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Keywords: *african catfish, pollution, nile delta, biomarkers, histopathology.*

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Effect of Water Pollution on Physiological and Histological Alterations of African Catfish, *Clarias Gariepinus* from Nile Delta

Ali A. Al-Halani[α], Ahmed E. Hagras[σ], Sherif H. Abdeen[ρ], Abeer E. Abdrabbuh[Ѡ] & Mohamed I. Mashaly[¥]

Abstract- The present study aimed to assess the effect of water pollution on physiological and histological alterations of African catfish, Clarias gariepinus collected from spring 2015 to winter 2016, in two differing-quality aquatic ecosystems in Nile Delta, Egypt, namely Damietta Branch of River Nile and Ammar Drain. Results showed significant differences ($p \leq 0.001$) in all estimated physicochemical environmental factors in study areas. In addition, the results showed that the bad water quality in Ammar Drain due to pollution, the vast majority of the hematological parameters recorded lower levels in Ammar Drain than in River Nile. Because Ammar Drain receives greater agricultural, industrial and domestic wastes than the second one. Antioxidant enzyme activities were lower in fish from the contaminated site compared with fish from the reference site, suggesting a deficiency of the antioxidant system to overcome the oxidative stress. Lesions of histological deformations (skin, muscles, and gonads) were detected and analyzed to clarify the effect of water pollution on fish organs. The present study recommended treatment of the waste before being discharged into the waterways.

Keywords: african catfish, pollution, nile delta, biomarkers, histopathology.

I. Introduction

Fish spend their lifespan in water (from egg to adult), so, they are subjected to the full force of the aquatic environment, regarding physical, chemical and biological parameters. Successful survival of fish in fluctuating conditions of the aquatic ecosystem necessitates the possession of efficient hematological, physiological and immunological mechanisms to cope with the dramatic fluctuations of constituent elements of the ecosystem (Osman, 2010 & Ben Ameur et al., 2012). Point and non-point contamination sources affect water physicochemical features, natural ingredients of the sediment and biological components that in turn alter the quality and quantity of fish stocks (Mantovi et al., 2005 & Singh et al., 2006).

Field and experimental studies revealed that the buildup of pollutants in tissue is chiefly dependent on the amount of pollutant and duration of the exposure time. However, other physicochemical parameters such as temperature, hardness, pH and salinity can play an important role in the accumulation of pollutants in fish (Jeffree et al., 2006 & Has-Schon et al., 2007). Demonstration of the histopathology of the fish has been found to be a consistent bioindicator of the environmental stress (Elahee and Bhagwant, 2007), and a sensitive tool in demonstrating the interplay between the performance of fish and contamination of their natural habitats (Costa et al., 2009 (Evaluation of the biochemical and hematological profiles of fish has become a key indicator of the health and means of comprehending normal and pathological progressions, and toxicological influences (Saravanan et al., 2011). Fish represent one of the most nutritive and cheap sources of the animal protein. Fish contribute to about 6% of the world supply of protein and about 24% of the animal protein (El-Badry, 2010). African catfish, Clarias gariepinus (Burchell, 1822) are highly consumed by a large sector of Egyptians. These fish are more abundant throughout the year than much other Egyptian fish. They are important and cheap sources of the animal protein (Kime et al.,1996 & El-Badry, 2010). A promising development program of fish resources could solve the problem of animal protein deficiency at qualitative and quantitative levels (Nguyen, 2009). In this respect, the proper investigation of different aspects of the fish biology and ecology are strongly recommended (El-Etreby et al., 1993). The present study aimed at evaluating the effect of water pollution in Nile Delta on physiological and histological alterations of African catfish, Clarias gariepinus.

II. Materials And Methods

a) Study Area

Two different aquatic ecosystems were selected. Ammar Drain at Belqas city as a polluted environment and Damietta Branch of River Nile at Al-Tawailah village as a reference site50 km north Mansoura City and has the following coordinates: 31°22'46.4556" N 31°29'13.2432" E (Figure 1). The present study was conducted during the period spring March 2015 to winter 2016.

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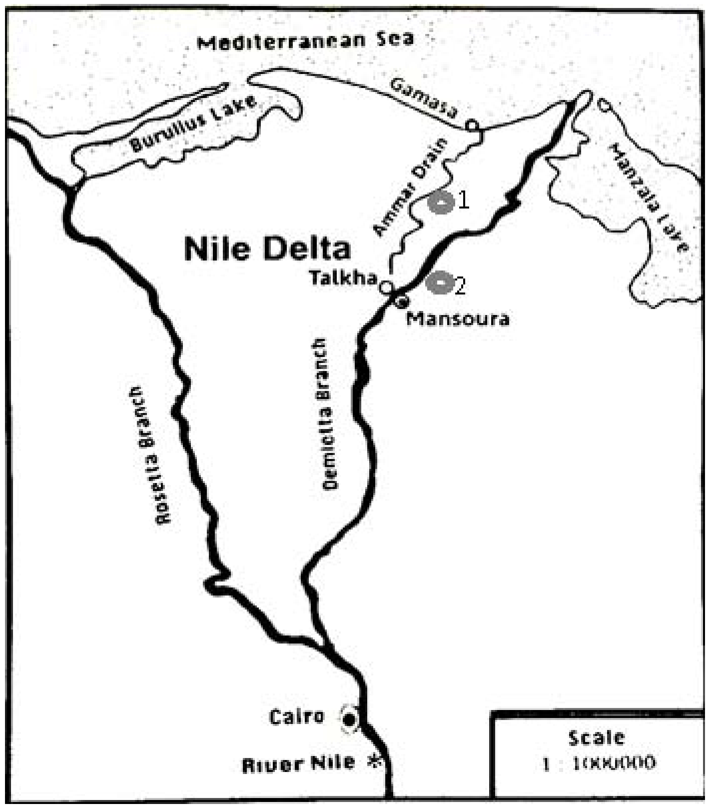

