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Heavy Metal Concentrations and Liver Histopathology of *Oreochromis niloticus* in Relation to Aquatic Pollution

M.M.N. Authman, E.M. Bayoumy and Amany M. Kenawy

Department of Hydrobiology, National Research Center, Dokki 12622, Giza, Egypt

Abstract: Concentrations of heavy metals were determined in water and liver of *Oreochromis niloticus* obtained from Sabal drainage canal, Almenofiya, Egypt, during the period from January to December 2006. Also, the liver condition (Hepatosomatic index, HSI) and the histopathological alterations were investigated as bio-indicators of environmental pollution. It was found that the drainage canal water was heavily polluted with heavy metals, which accumulated in liver of *O. niloticus* in concentrations higher than that of the canal water. The concentrations of the heavy metals in water and fish livers were higher than the world permissible limits and in comparison to other localities. Fishes from this canal had higher hepatosomatic index and revealed various liver histopathological lesions as well as parasitic infection. It was suggested that the hepatosomatic index and histopathological changes can be used as sensitive indicators for aquatic environmental pollution.

Key words: *Oreochromis niloticus* · hepatosomatic index · heavy metals · bioaccumulation · histopathology · Egypt

INTRODUCTION

The ecological damage of the aquatic environment is mainly related to anthropogenic factors as well as the presence of hazardous, infectious and parasitizing agents [1].

Most of the field studies relevant to the effect of various contaminants on fish are concentrated on the detection of concentration of pollutants either in the ecosystem (water and sediments) and/or in different fish organs [2-4].

On the other hand, surveys of the histopathological lesions in wild fish populations is a valuable tool for identifying ecosystems pollution [5]. Additionally, fish diseases and pathologies bioindicators provide powerful tools to detect environmental stress and characterize the biological end points of toxicant [6, 7].

Liver is the central metabolic organ of fishes and has numerous anabolic and catabolic functions [8] and it is widely recognized as a valuable indicator of pollution [9]. So, the major objective of this field study was to assess the heavy metals concentrations in fish liver and to document histopathological conditions of fish liver that may associate with the presence of pollutants.

MATERIALS AND METHODS

Sabal drainage canal, area of study, is the largest drain in Al-Menoufiya Province, Egypt, which extends for more than 62 km, through Menouf and Al-Shouhada cities and pours into Rossetta Branch of the River Nile, at Tamalay village. This drain receives untreated domestic sewage from numerous towns and villages in addition to the agricultural and industrial wastes. Water samples were collected monthly during the period between January to December 2006, at four sites by a water sampler. Samples were preserved and heavy metals were extracted [10]. Thirty fish were collected monthly by the fishermen using bottom nets. Fish were transported alive to the laboratory in tanks provided with oxygen pump (battery operated). Their body total length (cm) and weight (gm) were measured (11.0-23.0 cm and 25-275 g, respectively). Fish abdomen was incised from the anus to the isthmus and the unusual features of the liver were noted and recorded [11]. The liver was dissected out and weight to the nearest gram was recorded. The hepatosomatic index (HSI) was calculated as follows: HSI = Weight of liver (gm)/Whole body weight (gm) X 100[12]. Small pieces of the lesioned liver were fixed in Bouin's solution for 24 hrs. The

Corresponding Author: Mohammad M.N. Authman, Department of Hydrobiology, National Research Center, Dokki 12622, Giza, Egypt E-mail: mmauthman@yahoo.com

tissues were prepared for light microscopy and stained with hematoxylin-eosin stain and the histopathological conditions of the liver were defined [13]. Samples of the liver tissue were collected and stored in a deep freezer (-20°C) until processing for metal analysis. Heavy metals were extracted from the fish liver samples according to the method of [14]. Heavy metal concentrations in water and liver samples were determined using a flame atomic absorption spectrophotometer (Model 2380, Perkin Elmer, USA). Statistical analyses were performed using a computer program SPSS, version 14 for Windows. The statistical significance of the correlation was reported at both the $P \leq 0.01$ and $P \leq 0.05$ levels.

