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Impact of watercourse lining in Egypt on the gills of the Nile tilapia *Oreochromis niloticus*: Histopathological and biochemical study

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Abstract
The present study was aimed to investigate the impact of lining of the watercourses on histology and function of the gills of the tilapia fish. The investigated sites were divided according to the degree of the pollution into two study areas. The first study area was less polluted than the second one. Both areas were divided into two sites according the existence and the kind of the lining that they have. The study area 1 has site I: El-Bostan canal lined with cement and site II: unlined Nasser canal that has a sandy soil, sites I and II are in El-Beharia governorate. The study area 2 has site III: River Nile lined with rocks, in Giza governorate and site IV: unlined Ismailia Canal with a muddy soil, in Al-Qaluobia governorate. The microscopical examination showed presence of marked histopathological alterations of the gills of fish collected from the lined and the unlined sites. The severity of these changes ranged from mild – as in the samples of the lined sites – to severe in those of the unlined sites. These findings included atrophy and curling in the gill lamellae, interstitial odema, necrosis of the epithelial lining, rupture of the pillar cells and capillaries, telangiectasis and aneurism. The biochemical results revealed an increase of the values of urea and creatinine in the serum of the fish collected from the unlined, highly polluted study area. These biochemical changes may be attributed to the gill dysfunction that is due to the recorded histopathological alterations.

Keywords: Lining of watercourse, *Oreochromis niloticus,* histopathology, gills, urea, creatinine, tilapia, Egyptian canals

1. Introduction
The issue of water loss through irrigation systems has major impacts on the surface water supplies so, it needs management. Seepage loss could be effectively minimized by the use of an impervious medium as a canal lining between the porous soil and the water flow [1]. Lining of watercourses is one of the environmental modifications used in irrigation schemes to reduce the water loss. Also, it could reduce the bacterial growth and thus preserving the water with a good quality [2]. The water quality is one of the most important conditions that are controlling life in the aquatic ecosystems. The quality of water and the well-being of the aquacultures are interconnected and directly proportional [3]. Thus, the maintenance of good water quality is essential for both survival and optimum growth of the aquatic fish [4]. Also, the diversity, distribution and the population size as well as the state of health in fishes are affected mainly by the water quality [5]. The fluctuations, even slight, in any of the water parameters, severely affect the dwelling of fish [6]. Fish are one of the most indicative species in freshwater systems because they respond with great sensitivity to any change of their surroundings. Fish are usually considered as organisms of choice for assessing the effects of environmental changes and they are used as bio-indicators in monitoring the water pollution of the aquatic ecosystem [7]. River Nile is the main water source for Egypt. It constitutes over 98 % of the fresh water resources. Many studies proved that the polluted water caused a severe damage to the cells and different tissue of the fish inhabiting these places [8, 9]. It is believed that the gills of fish exposed to irritants are of the most important organs that are highly affected [10-13]. The River Nile is receiving huge amounts of pollutants including the agricultural and domestic sewage [14] as well as the industrial wastes [15] which find their way into the river and its branches.
So, the objective of the present study is to investigate the impact of lining of water bodies on the gills of the Nile tilapia; *Oreochromis niloticus* (Linnaeus, 1766).

2. Materials and Methods

2.1 Study areas (Figure 1):

The investigated sites were divided according to the degree of the pollution into:

2.1.1 Study area 1; (less polluted):

Site I: El-Bostan canal lined with cement.
Site II: unlined Nasser canal (sandy soil).
These two canals radiate from the Nubaria canal of the River Nile in El-Beharia governorate. Nubaria canal is the largest main canal in West Delta region, as it extends to 100 km and irrigates 470 thousand hectares [16].

2.1.2 Study area 2; (more polluted):

Site III: River Nile lined with rocks and at Warraq El-Haddar region in Giza governorate.
Site IV: unlined Ismailia Canal (muddy soil) at Mostorod region in Al-Qaluobia governorate.
Ismailia canal is the main source of drinking and irrigation water for many cities. It is one of the most important canals of the River Nile in Egypt. The canal transports fresh water from River Nile at north of Cairo to the Suez Canal zones; Ismailia, Port Said and Suez governorates [17]. On the two banks of the canal, there are several factories that discharge their wastes into the canal's water, making a change in the water quality of the canal [18].