Fig 1: Map showing the study areas: Ammar Drain (1) and the River Nile (2)

b) *Water and Fish Samples*

For studying the physicochemical parameters in Damietta Branch of River Nile and Ammar drain, three sites were selected for each ecosystem with appropriate distances. However, ten water samples were collected monthly at each sampling site at 50-cm depth (from March 2015 to February 2016). In a one-liter plastic container, primary treatment of the samples was carried out in the field at the time of sampling. Concurrently, water samples were kept according to the protocol designed by El-Naggar et al. (2016)& Al-Halani et al. (2018) The procedures were implemented according to the standard methods (APHA, 1998).

About 20-30 samples of C. gariepinus were collected seasonally from each sampling site from River Nile and Ammar Drain. Fish samples were transported alive in aerated tanks with a plentiful amount of water to the laboratory for different investigations. Fish were placed in large plastic containers accommodating natural water to minimize stresses and injuries. Records were kept of the total body length, weight, and sex. Moreover, the gonads were weighed.

c) *Fish Measurements*

i. *Hematological and Biochemical Analysis*

Before fish dissection, blood samples were obtained from the caudal vein by a hypodermic syringe. Then, each sample was immediately divided into two portions; the first one used EDTA as an anticoagulant for measuring complete blood counts, while the second one was centrifugedat 3000 rpm for 10 minutes without anticoagulant. The collected sera were kept at -20 °C till analysis.

The whole blood was immediately used for the estimation of RBCs, and total WBCs counts under the light microscope using Brand count chamber (hemocytometer) following dilution of the blood in phosphate buffer at pH of 7.2 (Dacie and Lewis, 1984). Hemoglobin (Hb) content was determined colorimetrically by measuring the forming of cyanomethaemoglobin according to Van Kampen and Zijlstra (1961). Haematocrit (Ht) ratio was directly estimated after blood collection, by transferring a small amount of blood into a capillary tube and centrifuging them for 5 min in an ABX Micros 60 device, manufactured by HORIBA ABX SAS.

Levels of superoxide dismutase (SOD) enzyme were estimated with the aid of Cayman Kit (Biodiagnostic Company, Mansoura, Egypt), according to the method described by Nishikimi et al. (1972).Serum levels of catalase (CAT) activity were determined using Cayman Kit (Biodiagnostic Company, Mansoura, Egypt), according to the procedure estimated by Aebi (1984).

ii. *Histopathological Manifestations*

Following dissection, appropriate segments of fish muscles, gonads (testes and ovaries), and skin were preserved in 10% formaldehyde and processed for embedding paraffin, then sectioned at five µm thickness

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and stained in hematoxyl in and eosin according to Roberts (2012). Histological investigations were studied with the high aid power Leitz Labor Lux light microscope, and the imperative histological features were captured by a digital camera connected to the microscope.

d) Statistical Analysis

All values are given as (Mean ± SD). Data presenting monthly levels of the physicochemical parameters were analyzed using the Student's t-test to demonstrate whether the elements of the water quality are variant between the two investigated localities. On the other hand, the seasonal differences of the physicochemical parameters, blood parameters and water quality indices in each locality were tested using One-way ANOVA test. Furthermore, Tukey HSD was used as a post hoc test to detect significant differences between seasons at $P < 0.05$.

III. Results

a) Water Factors

Tables 1 and 2 show the seasonal fluctuations of water physicochemical parameters from River Nile and Ammar Drain, respectively. The results obtained from the analysis of water sampled from the two aquatic environments indicate that the quality of water varies greatly between the two study areas. From tables 1 and 2, it is clear that the pH of the River Nile and Ammar Drain was slightly alkaline. It ranged from 7.447 to 7.970 in the River Nile (Table 1) and from 7.093 to 7.557 in Ammar Drain (Table 2).

Tables 1 and 2 show seasonal variations of water temperature in the two investigated areas. The values of water temperature ranged between 17.70 °C in Ammar Drain during winter and 32.06 °C in River Nile during summer. From Figure 6, it, and is obvious that water temperature gradually increases in spring, reaching its maximum levels in summer.

Tables 1 and 2 show a very wide range of variation in water EC between River Nile and Ammar Drain. The EC values varied from 0.40 µs/cm in River Nile during winter to 2.49 µs/cm in Ammar Drain during spring. The highest EC values were estimated during spring in Ammar Drain, while the lowest value was recorded during winter in River Nile.

Seasonal fluctuations of TDS of water in the two study sites are recorded in Tables 1 and 2. The TDS values ranged between 246.720 mg/l in River Nile during spring and 1590.400 mg/l in Ammar Drain during the same season. From Figure 10, it is obvious that TDS reached its maximum value during spring in Ammar Drain; however, the minimum value was recorded during the same season in the River Nile.

Tables 1 and two show marked seasonal variation of bicarbonates values between River Nile and Ammar Drain. It varied from 90.28 mg/l in River Nile during summer to 356.607 mg/l in Ammar Drain during winter.

