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Biochemical and histological biomarker approaches in the assessment of the water pollution in some lined and unlined watercourses of Egypt

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Abstract
The present study was aimed to apply different approaches of biochemical and histological biomarkers on the Nile tilapia fish to assess the water pollution referring to the lining of the water canals in Egypt that may affect the health state of the inhabiting organisms of these canals. The field study areas were divided according to the degree of pollution into two study areas; with low or high pollution. Each study area has lined and unlined sites, respectively (the low-pollution study area (1) has sites I, II and the high-pollution study area (2) has sites III, IV). The present analysis of the water of the unlined site IV of the study area (2) recorded the highest value of pollution with heavy metals. The results indicated that when the water quality is affected, the physiological functions of the fish were affected, as well. These changes were reflected in the values of one or more of the biochemical parameters of the fish. The biochemical analysis of the serum revealed significantly ($P < 0.05$) higher values of the activities of the alkaline phosphatase, the alanine transaminase and the aspartate transaminase (ALP, ALT and AST, respectively) in the fish collected from the highly polluted unlined sites than those in the samples collected from the lined sites; with less degree of pollution. The liver tissue of fish collected from the highly polluted unlined sites had marked histopathological alterations such as cytoplasmic vacuolation of the hepatocytes, pyknosis of the nuclei and necrosis of many liver cells. In addition to loss of architecture, distortion, congestion and dilation of the blood vessels were observed. According to these findings, it was concluded that the biochemical and histological investigation of the Nile tilapia fish can be used as biomarkers for assessment of the environmental pollution of the watercourses.

Keywords: Lining of watercourses, Nile tilapia, *Oreochromis niloticus*, liver, biochemical analysis, histopathology, biomarkers, heavy metals

1. Introduction
Water pollution occurs when it has pathogens, toxins or/and heavy metals in excess amount exceeding the permissible limits [1]. Although water pollution is usually caused by human activities, polluted water has harmful impact on human health directly by drinking it or indirectly by eating the polluted food including fish [2]. Chemical and physical changes in the aquatic environment often induce various alterations in both blood and tissue causing physiological disturbances in fish [3].
Recently, different types of biomarkers have been studied and evaluated for their acceptability to detect the biological effects as a biomonitoring tool [4]. These tools evaluate not only the presence of the pollutants, but also the response of the organisms to them by the assessment of various biochemical and histopathological biomarkers [5].
Biochemical biomarkers are important indices used in fish for field monitoring of the status of the aquatic habitat [6]**.** Blood is a very good medium for assessing the health status of animals and it reveals conditions within the body of the fish before there is any visible sign of disease [7]. Changes in the environment of fish adversely affect the blood constituents; the fishes can tolerate these conditions, to a certain extent, by some physiological mechanisms [8]. Also, tissue biomarkers, 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. For field investigations, histopathology is often the easiest method assessing both short and long term toxic effects. Therefore, histopathological evaluation remains an important part of the monitoring programs [9].
In fish, the liver is the main organ of metabolism and concerned with the urea production,

detoxification and biotransformation processes of pollutants in the blood that coming from the intestine. So, it is considered as an indicator to assess the impact of xenobiotic on the aquatic animals in the field as it is one of the most affected organs by contaminants in the water due to its multiple functions, position and blood supply. The liver has the ability to degrade toxic compounds, but when their concentrations are elevated; its regulating mechanism can be overwhelmed resulting in histological damage [10-11].

Wide areas of the River Nile are highly polluted with the agricultural and industrial chemicals that severely affected on many tissues of the fish [12-13]. Taking into consideration the analysis of the heavy metals (HM) such as copper, lead and cadmium (Cu, Pb, Cd, respectively) in the aquatic system can be very important for providing information on the degree of the environmental pollution [14]. Generally, the field studies are a realistic assessment that is representing a real state of the surrounding environment and the health of the aquatic organisms [15]. As a standard bioassay fish and one of the most marketable freshwater species, the Nile tilapia, *Oreochromis niloticus* fairly satisfied selection criteria for the environmental assessment. These fish are of economic importance and they support the fishing industry of inland and lake water in Egypt. Therefore, the present study was conducted on the Nile tilapia fish, *O niloticus* as a bioindicator species to assess the impacts of the lining of the watercourses as a mean of the environmental modification on the water quality, using two types of biomarkers; i) The biochemical analysis of the blood for the activity of three enzymes; the alkaline phosphatase, the alanine and the aspartate transaminases (ALP, ALT and AST, respectively) and ii) The histological examination of the liver.

Materials and methods

Study areas

The investigated sites were divided according to the degree of the pollution into two study areas. Each area has two sites (I, II, III and IV, respectively) according to their lining as explained previously by the present authors [16].

Study area 1

It is in El-Beharia governorate and has less degree of pollution than the second study area (2).

