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Using *Oreochromis niloticus* for Assessment of Water Quality in Water Treatment Plants

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Abstract: The present study was suggested in order to explore the capability of water treatment plants to induce changes in organisms using the fish as a model. To achieve such a purpose, *Oreochromis niloticus* were chosen as a test material for the study. Two hundred apparently healthy *Oreochromis niloticus* were divided into 7 groups (control and 3 different WTPs) to assess differences developed from water treatment and the effect of these differences on the histological aspects of the gills of each group. From the beginning to the end of experiment, water samples were collected and daily analyzed. At the end of experiment, fish from each group were sacrified to assess the heavy metals and histological changes to the gill after 90 days of exposing to different kinds of water from different WTPs. Some heavy metal concentrations (Fe, Mn, Cu, Zn, Pb and Cd) were determined in both water and gill samples. Heavy metals were measured by Flame Atomic Absorption Spectrophotometer. Results of water were compared with national and international water quality guidelines. Also, gill accumulated heavy metals concentrations go parallel with that of water metals. The histological results revealed that the gill tissue of the control and input groups has many histopathological alterations while that of the WTPs output groups were characterized by improvement in the gill histology. The main WTP show normal structure of the gill and the developed one showed irregular shape of the filament, while the compact one were characterized by two states; normal and mucoid metaplasia. The histological alterations nearly agree with the heavy metals measurements. In conclusion, water quality differed by the difference in the design of WTPs. Also, the quality of water treatment affected the histology of the gills of the investigated samples.

Key words: Oreochromis niloticus · Heavy metal · Gill · Histology · Water treatment plant · Water quality

INTRODUCTION

Safe and good quality drinking water is the basis for good human health. So, Water Treatment Plants (WTPs) come in all shapes and sizes and no two are exactly the same. While the design of the water treatment plants may vary, they all share the same goal - providing safe, reliable drinking water to the communities they serve.

WTPs use a variety of treatment processes to remove contaminants from drinking water. These individual processes may be arranged in a series of processes applied in sequence. The most commonly used processes include filtration, flocculation, sedimentation and disinfection for surface water. Some treatment trains also include ion exchange and adsorption.

Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms [1].

Copper and iron are essential metals since they play an important role in biological systems whereas cadmium and lead are non-essential metals, as they are toxic, even in trace amounts [2]. These essential metals can also produce toxic effects when the metal intake is excessively elevated [3].

The fish, as a bioindicator species plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fish indicates heavy pollution; the effects of exposure to sub lethal levels of pollutants can be measured in terms of biochemical, physiological or histological responses of the fish organism [4].

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Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems [5].

Various studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood [6-8].

Nile tilapia, *Oreochromis niloticus*, is one of the most common freshwater fishes used in toxicological studies [9-11] because it presents a number of characteristics that may make it an appropriate model that can be used as indicator species in biomonitoring programs [12].

Bioaccumulation of heavy metals does not only depend on the structure of the organ, but also on the interaction between metals and the target organs [13].

Gills are the first target of waterborne pollutants due to the constant contact with the external environment [14]. It is well known that changes in fish gill are among the most commonly recognized responses to environmental pollutants [15,16].

Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the over all health of the entire population in the ecosystem. Many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the organism [17]. As an integrative parameter, histological changes provide better evaluation of an organism's health than a single biochemical parameter [18, 19].

The objective of this study was to find out if there are differences in water quality between different water treatment plants evaluating these differences chemically and histologically using *Oreochromis niloticus* fish as a bio-model that affect mostly by water changes.

MATERIALS AND METHODS

Study Area: Three different types of water treatment plants were selected along the River Nile, Damietta branch covering the different designs in Damietta governorate. These water treatment plants were El-Adlia as a main WTP, Dakahla-2 as a developed WTP and El-Serw as a compact WTP.

Experimental Design: Two hundreds of *Oreochromis niloticus* were collected from a private fish farm in Manzala Lake with average body weight of 40-50 g and 14-17 cm in the length. All fish were transported immediately in double walled polyethylene bags. Fish were divided into 7 groups in 7 equal glass aquaria. Each group includes 30 fish except the control group 20 fish. All conditions were the same, only the difference is the water.