Seasonal changes of Cl contents of water are documented in Tables 1 and 2. The chloride content of water in Ammar Drain is higher than that in River Nile. The maximum value of the chloride content of water (578.413 mg/l) was recorded in Ammar Drain during winter; however, the minimum value was 32.260 mg/l and obtained in River Nile during the same season.

Seasonal differences of the sulfate content of water are shown in Tables 1 and 2 . The recorded values of sulphate varied from 9.28 mg/l at River Nile during autumn to 910.85 mg/l at Ammar Drain during spring.

Tables 1 and 2 document the seasonal changes of calcium, magnesium, sodium and potassium at River Nile and Ammar Drain. The concentrations of these minerals are generally lower in River Nile than Ammar Drain. The mineral calcium varied from 38.133 mg/l at River Nile during summer to 112.933 mg/l at Ammar Drain during winter. The concentration of magnesium varied from 6.257 mg/l in River Nile during autumn to 68.607 mg/l at Ammar Drain during winter. The concentration of sodium ranged between 1.820 mg/l at River Nile during winter and 442.165 mg/l at Ammar Drain during spring. The concentration of potassium ranged between 5.5 mg/l at River Nile during spring and 21.833 mg/l at Ammar Drain during autumn. From Tables 1 and 2, it can be seen that the concentrations of minerals have higher values in Ammar Drain during the study period.

Table 1 and 2 show the seasonal differences in nitrogen and phosphorous concentrations between River Nile and Ammar Drain. It is noticed that the concentration of these elements are generally lower in River Nile than Ammar Drain. Regarding the concentration of nitrogen, it varied from 3.15 mg/l at River Nile during spring to 10.5 mg/l at Ammar Drain during winter. On the other hand, the concentration of phosphorous varied from 0.107 mg/l at River Nile during summer to 0.16 mg/l at Ammar Drain during spring.

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Table 1: Seasonal changes of physicochemical parameters (Mean ± SD) of water sampled from River Nile.

Parameters	pH	Temp.°C	EC(µs/cm)	TDS	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+} (mg/l)	Mg^{2+}	Na^+	K^+	N	P
Season													
Spring	7.447	27.33	0.413	246.720	94.795	58.400	31.485	41.800	13.320	11.830	5.500	3.150	0.130
	± 0.349	±1.53	±0.006	±2.240	±13.545	±8.340	±2.305	±6.600	±0.120	±8.460	±0.500	±0.630	±0.000
Summer	7.970	32.067	0.447	274.560	90.280	42.270	77.727	38.133	15.393	21.393	6.667	3.780	0.107
	±0.416	±0.416	±0.006	±10.652	±20.687	±5.096	±15.385	±2.540	±2.665	±1.529	±1.607	±0.630	±0.032
Autumn	7.800	22.133	0.463	282.027	180.560	44.493	9.277	68.933	6.257	4.990	8.667	7.560	0.127
	±0.191	±3.544	±0.006	±29.254	±20.687	±5.102	±8.758	±14.144	±4.098	±1.866	±0.577	±1.890	±0.018
Winter	7.500	22.033	0.403	257.920	176.047	32.260	11.297	48.400	16.273	1.820	6.833	9.450	0.127
	±0.027	±1.779	±0.023	±17.209	±13.545	±0.096	±12.002	±11.641	±4.671	±1.210	±1.258	±1.890	±0.015

pH = Hydrogen ion conc. TDS= Total dissolved solids
SO_4^{2-} = Sulphates Na^+ = Sodium P= Phosphorous
HCO_3^- = Bicarbonates Ca^{2+} = Calcium K^+ = Potassium
N = Nitrogen EC = Electric conductivity Cl^- = Chlorides
Mg^{2+} = Magnesium

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Table 2: Seasonal changes of physicochemical parameters (Mean ± SD) of water sampled from Ammar Drain.

Parameters / Season	pH	Temp.°C	EC(µs/cm)	TDS	HCO_3^-	Cl−	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+	N	P
				(mg/l)									
Spring	7.093	25.750	2.490	1590.400	162.503	91.765	910.845	83.600	15.120	442.165	7.250	4.290	0.160
	±0.340	±1.750	±0.300	±277.200	±13.545	±5.005	±194.880	±22.00	±3.600	±63.105	±0.250	±0.750	±0.02
Summer	7.557	31.933	2.313	1420.803	162.503	78.977	830.943	60.427	27.617	384.260	6.667	6.090	0.127
	±0.197	±0.757	±0.335	±214.091	±13.545	±13.489	±184.876	±7.113	±7.743	±62.880	±1.041	±0.962	±0.015
Autumn	7.410	22.533	2.333	1339.400	334.037	493.873	66.177	85.067	35.743	289.453	21.833	8.820	0.156
	±0.062	±2.363	±0.199	±174.274	±61.065	±105.947	±69.838	±17.782	±16.954	±71.764	±3.055	±1.667	±0.038
Winter	7.300	17.700	2.377	1521.067	356.607	578.413	78.110	112.933	68.607	355.967	8.833	10.500	0.140
	±0.121	±1.652	±0.618	±395.577	±47.556	±222.188	±24.335	±26.522	±17.264	±169.951	±2.255	±0.962	±0.01

pH = Hydrogen ion conc. TDS= Total dissolved solids
SO_4^{2-} = Sulphates Na^+ = Sodium P= Phosphorous
HCO_3^- = Bicarbonates Ca^{2+} = Calcium K^+ = Potassium
N = Nitrogen EC = Electric conductivity Cl^-= Chlorides
Mg^{2+} = Magnesium