Site I: El-Bostan canal lined with cement.

Site II: unlined Nasser canal (sandy soil).

Study area 2

It has a higher degree of pollution than the first study area (1).

Site III: River Nile lined with rocks. It is in Warraq El-Haddar region of Giza governorate.

Site IV: unlined Ismailia canal (muddy soil) in Mostorod region of Al-Qaluobia governorate. Ismailia canal supplies fresh water to the Suez Canal cities [17]. It is highly polluted with industrial wastes [18].

Analysis of the heavy metals in the water

Water samples were collected in sterilized one-liter polyethylene bottles from each investigated site [19]**.** The samples were transported in an ice box to the laboratory for further analysis within 12 hours by using the Atomic Absorption Spectrophotometer (AA 6200, SHIMADZU, Kyoto, Japan) according to the method of AOAC [20].

Collection of the fish samples

The Nile tilapia Fish (*O niloticus*) samples from all sites (I, II, III and IV) of the study areas (1 and 2) were caught by fishermen using "gobiah" net during the four seasons starting at 2012 until the end of 2015. The control fish samples were grown in the aquaria of the lab under suitable conditions [21].

Blood collection from the fish for the biochemical analysis

The blood samples were collected, individually in a 3 ml Wesherman tubes by the cardiac puncture method and maintained in an ice box and left to coagulate during its transferring into the lab, then centrifuged at 3000 rpm for 15 min, serum is preserved in 1.5 ml Eppendorf tubes at 2-8 ˚C for the biochemical assays [16].

Biochemical assays

Determination of the serum alkaline phosphatase (ALP) activity

The method was dependent on degradation of the phenyl phosphate by ALP (at pH = 10) into phenol and phosphate. Then, the liberated phenol was measured calorimetrically as international units per liter (IU/L) at 510 nm in the presence of 4-aminophenazone and potassium ferricyanide [22]. Enzyme activity $= A_s / A_{st} X_n$

Where (A_s) is the absorbance of the samples under the investigation, (A_{st}) is the absorbance of the standard sample and (n) is a constant (n = 75).

Determination of the serum aspartate and alanine transaminase activities

Activities of alanine transaminase (ALT, formerly called serum glutamic-pyruvic transaminase, SGPT) and aspartate transaminase (AST, previously named serum glutamic-oxaloacetic transaminase, SGOT) were determined using a colorimetric technique [23] according to the following reactions:

ALT catalyses the reaction: Alanine + α-Ketoglutarate >>>>>>>>>>>>> Pyruvate + Glutamate

AST catalyses the reaction: Aspartate + α-Ketoglutarate >>>>>>>>> Oxaloacetate + Glutamate

Both pyruvate and oxaloacetate react with 2,4dinitro-phenyl hydrazine in an alkaline medium to produce a reddish brown color whose intensity is proportional to the activity of the respective enzyme. The colorimetric activities of the enzymes (ALT and AST) were measured at 490-520 nm and the number of u/ml (units per milliliter) of the tested samples was calculated from the standard curve corresponding to the used kits.

Data analysis

SPSS (2001) software [24] was used for statistical analysis of the results of the biochemical assays and to determine differences among means, least significant difference (LSD) test was used [25]. The differences between all biochemical values were considered significant at *P*-value < 0.05.

Preparation of the histological samples

Each fish was dissected to obtain the liver tissues that then, were cut into small pieces (about 0.5 cm in diameter), they were rapidly washed in 0.9% NaCl saline solution and then fixed in 10% formalin. Then, all samples were transferred in an ice box to the laboratory for later histological preparation. After 24 hours in the formalin, the pieces of liver were dehydrated by using an ascending series of the ethyl alcohol

starting by 70, 80, 90, 95 and finally, 100%. Xylene was then used as a clearing agent before embedding into the paraffin wax. By using the normal rotary microtome, "5 µm" thick sections were obtained and then stained routinely, with hematoxylin and eosin [26-27]. These liver sections were examined by the light microscope and photographed for documentation.

In the present study all used fine chemicals and kits were purchased from Sigma-Aldrich Co, New York, USA. All other routine chemicals were ordered from Nasr Co, Cairo, Egypt. The plan of the current research was carried out under supervision of the Department of Zoology, Faculty of Science, according to the protocols of the scientific ethics of Ain-Shams University, Cairo, Egypt.

Results

Results of analysis of the heavy metals in the water; (Table 1):

The concentrations of the heavy metals (Cu, Pb and Cd) in the investigated waterbodies were zero or traces during all seasons in the study area (1) and highly elevated in the study area (2) especially during summer. The statistical analysis of the results revealed the presence of a significant ($P < 0.05$) difference between the two study areas. The unlined site IV of the study area (2) recorded the highest values of pollution with these three heavy metals (Cu, Pb and Cd) during all seasons compared to all other sites, and it was severely polluted during the summer.