Group (1) : Control from the farm.
Group (2) : Raw (input) water of El-Adlia WTP.
Group (3) : Treated (output) water of El-Adlia WTP.
Group (4) : Raw (input) water of Dakahla-2 WTP.
Group (5) : Treated (output) water of Dakahla-2 WTP.
Group (6) : Raw (input) water of El-Serw WTP.
Group (7) : Treated (output) water of El-Serw WTP.

To avoid seasonal variation, All groups were kept under observation for the whole period of the experiment through one season (from August to October 2010) .In the same time water samples were collected from the research groups sites and analyzed daily during the research period. The treated water that fish lived in it with free residual chlorine 0.1-0.2 ppm. After dissection of fish, parts of gills were carefully removed and prepared for heavy metals determination and other fish gills for histological studies.

Sampling and Sample Preparation

Water: The sampling bottles were pre-conditioned with 5% nitric acid and later rinsed thoroughly with distilled de-ionized water. Samples were acidified with 10% HNO_3, placed in an ice tank and brought to the laboratory. The samples were digested according to the method described by SMEWW [20]. The samples were measured by Atomic Absorption Spectrophotometer (Perkin Elmer Model, 2380 Germany). Results were expressed in ìg/ml

Gills: A representative sample of 0.5g dry weight of each gill sample was taken from fish specimens. These samples were digested according to the method described by Goldberg *et al.* [21] in which concentrated nitric and perchloric acids with ratio of 5:5 ml were used in Teflon beakers on a hot plate at 50°C for about 5 hours till complete decomposition of organic matter. The digested solutions were cooled to room temperature, filtered and diluted to a final volume of 50 ml using de-ionized distilled water. The concentration of iron, manganese, copper, zinc, lead and cadmium in gills of the selected fish were measured by Atomic Absorption Spectrophotometer (Perkin Elmer Model, 2380 Germany). Results were expressed in ìg/mg dry weight of the gill.

Histological Investigations: Specimens from gills of *Oreochromis niloticus* were immediately preserved in 10% neutral formalin then dehydrated in ascending series of alcohol and cleared in xylene and finally the fixed tissue were embedded in paraffin wax (M.P.60°C) after which paraffin sections of (5-6m thick) were prepared and stained with haematoxylin and eosin [22]. Changes induced by water treatment in the gill tissues were photographed and analyzed by light microscope.

Statistical Analysis: All data were presented as mean±standard deviation.

RESULTS

Concentration of Heavy Metals in Water: Table 1 shows the mean concentrations of the tested metals (Fe, Mn, Cu, Zn, Pb and Cd) in the water samples of the 7 selected studied groups. Fig.1. represents a comparison between the control and WTPs; raw and treated water samples.

Control Group: Water samples taken from the farm near to El-Manzala Lake that represents our control, recorded the highest concentrations of all studied heavy metals except the Copper in descending order Fe> Mn > Pb > Zn > Cd> Cu.

Input Groups: Although, the three water treatment plants take their raw water from the River Nile, the input of each one differs from the other in its concentrations of the heavy metals. El-adlia input followed the order Fe> Mn> Zn> Cd> Cu> Pb, Dakahla-2 heavy metal concentrations were in the sequence of Fe> Mn> Zn> Pb > Cu >Cd and El serw WTP input heavy metal concentrations were as follows Fe> Mn>Pb > Cu> Cd > Zn.

Output Groups: Each WTP differs from the other in their heavy metals removing efficiency hence, in output heavy metals concentrations. El- Adlia Fe> Zn> Mn> Cd> Cu> Pb. Dakahla -2 and El Serw output had the same sequence, Fe> Zn> Pb > Mn> Cd> Cu.

The WTPs heavy metals removing efficiency percentage calculated by The equation of removing percentage = [(input-output) ÷ (Input)] × 100 (Egyptian code, 2009) [23] and represented in Fig.2. The national and international guidelines of heavy metals in drinking and raw water in mg/l. showed in table 2

Gills: Table 3 and figure.3. Represent the comparison between heavy metals accumulation in gills that represented different types of water.