RESULTS

Table 1 illustrates the concentrations of heavy metals residues in water. Analysis of variance revealed significant ($P<0.01$) differences among the values of aluminium, iron and manganese during different studied months.

On the other hand, the concentrations of metals in the liver of *O. niloticus* are shown in Table 2. Analysis of variance revealed insignificant differences among the values of these heavy metals in liver tissues during the same months. It was found that, the levels of residues in livers are much higher than in the water.

Table 1: Monthly variations of average heavy metal concentrations (mg/l) in Sabal drainage canal water

Months	Al	Cd	Cu	Fe	Pb	Mn	Hg	Zn
January	37.96^{bc}	0.02^{ab}	0.15^{a}	24.18^{bc}	0.77^{a}	1.71^{c}	0.16^{a}	0.72^{ab}
February	31.56^{bc}	0.01^{ab}	0.13^{a}	31.27^{c}	0.51^{a}	1.75^{c}	0.16^{a}	0.38^{ab}
March	28.78^{b}	0.04^{ab}	0.12^{a}	31.22^{c}	0.66^{a}	1.47^{c}	0.18^{a}	0.51^{ab}
April	65.33^{c}	0.02^{ab}	0.16^{a}	24.35^{bc}	0.44^{a}	1.12^{bc}	0.21^{a}	0.62^{ab}
May	11.30^{a}	0.02^{ab}	0.08^{a}	25.48^{bc}	0.47^{a}	1.15^{bc}	0.12^{a}	0.49^{ab}
June	10.65^{a}	0.03^{ab}	0.13^{a}	19.38^{b}	0.71^{a}	0.71^{ab}	0.92^{a}	0.62^{ab}
July	41.73^{c}	0.04^{b}	0.11^{a}	19.51^{b}	0.64^{a}	0.78^{ab}	0.56^{a}	0.58^{ab}
August	22.88^{b}	0.00^{a}	0.15^{a}	18.29^{b}	1.13^{a}	0.47^{ab}	3.55^{b}	0.87^{b}
September	21.92^{b}	0.03^{ab}	0.05^{a}	7.22^{a}	0.42^{a}	0.17^{a}	0.34^{a}	0.29^{ab}
October	9.25^{a}	0.00^{a}	0.05^{a}	8.80^{a}	0.39^{a}	0.36^{a}	0.20^{a}	0.29^{ab}
November	20.20^{b}	0.02^{ab}	0.05^{a}	7.85^{a}	0.75^{a}	0.32^{a}	0.07^{a}	0.20^{a}
December	12.18^{a}	0.01^{ab}	0.04^{a}	6.53^{a}	0.56^{a}	0.66^{ab}	0.20^{a}	0.26^{ab}
Grand average	26.15	0.02	0.10	18.67	0.62	0.89	0.56	0.49
F-value	7.48**	1.17	1.31	9.13**	0.72	6.15**	1.22	1.27
Permissible limits	87.00$^{\bullet}$	0.25$^{\bullet}$	9.0$^{\bullet}$	1000.00$^{\bullet}$	2.50$^{\bullet}$	NA$^{\bullet}$	0.77$^{\bullet}$	120$^{\bullet}$

Means with the same letter in the same column are not significantly different ($P>0.05$)

**Highly significant ($P<0.01$), NA= not available, $^{\bullet}$µg/l [19]

Table 2: Monthly variations of average liver weight, Hepatosomatic Index (HSI) and heavy metal concentrations (µg/gm dry wt) in liver of *O. niloticus*