Table 1: The values of the heavy metals in water samples collected seasonally from the different sites.

Element		**Cu (µg/l)**			
Seasons Investigated sites		**Spring**	**Summer**	**Autumn**	**Winter**
Study area (1); less polluted	Site I (Lined with cement)	$0.00^{A, a}\pm 0.00$	$0.02^{A, b}\pm 0.001$	$0.00^{A, a}\pm 0.00$	$0.00^{A, a}\pm 0.00$
	Site II (Unlined; sandy soil)	$0.06^{A, a}\pm 0.03$	$0.05^{A, a}\pm 0.02$	$0.00^{A, b}\pm 0.00$	$0.00^{A, b}\pm 0.00$
Study area (2); highly polluted	Site III (Lined with rocks)	$6.53^{B, a}\pm 0.07$	$41.53^{B, b}\pm 5.92$	$2.18^{B, a}\pm 0.57$	$2.01^{B, a}\pm 0.58$
	Site IV (Unlined; muddy soil)	$21.00^{C, a}\pm 1.38$	$47.35^{B, b}\pm 1.71$	$4.85^{C, c}\pm 0.48$	$8.82^{C, d}\pm 0.02$
Element		Pb (µg/l)			
Seasons Investigated sites		Spring	Summer	Autumn	Winter
Study area (1); less polluted	Site I (Lined with cement)	$0.02^{A, a}\pm 0.004$	$0.05^{A, b}\pm 0.003$	$0.00^{A, c}\pm 0.00$	$0.09^{A, d}\pm 0.005$
	Site II (Unlined; sandy soil)	$0.02^{A, a}\pm 0.003$	$0.05^{A, ab}\pm 0.01$	$0.04^{A, ab}\pm 0.37$	$0.12^{A, b}\pm 0.03$
Study area (2); highly polluted	Site III (Lined with rocks)	$1.57^{B, a}\pm 0.30$	$5.13^{B, b}\pm 0.07$	$0.00^{A, c}\pm 0.00$	$1.19^{B, a}\pm 0.09$
	Site IV (Unlined; muddy soil)	$4.05^{C, a}\pm 0.61$	$7.03^{C, b}\pm 0.68$	$0.19^{B, c}\pm 0.08$	$1.44^{B, c}\pm 0.34$
Element		Cd (µg/l)			
Seasons Investigated sites		Spring	Summer	Autumn	Winter
Study area (1); less polluted	Site I(Lined with cement)	$0.01^{A, a}\pm 0.001$	$0.04^{A, b}\pm 0.004$	$0.00^{A, a}\pm 0.00$	$0.02^{A, c}\pm 0.00$
	Site II(Unlined; sandy soil)	$0.12^{A, a}\pm 0.06$	$0.08^{A, ab}\pm 0.00$	$0.01^{A, b}\pm 0.00$	$0.03^{A, ab}\pm 0.00$
Study area (2); highly polluted	Site III(Lined with rocks)	$2.24^{B, a}\pm 0.03$	$1.53^{B, b}\pm 0.39$	$1.65^{B, ab}\pm 0.20$	$0.51^{A, c}\pm 0.02$
	Site IV(Unlined; muddy soil)	$2.68^{B, a}\pm 0.40$	$4.67^{C, b}\pm 0.17$	$1.74^{B, a}\pm 0.14$	$4.06^{B, b}\pm 0.55$

* Data are presented as mean ± SE

* Mean values in the same column with different capital letters are significantly different ($p < 0.05$) & the mean values in the same row with different small letters are significantly different ($p < 0.05$).

Results of the biochemical assays of the serum of the collected tilapia fish:

Alkaline phosphatase, ALP; (Table 2):

The results of the activity of ALP of fish samples collected from the lined sites (I and III) of the both study areas (1 and 2) showed a significant ($P < 0.05$) reduction comparing to those of the unlined ones (sites II and IV) during the four seasons. The values of ALP activity of fish inhabited the study area (1) recorded the highest activity during the hot seasons and the lowest values were observed during the cold seasons in both the lined and unlined sites. However, the activity of ALP of fish samples of the area (2) was elevated during the different seasons in the lined and the unlined sites. The recorded mean value of ALP of the control fish was 51.37 ± 1.05 IU/L.

Alanine and aspartate transaminases, ALT & AST; (Table 2):

The activities of ALT and AST enzymes were observed to be high during the hot seasons and low during cold ones in both the lined and unlined sites of the two study areas (1 and 2). The transaminases (ALT and AST) were significantly ($P < 0.05$) higher in fish samples of the unlined sites (II and IV) than those of the lined sites (I and III) in the investigated study areas. The mean values of ALT and AST of the control samples were (27.50 ± 0.52) and (36.45 ± 0.62) u/ml, respectively.