Table 1: The residual analysis of trace metals in water samples (mg/l Mean±SD)

		El-Adlia		Dakahla-2		El-serw	
Heavy Metal	Control	Input	Output	Input	Output	Input	Output
Fe	0.312±0.153	0.073±0.001	0.066±0.012	0.157±0.006	0.058±0.006	0.188±0.12	0.080±0.001
Mn	0.115±0.004	0.0124±0.001	0.0067±0	0.029±0.005	0.0045±0	0.0287±0.01	0.007±0.003
Cu	0.00187±0	0.0009±0	0.0018±0	0.0028±0	0.0018±0	0.0028±0	0.0028±0
Zn	0.024±0.005	0.0108±0.003	0.007±0.003	0.013±0.004	0.015±0.002	0.006±0.002	0.011±0.002
Pb	0.046±0	0±0	0±0	0.0077±0.001	0.0077±0.001	0.01±0.001	0.01±0.001
Cd	0.009±0.0003	0.0023±0	0.0029±0.0003	0.0022±0.0003	0.003±0.0003	0.0023±0.0003	0.003±0.0006

Table 2: Showing national and international guidelines of heavy metals in drinking and raw water in mg/l.

Guidelines	Fe	Mn	Cu	Zn	Pb	Cd
Drinking water Egyptian Standards, 2007 [24]	0.3	0.4	2	3	0.01	0.003
WHO,1993 [25]	-	0.5	2	3	0.05	0.01
EPA,2002 [26]	0.3	-	1.3	-	0.05	0.01
River Nile, law,no.48, 1982 [27]	1	0.5	1	1	0.05	0.01

Table 3: The residual analysis of trace metals in gills of *Oreochromis niloticus* (mg/kg dry weight, Mean±SD)

		El-Adlia		Dakahla-2		El-serw	
Heavy metal	Control	Input	Output	Input	Output	Input	Output
Fe	42.045±0.012	28.62±0.019	25.942±0.112	34.888±0.16	15.207±0.022	33.994±0.016	26.837±0.27
Mn	197.56±2.2	104.26±2.2	38.414±2.2	93.292±2.2	27.439±2.2	98.780±2.2	43.902±2.2
Cu	1.8762±0.33	1.4072±2.2	0.9381±0.23	2.3453±0.24	1.4072±0.21	1.8762±0.12	2.3453±0.22
Zn	204.8±17.02	103.6±12.02	81.443±10.02	143.14±20.03	120.9±18.06	66.63±16.06	106.12
Pb	30.833±4.2	15.416±1.3	7.7083±0.66	15.416±0.9	15.416±1.2	15.416±0.9	15.416±1.3
Cd	2.048±0.1	0.4552±0.09	0.2276±0.08	0.6828±0.012	0.2276±0.07	0.9104±0.07	0.6828±0.08

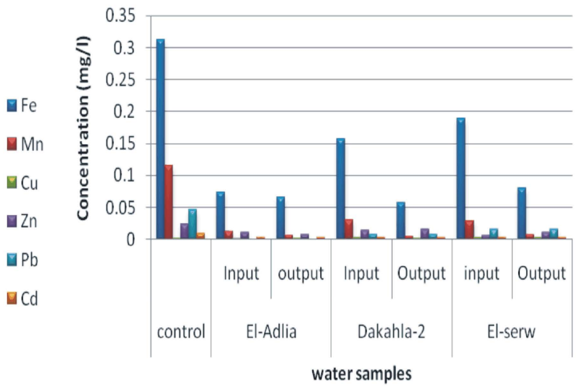
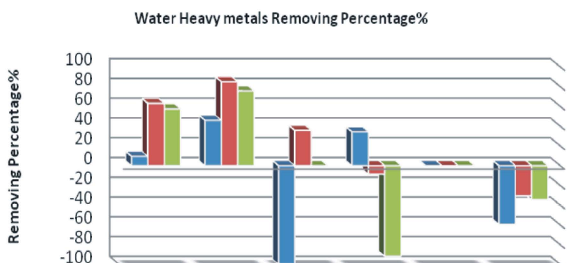


Fig. 1: Heavy metals in water samples



	Fe	Mn	Cu	Zn	Pb	Cd
El-Adlia	9.45	45.96	-100	34.25	0	-59.3
Dakahla-2	63.05	84.89	35.71	-8.69	0	-30.43
El-serw	57.44	75.6	0	-91.52	0	-34.34

Fig. 2: WTPs Heavy Metals Removing Percentage %.

