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Impact of environmental pollutants and parasites on the ultrastructure of the Nile bolti, *Oreochromis auruis*

Eman Hashem Radwan[1], Amel Abd El Rahman Hassan [1], Gehan Hassan Fahmy[2], Salma Sameh El Shewemi[2] and Sherine Abdel Salam[2]

1. Damanhour University, Faculty of Science, Zoology department, Egypt. El Gomhoria street, ElBehara, Egypt. 2. Alexandria University, Faculty of Science, Zoology Department, Egypt.

Corresponding author: dr_eman_hashem@yahoo.com, eman.radwan@Sci.dmu.edu.eg, 00201001089259

Abstract

Environmental variability has great impact on processes of ecological organization. Local variation probably accounts within population variation in death rate. Marine parasites are of great importance. Incorporating environmental variation into theories of life histories for a better understanding of how environmental factors influence physiology, and the resulting histories of individuals. Adult *Oreochromis auruis* were collected from, El Behiara, Egypt. Concentration of Pb, Cu and Cd have been detected in the biota samples collected from El Behara, Egypt. The plenty of heavy metal concentration in the fish samples was found in the order Cu > Pb > Cd. A significant correlation ($p<0.05$) were found for each of Cu, Cd and Pb in *Oreochromis auruis* . The mean level of Pb is highly positively correlated with mean concentration level of the activity of GPx and the mean level of the total protein as r= 0.51 and r= 0.61; while Cu is highly positively correlated with Cd, S‰ and the total protein as r=0.75, r=0.65 and r=0.74; respectively. Cd is only highly significantly correlated with the total protein as r= 0.64.The salinity was highly correlated with both pH and the total protein as r=-0.64 and r=0.6; respectively. The mean value of pH is highly negatively correlated with both GPx and with the total protein as r= - 0.50 and r=-0.52; respectively. The activity of GPx is highly correlated with only the activity of the SOD as r=- 0.701.

Key words: Heavy metals, physiology, Scanning Electron Microscope fish, parasites, fresh water .

Introduction

The chemical compounds are used for controlling pests and diseases of plants; to eliminate pests that damage agricultural products are called pesticides (Gruzdyev *et al*., 1983). The Nile has been subjected to different causes of pollution, which affect its physical, chemical, and biological characteristics. These causes include industrial, agricultural, and municipal wastes. Industrial wastewater pollutes the environment especially for drinking water. Pesticides are merged into the water runoff and soil erosion.

Pesticides can also drift during application and harm aquatic systems samples (El- Kabbany *et al*., 2000; Mansour *et al*., 2001; Shukla *et al*., 2002; Osibanjo, 2003; Jadwiga *et al.*, 2012). Wild animals are partially or completely damaged by pesticides and these animals make excellent "bio-indicator species" (Pimentel, 2005; Akhtar *et al*., 2009).

Pollution burden in the Nile system has increased because of population increases (Abdel-Satar, 2005; Abdel-Dayem *et al*., 2007). It is anticipated that the dilution capacity of the River Nile system will decrease as the program to enlarge irrigated agriculture moves forward and recently (MWRI, 2002). The major pollutants including oil and wastes from passenger and river boats. The most polluted part of Nile is the part located between Cairo and the Mediterranean Sea; the two branches of Nile are considered the highest pollutant part (Abdo, 2004; NAWQAM, 2003). In Egypt, climate conditions average has a wide range and the average daily temperature ranges from 13C to 38C (CAPMAS, 2007). Wastewater resulted from industry is considered the major sources of Nile water pollution because of the toxic chemicals and organic charge in this waste- water. Egyptian industry uses about 7.8 billion m3/year of water, of which 4,050 million m3/year are drained into the River Nile system. There is plenty of factories (129) discharging their waste- water into the River Nile system. Effluent wastewater is often partially treated. In spite of all official efforts to prevent this pollution source (NBI, 2005).

The toxicity of the fish is of a unique importance in view of the use of insecticides in the aquatic habitats. The present study aimed at pointing out the possible hazards resulting from heavy metals which affect the water fauna, of which fishes are considered as one of the most important group. The determination of the toxic effects of heavy metals on the Nile fish, *Oreochromis auruis* is of much importance and to report parasites fauna if present in the studied locations. Reporting the effect on pollution and parasites on the collected fish samples by using scanning electron microscope was of the important priority .

The contamination of sediment, water resources and biota by heavy metals is one of the main concerns that has a considerable effect on the environmental problems worldwide because these metals are everlasting and having toxic effects on living organisms when they override a certain concentration. This is particularly true in many of the countries due to their bioaccumulation (Schuurmann and Market, 1998; MacFarlane and Burchett, 2000; Abdel-Azeem *et al*., 2007; Kata and Ramana, 2013). Marine parasites are of great importance (Kinne, 1990; Sindermann, 1993). Many parasites affect marine fishes making them commercially less valuable and sometimes limit their populations or lead to mortalities (Rohde, 1993). Helminth parasites of marine fishes are mainly belonging to five taxa; Monogenea, Digenea, Cestoda, Nematoda and Acanthocephala. Pathological effects the the helminthes on their fish host were studied by several authors; (Whittington, 1990; Sindermann, 1993; Milinski, 1990; Hassanine, 2000 and Dezfuli et al., 2002). Many parasite species survive for several years even in fish migrating between the freshwater and the sea. These facilitable the use of parasites as biological markers (Margotis, 1992).

Infection of fish with helminth parasites may be is affected by pollution. Skinner (1982) stated that marine environment polluted with ammonia, trace metals or pesticides increase the infection of fish with monogenean trematodes. Khan and Kiceniuk (1983) and Moles and Narcoss (1998) stated that marine environment polluted with crude oil reduces the infection of fish with intestinal helminthes. They concluded that intestinal helminthes can serve as biomarker for petroleum exposure. There are good reasons for focusing on fish helminth parasites in the search for highly sensitive indicators of pollution in the marine environment (Mackenzie,1999). Helminth parasites reduce the marketability of some commercial marine fishes in several ways; the metacercariae of many species of digenean

trematodes, reduce the quality of the flesh in different species of mullets; (Kurochkin, 1985).

Material and Methods

Adult similar size and active specimen of the Nile fish were obtained from four locations from El Behara, Egypt sent to the laboratory. The salinity and pH of the water were determined biweekly (7 replicates) from the four locations in the study period (winter, December2016 to February2017).

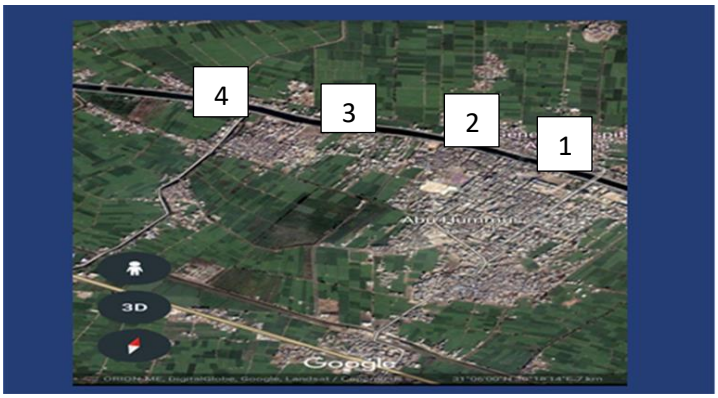

Fig. 1: The four locations of the present study El Mahmodia (El Behaira, Egypt)

Water samples (2.5 L) were collected in clean glass bottles at water surface and 50 cm below water surface. The bottles were capped with screw caps and the samples were immediately transferred to the laboratory for analysis .

The following physical and chemical parameters of all water samples were analyzed according to the Standard Methods for the examination of water and waste water (APHA, 1995) . Field instruments (pH and salinity) were measured in situ using the portable, model, and rechecked in laboratory using bench top equipment to ensure data accuracy (APHA, 1995). Using the Atomic Absorption spectrophotometer for elements being measured (Cu, Pb, and Cd) according to (Ediger, 1973) .

The fish samples were collected randomly from the studied locations, El Mahmodia, Egypt. Fishes were collected alive or in a good condition for examination. The collected samples were transported as soon as possible to the laboratory. At least 28 specimens of each location (7 replicate) were examined in search for the parasitic helminthes.

Determination of the total protein was according to Lowry et al. (1951). Determination of glutathione peroxidase activity was due to Paglia and valentine 1967).

