and Al-hamadawi (2021)** who recorded that, gills are the first site of water contact and accumulation center. Concentration of the studied metals in the tilapian muscles were within the permissible levels suggested by **WHO (2005)**. Similar observation has been reported by **Ghanem *et al*. (2015)**, **Ghanem (2019)** and **Elwasify *et al*. (2021)** stating that, muscles is not a target organ for metals accumulation. Those differences in metals accumulation in the edible organs may be attributed to physiological roles of the studied organ. Furthermore, the alterations in fish tissues leading to functional defect of organs could be due to the toxic effects of heavy metals in relation to their concentrations in water and sediments **(Coulibaly *et al*., 2012; Abarghoei *et al*., 2016; Junejo *et al*., 2019; Kouamenan *et al*., 2020)**.

The results indicate that, the biosedimentation factor of Cd, Fe and Mn in fishes were lower than bioaccumulation one and this implies that the fishes bioaccumulated these metals from water. This finding is matching with that of **Elwasify *et al*. (2021)** who concluded that this result may be attributed to the feeding behaviors of the studied fish. Moreover, copper and zinc concentrated in fishes from sediment with a higher level than that recorded from water may imply that the fishes accumulate these metals from sediment. This result agrees with the findings of **Abdel-Baki *et al*. (2011)**, **Asante *et al*. (2014)** and **Elwasify *et al*. (2021).**

Generally, biosedimentation factor for Cd, Cu, Fe and Mn have values less than 1 (BSF < 1). This means that there is almost no dangerous bioaccumulation of those metals in fish. This could be attributed to the content of metals in substrate sediment**.** This finding aligns with that of **Zhao *et al*. (2012**) who reported that, the biosedimentation is significant when occurred in the body of organisms with values of BSF > 1.

The increased rate of bioaccumulation and biosedimentation factors in the edible organs indicate the high risk to fish health and human consumption. Therefore, the current study recommends that precautionary measurements must be taken to prevent heavy metal pollution in the future.

2. Histopathological studies:

2.1. Kidneys histopathology:

The kidneys in the *Tilapia zillii* are similar in the two sexes. The compound tubular organ composed of a huge number of renal units (nephrons). Each one consists of Malpighian corpuscles, proximal, distal and collecting tubules. In between these tubules, there is a haemopoietic tissue, forming a connecting matrix. The Malpighian corpuscles are made up of a well vascularized glomerulus inside the Bowman's space. The latter is formed of squamous epithelial cells. Between the glomerulus and Bowman's capsule, there is a Bowman's space. The renal (Malpighian) corpuscles leads to the proximal segment through a short neck segment. The proximal convoluted tubules are lined with large cuboidal epithelial cells with a conspicuous easinophilic brush borders. The cytoplasm of these cells is easinophilic, while their nuclei were basophilic, large, oval, and centrally located. Each nucleus possessed a prominent basophilic nucleolus. The collecting tubules are lined with cuboidal cells which are slightly easinophilic and have spherical nuclei with prominent nucleolus. The lumen of these tubules is wider than that of the proximal and distal ones. The haemopoietic tissue, located inbetween the renal tubules, is composed of polygonal slightly basophilic cells with spherical nuclei. Few scattered blood corpuscles are seen in the haemopoietic tissue (Fig. 3).

In the present study, kidney was the most affected and sensitive target organ. It reacted strongly with the toxins which may be increased in the surrounding media. While, the renal lesions included tubular epithelial lining degeneration and absence of inter tubular spaces in most sections. Histopathological observations in the kidneys of *T. zillii* caught from Lake Qarun show congestion in the interstitial blood vessel and multifocal necrotic areas in renal tubules (Figs. 4 & 5). In the present study, the histopathological alterations were related to the exposure to the different heavy metals in the surrounding medium. Accumulation of heavy metals in fish kidney may be an important factor in their injury.