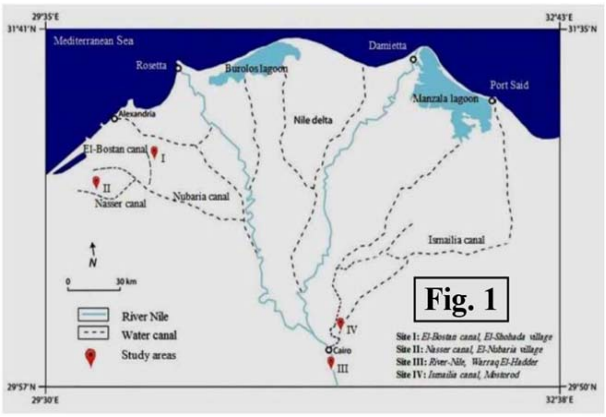

Fig 1: Map of the study areas (1 & 2) and sites (I - IV).

2.2 Collection of the tilapia fish:

Control fish were obtained from aquaria that were kept under ideal conditions in the lab for growing the tilapia fish. The field Fish samples were collected regularly from the different sites along different months during the four seasons from 2012 to 2015. Fish samples were caught by fishermen using "gobiah" net. The present study was approved and conducted in accordance with ethical guidelines set forth by the Department of Zoology, Faculty of Science, Ain-Shams University, Cairo, Egypt.

2.3 Preparation of tissue for the light microscopy:

Dissection of the caught fish was immediately carried out, and then the gills were rapidly removed and cut into small pieces (0.5 cm in diameter). These pieces were washed in 0.9 % NaCl saline solution and then fixed in 10 % formalin for 24 hours in the lab, then dehydrated through upgraded series of ethanol (70, 80, 90, 95 and 100 %), finally, cleared in xylene. After that, they were embedded in paraffin wax. Thin sections "5 µm" thick were prepared using the microtome (RM-2125-RTS, Leica, Nusslosh, Germany) and then stained with hematoxylin and eosin for routine histological examination [6]. These slides were examined by the light microscope (BX51, Olympus, New York, USA). Images were captured by a digital camera (PEN E-PM2, Olympus, New York, USA).

2.4 Blood collection from the tilapia fish for the biochemical assays (Figure 2):

Blood samples were collected by the cardiac puncture method using 3 cm^3 disposable plastic syringes. The use of the plastic syringe is a necessary precaution with fish blood because of contact with glass results in decreased coagulation time [19]. The needle was inserted perpendicular to the ventral surface of the fish in the center of an imaginary line between the anterior part and the base of the pectoral fins. It was then pushed gently down until blood started to enter as the needle punctured the heart. Blood was taken under aspiration until at least about 1 ml was obtained from each caught fish. Then, the needle was withdrawn and the blood was pooled in a 3 ml Wesherman tube and left to coagulate then centrifuged at 3000 rpm for 15 min, serum is preserved in Eppendorf vial (1.5 ml) at 2-8 °C till being subjected to the biochemical assays.

Fig 2: Blood collection from the tilapia fish.

2.4.1. Determination of the serum urea in the tilapia fish:

This test was done according to the urease-Berthelot method which is based on the following reaction: Urea + H_2O (by help of urease) >>>>>>> 2 NH_3 + CO_2
The ammonium ions formed are measured by the Berthelot reaction. The blue dye indophenol product reaction absorbs light between 530-560 nm proportional to initial urea concentration. **Urea concentration = (A_s / A_{st}) X St. conc.**
Where (A_s) is the absorbance of the samples under the investigation, (A_{st}) is the absorbance of the standard sample and (St. conc. = 50 mg/dL) is the standard concentration.

2.4.2. Determination of the serum Creatinine in the tilapia fish:

Creatinine test was carried out according to the colorimetric end point method [20] in which creatinine forms a colored complex with picrate in an alkaline medium. It was measured in mg/dl at 500-550 nm. Creatinine concentration = 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 = 2).
Statistical analysis for biochemical assays was performed using SPSS (2001) software [21]. Least significant difference (LSD) test was used to determine differences among means [22]. Differences were considered significant at *P*-value < 0.05 in all measurements.