Helminth collection: Fishes were dissected, and the different organs such as the gills, oesophagus, stomach, intestine and rectum were placed in different Petri dishes filled with water or normal saline solution (0.065% NaCl). Each organ was opened by a fine scissor and left for sometimes with occasional shaking. Helminth parasites if present, would become detached from the tissue of the respective organ; in some cases, the parasites were still attached to the host's tissue and by using fine needles, they get loose their grasp and can be easily picked up by using a fine glass dropper. The parasites are to be kept alive for sometimes in small Petri dishes filled with water (1% salinity) as recommended by Schroeder (1971), then examined under a compound research microscope. This step was carried out by putting the parasite between a slide and a thin coverslip (in case of Digenea and larval cestodes) or between two slides (in case of Acanthocephala), the applied pressure

is depending on the thickness of the specimen. Several fixatives were tried, but the fixative used in the present study is sublimate acetic (100ml saturated aqueous mercuricchloride+5ml glacial acetic acid). In this fixative, the helminthes were fixed for 24hrs. , washed in running water for 12hrs., and then placed in alchoholic iodine solution (5ml saturated solution of iodine in 70% alcohol+ 95ml of (70% ethyl alcohol). Staining by Alum Carmine stains (Weesner, 1968). The stained specimens were passed through an ascending series of ethyl alcohol (30, 40, 50, 60, 70, 80, 90, 100%), each concentration, then clearing by xylene then mounting which is the final stage by embedding of the parasite in suitable mounting medium (Canada balsam).

Scanning electron microscopical (SEM) study: Gills were cut into small pieces and fixed with 4% Formalin and 1% Glutaraldehyde (4F1G) fixative mixture in 0.1M phosphate buffer (pH 7.2) for 24 hours at 4°C, then the specimens were post fixed in 2% osmium tetroxide (OsO4) in the same buffer for 2 hours at 4°C, the fixed specimens were then washed in the buffer and dehydrated at 4°C through a graded series of ethanol. Specimens were dried by the critical point drier to prevent collapse and shrinkage, then mounted on an Al-stub and coated with gold in a sputter-coating (Jeol JFC-1100E ion sputtering device). The specimens examined and photographed were made using Jeol scanning electron microscope (JSEM-5300) of the Faculty of Science, Alexandria University operated at 20 kV.

Statistical analysis of the data:

The data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp).The Kolmogorov-Smirnov, Shapiro and D'agstino tests were used to verify the normality of distribution of variables, ANOVA was used for compare two groups for normally distributed quantitative variables for comparing the four studied groups and followed by Post Hoc test (Tukey) for pairwise comparison. Pearson coefficient was used to correlate between quantitative variables. Significance of the obtained results was judged at the 5% level.

Results

The present results showed the mean concentration level in freshwater, El Behaira, Egypt and the pH of the freshwater as well as the salinity. The mean concentration level of heavy metals was reported in the tissue of the Nile river *Oreochromis auruis* in four representing locations of lake El Mahmodia, El Behara, Egypt during the period of winter season of the year 2016-2017(December 2016-february2017).

Table (1) shows the comparison of the mean level of the heavy metals in freshwater as the mean of the highest level of Pb was reported in loc.#2,as 3±0.8 followed by 2.9± 0.4, 2.7±0.4 and 2.3±0.4 in loc.#1, loc.#4, loc.#3;respectively. The mean concentration level of Cu showed the highest level in loc.#4 as 4.1±0.3 and location#2 showed the lowest mean level of Cu as 3.4 ±0.3 whereas loc.#1 and loc.#3 were reported as 3.8±0.4 and 3.4±0.4. The mean concentration level of Cd showed the highest mean level was reported in loc.#2 and loc.#3 as 1.4±0.3 and 1.4±0.5 whereas the mean concentration level of Cd in loc.#1 and loc.#4 were reported as 1.0±0.3 and1.1±0.4.

Table (2) represents the mean level of the pH and the salinity of the fresh water that were collected from the four location representing lake El Mahmodia, El Behara, Egypt during the period of the study. For the salinity, the mean level ranged between 9.9±0.6 in location 2 to 9.2±0.5 in location 1. Location 3 and location 4 were reported as 9.5±0.8 and 9.8 ±0.6. The mean level of the pH concentration ranged between 7.7±0.3 in both loc.#3 and loc.#4 to 7.4 ±0.4 in loc.#1 and loc.#2 was reported as 7.6±0.4.

Table (1): Comparison between the four studied locations according to heavy metals in El Behaira fresh water, Egypt

	Loc.#1 (n = 7)	Loc.#2 (n = 7)	Loc.#3 (n = 7)	Loc.#4 (n = 7)	F	P
Pb Mean ± S.D. Median (Min.– Max.)	2.9±0.4 2.9(2.1–3.3)	3.0±0.8 3(2– 4.2)	2.3±0.4 2.4(1.6– 2.7)	2.7±0.4 2.8(2.1–3.1)	2.568	0.078
Cu Mean ± S.D. Median (Min.– Max.)	3.8±0.4 4(3.1 – 4.2)	3.4±0.3 3.4(3.1– 3.9)	3.4±0.4 3.5(2.9 – 3.9)	4.1^{bc}±0.3 4(3.8 – 4.6)	5.324*	0.006*
Cd Mean ± S.D. Median (Min.– Max.)	1.0±0.3 0.9(0.6 – 1.4)	1.4±0.3 1.5(1 – 1.8)	1.4±0.5 1.3(0.8 – 2)	1.1±0.4 0.9(0.7 – 1.9)	2.483	0.085

Table (2): Comparison between the four studied locations according to the salinity and pH of fresh water, El Behaira, Egypt

Physicochemical parameters of water, El Behara	Loc.#1 (n=7)	Loc.#2 (n=7)	Loc.#3 (n=7)	Loc.#4 (n=7)	F	P
S‰ Mean ± S.D. Median (Min. – Max.)	9.2±0.5 9.1(8.5– 9.8)	9.7±0.5 (9.3–11.2)	9.3±0.4 (8.8–10.8)	9.6±0.5 (9.2– 11.1)	1.606	0.214
pH Mean ± S.D. Median (Min. – Max.)	7.4±0.4 7.3 (7 – 8.1)	7.6±0.4 7.8 (7 – 8)	7.7±0.1 7.7 (7.5 – 7.9)	7.7±0.2 7.8 (7.3– 7.9)	1.170	0.342

In table (1), the mean Cu concentration level in location (4) was highly significantly different from that of loc.#2 and loc.#3, at F=5.324 and at P<0.006*. In table (2) the pH and the salinity did not show significant difference in the four representing locations for Lake El Mahmoudia, El Behara, Egypt during the period of study (winter 2016-2017).

Table (3) represented the SOD, GPx and the total protein level in the fish *Oreochromis auruis* collected from El Mahmodia Lake in winter (2016-2017) from the studied locations. The mean concentration level of SOD in fish whole tissue showed high significant difference level at F=6.194 and P<0.003*. The highest concentration level was reported in location 3 as 3.8±2.5. The lowest concentration level was reported as 0.7±0.1 in location 4 while location 1 and location 2 showed the mean concentration level as 0.8±0.1 and 3.3±0.4.

The mean concentration level of GPx in the fish collected from the study locations was represented as; 11.0±0.7, 12.4±0.3, 11.3±0.8 and 10.4±0.4 in loc.#1, loc.#2, loc.#3 and loc.#4; respectively. The loc.#2

showed the highest level of the activity of GPx and loc.#4 showed the lowest activity of the enzyme level of GPx as 10.4±0.4. The activity of the enzyme GPx showed a significant difference in the four studied locations representing El Mahmoudia lake at F= 15.5 and P<0.001*. The total protein level ranged between 4.4±0.3 in loc.#4 to 5.9±1.0 in loc.#2 whereas both location 1 and location 3 showed 5.5±0.5 and 5.5±0.8. The mean concentration level of total protein differed significantly between the four representing locations at F=5.414 and P<0.005*.