However, accumulation patterns of heavy metals depend on both uptake and elimination rates **(Gomaa *et al.*, 1995; Ghanem, 2019; Mohamed, 2019)**. Some elements such as copper, zinc and lead made changes in the ionic composition of the aquatic environment. The most significant changes observed in the kidney were vacuolar degeneration of the tubular epithelium and congestion of the blood vessels, as well as the presence of pigment granules in between the renal tubules and the nearest renal corpuscles. The appearance of histopathological lesions in kidney of examined fish may be due to the presence of stressors and pollutants in contaminated water with higher levels of Cd, Pb and Cu **(Pirbeigi *et al*., 2016).**

Results revealed that, histopathology of the kidney showed multifocal necrotic areas in renal tubules. Similar investigations has been reported by **Camargo and Martinez (2007)** in the case of *prochliodus lineatus* due to anthropogenic activities of agricultural, industrial and domestic effluents to Apertados stream. Necrosis of tubular epithelium might be attributed to toxin, injury and infection, which led to unregulated cell death as a primer to cause nephrotoxicity. Additionally, **Dhevakrishnan and Zaman (2012)** in case of *Labio rohita* declared that degeneration in renal tubules, necrosis and degeneration of glomerulus was due to untreated waste water. The current study agrees with that of **Mohamed (2009)** in case of *Tilapia zillii* and *Solea vulgaris* who observed the same lesions caused by agricultural, industrial and domestic wastes discharged into Lake Qarun in Egypt. Similar findings were also reported by **Ghanem (2006)** and **Mohamed (2019)** in case of *T. zillii* and *M. cephalus* when caught from untreated sewage and drainage waters of Lake Qarun. Similar findings have been exhibited by **Fathalla *et al.* (2001)** who revealed severe damage in the renal tubules including hamorrhage, vacuolation, degeneration and necrosis. On the other hand, **Tuan *et al.* (2003)** showed that no histopathological lesions were observed in the tilapia fed on diets containing either monilformin or fumonisin.

Several histopathological alterations in the kidney of *Tilapia spp.* from Lake Qarun were recorded a severe vacuolar degeneration with hypertrophy of epithelial cells at the renal tubules, severe degeneration and necrosis in the renal tubules with focal areas of necrosis between the renal tubules, haemorrhage, haemolysis and aggregations of inflammatory cells between the renal tubules, depletion in the haemopoietic areas, dilation of the tubular lumen, edema in Bowman's capsules with atrophy in the glomeruli as well as dilation and congestion in renal blood vessels (**Mohamed & Sabae, 2015; Mohamed, 2019; Dai *et al*., 2020)**.

2.2. Liver histopathology:

The liver is the largest digestive gland. It is very soft, easily lacerable and is of a jell-like consistency, due to the presence of oily-fluids. It appears as a continous mass of cells forming luminae that surrounds the sinusoids. The liver cells are polygonal in shape. The cytoplasm of hepatic cells has a granular appearance. Each hepatic cell has comparatively large nucleus with mostly one, two and rarely three distinct nucleolus and well defined nuclear membrane. Some of large veins that traverse the liver tissue run into tunnels. These are most probably branches of hepatic portal vein. They are surrounded with pancreatic islet tissue. The latter is not indirect contact with the liver cells. The same separation exists between the pancreas and the hepatic ducts, even if they appear very close to each other (Fig. 6).

Results showed that, histopathological changes in the liver of *T. zillii* inhabiting lake Qarun included vacuolated hepatocytes, dilated central vein, compressed blood sinusoids and congestion & vasculitis hepatoportal blood vessel (Figs. 7& 8). 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 harmfull chemicals.

These findings are matching with those of **Shahid *et al*. (2021**) who observed that hepatocytes damage may be attributed to vascular dilation, and necrotic changes in hepatic cells that were most probably a consequence of the cellular degenerations. The changes in histological features of the liver may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals. The cellular degeneration observed in liver of the studied fish is most probably due to vascular dilation, intravascular haemolysis and necrotic area in hepatic cells **(Ghanem, 2006; Mohamed, 2019)**.

The present observations suggests a strong link between heavy metals and lesions in the liver and symmetrical findings with those of **Sharaf (2004)** who studied the histopathological changes in the liver of *Tilapia zillii* collected from ponds near Lake Timsah and the Bitter Lakes and recorded the presence of blood vessels and sinusoids congestions and cloudy swelling of the hepatic cells. **Lumlertdacha *et al.* (1995)** noticed areas of vacuolated hepatocytes in the liver of channel catfish, *Ictaburus punctatus*. The vacuoles contain lipid which is suggestive of impaired lipid metabolism. Moreover, **Kamel and Fathalla (1995)** and **Tayel *et al.* (2013)** recorded many pathological alterations in the liver of *Tilapia zillii* inhabiting Lake Qarun included fatty degeneration and necrosis in hepatic cells and dilation, hemorrhage and hemosidrin in blood vessels.