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Statistical analysis (Independent samples t-Test on SPSS package version 20) indicated a very highly significant difference of the electrical conductivity, total dissolved solids, bicarbonate content, calcium content and sodium between the two investigated areas ($p \leq 0.001$). Independent samples t-There was a high significant difference in the hydrogen ion concentration, chloride content, sulfates content, magnesium content and dissolved oxygen between the River Nile and Ammar Drain ($p \leq 0.01$). The minerals potassium and phosphorous showed significant differences between the two investigated areas ($p \leq 0.05$). Other physicochemical environmental parameters showed non-significant differences between the Nile River and Ammar Drain ($p > 0.05$).

b) Hematological Parameters

Table 3 represents seasonal values of Hb content in fish inhabiting the two study areas. Significant variability in Hb content was observed between the two investigated areas, especially in winter. From Table 3, it is clear that the highest Hb level (17.33 g/dl) was detected during winter in fish sampled from River Nile, while the lowest value (7.13 g/dl) was recorded during summer in those sampled from Ammar Drain.

The seasonal changes of RBCs collected from C. gariepinus inhabiting River Nile and Ammar Drain. This blood parameter attained higher levels in River Nile than Ammar drain (Table 3). From Table 3, it is obvious that the highest RBCs count (2.87 106/μL) was recorded during winter in River Nile, while the lowest level (1.73 106/μL) was counted summer in, Ammar Drain.

Table 3 represents the seasonal values of Hct collected from C. gariepinus inhabiting River Nile and Ammar Drain. This blood parameter showed marked variations between the two study sites. From Table 3, it can be noticed that the highest Hct level (38.33 %) was measured during autumn in River Nile, whereas the lowest value (24.03 %) was recorded in summer during Ammar Drain.

Table 3 represents the seasonal variations of WBCs count of C. gariepinus sampled from the two investigated areas. Marked variability in WBCs was detected between River Nile and Ammar drain. From Table 3, it is clear that the highest WBCs value (214.13 103/μL) was recorded during autumn in Ammar drain, while the lowest value (161.23 103/μL) was counted in winter in River Nile.

Statistical analysis (Independent samples t-Test on SPSS package version 20) indicated the hemoglobin and RBCs content of the blood of catfish varied significantly between both sites ($p \leq 0.05$). A high significant difference in Hct values was observed in catfish between River Nile and Ammar Drain ($p \leq 0.01$). Marked variability in WBCs was detected between the two investigated areas. The WBCs count of C. gariepinus varied significantly between River Nile and Ammar Drain ($p \leq 0.05$).

Table 3: Seasonal changes in the blood parameters (Mean ± SD) of C. gariepinus sampled from River Nile and Ammar Drain.

StudyAreas	River Nile				Ammar Drain			
Parameters	Hb (g/dl)	RBCs ($\times 10^6/\mu L$)	Hct (%)	WBCs ($\times 10^3/\mu L$)	Hb (g/dl)	RBCs ($\times 10^6/\mu L$)	Hct (%)	WBCs ($\times 10^3/\mu L$)
Season								
Spring	9.93±3.42	2.21±0.74	34.37±8.87	207.13±27.39	9.73±0.40	2.06±0.02	28.47±2.25	209.43±20.59
Summer	9.83±1.79	2.34±0.28	30.33±5.15	179.77±16.54	7.13±2.14	1.73±0.48	24.03±6.38	201.63±13.68
Autumn	11.43±0.46	2.63±0.14	38.33±4.47	187.23±25.99	9.40±1.75	1.91±0.82	26.13±7.52	214.13±2.30
Winter	17.33±3.26	2.87±0.61	35.28±7.38	161.23±32.35	9.50±1.56	2.16±0.40	26.93±3.54	204.00±11.53

Hb = Hemoglobin
RBCs = Red blood cells
Hct = Hematocrit
WBCs = white blood cells

Table 4 shows the seasonal levels of SOD enzyme in blood samples of fish inhabiting River Nile and Ammar Drain. This parameter varied obviously between the two water environments. From Table 4, it can be seen that the highest SOD value (341.50 u/ml) was recorded during winter in River Nile, while the lowest level (137.50 u/ml) was detected in spring in the same environment.

Table 4 shows the seasonal variations of CAT enzyme in the blood collected from fish inhabiting River

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Nile and Ammar Drain. This parameter showed marked variations in CAT activity between the two study areas. From Table 4, the highest CAT value (869.10 u/l) was recorded during spring in Ammar Drain, while the lowest value (109.10 u/l) was recorded during winter in the same locality.

Statistical analysis revealed the significant variation of SOD value of catfish between River Nile and Ammar Drain ($p \leq 0.05$). There was no significant variation in the CAT levels of catfish between River Nile and Ammar Drain ($p > 0.05$). Seasonally, differences in CAT activity of catfish were very highly significant either in River Nile or Ammar Drain ($p \leq 0.001$ respectively).

Table 4: Seasonal changes in the serum parameters (Mean ± SD) of C. gariepinus at River Nile and Ammar Drain.