			Average heavy metal concentrations in liver (µg/gm dry wt)							
Months	Average liver weight (gm)	Average HSI	Al	Cd	Cu	Fe	Pb	Mn	Hg	Zn
January	2.84^{e}	2.52^{d}	719.5^{ab}	1.1^{a}	165.6^{ab}	922.1^{a}	33.0^{a}	12.8^{ab}	54.5^{a}	117.5^{a}
February	1.62^{bc}	2.62^{d}	909.2^{b}	15.9^{b}	200.0^{ab}	807.7^{a}	121.5^{b}	23.3^{ab}	50.1^{a}	164.4^{a}
March	1.66^{c}	2.29^{cd}	315.3^{a}	1.7^{a}	191.8^{ab}	838.0^{a}	26.8^{a}	15.3^{ab}	42.6^{a}	127.1^{a}
April	1.57^{cd}	1.88^{bc}	340.0^{a}	1.5^{a}	228.6^{ab}	887.8^{a}	12.5^{a}	7.9^{a}	50.2^{a}	134.6^{a}
May	1.21^{abc}	1.92^{bc}	357.1^{a}	1.7^{a}	110.5^{ab}	908.7^{a}	39.2^{a}	12.5^{ab}	38.4^{a}	169.0^{a}
June	0.81^{a}	1.61^{ab}	688.3^{ab}	0.0^{a}	79.4^{ab}	993.9^{a}	45.8^{a}	27.3^{ab}	73.4^{a}	109.4^{a}
July	0.68^{a}	1.20^{a}	251.9^{a}	2.8^{a}	186.9^{ab}	730.3^{a}	16.9^{a}	13.3^{ab}	32.9^{a}	92.6^{a}
August	2.86^{ab}	2.35^{cd}	921.2^{b}	0.0^{a}	291.0^{b}	754.0^{a}	13.9^{a}	10.2^{ab}	32.1^{a}	101.9^{a}
September	1.31^{bc}	1.99^{bc}	967.5^{b}	10.0^{ab}	168.3^{ab}	955.0^{a}	116.4^{b}	60.5^{b}	65.6^{a}	135.9^{a}
October	3.18^{abc}	2.19^{cd}	412.1^{a}	2.6^{a}	126.0^{ab}	935.5^{a}	14.2^{a}	8.7^{a}	24.4^{a}	85.7^{a}
November	2.44^{de}	2.33^{cd}	824.9^{b}	1.0^{a}	198.9^{ab}	665.4^{a}	29.5^{a}	3.9^{a}	16.3^{a}	62.6^{a}
December	2.81^{e}	3.10^{e}	927.0^{b}	8.5^{ab}	59.3^{a}	862.8^{a}	119.3^{b}	11.2^{ab}	35.3^{a}	77.4^{a}
Average	1.52	2.55	636.2	3.9	167.2	855.1	49.1	17.2	43.0	114.8
F-value	8.64**	12.59**	1.83	1.92	0.91	1.05	1.14	1.17	1.22	0.45
Permissible limits in fish tissue			NA	0.5$^{\cdot}$	30$^{\cdot}$	5.0$^{\cdot}$	2.0$^{\cdot}$	100.0$^{\cdot}$	0.3$^{\cdot}$	40.0$^{\cdot}$

Means with the same letter in the same column are not significantly different ($P>0.05$)