The same diagnosis has been detected by **Vasanthi *et al*. (2013)** in case of *Mugill cephalus* due to heavy metal pollution in Ennore Estuary. Parallelism in results is found by **Abdel-Moneim *et al*. (2012)** in case of *Orechomius niloticus* who reported hepatocyte vacuolization and necrosis in parenchymal tissues were induced due to contaminated water with heavy metals. The same observations have been obtained by **Ikechukwu and Ajeh (2011)**, **Marchand *et al*. (2012)** and **Rajeshkumar *et al*. (2015)**. In addition to several histopathological observations in the liver of *Tilapia spp*. from Lake Qarun included vacuolar degeneration of the hepatocytes, focal areas of necrosis, haemorrhage, haemolysis, aggregations of inflammatory cells as well as haemosiderin between the hepatocytes, dilation with intravascular haemolysis in hepatic, hepatoportal blood vessels and central veins as well as dilation and congestion in blood sinusoids **(Ghanem, 2006; Mohamed & Sabae, 2015; Mohamed, 2019).**

2.3. Muscle histopathology:

Muscle is the specific tissue of body motion. It is composed of elongated muscle fibers, each an individual muscle cell, held together by connective tissues. The body musculature is relatively simple in fishes. Muscles show an arrangement of fibers into bundles separated from each other by connective tissue portions. The nerves pass through a muscle on its side and branch out as they penetrate into the connective tissue. Segmentation or metamerism of vertebrate musculature is seen clearly in the lateral muscles of the fishes. They are divided into myotomes or muscle segments. Each myotome is divided into an upper (epaxial) and a lower (hypaxial) half by a groove running along the side of the fish. Successive myotomes are separated by obliquely oriented connective tissue partitions (myosepta). The epaxial portion is separated from the hypaxialmyotome by a fibrous septum running antero- posteriorly, parallel to the longitudinal axis. The fibers are unbranched. Each fiber is enclosed in a thin sarcolemma (specialized cell membrane). The protoplasm of the fiber, sarcoplasm contains five longitudinal myofibrils. Each myofibril is composed of two short myo-filaments which are giving the appearance of transverse banding (Fig. 9).

The present study determined some histological changes in the muscles of *T. zillii* including intermuscular edematous areas **(Figs. 10& 11).** These results coincide with those obtained by **Ahmed (2007)**, **Yacoub *et al*. (2008)** and **Saad *et al*. (2012)** who recorded hemorrhage, and hemosiderin. Moreover, fatty degeneration and necrosis in connective tissue of hypodermal layer as well as degeneration, necrosis and edema in the muscle fiber of *Tilapia zillii* were showed. Additionally, **Mohamed (2009)** studied the histopathological changes in *Tilapia zillii* and *Solea vulgaris* collected from Lake Qarun and found degeneration in the muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis. Furthermore, vacuolar degeneration in muscle bundles and atrophy of muscle bundles were observed. Edema within the muscle bundles and splitting of muscle fibers were showed. Hemorrahge, hemosidrin, fatty degeneration and necrosis in the connective tissue of hypodermal layer as well as degeneration in the muscles of *T. zillii*. Necrosis and edema in the muscle fiber layer were showed **(Saad *et al*., 2012; Tayel *et al.*, 2013)**

The present findings are in agreement with those of **Sabae and Mohamed (2015)** and **Mohamed (2019)** who observed some histopathological changes in the muscles of the *Tilapia sp* caught from Lake Qarun with markedly degeneration in the muscle bundles and atrophy, focal areas of necrosis and edema between the muscles bundles, vacuolar degeneration in the muscles bundles and splitting of the muscles fibers. On the other hand, **Shahid *et al*. (2021)** revealed that, muscles were found to be less affected because they were not directly exposed to pollutants.

CONCLUSION

Accumulation of heavy metals found in the target organs appeared the harmfull effects on different organs functions. The increasing of bioaccumulation, biosedimentation factors and histological changes in edible organs may be considered as an important signal for negatively healthy status of fish and human as well. In addition, it is necessary to be more cautious towards water resources in the aquaculture to reduce or prevent the future pollution in a way that would yield good fish production avoiding any harm that may threat the health of the end- users.