Study Areas	River Nile		Ammar Drain	
Parameters	SOD (u/ml)	CAT (u/l)	SOD (u/ml)	CAT (u/l)
Season				
Spring	137.50±57.28	422.67±32.13	175.00±78.06	869.10±137.04
Summer	270.73±36.08	150.60±33.90	145.76±95.43	115.27±55.33
Autumn	267.04±20.61	610.31±77.56	144.14±52.03	363.81±13.92
Winter	341.50±14.50	148.80±15.21	245.72±25.93	109.10±82.75

SOD = Superoxide dismutase enzyme
CAT = Catalase enzyme

c) Histopathological Investigations

i. *Sections of skin*

The normal architecture of the fish skin is illustrated in Figure 2 (A, B, and C). The skin comprises three main layers, namely the epidermis, dermis, and hypodermis (Figure 2 A, B and C). The epidermis is formed of the superficial, middle and inner layers. The superficial layer includes the stratified squamous epithelium that is arranged in some rows of polygonal cells (Figure 2 A, B, and C) and unicellular, oval to circular goblet (mucus-producing) cells (Figure 2 A, B, and C). As shown in Figure 2 (A, B, and C), each goblet cell possesses a storing vacuole and a small, flattened basal nucleus. The goblet cell evacuates on the outer surface through a terminal opening. The middle layer of the epidermis comprises the club cells (Leydig or alarm cells) (Figure 2 A, B, and C). This cell type is large in size, irregular in shape and is supported by epidermal cells. There is a pale pink cytoplasm in the club cells. The inner epidermal layer is formed of a single layer of cuboidal or columnar cells held on a thin basement membrane (Figure 2 A, B and C).

The corresponding histopathological changes of the skin of fish caught from Ammar Drain are shown in Figure 2 (D). It can be noticed that there is a reduction in the number of mucus-producing cells and Leydig cells. The basement membrane is two or three times thicker than normal. Moreover, the number of pigment cells is reduced. Regarding the inner epidermal histological features, there is a marked proliferation of the cuboidal cells. Furthermore, no lymphocytic cells were recognized among the constituent elements of the skin.

ii. *Sections of fish muscles*

Figure 3 (A and B) shows the normal histology of muscles fibers of C. gariepinus dwelling River Nile. It can be observed that the muscle bundles are polygonal, well–organized and formed of closely–packed units (Figure 3 A and B), during winter and summer, respectively. In contrast, the muscles bundles of C. gariepinus from Ammar drain (the polluted site) showed marked deformation; the muscles bundles are loosened and widely–spaced (Figure 3 C and D). A typical inflammatory response could be recognized around a likely parasitic form in Figure (3 C and D). Clumps of inflammatory cells (granulomas) and marked degeneration of the muscular tissue bordering the pathogen and encapsulating granuloma are evident (Figure 3 C and D).

iii. *Sections of the fish ovary*

The normal histology of the ovary of fish inhabiting River Nile is illustrated in Figure 4 (A and B). The oocytes are full– formed and the stages of gonadal development were completed (previtellogenic, postvitellogenic, and vitellogenic stages). The oocytes are loaded with vitellogenic cells and surrounded by stroma (Figure 4 A and B). As shown in Figure 4 (B), there is a greater number of follicles compared to the winter harvest, however, they are smaller in size and relatively irregular in shape. At Ammar Drain, there is a marked alteration of the gonadal development is recognized (Figure 4 C and D). There are a marked

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degeneration and resorption of oocytes at any point in development (Figure 4 C and D). The vitellogenin mass inside the mature oocytes and obvious patchiness dominates the ovarian tissues (Figure 4 D). From Figure 4 (C and D), it is clear that the summer preparations of the ovarian tissues are more adversely influenced than winter samples.

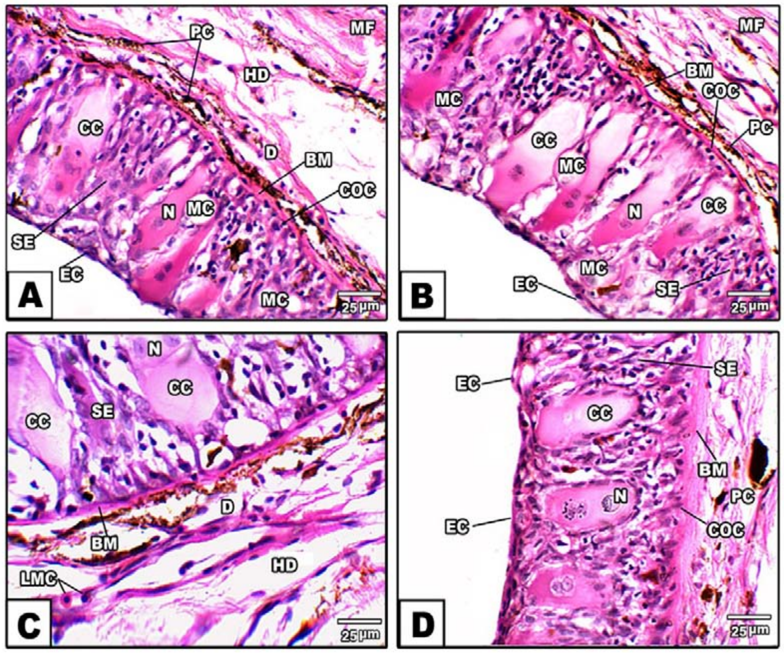

Fig. 2: Photomicrograph showing the normal histological features of the skin of *C. gariepinus* (A, B and C) inhabiting River Nile in comparison with the corresponding histopathological changes of the skin of catfish, (D) inhabiting Ammar Drain. Scale bar = 25 µm. BM, basement membrane; CC, club cell; COC, columnar cell; D, dermis; EC, epithelial cell; HD, hypodermis; LMC, lymphocytic cell; MC, mucus cell; MF, muscle fiber; N, nucleus; PC, pigment cell; SE, stratified squamous epithelium. (H & E X 400)