Table (3): Comparison between the four studied locations according to the stress enzymes and total protein in the fish *Oreochromis auruis* collected from water, El Behaira, Egypt

	Loc.#1 (n=7)	Loc.#2 (n=7)	Loc.#3 (n=7)	Loc.#4 (n=7)	F	P
GPx Mean ± S.D. Median(Min.–Max.)	11.0±0.7 11.1(9.8–11.9)	12.4±0.3[a] 12.5(12–12.7)	11.3±0.8[b] 11.6(10.2–12.3)	10.4±0.4 10.4(9.7–10.9)	15.487*	<0.001*
SOD Mean ± S.D. Median(Min.–Max.)	0.8±0.1 0.7(0.6 – 0.9)	3.3±2.4 1.9(1–5.9)	3.8±2.5[a] 5.7(0.9–5.9)	0.7±0.1[bc] 0.7(0.6–0.8)	6.194*	0.003*
Total protein Mean ± S.D. Median(Min.–Max.)	5.5±0.5 5.6(4.7–5.9)	5.9±1.0 6(4.5–7.1)	5.5±0.8 5.5(4.7–6.7)	4.4±0.3[bc] 4.5(4.1–4.9)	5.414*	0.005*

Table (4): Comparison between the four studied locations according to heavy metal in tissue of *Oreochromis auruis* collected from El Behaira, Egypt

	Loc.#1 (n = 7)	Loc.#2 (n = 7)	Loc.#3 (n = 7)	Loc.#4 (n = 7)	F	P
Pb						
Mean ± S.D.	3.1 ± 0.3	3.1 ± 0.4	3.2 ± 0.5	4^{abc} ± 0.5	6.633*	0.002*
Median (Min.–Max.)	3.1(2.7 – 3.5)	3(2.6 ± 3.8)	3.4(2.3 – 3.7)	3.8(3.5 – 5)		
Cu						
Mean ± S.D.	4.9 ± 1	3.7 ± 0.8	4.2 ± 0.6	5.4^{b} ± 1.1	5.053*	0.007*
Median (Min.– Max.)	5.3(3.5 – 6.1)	3.9(2.6 – 4.6)	4.3(2.9 – 4.9)	5.8(4 – 6.6)		
Cd						
Mean ± S.D.	1.3 ± 0.4	1.3 ± 0.4	1.9 ± 0.5	1.6 ± 0.6	2.388	0.094
Median (Min.– Max.)	1.4(0.8 – 1.8)	1.2(1 – 2)	1.9(1.4 – 2.9)	1.7(0.8 – 2.3)		

F, p: F and p values for ANOVA test, Sig. bet. locations was done using Post Hoc Test (Tukey)

a: Statistically significant with loc.#1 -b: Statistically significant with loc.#2 -c: Statistically significant with loc.#3. *: Statistically significant at $p \leq 0.05$

Table (4) represents the comparison between the studied locations according to the heavy metals in tissues of the Nile fish *Oreochromis auruis* collected from four representing locations in El Mahoudia, El Behara, Egypt. The mean level of Pb in tissue showed 3.1±0.3, 3.1±0.4, 3.2±0.5 and 4.0±0.5 in loc.#1, loc.#2, loc.#3 and loc.#4; respectively. The level of Pb in tissue in location 4 differs significantly than loc.#1, loc.#2 and loc.#3 at F=6.633 and P<0.002*. The Cu concentration level in the tissue was reported as 4.9±1.0, 3.7±0.8, 4.2±0.6 and 5.4±1.1 in loc.#1, loc.#2, loc.# and loc.#4; respectively. The level of Cu in location 4 differs significantly from loc.#1, loc.#2 and loc.#3 at F=5.053 and P< 0.007*. The mean level of Cd was reported in table 4 as 1.3±0.4,1.3±0.4,1.9±0.5 and 1.6±0.6 in loc.#1, loc.#2, loc.#3 and loc.#4 ; respectively whereas the highest level of Cd was reported in loc.#3 at F=2.388 and P<0.094.

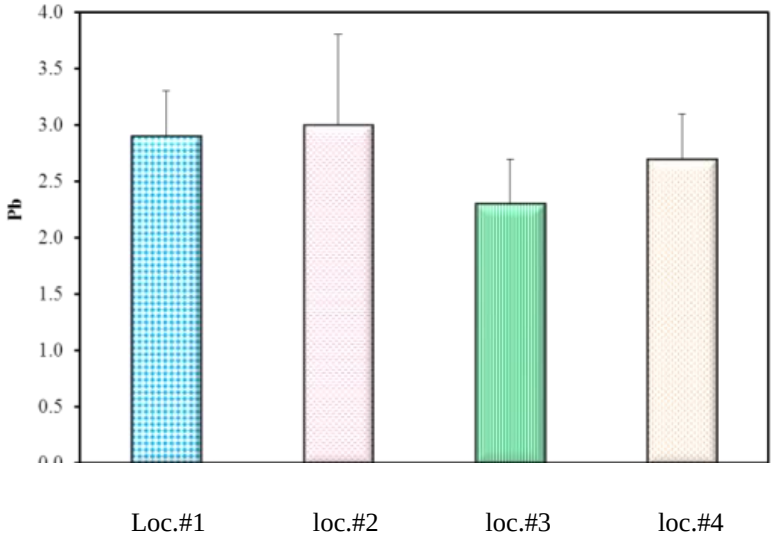

Fig. (2): Comparison between the four studied locations according to Pb in freshwater, El Behara, Egypt.

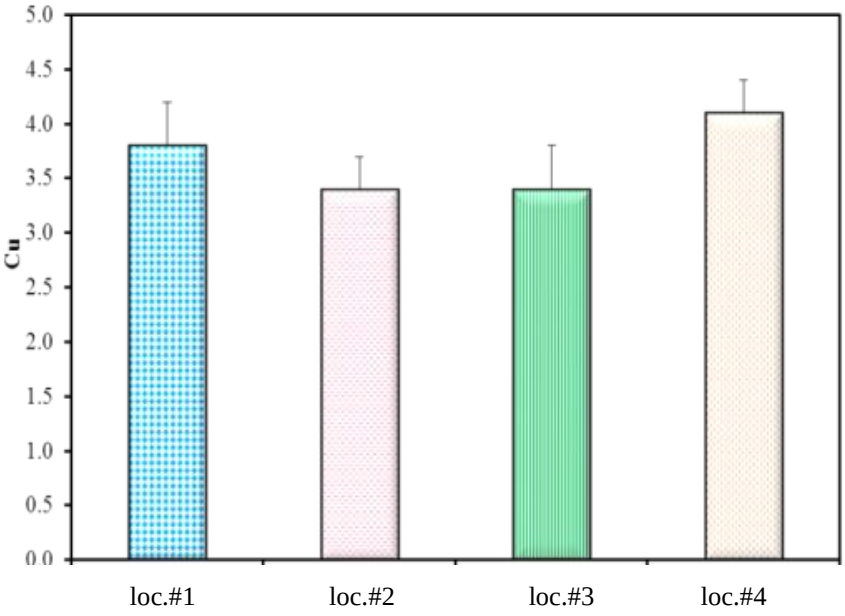

Fig. (3): Comparison between the four studied groups according to Cu in freshwater, El Behaira, Egypt.

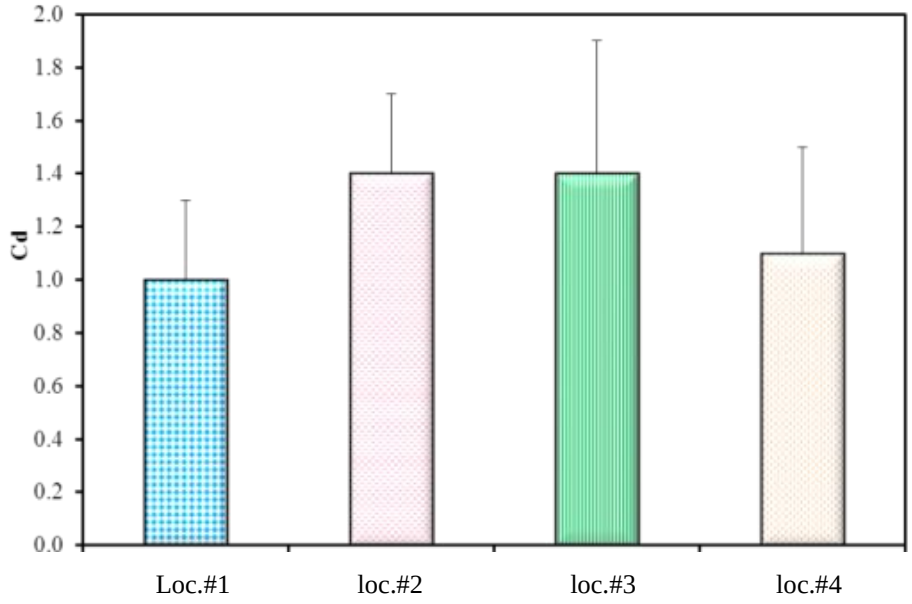

Fig. (4): Comparison between the four studied groups according to Cd in freshwater, El Behaira, Egypt.