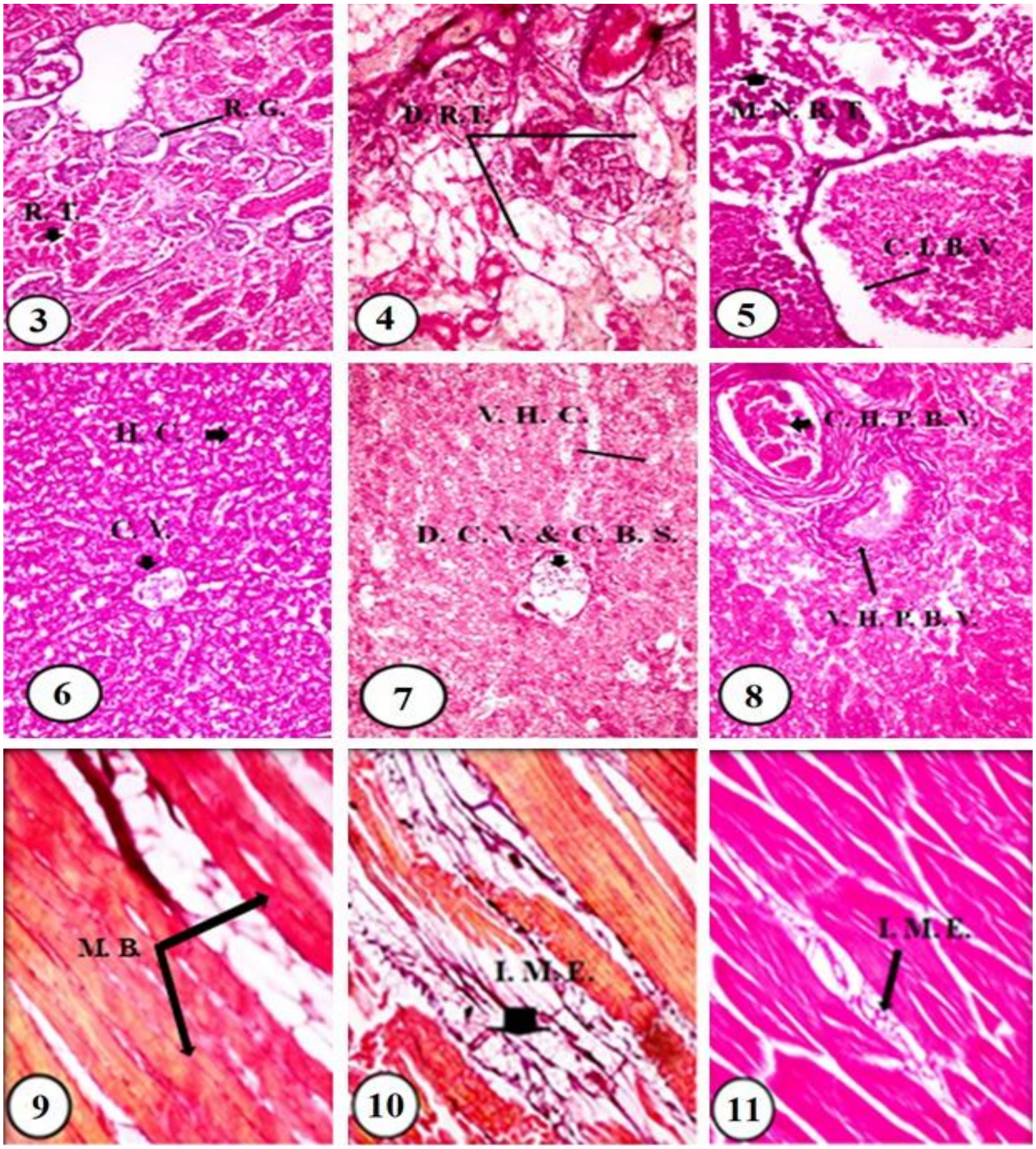

Fig. (3): Photomicrograph in the normal structure (control) in the kidneys of *T. zillii* showing normal renal glomeruli (arrow) and renal tubules (arrow head).

Fig. (4): Photomicrograph in the Kidneys of *T. zillii* showing normal diffuse degeneration (arrows) in renal tubules (H&E X 400).

Fig. (5): Photomicrograph in the kidneys of *T. zillii* showing congestion in the interstitial blood vessel (arrow) and multifocal necrotic areas (arrow head) of renal tubules (H&E X 400).