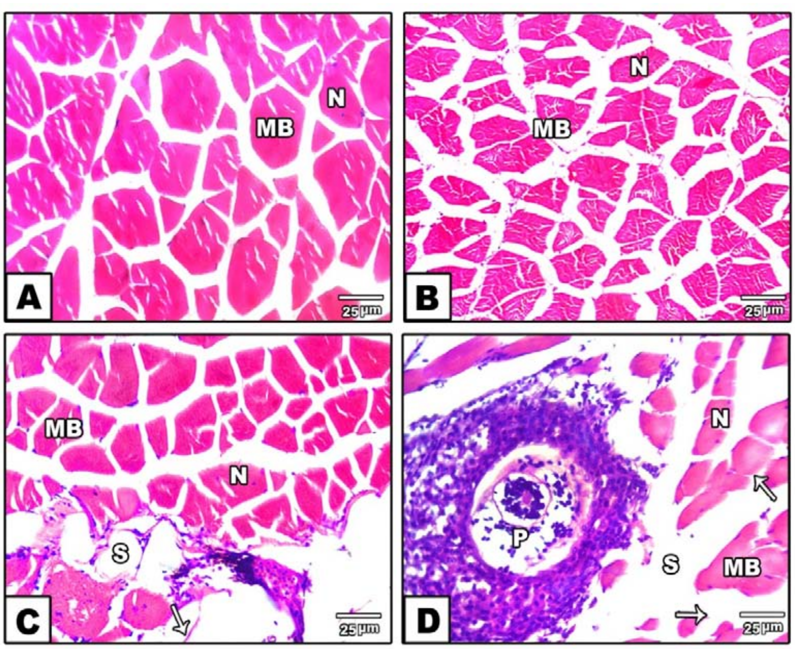

Fig. 3: Photomicrograph showing the histological features of muscles fibers of *C. gariepinus* inhabiting River Nile (A and B) and Ammar Drain (C and D), during winter and summer, respectively. Scale bar = 25 µm. MB, muscles bundles; S, spaces between muscle bundles; N, the nucleus of muscle bundle; P, parasitic cyst; arrow, degenerated fibers. (H & E x 400)

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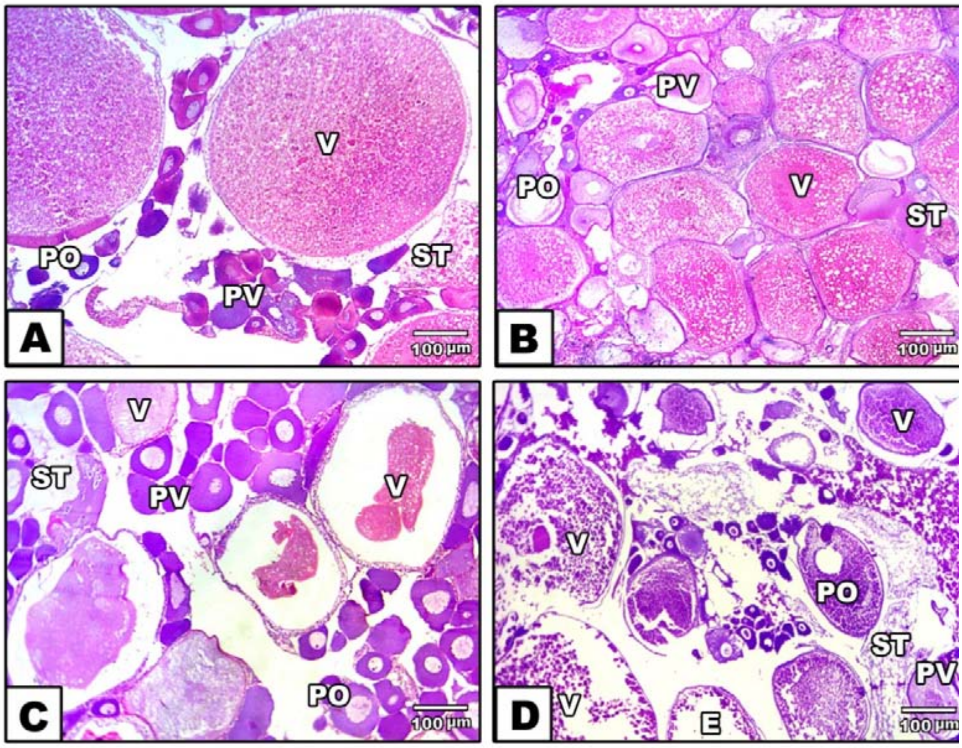

Fig. 4: Photomicrograph showing the histological features of the ovary of *C. gariepinus* dwelling River Nile during winter (A) and summer (B), and the histopathological features of some fish from Ammar Drain during winter (C) and summer (D). Scale bar = 100 µm. E, empty follicle; PO, postvitellogenic stages; PV, previtellogenic stages; ST, stroma; V, vitellogenic stages. (H & E X 100).