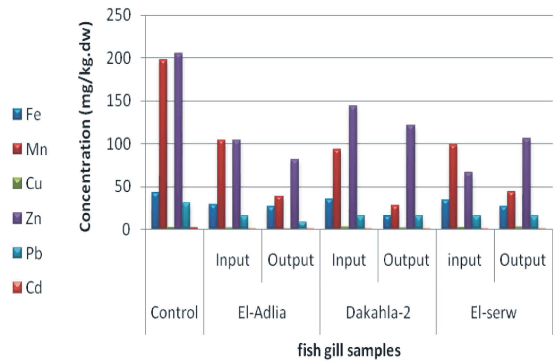


Fig. 3: Accumulation of heavy metals in gills

The accumulation of Iron, Manganese, Copper, Zinc, Lead and Cadmium in gills of control *Oreochromis niloticus* groups has the sequences as Zn>Mn > Fe > Pb> Cd > Cu.

Input Groups: There are bioaccumulation in the gills of fish that lived in raw water from different WTPs input. This bioaccumulation differs from WTP to another; AL-adlia WTP input fish had the sequence Mn > Zn >Fe> Pb >Cu >Cd.

Dakahla-2 WTP input fish had the sequence Zn>Mn > Fe > Pb> Cu >Cd.

El-serw WTP input fish had the sequence Mn > Zn > Fe >Pb >Cu >Cd. All

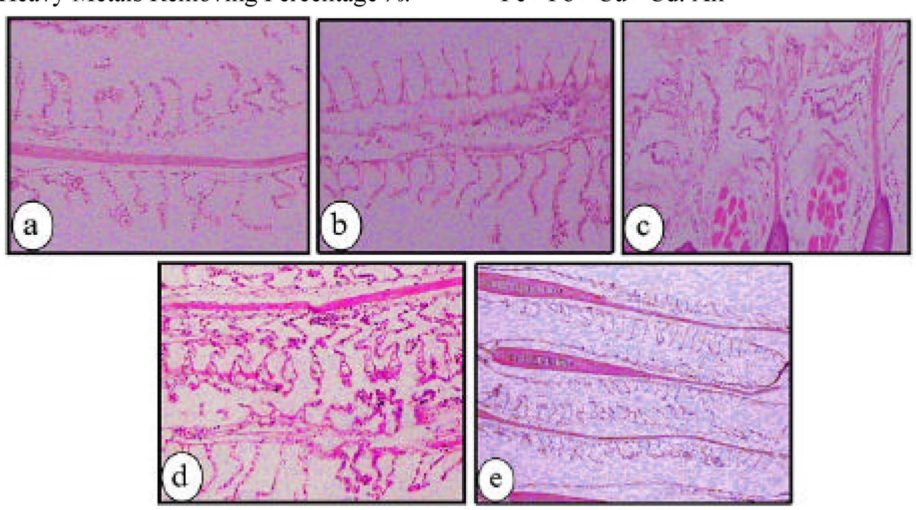


Fig. 4: Photomicrographs of the gills of *Oreochromis niloticus* (control group)a-Epithelial interstitial edema in the filament near the lamellar axis and aneurisms (HandE. X100)
b-damage of central venous sinus and edema (HandE. X100).
c-degenerative and necrotic changes in the epithelium of gill filament and secondary lamellae, lamellar epithelium lifting and loss of regular shape of secondary lamellae (HandE. X40)
d-clubbing at the tips of the secondary lamellae, lamellae with the marginal channel dilated and blood congestion (HandE. X100).
e-atrophy, absence and disorganization of secondary lamellae (HandE.X40).