Fig. (6): Photomicrograph of the normal structure (control) in the liver of *T. zillii* showing normal hepatocytes, central vein and blood sinusoids (H&E X 400).

Fig. (7): Photomicrograph of the liver of *T. zillii* showing vacuolated hepatocytes (arrows), dilated central vein (arrow head) and compressed blood sinusoids (H&E X 400)

Fig. (8): Photomicrograph of the liver of *T. zillii* showing congestion (arrow head) and vasculitis (arrows) of the hepatoportal blood vessel, (H&E X 400).

Fig. (9): Photomicrograph of the normal structure (control) in the muscle of *T. zillii* showing normal muscle bundles with normal striations and nucleation (H&E X 400).

Fig.(10): Photomicrograph of the histopathological changes in the muscles of *T. zillii* showing intermuscular edematous areas (arrow head), (H&E X 400).

Fig. (11): Photomicrograph of the histopathological changes in the muscles of *T. zillii* showing intermuscular edematous (arrow) (H&E X 400).

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ARABIC SUMMARY

تأثير معاملى التراكم الحيوى والترسيب الحيوى لبعض العناصر الثقيلة على السمات الهستولوجية فى الأعضاء المختلفة لسمكة البلطى الأخضر القاطنة لبحيرة قارون، مصر.

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تم إجراء هذه الدراسة خلال الفترة من أكتوبر، 2015 إلى سبتمبر، 2016م، لرصد وتقييم معاملى التراكم والترسيب الحيوي لبعض العناصر الثقيلة داخل أسماك البلطى الأخضر، القاطنة لبحيرة قارون، مصر. حيث تم تجميع عينات المياه ورسوبيات القاع وكذلك عينات الأسماك خلال عام 2015- 2016.

أظهرت الدراسة الحالية أن أعلى قيمة لكلاً من معاملى التراكم الحيوى والترسيب الحيوى قد سجلت داخل أكباد البلطى ماعدا عنصر المنجنيز، فقد سجلت الخياشيم أعلى قيمة له وأقل قيمة لكلا المعاملين قد سجلت فى عضلات هذه النوعية من الأسماك. كما سجلت النتائج وجود إختلافات ملحوظة فى تراكم العناصر الثقيلة داخل الأنسجة المختلفة للأسماك، كما تزايد معامل التراكم الحيوى للعناصر الثقيلة داخل أكباد هذه الأسماك خلال موسمى الشتاء والربيع مقارنة بالمواسم الأخرى، ماعدا عنصر الزنك، حيث سجل أعلى معدل له من معامل التراكم الحيوى داخل الكبد خلال فصل الخريف بينما كان أعلى معدل لمعامل الترسيب الحيوى داخل نفس العضو خلال فصل الربيع. تعد الكبد أكثر الأعضاء تركيزاً لعناصر الكادميوم، النحاس، الحديد والزنك عن باقى الأعضاء. كما أوضحت النتائج أن معامل التراكم الحيوى لعناصر الكادميوم، الحديد والمنجنيز داخل أعضاء الأسماك أعلى من معامل الترسيب الحيوى لها داخل نفس الأعضاء، مما يشير إلى أن هذه العناصر تتراكم داخل الأسماك عن طريق المياه. بينما أشارت النتائج أن معامل الترسيب الحيوى لعنصرى النحاس والزنك أعلى من معامل التراكم الحيوى لها وهذا يشير لتركيز الأسماك لهما عن طريق رسوبيات القاع.

من الناحية النسيجية، تظهر النتائج وجود إحتقان بالأوعية الدموية الخلالية والعديد من المناطق النخرية داخل الأنابيب الكلوية. بينما شوهدت خلايا كبد مفرغة، وريد مركزي متضخم، وظهور إحتقان داخل الجيوب الدموية. علاوة على ذلك، أظهر الفحص الميكروسكوبى ظهور تورم بين الحزم العضلية.

تشير زيادة معدلات التراكم الحيوى، الترسيب الحيوي وكذلك التغيرات النسيجية داخل الأعضاء المستهدفة إلى المخاطر الكبيرة على صحة الأسماك والإنسان. لذلك تظهر الدراسة الحالية وجوب إتخاذ التدابير الإحترازية لمنع التلوث بالمعادن الثقيلة في المستقبل.