Table 2: Seasonal investigation of serum ALP, ALT and AST of *Oreochromis niloticus* collected from the different lined and unlined sites.

ALP (IU/L)					
Seasons Investigated sites		**Spring**	**Summer**	**Autumn**	**Winter**
Study area (1); less polluted	Site I(Lined with cement)	$55.33^{A, a}\pm 2.55$	$52.43^{A, a}\pm 1.12$	$45.39^{A, b}\pm 2.88$	$40.31^{, b}\pm 2.38$
	Site II(Unlined; sandy soil)	$73.59^{B, a}\pm 3.13$	$68.93^{B, ab}\pm 3.39$	$66.69^{B, ab}\pm 2.84$	$60.55^{B, b}\pm 3.20$
Study area (2); highlypolluted	Site III(Lined with rocks)	$86.29^{C, a}\pm 3.08$	$89.98^{C, a}\pm 3.41$	$73.79^{B, b}\pm 3.50$	$83.60^{C, ab}\pm 3.65$
	Site IV(Unlined; muddy soil)	$103.69^{D, a}\pm 4.52$	$102.33^{D, a}\pm 3.65$	$97.54^{C, a}\pm 3.61$	$107.84^{D, a}\pm 3.91$
ALT (Units/ml)					
Seasons Investigated sites		Spring	Summer	Autumn	Winter
Study area (1); less polluted	Site I(Lined with cement)	$35.28^{A, a}\pm 1.27$	$36.32^{A, a}\pm 1.41$	$30.55^{A, b}\pm 1.36$	$24.30^{A, c}\pm 1.24$
	Site II(Unlined; sandy soil)	$46.94^{B, a}\pm 1.77$	$48.91^{B, a}\pm 1.43$	$39.11^{B, b}\pm 1.40$	$31.21^{B, c}\pm 1.27$
Study area (2); highly polluted	Site III(Lined with rocks)	$56.31^{C, a}\pm 1.81$	$67.68^{C, b}\pm 1.47$	$45.00^{C, c}\pm 1.43$	$39.44^{C, d}\pm 1.30$

	Site IV(Unlined; muddy soil)	64.02 $^{D, a}$±1.87	85.62 $^{D, b}$±1.59	61.75 $^{D, a}$±1.65	54.84 $^{D, c}$±1.39
AST (Units/ml)					
Seasons Investigated sites		Spring	Summer	Autumn	Winter
Study area (1); less polluted	Site I(Lined with cement)	42.43 $^{A, a}$±1.30	49.68 $^{A, b}$±1.31	34.95 $^{A, c}$±1.29	30.58 $^{A, d}$±1.30
	Site II(Unlined; sandy soil)	49.68 $^{B, a}$±1.34	60.20 $^{B, b}$±1.40	44.64 $^{B, c}$±1.30	42.42 $^{B, c}$±1.28
Study area (2); highly polluted	Site III(Lined with rocks)	56.57 $^{C, a}$±1.35	72.88 $^{C, b}$±1.42	49.54 $^{C, c}$±1.30	50.08 $^{C, c}$±1.35
	Site IV(Unlined; muddy soil)	77.79 $^{D, a}$±1.45	86.45 $^{D, b}$±1.51	66.88 $^{D, c}$±1.39	54.12 $^{D, d}$±1.35

* Data are presented as mean ± SE, n=5.

* Mean values in the same column with different capital letters are significantly different ($p < 0.05$) & the mean values in the same row with different small letters are significantly different ($p < 0.05$).

Results of the histological examination by using the light microscope

Normal structure of the liver of the control tilapia fish (Figure 1)

The histological examination of the normal liver tissue shows that the hepatocytes are polygonal cells with a central spherical nucleus and a densely stained nucleolus. These liver cells form cords that are separated by capillary sinusoids radiating from a central vein. The cytoplasm of the hepatocytes may normally contain vacuoles to store the lipids and glycogen which are related to the normal metabolic function of the liver. There are also, the pancreatic tissues in the form of randomly-scattered pancreatic acini that are embedded in the hepatic tissue and in close contact with the hepatocytes.