3. Results

3.1. Microscopic examination results

3.1.1. Normal structure of the gills of the control tilapia fish (Figure 3):

The gill consists of gill filaments covered by a stratified epithelium called; filamental epithelium. Each filament has a series of parallel thread-like structures called gill lamellae that are located perpendicular to the filament; they represent the actual respiratory surfaces. The gill lamellae are lined with the lamellar epithelium which consists of a single or double layer of simple squamous epithelial cells; pavement cells and narrow supporting cells; pillar or pilaster cells which enclose capillary blood channels that are filled with erythrocytes. These are contractile cells to regulate the blood flow. The epidermis of the gill filament contains numerous mucous cells with opaque cytoplasm. Chloride cells or iono-cytes with light cytoplasm, are common at the bases of the lamellae. They are involved in ion regulation with a possible role in detoxification.

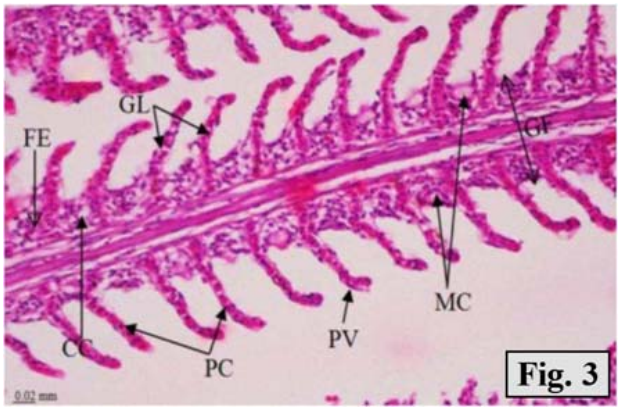

Fig 3: A photomicrograph of a transverse section showing the normal structure of the gills of *Oreochromis niloticus*. Notice the gill filament (GF), the gill lamellae (GL), the chloride cells (CC), the pillar cells (PC), the pavement cells (PV) and the mucous cells (MC). Hx and E.

3.1.2. Histopathological alterations of the gills of the tilapia fish that were collected from the four sites I, II, III and IV during the four seasons (Figures 4 - 7):

The light microscopy examination of the sections of the gills of fish samples collected during the four seasons from the lined and unlined sites of the study areas (1) and (2) showed some structural changes. It was obvious that the histopathological alternations of the gills were more or less similar in the investigated sites, however they differ in their degree of severity from mild as it was recorded in sections of gills obtained from the fish samples of the study area (1) to totally damaged like that was noticed in the samples from the unlined site of the area (2). These lesions included cellular and circulatory changes. The cellular alterations revealed shortening, atrophy and curling in the lamellae. Lamellar interstitial oedema was observed. This oedematous change led to the epithelial lifting of the gill lamellae. Hypertrophy of the chloride and mucous cells was also observed. Epithelial hyperplasia of the gill filament was clearly seen. These alterations led to fusion of the neighboring lamellae. There was also desquamation of the gill lamellae. Necrosis and epithelial degeneration occasionally occurred. However, the circulatory disturbances showed lamellar clubbed tips, vasodilation in the gill filaments with blood congestion. Rupture of the pillar cells and capillaries resulted in small red or purple clusters showing telangiectasis. Also, aneurism was observed in the gill sections. Aneurism was resulted due to the abnormal widening or ballooning of the marginal blood capillaries of the gills.

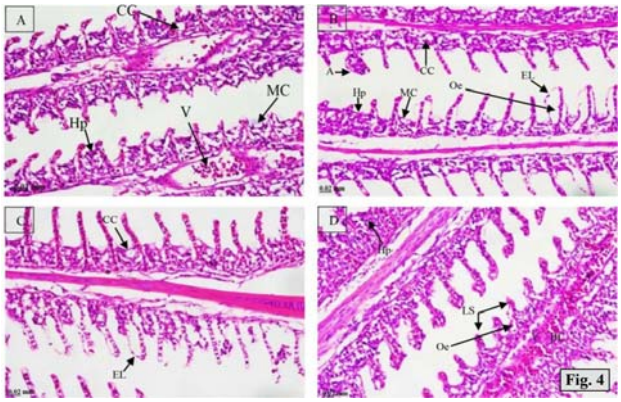

Fig 4: Photomicrographs of transverse sections through the gills of *Oreochromis niloticus* collected during the four seasons from site I: El-Bostan canal; lined with cement. Hx and E.