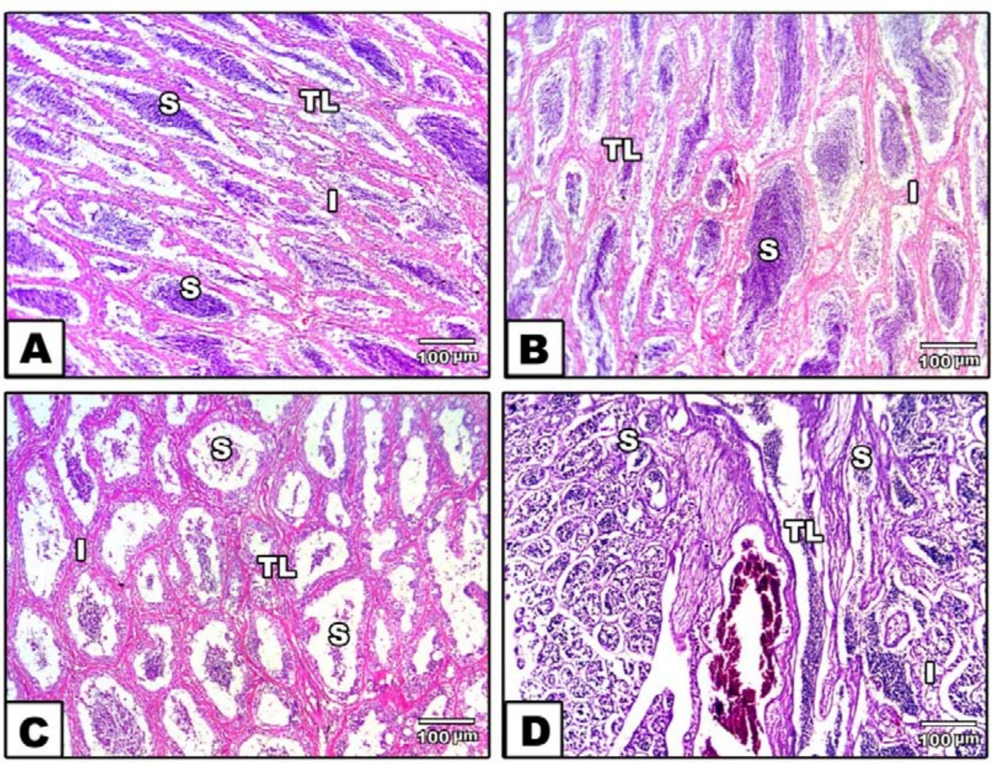

Fig. 5: Photomicrograph showing the histological features of the testis of *C. gariepinus* dwelling River Nile during winter (A) and summer (B), and the histopathological features of some fish from Ammar Drain during winter (C) and summer (D). Scale bar = 100 µm. TL, testicular lobule; S, spermatozoa; I, interstitium. (H & E X 100)

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iv. *Sections of the testis*

Regarding the normal testis (Figure 5 A, and B) of C. gariepinus inhabiting River Nile, the distended testicular lobules showed high variation in their shapes and sizes. They are surrounded by interstitium (Figure 5 A, and B). Spermatozoa are centrally located and tubules are surrounded by blood vessels. In affected areas during winter (Figure 5 C), the testicular lobules showed different activity where most of lobules were distended with spermatozoa and some the lobules appeared empty as they discharged their spermatozoa. Spermatozoa were scattered in the testes in an eccentric location. During the summer season, effects were less evident (Figure 5 D), where other lobules appeared partially empty or empty from spermatozoa.

IV. Discussion

The Damietta Branch of the River Nile is regarded as a control or lightly polluted ecosystem. However, Ammar Drain is considered as a stressed ecosystem as it accommodates huge amounts of agricultural and domestic discharges. The water body of Ammar Drain is rich in its chemical nature, where it incorporated higher levels of total dissolved solids, electrical conductivity, bicarbonates, chlorides, sulfates, calcium, magnesium, and sodium.

The acceptable pH for fish culture ranges between 6.5 and 8.0 (Meade, 1998). The study found that the pH values of all sampling sites were within the standard limit, indicating that the two aquatic ecosystems are valid for aquatic life. The pH limit of protection between 6 and ten was adopted for fisheries and aquatic life by the European Community (Chapman, 1993). Elevation of the water temperature affects the amounts of dissolved oxygen in the water column where DO is inversely proportional to the thermal regime (Adeogun, 2012). Evans et al. (1965) found that fish can produce an immune response at temperatures from 5 to 8oC.The relative increase in the water temperature of Ammar Drain in some seasonals had potential implications on the oxygen retention capacity of water (UNEP, 2006). The electrical conductivity (EC) reflects the number of dissolved salts in water samples(Moore et al.,2008). EC in the present investigation showed a very wide range of variation between the River Nile and Ammar Drain. The electrical conductivity lied within the non-desirable scale at Ammar Drain. The high conductivity values recorded in Ammar Drain suggested possible sources of run-off from adjacent landscapes and strongly implicates agricultural and sewage sources.

The levels of the total dissolved solids showed significant variation between the two investigated areas. The water of the River Nile showed lower levels of TDS than Ammar Drain. This might be due to variations in agricultural activities or drainage water. TDS comprise primarily chlorides, bicarbonates, phosphates, sulfates and conceivably nitrates of magnesium, potassium, sodium, calcium, in addition to hints of manganese, iron and further ingredients (Akan et al.,2012). The total dissolved solids recorded in Ammar Drain were above the WHO guideline of 1000 mg/l for the protection of fisheries and aquatic life, and for domestic water supply (WHO, 2004).