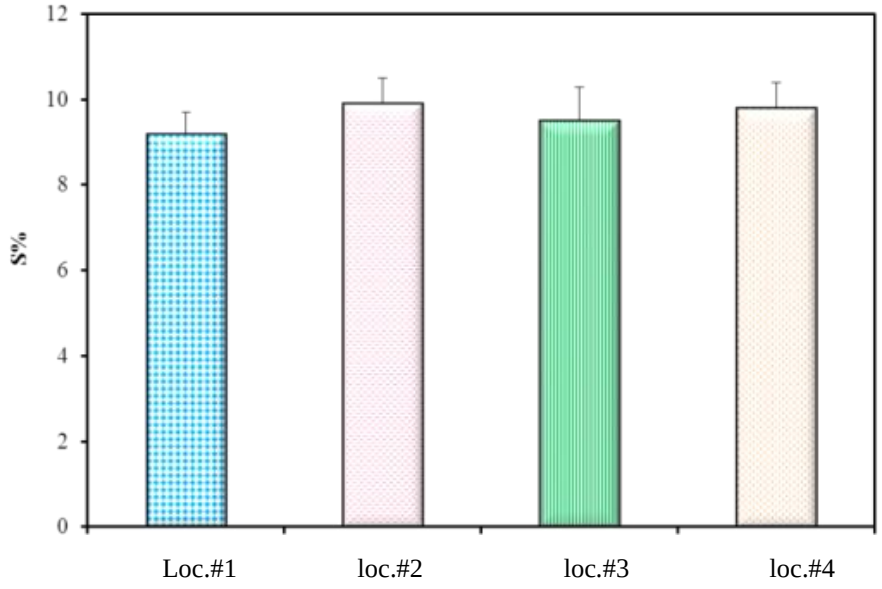

Fig. (5): Comparison between the four studied groups according to S‰ in freshwater, El Behaira, Egypt.

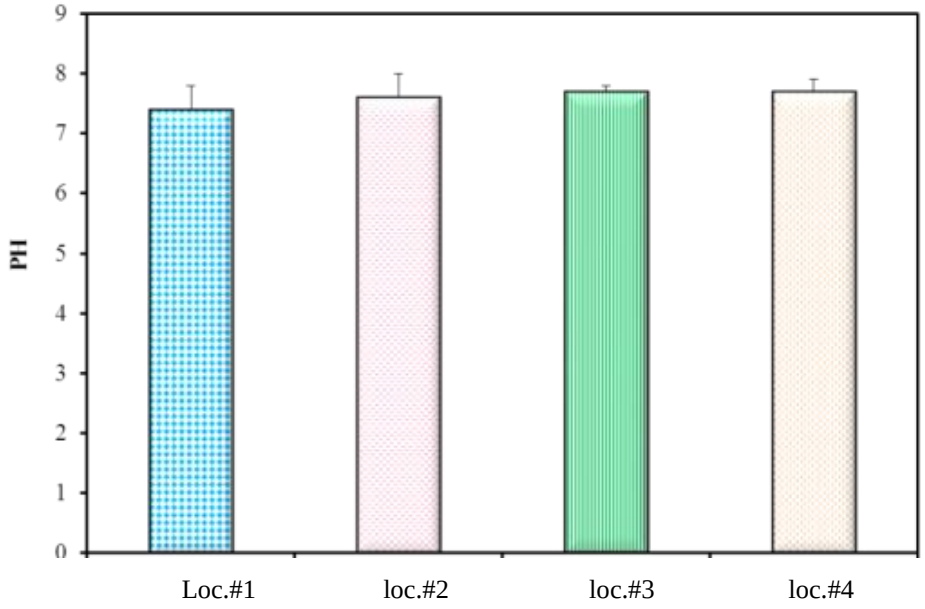

Fig. (6): Comparison between the four studied locations according to pH in freshwater, El Behaira, Egypt.

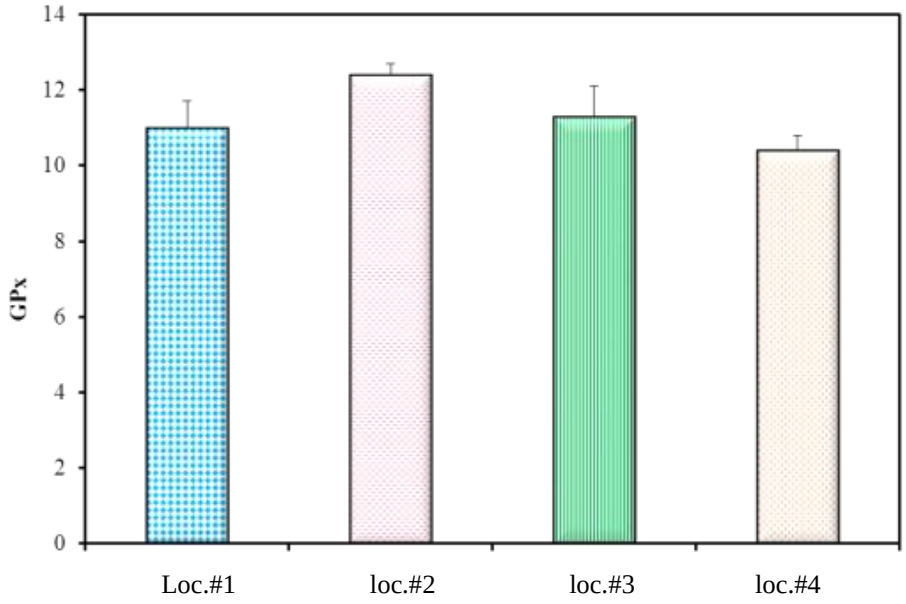

Fig. (7): Comparison between the four studied locations according to GPx in tissue of *Oreochromis auruis* collected from El Behaira, Egypt.

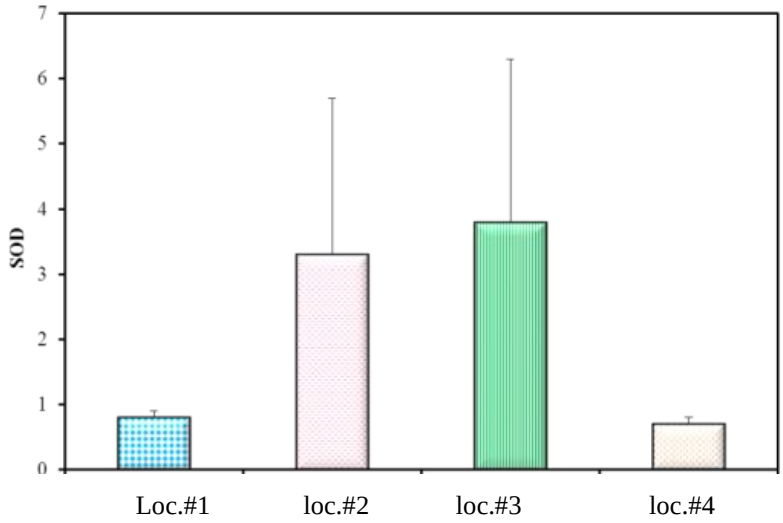

Fig. (8): Comparison between the four studied groups according to SOD in tissue of *Oreochromis auruis* collected from El Behaira, Egypt.

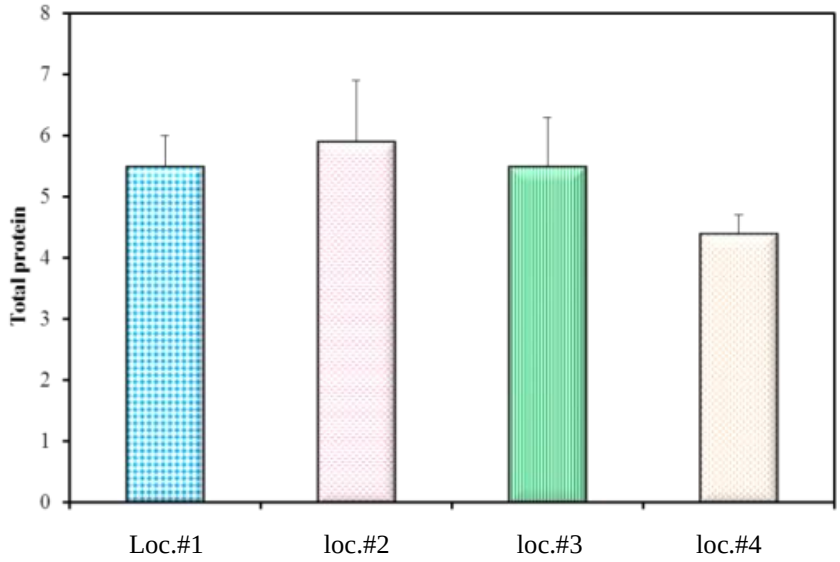

Fig. (9): Comparison between the four studied locations according to total protein in tissue of *Oreochromis auruis* fish collected from El Behara, Egypt.