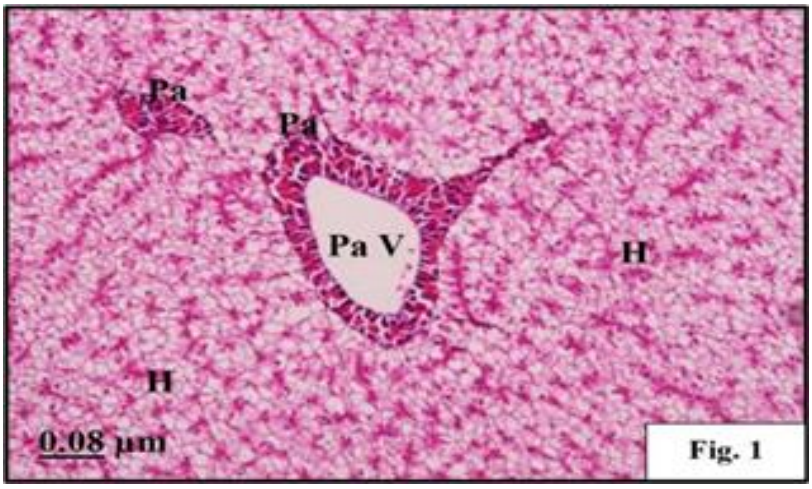

Fig 1: A photomicrograph showing the normal histological structure of the liver of *Oreochromis niloticus*. Notice the normal hepatocytes (H) and the pancreatic tissue or acini (Pa) embedded in the liver tissue and the pancreatic blood vessel (Pa V). Hx & E.

Histopathological alterations of the liver of the Nile tilapia collected from the sites I, II, III and IV during the four seasons; (Figures 2, 3, 4 and 5)

The most prominent alterations of the collected liver samples were distortion, dilation and congestion of the blood vessels and sinusoids. In addition to irregular arrangements of hepatocytes that resulted in loss of its characteristic architecture. There was cytoplasmic vacuolation in the liver cells. Different levels of degenerative and necrotic changes were also observed. The presence of the pyknotic nuclei was one of the most frequent and marked changes revealed almost in liver tissues of all samples collected from the four different sites of the first and second study areas. The extracellular spaces showed marked wideness and loss of the contact between hepatocytes and pancreatic acini due to degeneration of the hepatocytes around the pancreatic tissue, degeneration of many pancreatic cells and congestion of the pancreatic blood vessel, especially in the liver of the fish samples collected from the unlined site IV (Ismailia canal in Mostorod region) of the second study area. Infiltration of inflammatory cells, hemorrhage inside the blood vessels and hypertrophy of Kupffer cells were also noticed in the liver of the Nile tilapia fish samples collected from Ismailia canal (site IV). These histopathological changes were markedly severe in the unlined site IV of the study area 2 more than the alteration that were observed in any other site of both study areas (1 and 2).

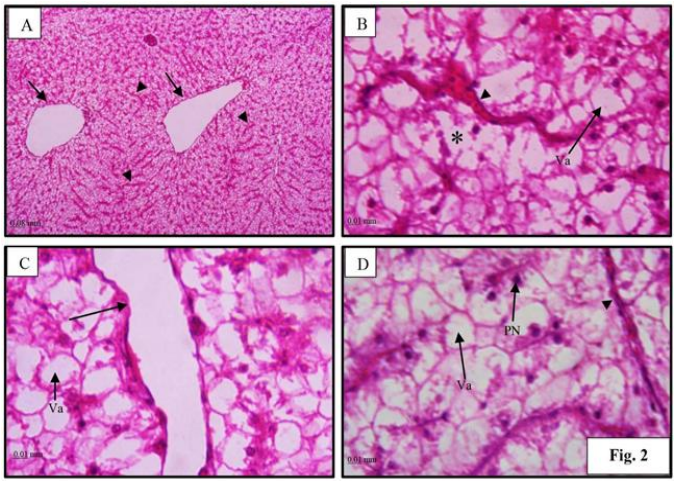

Fig 2: Photomicrographs of sections of liver of *Oreochromis niloticus* collected during the four seasons from site I: El-Bostan canal; lined with cement. Hx & E.

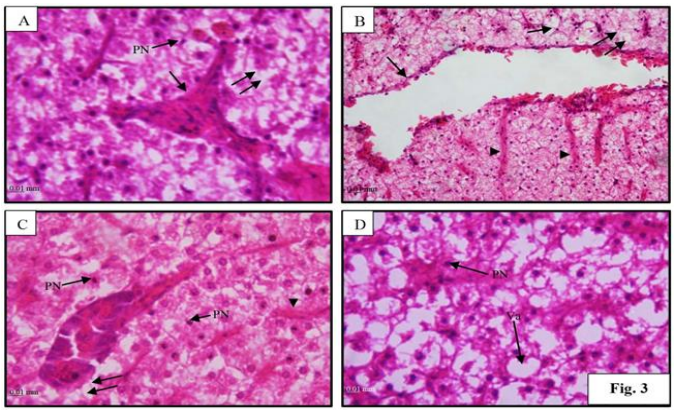

Fig. 3: Photomicrographs of sections of liver of *Oreochromis niloticus* collected during the four seasons from site II: Nasser canal; unlined sandy soil. Hx & E. **Fig 3A:** During spring showing degenerative change (double arrow), pyknotic nuclei (PN), dilated and congested blood vessel (arrow). **Fig 3B:** During summer showing degenerative change (double arrow), pyknotic nuclei (PN), dilated and congested blood sinusoids (arrowheads), dilated blood vessel (arrow) and degeneration of some hepatocytes (double arrow). **Fig 3C:** During autumn showing degenerative change (double arrow), pyknotic nuclei (PN) and dilated and congested blood sinusoids (arrowhead). **Fig 3D:** During winter showing vacuolation (Va) and pyknotic nuclei (PN).