**Highly significant ($P<0.01$). $^{\cdot}$µg/gm [22]. NA= not available

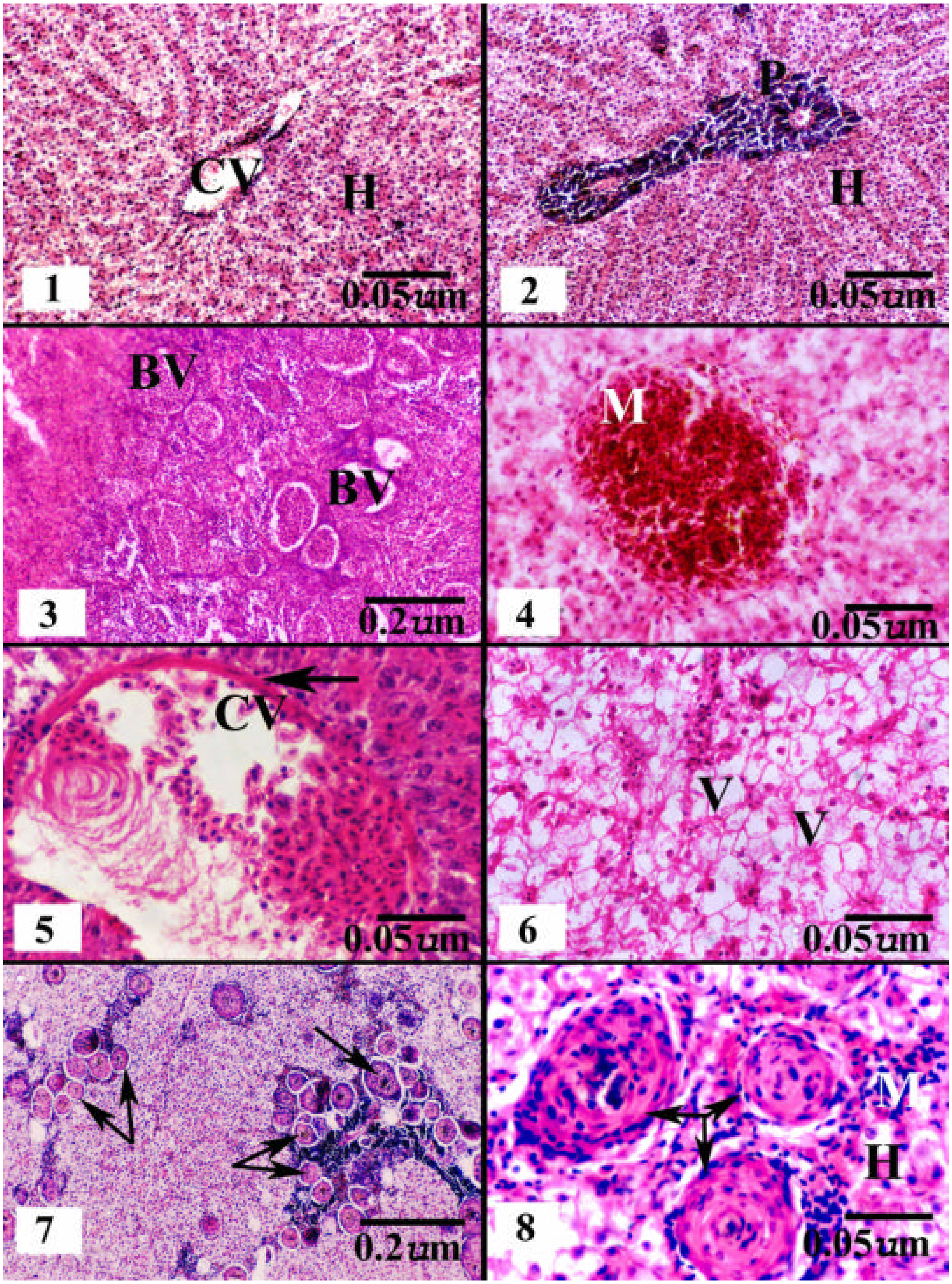

Fig. 1: Normal structure of the hepatocytes with cytoplasm (H) and central vein (CV)
Fig. 2: Normal structure of the pancreatic tissue in the portal area (P) with surrounding hepatocytes (H)
Fig. 3: Sever congestion in the hepatic blood vessels (BV)
Fig. 4: Hemorrhage and numerous numbers of haemosidrin laden macrophages aggregations (M)
Fig. 5: Fibrinous thrombosis in the central vein (CV)
Fig. 6: Vacuolar degeneration: vacuoles (V) clearly visible as white unstained areas within the hepatic cells
Fig. 7: Heavy infestation with parasitic granuloma (arrows) in different size
Fig. 8: Calcium deposition inside the parasitic granuloma (old granuloma) (arrows)