Table (5): Correlation between the different parameters in loc. # 1

		Cu	Cd	S‰	pH	GPx	SOD	Total protein
Pb	r	0.256	0.352	0.352	-0.114	0.510	-0.398	0.614
	p	0.580	0.439	0.438	0.807	0.242	0.377	0.143
Cu	r		0.752	0.653	-0.013	-0.185	0.005	0.744
	p		0.051	0.112	0.979	0.691	0.992	0.055
Cd	r			0.446	-0.180	-0.289	0.430	0.644
	p			0.316	0.700	0.530	0.336	0.119
S‰	r				-0.642	-0.350	-0.116	0.600
	p				0.120	0.442	0.805	0.155
pH	r					0.351	-0.287	0.007
	p					0.440	0.532	0.989
GPx	r						-0.388	0.073
	p						0.389	0.876
SOD	r							-0.052
	p							0.913

r: Pearson coefficient

*: Statistically significant at $p \leq 0.05$

Table (5) shows the correlation coefficient between the heavy metals and pH and the salinity in the fresh water of lake El Mahmodia and the oxidative stress enzymes in the Nile fish *Oreochromis auruis* collected in winter (Decermber2016-February2017). Pb in table 5 is highly positively correlated with GPx and the total protein as r= 0.51 and r= 0.61; respectively Cu is highly positively correlated with Cd, S‰ and the total protein as r=0.75, r=0.65 and r=0.74; respectively. Cd is only highly significantly correlated with the total protein as r= 0.64. The salinity is highly correlated with both pH and the total protein as r=-0.64 and r=0.6; respectively. The pH is highly negatively correlated with both GPx and with the total protein as r= - 0.50 and r=-0.52; respectively. The activity of GPx is highly correlated with only the activity of the SOD as r=- 0.701.

Table (6): Correlation between the different parameters in loc.# 2

		Cu	Cd	S‰	PH	GPx	SOD	Total protein
Pb	r	0.223	0.828*	-0.618	0.395	0.438	-0.759*	-0.152
	p	0.631	0.021*	0.139	0.380	0.326	0.048*	0.745
Cu	r		0.500	-0.597	0.887*	0.000	-0.238	-0.345
	p		0.253	0.157	0.008*	1.000	0.607	0.448
Cd	r			-0.625	0.436	0.161	-0.430	-0.250
	p			0.133	0.329	0.731	0.335	0.589
S‰	r				-0.750	-0.727	0.693	0.547
	p				0.052	0.064	0.085	0.204
pH	r					0.286	-0.509	-0.523
	p					0.534	0.244	0.229
GPx	r						-0.701	-0.219
	p						0.079	0.637
SOD	r							0.006
	p							0.989

r: Pearson coefficient ,*: Statistically significant at $p \leq 0.05$

Table (7): Correlation between the different parameters in loc.# 3

		Cu	Cd	S‰	pH	GPx	SOD	Total protein
Pb	r	0.822*	0.789*	-0.746	-0.373	0.623	-0.531	0.747
	p	0.023*	0.035*	0.054	0.410	0.135	0.220	0.054
Cu	r		0.374	-0.517	-0.161	0.512	-0.604	0.592
	p		0.409	0.234	0.730	0.240	0.151	0.162
Cd	r			-0.795*	-0.607	0.530	-0.433	0.394
	p			0.033*	0.148	0.222	0.332	0.382
S‰	r				0.494	-0.369	0.627	-0.356
	p				0.259	0.416	0.132	0.433
pH	r					-0.240	0.716	-0.048
	p					0.604	0.070	0.919
GPx	r						-0.023	0.672
	p						0.961	0.098
SOD	r							-0.051
	p							0.913

Table (8): Correlation between the different parameters in loc. # 4

		Cu	Cd	S‰	pH	GPx	SOD	Total protein
Pb	r	0.400	-0.492	-0.410	0.340	0.690	0.464	-0.385
	p	0.373	0.262	0.361	0.456	0.086	0.294	0.393
Cu	r		0.269	-0.140	0.441	0.528	0.197	0.006
	p		0.559	0.764	0.322	0.223	0.672	0.990
Cd	r			0.010	0.590	-0.558	-0.651	0.614
	p			0.983	0.163	0.193	0.113	0.143
S‰	r				-0.565	-0.471	-0.543	0.435
	p				0.187	0.286	0.208	0.330
pH	r					0.025	-0.193	0.245
	p					0.958	0.678	0.596
GPx	r						0.895*	-0.599
	p						0.006*	0.155
SOD	r							-0.635
	p							0.125

r: Pearson coefficient , *: Statistically significant at $p \leq 0.05$

Table (9): Correlation between the different heavy metals in each location

		Loc.#1		Loc.#2		Loc.3		Loc.#4	
		Cu	Cd	Cu	Cd	Cu	Cd	Cu	Cd
Pb	R	0.344	0.460	-0.248	0.134	0.658	-0.880	0.558	0.351
	P	0.449	0.299	0.593	0.774	0.108	0.009	0.193	0.440
Cu	R		0.199		-0.712		-0.792*		0.781*
	P		0.668		0.073		0.034*		0.038*

r: Pearson coefficient

*: Statistically significant at $p \leq 0.05$

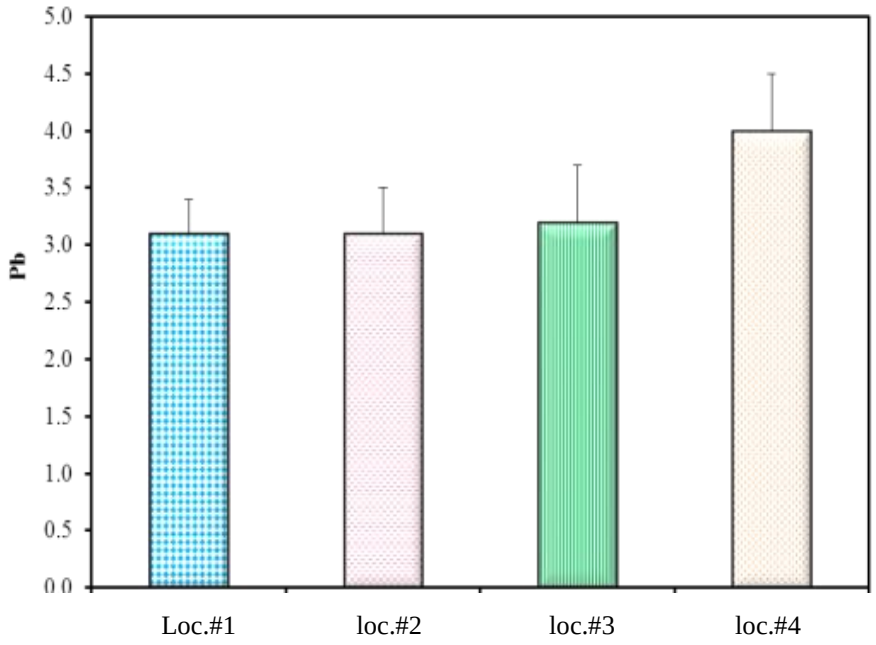

Fig. (10): Comparison between the four studied locations according to Pb in tissue of *Oreochromis auruis*

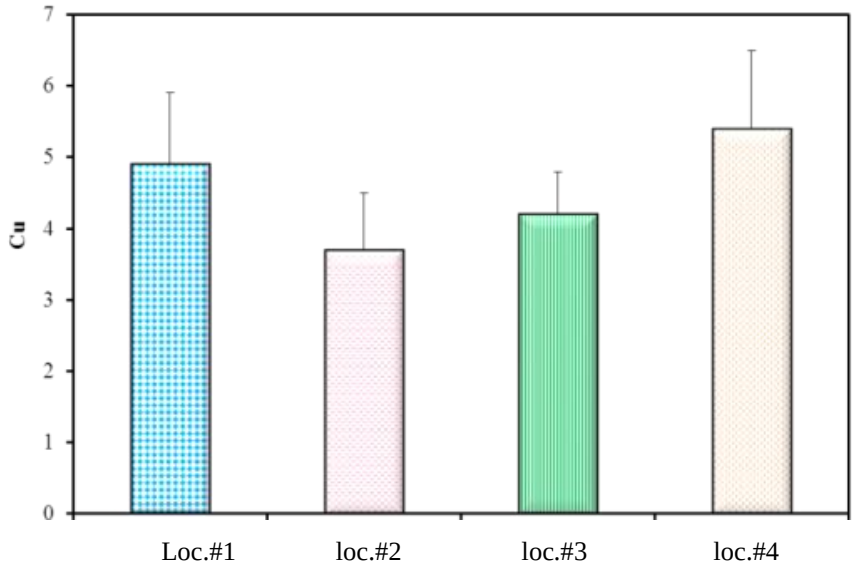

Fig. (11): Comparison between the four studied locations according to Cu in tissue of *Oreochromis auruis*