Fig 4A: During spring showing hypertrophy of chloride cells (CC) and mucous cell (MC), filamental vasodilation (V), epithelial hyperplasia of the gill filament (Hp).

Fig 4B: During summer showing aneurism (A), epithelial hyperplasia (Hp), hypertrophy of mucous cells (MC) and chloride cells (CC), lamellar oedema (Oe) and epithelial lifting (EL).

Fig 4C: During autumn showing hypertrophy of chloride cells (CC) and epithelial lifting (EL).

Fig 4D: During winter showing hyperplasia (Hp), lamellar shortening (LS), oedema (Oe) and filamentous vasodilation (V) and blood congestion (BC)

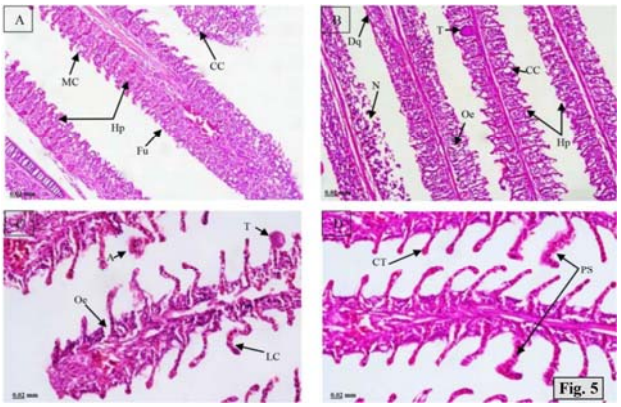

Fig 5: Photomicrographs of transverse sections through the gills of *Oreochromis niloticus* collected during the four seasons from site II: Nasser canal; unlined sandy soil. Hx and E.

Fig 5A: During spring showing hyperplasia of mucous (MC) and chloride cells (CC), epithelial hyperplasia (Hp) and fusion (Fu) of gill lamellae. (Hx and E)

Fig 5B: During summer showing necrosis (N) in the epithelium of the gill filament, desquamation (Dq) of gill lamellae, oedema (Oe) in the epithelium of gill lamellae, telangiectasis (T), hyperplasia and hypertrophy of chloride cells (CC) and epithelial hyperplasia (Hp) of gill filament.

Fig 5C: During autumn showing oedema (Oe) in the epithelium of gill filament, aneurism (A), lamellar curling (LC) and telangiectasis (T).

Fig 5D: During winter showing clubbed tips (CT) in the secondary lamellae and deformed pillar system (PS).

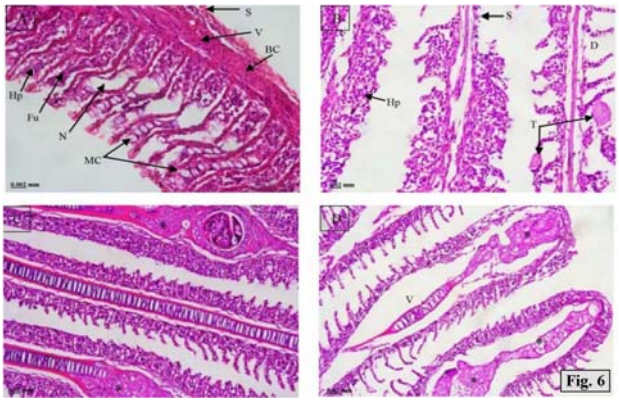

Fig 6: Photomicrographs of transverse sections through the gills of *Oreochromis niloticus* collected during the four seasons from site III: River Nile at Warraq El-Haddar; lined with rocks. Hx and E.

Fig 6A: During spring showing hyperplasia (Hp) of the filamental and lamellar epithelium, fusion (Fu) of the secondary lamellae, hyperplasia of mucous cells (MC), necrosis (N) in the gill filament and sloughing (S) of the gill lamellae.

Fig 6B: During summer showing hyperplasia (Hp) of the filamental and lamellar epithelium, epithelial degeneration (D) of the filamental and lamellar epithelium, telangiectasis (T) and sloughing (S) of the gill lamellae.

Fig 6C: During autumn showing deformation of the cartilaginous support (star) of the gill filament.