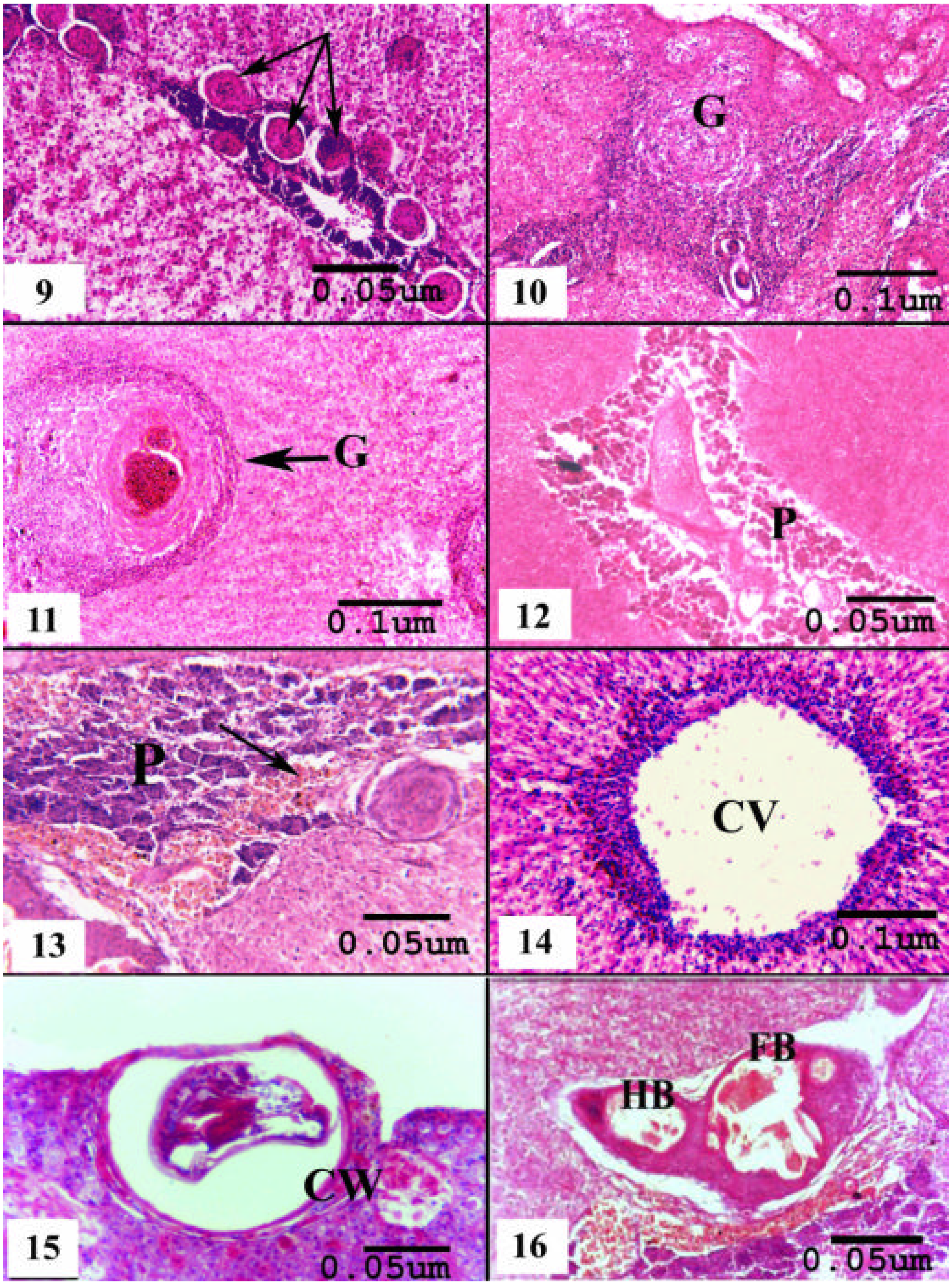

Fig. 9: Parasitic granuloma (arrows) within the pancreatic acini (P)

Fig. 10: Granuloma (G) like formation

Fig. 11: Granuloma (G) like formation surrounding the blood vessel

Fig. 12: Destruction within the epithelial cells lining the pancreatic acini (P)

Fig. 13: Hyperactivation and focal aggregation of melanomacrophage cells (arrow) in between pancreatic tissues (P)