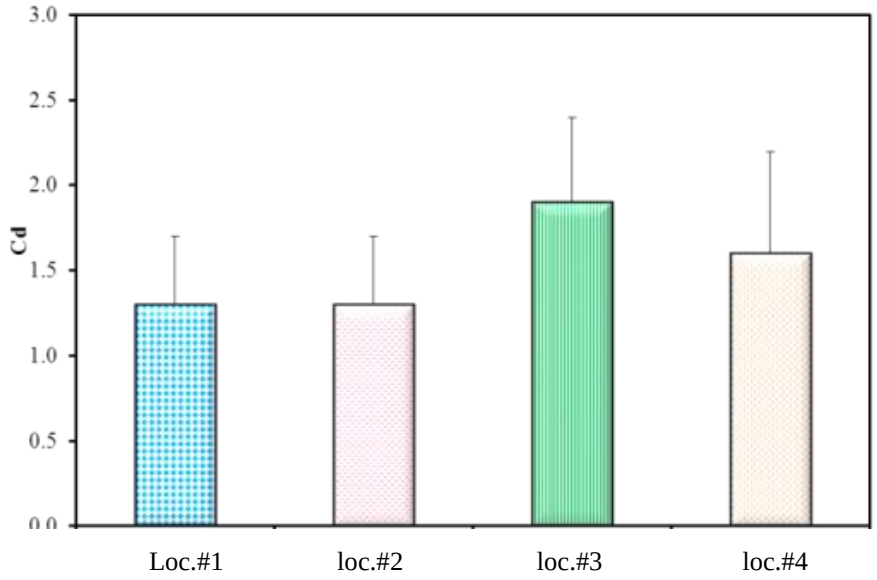

Fig. (12): Comparison between the four studied groups according to Cd in tissue of *Oreochromis auruis*

Scanning electron microscopy (SEM) results:

The Scanning electron microscopy (SEM) of the *Oreochromis auruis*'gills collected from the four locations of El Mahmodia canal revealed normal features for both primary and secondary gill lamellae at lower and higher magnifications in loc.#1(Fig. 13A, 14A respectively). Gill from the other sites showed some abnormal features in the form of clumping, curling, loss of alignment and disorganization of the primary lamellae in loc.#2 (Fig. 13B); breakage of secondary lamellae in loc.# 3 (Fig. 13C) and fusion of the filaments in loc.#4 (Fig. 13C). In closer point of view, another finding can be observed in locations from 2 to 4 as follow: fusion of the secondary gill lamellae and epithelial hypertrophy of the primary filaments, along with a loss of the filaments 'cell border and obliteration of the space between secondary gill lamellae (Fig. 14).

It is well known that pavement cells are the most abundant cell type covering the gill epithelium and its apical surface bears microridges. The SEM revealed that these microridges appeared more intensive in the distal portion of the filament when compared to the proximal one (Fig. 15). In addition, chloride cells can be observed also in between the pavement cells (Fig. 15). It is must notice that the microridges of loc.# 4 exhibited sharp structure, but it was agglomerated, disorganized and appeared in a different form comparing with other locations (Fig. 15). However, the microridges exhibited the uniform architecture and organized in loc.#3, while, it appeared less sharpened and not well defined in loc.#1. Moreover, the mucous secretion housing the inter-lamellar space between the secondary lamellae, and it was interesting to note that loc.# 4 obtain a huge amount of these secretions (Fig. 16).

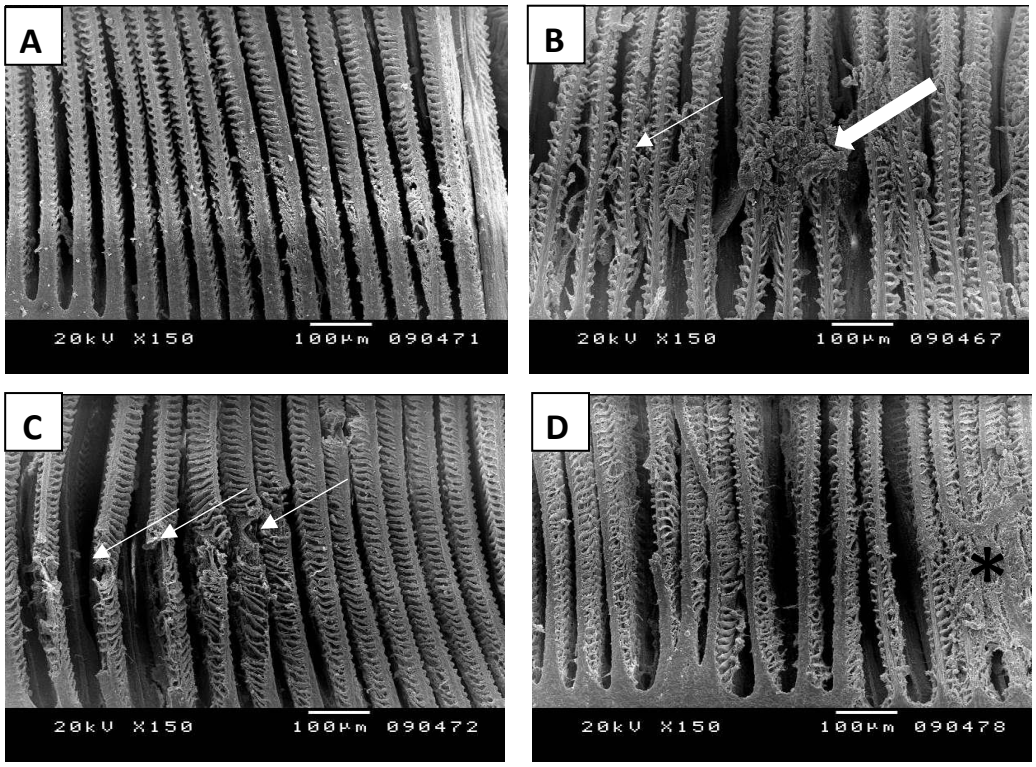

Fig. 13: Low power scanning electron micrograph showing the architecture of the gills in the different locations. A) Loc.#1, showing the approximate normal appearance of gill filaments. B) Loc.#2, showing clumping and curling filaments (thick arrow) and loss of their alignments of (thin arrow). C) Loc.#3, showing breakage of secondary lamellae (arrow). D) Loc.# 4, showing fusion of the filaments (*) (X150).