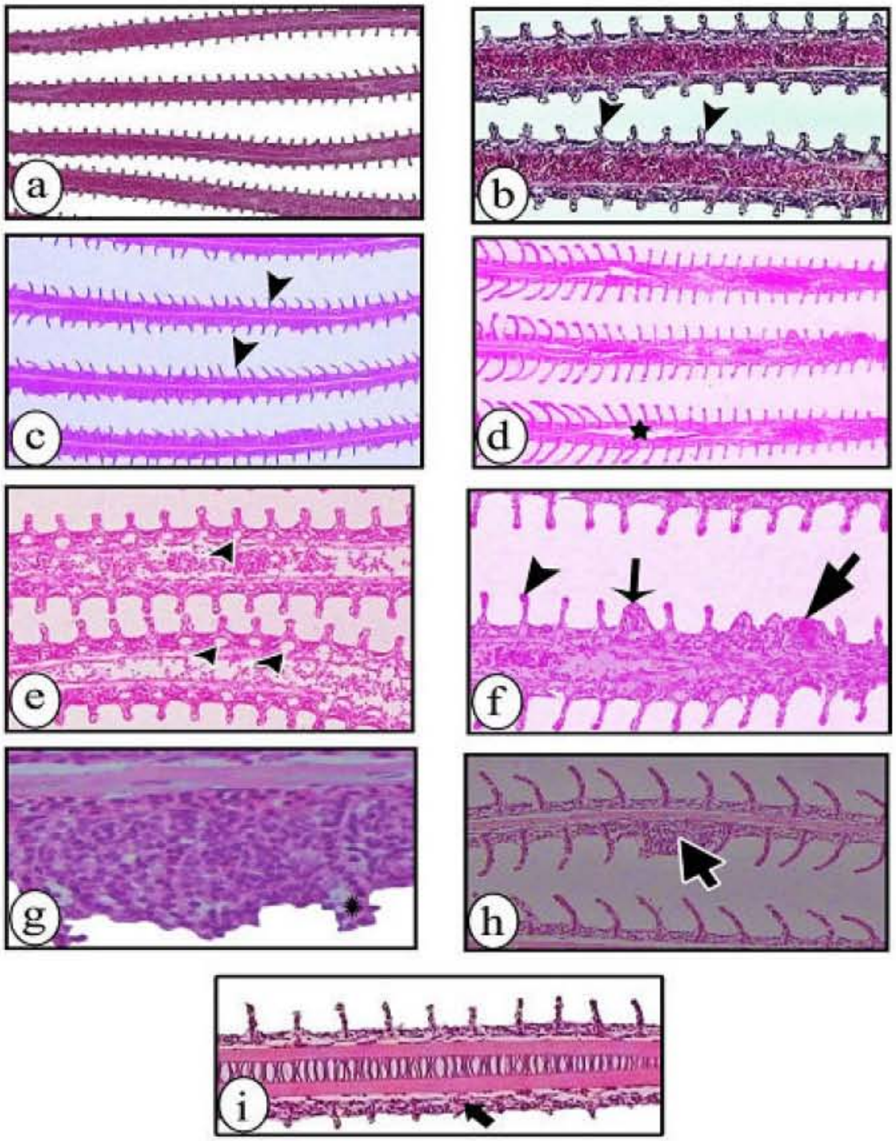


Fig. 5: Photomicrographs of histological alterations of gills in *Oreochromis niloticus of* the input water group samples.

Main WTP (El Adlia):

A, b- Hypertrophy of lamellar epithelium, aggregations of inflammatory cells in gill filaments and interstitial edema(a-HandE.X40)and(b-HandE.X100).

Developed Wtp (Dakahla-2):

c- Mucoid metaplasia and lamellar fusion (HandE.X40).

d- Degenerative and necrotic changes in the epithelium and congestion in blood vessels of gill filaments (star) (HandE. X40)

e- Irregular structure of filament epithelium, interstitial edema (arrow) and atrophy of secondary lamellae-(HandE.X100).

f- Lamellar disorganization (arrow) and blood congestion (head arrow), atrophy of secondary lamellae and partial fusion of some lamellae (HandE.X100).