Fig. 14: Hepatitis, numerous numbers of leucocytes around hepatic blood vessel (CV)

Fig. 15: Transverse section through an encysted digenian metacercaria in liver tissue (note the double wall of the cyst CW)

Fig. 16: longitudinal section through a migratory larva in liver tissue (note the fore FB and hind HB body)

Table 3: Comparison of heavy metals concentration in water and *O. niloticus* liver in Sabal drainage canal and other localities

	Al	Cd	Cu	Fe	Pb	Mn	Hg	Zn	Author
Water (mg/l):									
Sabal Drainage Canal	26.15	0.02	0.10	18.67	0.62	0.89	0.56	0.49	Present study
Fish farms in Sabal Canal	NM	NM	0.114	0.536	0.163	NM	0.218	0.055	[15]
Shanawan drainage canal	NM	0.06	0.013	23.03	0.65	1.42	1.87	0.59	[16]
River Nile	NM	0.003	0.26	6.33	0.002	NM	NM	0.65	[26]
Lake Edku	NM	0.01	0.17	1.3	0.21	NM	NM	0.08	[26]
Lake Borollus	NM	ND	0.11	3.3	ND	NM	NM	0.04	[26]
Lake Mariut	NM	0.18	0.24	11.9	ND	NM	NM	0.33	[26]
Lake Qarun	NM	0.0097	0.23	5.53	ND	NM	NM	1.16	[26]
O. niloticus fish liver *:									
Sabal Drainage Canal	636.2	3.9	167.2	855.1	49.1	17.2	43.0	114.8	Present study
Shanawan drainage canal	NM	12.6	205.5	1489.3	108.0	39.6	191.5	203.7	[16]
River Nile	NM	0.52	13.5	450.0	4.59	NM	NM	107.0	[26]
Lake Manzalah	NM	0.64	38.1	680.0	18.0	NM	NM	214.0	[26]
Lake Edku	NM	0.995	41.9	305.0	15.9	NM	NM	133.0	[26]
Lake Borollus	NM	0.58	44.8	830.0	2.9	NM	NM	162.0	[26]
Lake Mariut	NM	2.47	8.6	1017.0	2.1	NM	NM	130.7	[26]
Lake Qarun	NM	0.496	2.96	807.0	1.53	NM	NM	124.3	[26]

NM= not measured, * (µg/gm dry weight), ND= not detected

Analysis of variance revealed significant ($p<0.01$) difference among the values of liver weight and hepatosomatic index during different months. Liver weights were found to increase irregularly. However, these irregularities were more apparent in the changes of the hepatosomatic index (Table 2).

Liver of fish accumulated high heavy metal concentrations showed yellowish, whitish or dark green color, irregular pale spots and nodular or cystic protrusions. The histological picture of normal liver of *O. niloticus* is shown in Fig. 1, with embedded pancreatic tissue between the hepatocytes forming the hepatopancreas (Fig. 2). The main histopathological findings in the livers of fish showed high heavy metal concentrations were sever congestion (Fig. 3), associated with hemorrhage and numerous numbers of haemosidrin laden macrophages aggregation (Fig. 4). Some cases showed fibrinous thrombosis in the central vein (Fig. 5). Degenerative changes besides fatty degeneration in the hepatocytes (Fig. 6) were detected. In some cases, parasitic granulomas were seen within the hepatic tissues (Fig. 7) and appeared calcified in many cases (Fig. 8). The same granulomatous reactions were seen within the pancreatic acini (Fig. 9). Circumscribed round area of epithelioid with chronic inflammatory cells was noticed in the hepatic parenchyma with capsulation looks like granuloma (Fig. 10). The granuloma like formation was observed also surrounding the blood vessels (Fig. 11). Destruction within the epithelial lining the pancreatic acini (Fig. 12) was observed. Hyperactivation of melanin carrying cells (melanomacrophages) were demonstrated as focal aggregation in between the hepatic parynchyma and pancreatic tissue (Fig. 13). Hepatitis was observed around hepatic blood vessels (C.V.) (Fig. 14). The livers of fishes were found to be infected by digenian trermatode as migratory larvae and encysted metacercaria (Fig. 15 and 16). It is worth mentioning that, the liver tissue showed more than two or three abnormal histopathological features in the same time.