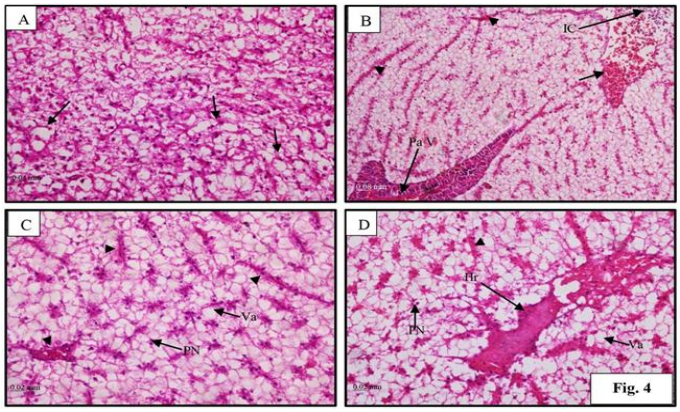

Fig. 4: Photomicrographs of sections of liver of *Oreochromis niloticus* collected during the four seasons from site III: River Nile in Warraq El-Haddar; lined with rocks. Hx & E. **Fig 4A:** During spring showing vacuolar degeneration (arrows). **Fig 4B:** During summer showing dilated and congested blood sinusoid (arrowheads), blood vessel (arrow) and pancreatic blood vessel (Pa V). Notice infiltration of inflammatory cells (IC). **Fig 4C:** During autumn showing dilated and congested blood sinusoid (arrowheads), vacuolation (Va) and pyknotic nuclei (PN). **Fig 4D:** During winter showing dilated and congested blood sinusoids (arrowheads), hemorrhage in the blood vessel (Hr). Notice vacuolation (Va) and pyknotic nuclei (PN) in the liver cells.