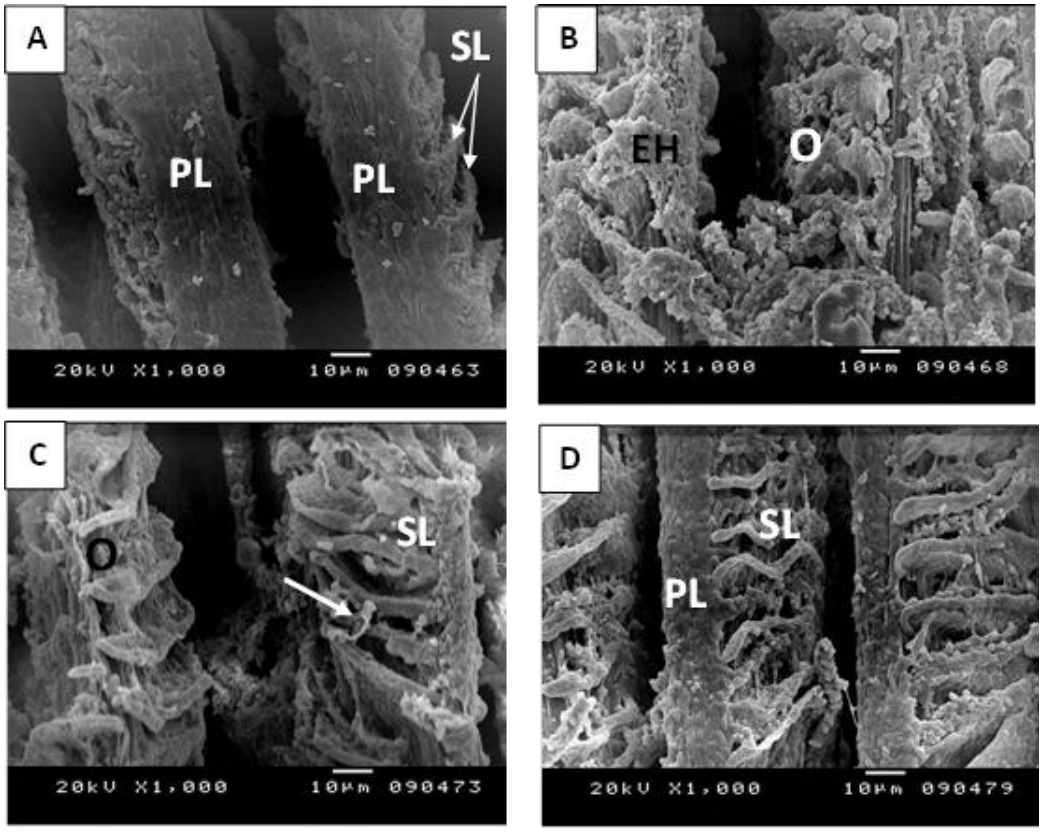

Fig. 14: Scanning electron micrograph showing the architecture of the gills in the different locations. A) Loc.# 1, showing the approximate normal appearance of gill filaments; primary lamellae (PL) with normal gill epithelium and secondary lamellae (SL). B) Loc.#2, showing epithelial hypertrophy (EH), loss of cell border, disorganization and obliteration (O) of space between secondary gill lamellae. C) Loc.#3, showing mucous secretion (arrows), obliteration (O) of space between secondary gill lamellae and secondary lamellae (SL). D) Loc.#4, showing sever disorganization of secondary lamellae (SL), primary lamellae (PL) (X1000).

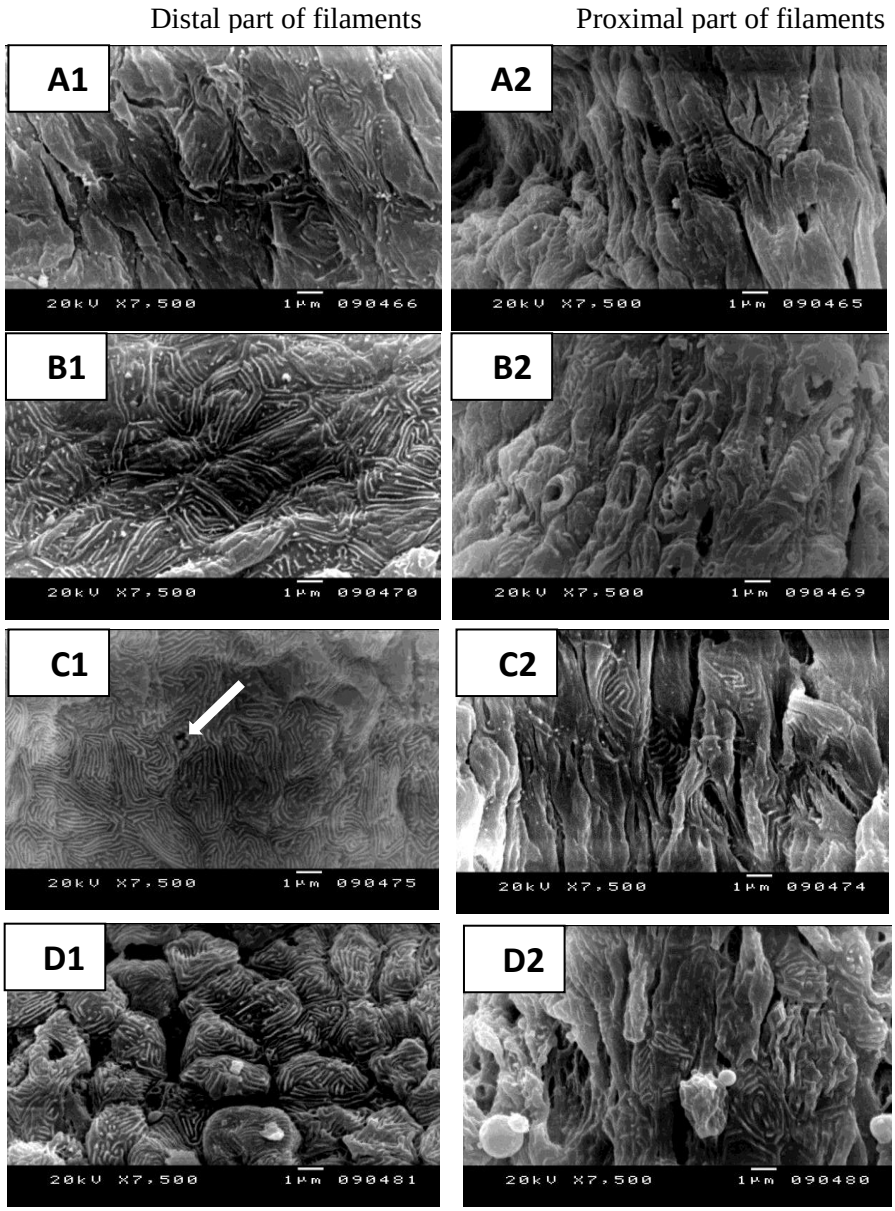

Fig. 15: Scanning electron micrograph showing Pavement cells of the proximal and distal parts of the primary gill filaments in the four different locations. Note: the microridges abundant in the distal part and gradually disappeared in the proximal part. A1, 2) Loc.#1 showing a not defined microridges. B1, 2) Loc.#2 showing defined microridges. C1, 2) Loc.#3 showing uniform, organized architecture of microridges. D1, 2) Loc.#4 showing agglomeration of sharp microridges, Chlorid cell (arrow) (X7500).

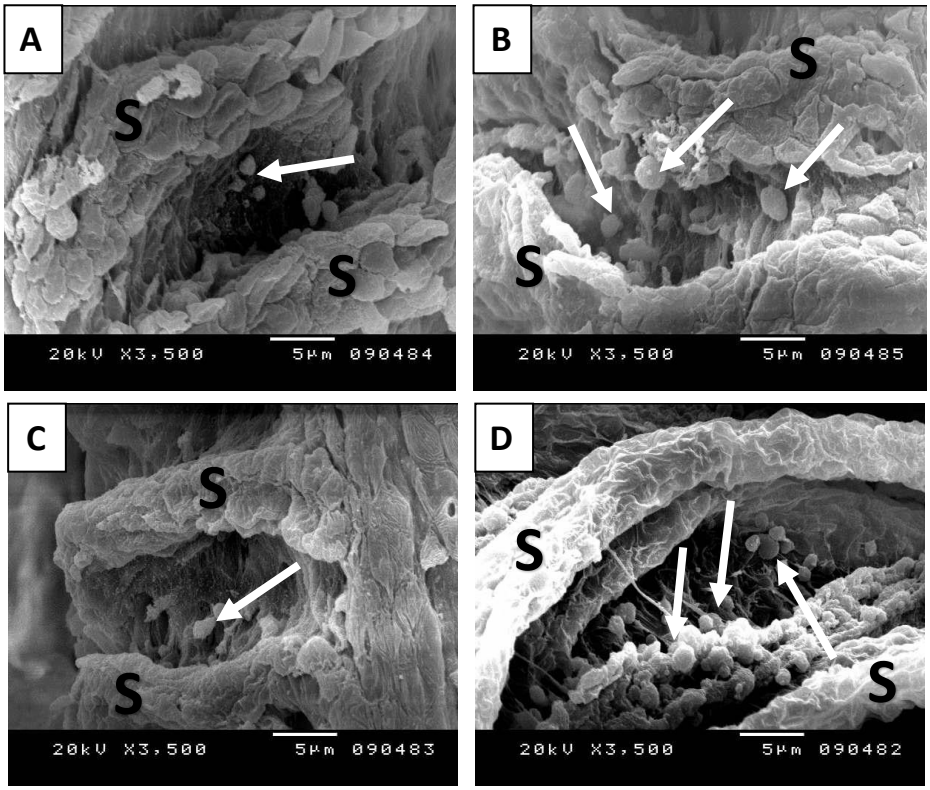

Fig. 16: Scanning electron micrograph showing the mucous secretions in the inter-lamellar area between two secondary lamella in the different locations. A) Loc.#1. B) Loc.#2. C) Loc.#3. D) Loc.# 4. Arrows indicate the mucous secretion, secondary lamellae (SL) (X3500).