Compact WTP (El Serw):

g- Absence of lamella and epithelium rupture with hemorrhage (star) (HandE.X400).

h- Proliferation in the epithelium of gill filaments and secondary lamellae (arrow)(HandE.X100)

i- Atrophy and lamellar fusion-(HandE.X100)

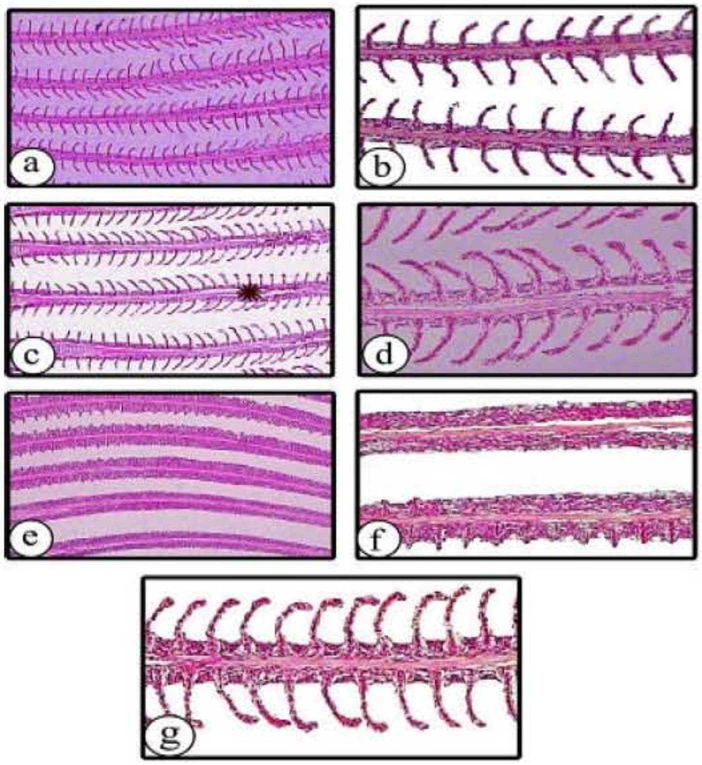


Fig. 6: Photomicrographs of histological alterations of gills in *Oreochromis niloticus* of the output water group samples
Main WTP (El Adlia):
a,b- The normal histological structure (HandE. X40, X100)
Developed Wtp (Dakahla-2)
c- Rupture of gill filament blood vessels-(HandE.X40).
d- Normal with thickening of primary lamellar epithelium-(HandE.X100).
Compact WTP (El Serw):
e,f- Mucoid metaplasia and absence of secondary lamellae (HandE.X40, X100).
g- Normal gill structure (HandE.X100).

Output Groups: Al-adlia output Zn> Mn> Fe>Pb>Cu>Cd.
Dakahla-2 output Zn> Mn> Pb > Fe >Cu>Cd.
El-serw output Zn> Mn> Fe>Pb>Cu>Cd.

Nearly all output has the same sequence Zn> Mn> Fe>Pb>Cu>Cd. The results confirmed the differences of accumulation of heavy metals in gills that lived in different WTPs water.

The Histological Studies: The gills of *Oreochromis niloticus* from the farm that represents our control, was characterized by several histopathological changes; epithelial interstitial edema in the filament near the lamellar axis (Fig.4a), damage of central venous sinus and edema (Fig.4b), degenerative and necrotic changes in the epithelium of gill filament, lamellar epithelium lifting and loss of regular shape of secondary lamellae (Fig.4c), clubbing at the tips of the secondary lamellae, lamellae with the marginal channel dilated and blood congestion (Fig.4d) and atrophy and disorganization of secondary lamellae (Fig.4e).

The gills of *Oreochromis niloticus* from the input groups was characterized by hypertrophy of lamellar epithelium, aggregations of inflammatory cells in gill filaments and interstitial edema in main WTP (El Adlia) (Fig.5a,b). In Developed WTP(Dakahla-2); mucoid metaplasia and lamellar fusion, degenerative and necrotic changes in the epithelium and congestion in blood vessels of gill filaments, irregular structure of filament epithelium, interstitial edema and atrophy of secondary lamellae and lamellar disorganization and blood congestion, atrophy and partial fusion of some lamellae (Fig.5c-f). Several epithelial alterations are found in compact WTP (El-Serw) such as absence of lamella and epithelium rupture with hemorrhage, proliferation in the epithelium of gill filaments and secondary lamellae and atrophy and lamellar fusion (Fig.5. g-i)

The gills of *Oreochromis niloticus* of the output water group samples showed the normal histological structure of the gill which consists of double rows of filaments from which arise perpendicularly the lamellae. The lamellae are lined by a squamous epithelium composed by pavement and non differentiated cells. Below that epithelium are lamellar blood sinuses separated by pillar cells. Between the lamellae, the filament is lined by a thick stratified epithelium constituted by several cellular types, such as chloride, mucous and pavement cells. The present observations are found on the histology of the gills of output groups (Fig.6), except (fig.6. c, e and f) which have some alterations.