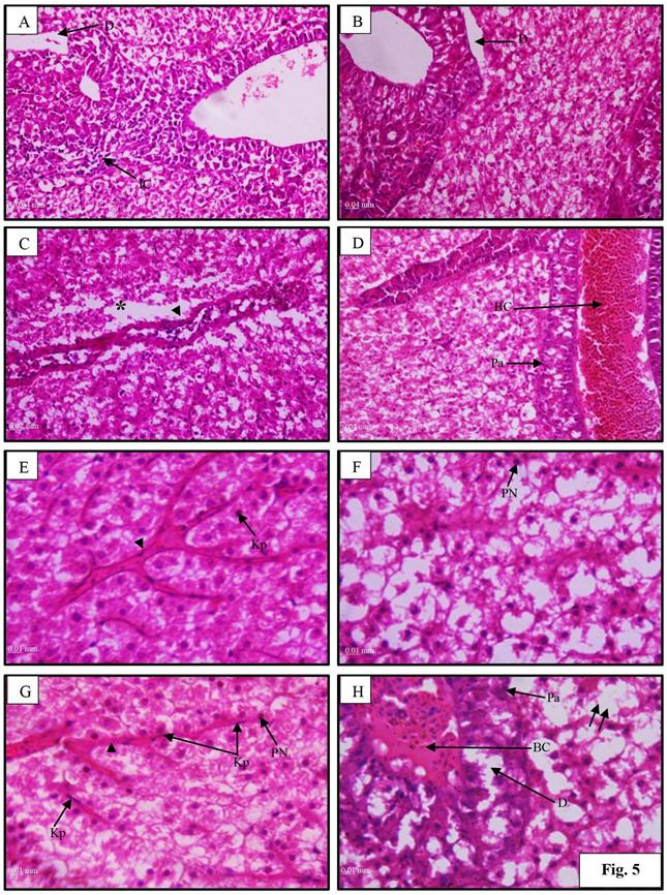

Fig. 5: Photomicrographs of sections of liver of *Oreochromis niloticus* collected during the four seasons from site IV: Ismailia canal in Mostorod region; unlined muddy soil. Hx & E. Fig. 5A: During spring showing infiltration of inflammatory cells (IC) and degeneration of the hepatocytes around the pancreatic tissue (D). **Fig. 5B:** During spring showing degeneration of the hepatocytes around the pancreatic tissue (D) and degenerative change in the hepatocytes. Fig. 5C: During summer showing distorted blood sinusoids (arrowhead) and necrotic hepatocytes (star) **Fig. 5D:** During summer showing blood congestion (BC) in the blood vessel inside the pancreatic tissue (Pa) and degenerative change in the hepatocytes. **Fig. 5E:** During autumn showing dilated and congested blood sinusoids (arrowheads) and pyknotic nuclei (PN) of liver cells. **Fig. 5F:** During autumn showing vacuolar degeneration and pyknotic nuclei (PN) of liver cells. **Fig. 5G:** During winter showing dilated and congested blood sinusoids (arrowheads), pyknotic nuclei (PN) of liver cells and hypertrophied Kupffer cells (Kp). **Fig. 5H:** During winter showing degeneration of the hepatocytes (double arrows) and pancreatic cells (D) and blood congestion (BC) of the blood vessel inside the pancreatic tissue (Pa).

Discussion

The present results of the analysis of heavy metals (Cu, Pb, Cd) found in the different watercourses of the two study areas revealed that the unlined site IV of the study area (2) recorded the highest values of pollution with these three heavy metals during all seasons compared to other sites and during summer, it was severely polluted. Among the most toxic heavy metals, are Cu, Pb and Cd. Small amounts of copper (Cu) may regulate multiple processes in the body metabolism especially in fish. However elevated levels of Cu concentrations disrupt the biological and the biochemical functions and could also induce cellular alterations [28]. Lead (Pb) is considered as a neurotoxin that causes persistent behavioral deficits in fish and also causes decreases in the survival, growth rates, development and metabolism. Lead (Pb) exerts its effect through substituting the essential elements (calcium, iron and zinc) that are participating in metabolism [29]. Cadmium (Cd) is a serious heavy metal, in fish, it can cause anemia and vertebral fractures, osmoregulatory problems, decreased the digestive efficiency, hematological and biochemical effects, growth deficits, erratic swimming, and mortality. The toxic effect of Cd is exacerbated by the fact that it has an extremely long biological half-life and is therefore retained for long periods of time in organisms after bioaccumulation [30].

Generally, the present analysis of the heavy metals (Cu, Pb and Cd) of water samples collected from the different sites revealed that the study area (2) possessed poor-quality water compared to those of the study area (1). This may be attributed to the industrial wastes discharged into the sites of the area (2) causing elevated levels of heavy metals than those recorded in the study area (1) that has less human activities. Other researchers reported that copper could harm fish at levels below that may cause mortality and at concentrations below the accepted criterion (< 9 μg/L) for aquatic life in Alaska [31]. This means that lower concentrations of heavy metals can cause a great damage to the fish.

In the present work, the activity of Alkaline phosphatase (ALP) in the blood of the fish specimens collected from the unlined sites of both study areas 1 and 2 showed a significant elevation compared to those of the lined sites during the four seasons. At the study area (1), it was obvious that the high values were recorded during spring and summer seasons, while the low values were noticed during autumn and winter in the lined and unlined sites. This could be attributed to the fact that during spring and summer seasons the metabolic activities are high and the food availability increases so the fish growth increases. Since ALP is related to growth in fish [32], it is normally to find these high values during spring and summer rather than the other seasons. On the other hand, the unlined site (IV) of the study area (2) showed the most elevated activity of this enzyme during the four seasons. This is may be due to the liver damage of the fish in this highly polluted site, especially with the heavy metals. This is in agreement with other studies that referred the increase of the activity level of ALP is normal in some cases such as damage of bone and liver cells [33]. Also, it was reported that ALP is a polyfunctional enzyme which is involved in the synthesis of nuclear protein, nucleic acid and phospholipids. Therefore, increase in its activity may be related to tissue damage [34].

The present biochemical results showed the presence of a significant increase in the activity of the transaminases; alanine transaminase (ALT) and aspartate transaminase (AST) in the serum of the tilapia fish caught from the area (2). This study area (2) possessed increased levels of pollutants like heavy metals as recorded in the results of the current work. These increased levels of the ALT and AST transaminase enzymes may reveal their possible leakage from different injured tissues across their damaged plasma membranes that became more permeable for these enzymes and other cellular components, as well. Therefore, the recorded high levels of the enzymes in serum of caught fish can be explained as the enzymes leaked into the extracellular fluid and the serum and/or the increased synthesis of these enzymes by the liver following the hepatic cell damage [35]. It was reported that the transaminases; alanine transaminase (ALT) and aspartate transaminase (AST) are enzymes that play a significant role in proteins and amino acid metabolism in different body organs [36]. These transaminases belong to the serum non-functional or tissue-specific enzymes which are normally localized within the liver and other organs. Therefore, their presence in the serum of the collected fish may give information and evidence for injury of the tissue or organ dysfunction [37]. In addition the present results showed that these ALT and AST enzymes were significantly higher in the serum of the tilapia specimens that were collected from the unlined sites than those of the samples that were collected from the lined sites of the two study areas. However, the increased activity of these enzymes in the fish of the study area (1) that has a less degree of pollution, during spring and summer may be related to the increased metabolic activities due to the increase in the food availability during these periods as previously reported [36].