Fig 6D: During winter showing vasodilation (V) of the gill filament and deformation of the cartilaginous support (star) of the gill filament.

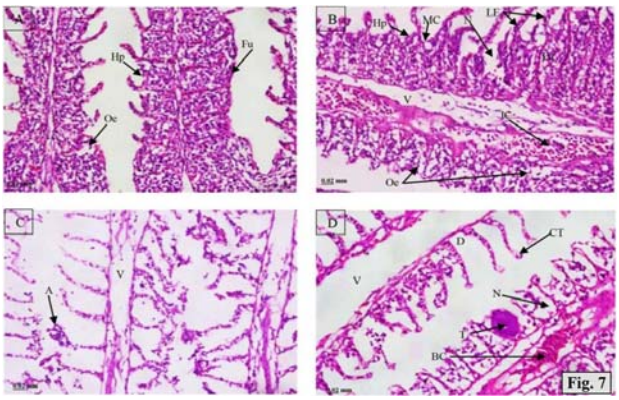

Fig 7: Photomicrographs of transverse sections through the gills of *Oreochromis niloticus* collected during the four seasons from site IV: Ismailia canal at Mostorod; unlined muddy soil. Hx and E.

Fig 7A: During spring showing hyperplasia (Hp), fusion (Fu) of the gill lamellae and interstitial oedema (Oe) of the gill lamellae

Fig 7B: During summer showing vasodilation (V) in the gill filament, hyperplasia (Hp) and fusion (Fu) of the filamental and lamellar epithelium, interstitial oedema (Oe) in the epithelium of the gill filament, aggregation of inflammatory cells (IC), lamellar elongation (LE), hypertrophy of the mucous cells (MC) and necrosis (N).

Fig 7C: During autumn showing filamental vasodilation (V), necrosis and degeneration of the filamental and lamellar epithelium and aneurism (A).

Fig 7D: During winter showing filamental vasodilation (V), congestion (BC) in the gill filament, degeneration (D) of the filamental and lamellar epithelium, necrosis (N) of the epithelium cells, clubbed tips (CT) and telangiectasis (T).

3.2. Results of the biochemical assays of serum of the tilapia fish that were collected from the sites I, II, III and IV during the four seasons:

Urea and creatinine (Tables 1 & 2)

The values of urea and creatinine showed seasonal variations during the entire period of the current study. It was found that there was a significant (P<0.05) increase in the serum urea and creatinine of the fish samples of the unlined site (II) in study area (1) relative to those of the lined one (I) during all seasons. It was also observed that values of urea and creatinine were high during the cold seasons; autumn and winter, whereas the low values were recorded during spring and summer seasons. In contrast, at the study area (2), the highest values of these tested parameters were detected during summer and the lowest values were recorded during winter for fish sample of both lined (III) and unlined (IV) sites. However, there was a significant (P<0.05) difference in most recorded values between samples of the lined and unlined sites during the different seasons. Generally, all values were higher than those of the control fish (16.35 ± .045 and 0.81 ± 0.02 mg/dl for urea and creatinine, respectively) recorded by the present authors.

Table 1: Seasonal investigation of serum urea *Oreochromis niloticus* collected from the different lined and unlined sites.

Urea (mg/dl)					
Investigated sites \ **Seasons**		**Spring**	**Summer**	**Autumn**	**Winter**
Study area (1)	Site I (Lined)	19.96 $^{A, a}$ ±1.15	18.56 $^{A, a}$ ±1.09	23.94 $^{A, b}$ ±1.10	26.57 $^{A, b}$ ±1.13
	Site II (Unlined)	27.50 $^{B, a}$ ±1.16	24.60 $^{B, a}$ ±1.13	31.88 $^{B, b}$ ±1.15	35.23 $^{B, b}$ ±1.16
Study area (2)	Site III (Lined)	41.67 $^{C, a}$ ±1.20	45.02 $^{C, a}$ ±1.20	36.74 $^{C, b}$ ±1.18	37.96 $^{B, b}$ ±1.19
	Site IV (Unlined)	42.98 $^{C, a}$ ±1.23	50.12 $^{D, b}$ ±1.24	49.94 $^{D, b}$ ±1.32	37.00 $^{B, c}$ ±1.19

- Data are presented as mean ± SE, n = 5
- Mean values in the same column with different capital letters are significantly different (p<0.05) and the mean values in the same row with different small letters are significantly different (p<0.05).
- **Site I**: El-Bostan canal, **site II**: Nasser canal, **site III**: River-Nile and **site IV**: Ismailia canal.