DISCUSSION

Domestic sewage, agricultural drainage water and other wastes heavily contaminate the drainage canals in the Egyptian Delta [15]. These polluted drainage water is heavily loaded with different contaminants such as pesticides and heavy metals [15-17]. It was determined that the current canal water is contaminated by different kinds of heavy metals, which were obviously high, mainly due to the decomposition of the organic matter and/or the use of fertilizers and other chemicals in agriculture [18]. Mean metal concentrations in Sabal drainage canal water are generally higher than metal concentrations in other regions (Table 3) with the exception of Shanawan drainage canal [16]. By comparing measured concentrations of metals with water quality standards [19], it was found that all metal concentrations were higher than the permissible limits.

Heavy metals accumulate mainly in metabolic organs such as liver for detoxification, thus, the liver in fish is more often recommended as environmental indicator organ of water pollution than any other fish organs [20, 21]. The values of metals in fish livers obtained in the present study were higher than those found in other localities (Table 3), except that of fishes from Shanawan drainage canal [16]. It was found that concentrations of metals in livers of the studied species were higher than those reported by [22].

Hepatosomatic index (HSI) is general measurement of the overall condition of fish or the growth status of liver [23] and can be an excellent predictor of adverse health in fish [24]. The present results showed an increase in the weight of the liver of *O. niloticus* living in the polluted water and this may be attributed to the accumulation of pollutants and lipid in liver tissue of fishes exposed to pollutants. The fatty degenerative changes that were detected in histological sections may confirm this suggestion. A relationship between hepatosomatic index and levels of contamination was reported by [25]. The HSI value found in the present study were higher than those found in *O. niloticus* captured in River Nile (1.2%), lake Manzalah (0.89%), lake Edku (0.77%), lake Burullus (0.83%), lake Qarun (1.8%) and lake Mariut (1.05%), as detected by [26].

Fish diseases and histopathology, with a broad range of causes, are increasingly being used as indicators of environmental stress since they provide a definite biological end-point of historical exposure [27, 28]. In the present study, the high frequency of grossly abnormal livers may directly attribute to water contamination. A large number of serious pathological liver conditions in fishes as a consequence of pollution were diagnosed by [29]. The highly prevalence of pale colored livers may be attributed to anemia as well as the homogeneous deposition of intrahepatocellular lipid (lipoid liver disease). Vaculation of the hepatocyte, pycnosis in many of the necrotic cells, necrosis of the pancreatic tissue and disintegration of blood sinusoids [30] characterized the degenerative changes. Among the observed histopathological lesions of liver, the macrophage aggregates have been shown to be involved in a number of fish diseases and as phagocytic cells [31]. It was noticed by [27] that, the prevalence of melanomacrophage centers was highest in flounder captured from the sites with the highest PAH contamination. The prevalent of this pathologic condition in the present study may be due to expenditure of energy in the detoxification process, more macrophage aggregates appeared associated with increased metabolic byproducts.

Presence of granuloma in the livers of fishes from the studied area may be attributed to the abundance of different infectious agents. Multi-focal inflammatory lesions (e.g. granuloma) observed are likely to be remnants of parasitic infections [27]. The exposure of fish in Sabal canal to toxicants can lower their resistance to infectious diseases. Therefore, the higher prevalence of liver parasitism in this study may be attributed to sewage discharges that reduce water quality and lead to microbial growth. In this work, the migratory digenian trematode larvae and encysted metacercariae infections were found in livers. A positive correlation between water pollution and the prevalence of encysted metacercariae in *Oreochromis spp.* and *Clarias gariepinus* was indicated by [32].

In conclusion, liver of fish from polluted drainage canals contains a tremendous amount of heavy metals. The pollution of the aquatic environment causes an increase in the hepatosomatic index which could be used as a powerful valid indicator for pollution.

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