The present histopathological alterations included many indicative features that reflected the effect of the high degree of pollution in the unlined sites on the liver of the collected fish samples. The cytoplasmic vacuolization of the hepatocytes was one of the most prominent changes observed in the present liver samples. It might be attributed to the imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulation system [38]. It was also stated that the vacuolation is a cellular defense mechanism against toxic substances to hepatocytes; as this mechanism collects the injurious elements to prevent them from interfering with the biological activities of these cells [39]. However, other studies described the increased occurrence of such alteration as a sign of degenerative process that suggests a metabolic damage [40]. On the other hand, it was reported that the cytoplasmic vacuolation of the hepatocytes is a non-specific response of fish due to toxic conditions [41].

The present investigation showed alterations in size and shape of nucleus including pyknosis. This type of alteration indicates that the cells became hypofunctional [42]. Necrotic and degenerative changes of the hepatocytes were also recorded in the present work. Necrosis was previously described – in other studies – as irreversible alteration of the tissue [43]. Necrosis was also reported in the liver of Brazilian tropical and subtropical fish species exposed to a variety of contaminants in different regions [44]. Necrosis is resulted from the presence of chemicals within cells that disturb the physiological process such as; inhibition or failure in the synthesis of enzymes, proteins and ATP molecules, also impaired carbohydrate metabolism and production of reactive oxidative species as well as damage in the cell membrane[45]. The observed histological changes may be due to the anoxia that was due to the gill damage affecting the oxygen exchange and tissue respiration resulting in tissue hypoxia consequently leading to the degeneration and necrosis of these tissues as referred in another study [46]. Other scientists referred these

changes to the influence of the pesticides and other toxic substances [47]. Other histological changes such as the dissociation between hepatocytes and disorganization of hepatic cords, loss of the hepatic architecture, marked wideness of the extracellular spaces and loss of the contact between hepatocytes and pancreatic acini were observed in the present study. These alterations are probably due to cell necrosis and degeneration of structural proteins in the membrane of the hepatocytes [40].

The present study showed dilation and congestion of the blood vessels and sinusoids of the liver tissue, as well as hemorrhage inside the blood vessels. However, some other authors attributed the hepatic degeneration and necrosis to the vascular engorgement and congestion of the blood vessels and sinusoids that resulting in the stasis of the blood with impaired blood flow to all tissues [48].

Infiltration of inflammatory cells was another morphological disturbance found in liver samples of the current research. This was thought to be the first line of immunological response that was classified by other researchers as a moderate injury and it is often associated with neutralization and destroying the source aggressor, cleaning the tissue by removing dead cells, as well as inducing the recovery of damaged tissue [43].

In the current research, there was a biochemical changes that come in agreement with the observed histopathological alterations in the liver. As there were significant increased levels of the ALP, ALT and AST enzymes in the serum of the fish collected from highly polluted and unlined sites, also there was a marked necrosis of the liver tissue obtained from the same fish samples. It was found that the necrosis of the liver tissue is associated with the elevation of the activities of some enzymes such as ALT and AST [49].

The present study suggests that the hepatic damage may be attributed to direct toxic effects of pollutants; especially the heavy metals on the hepatocytes, since the liver is the site of detoxification of all types of chemicals [50]. However, other authors explained that the alterations in different tissues are due to xenobiotic in water [51], oxygen depletion [52], parasitic infection [53], elevation in ammonia [54] or heavy metals [55]. Indeed, the present authors found that the fish exposed to different pollutants had a severe damage in their gills [16] that may lead into damage of other organs due to oxygen depletion.

Conclusion

The biochemical analysis of the serum showed that the activities of the enzymes (ALP, ALT and AST) revealed significantly ($P < 0.05$) higher levels in the Nile tilapia fish collected from the highly polluted unlined sites than in the samples collected from lined sites with less degree of pollution. These changes can be related to the histopathological alterations observed in the liver tissue of the same samples. The present authors suggested that these biochemical and histopathological changes in the fish collected from the study area (2) are due to one, more or all of the following reasons; i) the anoxia as a result of the gill damage. ii) The water pollution by heavy metals (Cu, Pb and Cd). iii) The effect of other pollutants such as pesticides that were discharged into the watercourses.

Recommendations

The biochemical assays of certain enzymes (such as ALP, ALT and AST) and the observation of the histopathological alterations of some vital organs such as liver can be considered as good biomarker tools, solely or together to assess the water pollution referring to the lining of the water canals that may affect the health state of the inhabiting organisms of these canals.

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