Table 2: Seasonal investigation of serum creatinine *Oreochromis niloticus* collected from the different lined and unlined sites.

Creatinine (mg/dl)					
Investigated sites \ **Seasons**		**Spring**	**Summer**	**Autumn**	**Winter**
Study area (1)	Site I (Lined)	0.82 $^{A, ab}$ ±0.02	0.77 $^{A, b}$ ±0.01	0.84 $^{A, a}$ ±0.02	0.84 $^{A, a}$ ±0.02
	Site II (Unlined)	0.91 $^{B, a}$ ±0.02	0.89 $^{B, a}$ ±0.02	0.93 $^{B, a}$ ±0.02	1.01 $^{BC, b}$ ±0.02
Study area (2)	Site III (Lined)	1.07 $^{C, a}$ ±0.02	1.08 $^{C, a}$ ±0.02	0.97 $^{B, b}$ ±0.02	0.97 $^{B, b}$ ±0.02
	Site IV (Unlined)	1.14 $^{D, a}$ ±0.02	1.14 $^{D, a}$ ±0.02	1.10 $^{C, ab}$ ±0.03	1.05 $^{C, b}$ ±0.02

- Data are presented as mean ± SE, n = 5.
- Mean values in the same column with different capital letters are significantly different (p<0.05) and the mean values in the same row with different small letters are significantly different (p<0.05).
- **Site I**: El-Bostan canal, **site II**: Nasser canal, **site III**: River-Nile and **site IV**: Ismailia canal.

4. Discussion

Histopathological alterations are considered as a reflection of the overall health of the entire population in the ecosystem [8]. Histopathological assessment of fish tissues allows for early warning signs of disease and detection of long-term injury in

cells, tissues, or organs [9]. Therefore, the histological studies on the fish are used widely in the evaluation of the water pollution.

In fish, gills are a multifunctional organ; involved in the transport of respiratory gases and homeostatic activities such as osmoregulation, metabolism and circulation of hormones as well as excretion of the nitrogenous products, mainly ammonia, and acid-base balance [23]. Since the gills are in permanent contact with the external environment they are particularly sensitive to chemical and physical changes of the aquatic environment; thereby it is frequently the first system to be affected and the most vulnerable target organs for the irritants [10]. External irritant (especially heavy metals; HMs) are the most frequent causes of significant gill pathological changes and retard the respiratory function of the organ by reducing its surface area [11].

Hyperplasia and lamellar fusion in gills found in *Oreochromis niloticus* from the study area (1) are considered as moderate changes compared to the severe lesions found in the fish of the study area (2), according to earlier classification [24]. This cell proliferation with thickening of the gill filament epithelium is one of the histological changes that most frequently occurred. The increase of cellular layers (hyperplasia) of gill epithelium may be due to an increase in the number of mitotic divisions of the lamellar epithelium resulting in increasing the epithelial thickness that may lead to fusion of the gill lamellae [12]**.** Also, the fusion of the gill lamellae may be induced by the effect of the toxins (including the HMs) that alter the polarity of the glycoprotein in the mucous coat, thus affecting the negative charge of the epithelium leading to the adhesion of neighboring lamellae. This is resulting in reducing the branchial superficial area in contact with the external environment [12]. The lifting of lamellar epithelium was another lesion recorded in fish samples collected in the present study. This is probably induced by the incidence of severe oedema; infiltration of fluids due to toxicity by heavy metals [13]. These oedematous changes in gill filaments are probably due to increased capillary permeability. Also, the lamellar oedema could result in the desquamation of the epithelium as a consequence of the pressure exerted on the epithelium [25]. A similar condition was observed following chronic exposure of *Oreochromis mossambicus* to copper that caused desquamation of some of the gill filaments leading into gradual separation of epithelial cells from pillar cells and consequent disintegration [26].