DISCUSSION

The Fe, Mn, Cu, Zn, Pb, Cd concentrations in WTPs input, output and control samples were compared with national and international standards. The control samples of water from the farm near El-Manzala Lake recorded the highest concentration of all heavy metals except copper; this may be due to industrial and sewage wastes in the Lake. While, these concentrations not exceeded the national standards. These metals accumulated in the gills more than hundreds folds, this agree with Popek *et al.* [28] who said that heavy metals are known to be accumulated in fish tissues; reaching concentrations of up to 20000 fold higher than surrounding water environment, this accumulation occurred in the histopathological alterations, this go parallel with previous studies [29,30]. According the gill is an important site for the entry of heavy metals that provokes lesions and gill damage.

The 3 WTPs intake from the same source; The River Nile, this improve that the highest metals in raw water is Fe>Mn this may be related to the increase of total dissolved iron in Nile water and consequently increase the free metal iron concentration and thereby lead to an increase in metal uptake by different organs [31,32]. although each input differs from another in its some heavy metals concentration; El-adlia input give the lowest concentration of Fe, Mn, Cu and Pb. This may be due to the existence of the El-Slam canal that renews the water before entrance El-adlia WTP intake which is far from any source of contamination. Dakahla-2 has the lowest Cd concentration also; El-serw WTP recorded the lowest Zn concentration.

Dakahla-2 and El-serw input near to each other in heavy metals concentration, this may be due to the short distance between them and as a result of the presence of El-serw highest drainage in the time of experiment that now removed, this cause increase of Cu, Pb and Cd, but in permissible limits, however, this metals accumulated in the gills resulting in alterations. The observed alterations in the control and input groups of fish like proliferation of the epithelial cells, partial fusion of some secondary lamellae and epithelial lifting are defense mechanisms because these result in the increase of the distance between the external environment and the blood and thus serve as barrier to the entrance of the contaminants [33, 34]

The gills, which participate in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants [2,35].

The obtained results showed that, the heavy metals concentrations of output water below national and international guidelines showed in table.2.

Treatment efficiency results showed that WTPs have a different efficiency for metals removal where Fe concentration was reduce by 9.45, 63.05and 57.44% in main, developed and compact WTP, respectively, also, Mn reduced by 45.96, 84.89 and 75.6% in main, developed and compact WTPs, respectively, Cu was reduced only in the developed WTP by 35.71%, Zn was reduced only by the main WTP by 34.25%. In the same time there are no treatments or removal of Pb metal by any type of WTPs. In contrast, Cd increased in treated water this may be due to the decrease of treated water hardness. The copper removal we agree with [36] and disagree with him in lead and cadmium removal.

Histological study of the gills shows of tilapia of the output group if compared with that of control and input. However, fish exposed to input groups shows several histological alterations, namely lamellar epithelium lifting, epithelium proliferation, lamellar axis vasodilation, edema in the filament and lamellar aneurisms due to the constant contact with the external environment, as well as the main place for copper uptake [37,38]. It is well known that changes in fish gill are among the most commonly recognized responses to environmental pollutants [39].

In conclusion, this is the first study that tried to assess water treatment quality and its effects on health using heavy metals measurements before and after treatment in WTPs. Also, using *Oreochromis niloticus* as a testing model to study the responses to various water treatment changes. The water treatment plants affect on heavy metals accumulation in gills and the histological aspects of the gills. The developed water treatment plant type (design) is preferred more than others as it gives the most heavy metals removing percentage. Further studies on this type would generalize these results confirming that it may be the future WTP.

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