Discussion

In the present study, the gills were selected for study because they perform many important functions such as respiration, acid-base balance, excretion and osmoregulation, and they are continuously and directly exposed to the external environment. They are indicators of water quality and used for studying the effects of environmental stressors on fish (Tkatcheva *et al*., 2004; Vigliano *et al*., 2006). The gill structure alterations affect their physiological functioning (Wendelaar Bonga and Lock, 2008). Gills have frequently been used in the assessment of impact of aquatic pollutants in marine as well as in fresh water habitats (Femanders *et al*., 2007; Miron *et al*., 2008; Nwani *et al*., 2010). Electron microscopy is proven to be reliable for examining the adverse effects of pollutants on the fish tissues and for assessing the effects of environmental stressors on fish structures (Palamiappan *et al*., 2008; Mir and Channa 2009).

In the present study the loss of alignment in the primary lamellae, as detected by SEM is indicative of increase in volume of non-tissue spaces of the lamellar epithelium resulting in increased diffusion distance (Fernandez and Mazon, 2003). The morphological abnormalities observed in the present study can lead to influx of ions, inhibition of active reception of ions and interference in the gaseous exchange. The phenomenon of epithelial hypertrophy of the primary filaments was also observed in the fish exposed to adverse water quality (Mallat, 1985) leading to the increase in the diffusion distance affecting gaseous exchange (Nowak, 1992). Cellular hypertrophy observed, could lead to a decrease in the respiratory capacity between the lamellae, impairing the diffusion of oxygen across the gills due to swollen condition of the epithelium (Ayoola, 2008a, b).

The disorganization and obliteration of the space between secondary gill lamellae is in agreement with Scwaiger *et al.* (2004) which revealed that this finding is probably induced by the incidence of severe oedema. This alteration is more often encountered in freshwater fishes than in marine fishes (Mailatt, 1985), which could be because freshwater fishes are hyper-osmotic in relation to the environment, increasing the volume in the edema (Machado and Fanta, 2003). Since the secondary lamellae are the site of gaseous exchange, with the blood-to-water diffusion distances less than one micrometer in active species (Evans, 1987), this damage could interfere with the efficiency of gas exchange (Jagoe *et al*., 1996a.). Nonetheless, such alteration is also an example of defense mechanism because lifting of lamellar epithelium increases the distance between the external environment and the blood, thus serving as a barrier to the entrance of contaminants (Fernandes and Mazon, 2003). These modifications can produce adverse effects on fish health and may increase their susceptibility to secondary infectious diseases and even may cause death (Hawkins *et al*., 1984).

Fusion of gill lamellae along with oedematous epithelial cells as observed in the present study have been also observed in fish exposed to pesticides, industrial wastes and other organic wastes (Venkataraman *et al*., 2007). Oedematous changes in gill filaments and secondary lamellae probably are due to increased capillary permeability (Kakuta and Murachi, 1997; Olurin *et al.*, 2006). However, the fusion of the secondary lamellae causes a decrease in free gas exchange and may play a defensive role against contamination (Khoshnood *et al*., 2011).

Mucous cells can be efficient in seizing the toxic agents and help in preventing the entrance of these agents into the gills. In the present study proliferation of mucous secretions was seen, indicative of the function of mucous in protecting the gill epithelium from environmental impacts, infectious agents, toxic agents and particles in suspension (Powell *et al*., 1992; Biagini *et al*., 2009). In addition, abundant Pavement cells are covering the primary filaments and its apical surface bears microridges was notice in our scanning micrographs, this is in agreement with Wilson and Laurent (2002) which stated that, Pavement cells are the most abundant cell type covering the gill epithelium and its apical surface bears microridges or microvilli and are the sites of proton pump-driven sodium uptake. Besides this, the microridges increase the functional surface of the epithelium (Mallat, 1985). The abundance of microridges or the reduction of it indicates the protective ability of the gills in relation to the quality and quantity of pollutants in the environment. Similar observations were made by different authors from time to time (Wong and Wong, 2000; Mazon *et al*., 2002; Biagini *et al*., 2009).

The variability of the environment affects processes at all levels of the organization ecology (Dunham *et al*., 1989). The main source of Cu and Pb in the Egyptian irrigation system, are industrial wastes (Mason, 2002). Metal levels are increased due to agricultural, industrial, and domestic activities (Kalay and Canli, 2000; Santoe *et al*., 2005). Water quality reflects inputs from the atmosphere (Ayazi *et al*., 2010; Zhang *et al*., 2011).

The pH is a limiting factor for aquatic organisms. The severe changes of pH of the water may cause a harmful effect on aquatic organisms and affect the human health. Any change in the pH affects aquatic biota. If the pH increases above this range, smaller amounts of ammonia are needed to reach a level that is toxic to fish, while when pH decreases, acidity of the water increases affecting the fish (Murdoch, 1991).

The stream has pH values within the permissible limits of law 48/1982 (7.94–8.50) and are not harmful for aquatic life and irrigation, where the pH of most natural water ranges between 6 and 8.5 (WHO, 1993). The normal pH for irrigation water is from 6.5 to 8.4 (FAO, 1985). In Egypt and other developing countries, where environmental protection laws have not been enforced, industrial and domestic wastes are dumped randomly into water bodies. These wastes have been reported to contain toxic and hazardous substances including metals. The contamination of water resources by trace metals is of important concern because of their toxicity, persistence and bio accumulative nature (Ikem *et al*., 2003).The primary sources of Cu are domestic wastewater, manufacturing processes

involving metals, steam electrical production, the dumping of sewage sludge, and atmospheric deposition. The high levels of Cu in water can be attributed to industrial and agricultural discharge (Mason, 2002).

Aquatic pollution causes threat to the survival of aquatic organisms (Saeed and Shaker, 2008). The present results of winter season (2016-2017) showed that the significant effect of season on water samples in all studied ecosystems as water in EL Mahmoudia stream in winter comparing with summer season data of Azab *et al*. (2012). Stephenson (1987) attributed the effect of pollution to be neurotoxicity of the pollutants on the fish. The data of the present work and other data from the literatures previously cited show the hazards of pollutants to fishes. From the eco-physiological point of view, the effects of pollutants on non-target species must be carefully evaluated.

In the present study there were no parasites reported in the selected fish in El Mahmoudia, ElBehara, Egypt in the four location represented in the present study. Hassan *et al.* (1990) described *Licithobotyrs aegyptiacus* from the intestine of *Mugil capito* (fish) caught from the Egyptian Mediterranean waters. Gupta and Tandon (1985) described *Gyliauchen indicum* from the intestine of *Engraulis hamiltoni*, a fish from India. Hassanine (2000) described *Gliauchen volubilis* Nagaty, 1956 from the intestine of *Siganus rivulatus*, a common fish in the northern Red Sea, Egypt.

Ramadan (1986) described *Apparyngogyliauchen callyodontis* Yamaguti, 1942 from the intestine of two fish species from Red sea, Egypt. Ramadan (1983) described *Proctoeces gohari* from the intestine of *Acanthopagrus bifasciatus*, a fish from Red Sea, Egypt. Ahmad and Dhar (1987) described *Lasiotocus guptai* from *Cynoglossus dubius*, a fish in the Arabian Sea. Viozzi *et al.* (2000) described *Steganoder szidati* from the intestine of freshwater fishes, (*Galaxias maculates* and *G. platei*) from Patagonia, Argentina. Etchegoin *et al.* (2002) described *Steganoderma valchetensis* from the intestine of *Gymnocharacinus bergi*, a freshwater fish from Patagonia, Argentina.

Thelma (2003) described *Allopodocotyle skoliorchis* from the intestine of *Parequula melbourensis*, a fish in Australia water. Shen (1985) described *Lecithocladium dongshanensis* from the stomach of *Pseudosciaena crocea*, a fish from the East China Sea. Toman (1992) recoded *Lecithocladium chingi* Manter and Pritchard, 1960 from the stomach of *Naso vlamingli* from the Indian Ocean. Chamber *et al.* (2001) described *Lecithocladium invasor* from the intestine of *Naso vlamingi*, a fish from Australia. Nadakal *et al.* (1991) described *Dinurus hippuri* from the stomach of *Coryphaena hippurus*, a fish from India.

Poulin (2000) described that the intensity of infection with helminth parasites is directly related to the host's size. They believed that chances for certain parasitism may be greater for larger individuals which having greater surface areas, consuming more potentially parasite-laden food and have lived longer than smaller ones. Mordvinova (1988) described *Neorhadinorhynchus myctophumi* from the intestine of *Myctophid sp*., a fish from the World Ocean, Russia. Martens and Moens (1995) and Geets and Ollevier (1996) reported *Sclerocollum rubimaris* Schmidt and Paperna, 1978 from the fish *Siganus sutor* at Kenyan coast.

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