Moreover, oedema may lead to telangiectasis which is seen in the present study. Telangiectasis is a circulatory lesion that resulted from rupture of pillar cells and capillaries under the effect of irritants. It is characterized by its appearing as small red or purple clusters due to the accumulation of the erythrocytes in the distal portion of the gill lamellae [27]. Lamellar aneurism, another circulatory lesion, was prevalent in the present study. Alterations in the blood vessels may also occur, when fishes suffer from severe stress. In this case, abnormal widening or ballooning of the marginal channel is due to weakness in their wall. The wall becomes thinner until it is no longer strong enough to contain the pressurized blood as a result of the damaged pillar system because of the direct effects of contaminants on these cells [28]. These lesions affect the blood circulation and causes respiratory impairment [29].

Alterations like oedema, epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides the fusion of the gill lamellae are examples of defense mechanisms since they increase the distance between the external environment and the blood and thus serve as a barrier to the entrance of toxins including HMs [30]. As a consequence of the alterations, the oxygen uptake is impaired. These histopathological changes of the gills likely resulted in hypoxia, respiratory failure problems with ionic and acid-base balance [31].

The chloride cells of the gill play a prominent role in the osmoregulation of the teleostean fish [32]. Chloride cell proliferation was observed in the current investigation. It seems that this change reflects an important role for chloride cells in toxicant extrusion or neutralization which may indicate osmoregulatory dysfunction. Also, the increased number of chloride cells, resulting from hypertrophy led to increased necrosis and apoptosis [33].

Hypertrophy of mucocytes was also seen in the present study. Mucous secretion is considered to be a common protective phenomenon and can temporarily protect the underlying epithelium from injury by preventing further entrance of toxins [34]. Gill clubbing was another lesion observed in the present work. It may be due to excess mucous production. This is because in the presence of pollutants, the epithelium of the gill lamellae has a tendency to increase the number of mucous cells [34].

The current study showed the presence of curling and abnormal elongation of the gill lamellae, as well as deformed pillar system. Similar findings of short-term copper exposure on gill structure of rainbow trout fish were revealed in previous studies by other authors [30]. In addition, there were vasodilation and blood congestion in the gill filaments and lamellae. Such alterations are thought to be a protective mechanism against the loss of osmotic stability, which normally is maintained by the epithelium [35].

In the present investigation, the gills showed mild lesions in the study area (1) especially at the lined site. However, at the study area (2); the alterations were severe in samples collected from the unlined site. Such histopathological alterations reinforce the concept that these lesions are indicators of the water quality and the health status in fish [36].

Similar lesions were also reported by other authors in fish exposed to different kinds of pollutants such as endosulfan [37], arsenic [38], and drugs [39]. This means such alterations are non-specific and may be induced by different types of contaminants [40].

Regarding the serum urea and creatinine in the present study, they showed seasonal variations during the entire period of study. At the study area (1), a significant increase was recorded in these parameters of the fish collected from the unlined sites compared to the lined ones during all seasons. It was observed that both urea and creatinine were found to be high during autumn and winter while the higher values were recorded during spring and summer. This was correlated with the increase of temperature which leads to the increase of the urine flow consequently, leads to the low values of urea and creatinine in the blood and vice versa [41]. In contrast, at the study area (2) the significant difference in most of the values between the lined and unlined sites during the different seasons in this area may be attributed to the gill dysfunction [42]. It was stated that the excretory system in fish is unique and most of the nitrogenous wastes are excreted via the gills with only a small fraction excreted by the kidney [43]. Also, the high blood urea concentration may be a sign of stress associated with the increase in the cortisol levels [44]. On the other hand, the changes obtained in the concentration of urea may be attributed to the action of stress (induced by the increased HMs as well as deficiency of the oxygen content

beside the wastes from the industrial processing) on the glomerular filtration rate [45]. Another explanation of the present findings is that the increase of creatinine level might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of the carbohydrates metabolism [46].

5. Conclusion and recommendation:

The lining of the watercourses could help in maintain the water with a good quality. It is supposed that the soil nature – especially earthen one with the increasing the pollution factors today – plays a non negligible role in maintaining the suitable habitat for bacteria, viruses and parasites which are harmful to both aquaculture life and human who is the main consuming of the freshwater generally and fish especially. So, it is recommended to apply lining of the watercourses in a wide scale.

6. Acknowledgement

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