Water pollution is one of the most crucial environmental problems and public health concerns in the River Nile (Mohamed et al.,2013; El-Amier et al.,2015). The highest mean value of total dissolved solids, bicarbonates, chlorides and sulfates was recorded in Ammar Drain. This could be attributed to drainage water in this area, and the decomposition of the organic matter or may be due to the effect of the interconnecting drains which increase sulfates in this area. Chloride concentrations higher than 200 mg/l are considered to be a risk for human health (Versari et al.,2002). However, sulfates in high levels cause water hardening. The present data indicated that sulfates in Ammar Drain exceeded the WHO permissible limit which is 200 mg/l (WHO, 1993).

The concentration of the monovalent cations (sodium and potassium) and the divalent cations (calcium and magnesium) were evaluated. The results showed that the levels of these minerals were generally higher in Ammar Drain than the River Nile. El-Amier et al., (2015) found that the cations of water take the following sequence; $Na+ > Ca+2 > Mg+2 > K+$.Sodium is the most abundant among other cations and this could be attributed to the drainage water from different agricultural and cultivated lands (Arain et al.,2008).

Exposure to interactive effects of the multisource pollutants leads to negative impacts on the hematological indices (Vutukuru, 2005).The present study showed that all hematological parameters of African catfish sampled from Ammar Drain were lower than those sampled from River Nile. This could be a reflection of fish response to environmental pollution (El-Naggaret al.,2016 and Tayel et al.,2007). The decreases of these parameters go in line with elevation in the levels of chemical parameters studied such as nitrogen and phosphorous, DO depletion and the increase in TOC as a result of pollution stress in that area (Al-Halani et al., 2018)

There was a positive correlation between DO and RBCs, Hb, Hct values in African catfish. Under oxygen depletion condition, the liver probably revives erythropoiesis to recompense the desire by elevated oxygen transportation to marginal tissues (Rifkind et al.,1980). Singh (2008) suggested that different water pollutants are the possible chief source of the

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physiological disorders of fish. The decrease in RBCs, Hb and Hct values in fish dwelling Ammar drain may be related to water pollution.

The WBCs are the regulator of the immune system; its count was elevated in the blood of African catfish sampled from Ammar drain. This could be attributed to the exposure to chronic sewage and domestic discharges, and agricultural wastes. This is in agreement with the results of Gaber et al. (2013) who suggested that the increase of WBCs in the blood of fish caught from El-Rahawy Drain could be a result of exposure to chronic sewage. In the present study, the increase of WBCs count may be attributed to a general immune response to pollution (Nussey et al., 2002). According toEl-Sayed et al.(2007), increased WBCs count in fish subjected to pollutants indicates leukocytosis or an increase in the total WBCs value over the normal range. Fish sampled from River Nile was found to be healthier than those from Ammar Drain. Fish sampled from Ammar drain seemed darker and unhealthy in appearance. This might be due to the high pollution level in Ammar Drain compared to River Nile.

Oxidative stress biomarkers such as SOD and CAT enzymes are commonly employed for environmental hazard impacts assessment (Livingstone, 2001& Tsangaris et al.,2011). The present work revealed lower SOD and CAT activities in the blood of African catfish sampled from Ammar Drain compared to those from River Nile. The changes of Oxidative stress biomarkers in Ammar drain can be interpreted due to water pollution. Different water pollutants are the possible main source of the physiological disorders in fish (Adams et al.,2001& Viarengo et al.,2007). In the present investigation, decreased oxidative stress enzyme activity in fish sampled from Ammar Drain can be correlated to the deficiency to recompense for imbalance of the release of free radicals and oxidative defense mechanisms, probably a consequence of intense pollutant gradient. Owing to the present findings, the levels of SOD and CAT activities were lower in fish sampled from Ammar Drain than those of River Nile. The drop in those enzyme activities may be correlated to long-term exposure of fish to environmental contaminants. Similar response patterns were documented in previous studies (Lenartova et al.,1997). Previous studies in sea bass, Dicentrarchus labrax and grey mullet, M. cephalus revealed low SOD and CAT actions in fish dwelling polluted water (Ben Ameur at el., 2012).

The histopathological changes of the skin of African catfish caught from Ammar Drain included a reduction in the number of mucus-producing cells, Leydig (alarm) cells and the pigment cells. The basement membrane appeared thicker than that illustrated in the normal skin of the catfish inhabiting River Nile. The skin is an outer wrapper of fish; hence, it is in-full-force of environmental fluctuations. As a consequence, the skin plays a key role as the first line of defense in physical and chemical means (Vernerey and Barthelat, 2014). Concerning the inner epidermis, there was a marked proliferation of the cuboidal cells. Moreover, no lymphocytic cells were observed in the histological preparations of the skin of African catfish inhabiting Ammar Drain. The epidermis is a fragile layer, which is regularly sloughed off and regenerated. The scales covering the skin of African catfish act as an additional, physical barrier separating the skin and underlying tissues from the flowing water currents (Helfman et al.,2009). The dermis lies beneath the epidermis. This layer contains blood vessels, nerves, connective tissues and sense organs. The dermis is well supplied by blood vessels; hence, it also provides nourishment to the epidermis.

The present study revealed that fish inhabiting Ammar Drain exhibited some histopathological signs where their muscles bundles were obviously loosened and showed marked spacing. The tissues changes in fish sampled from Ammar Drain can be contributed to water pollution. The current study illustrated that histopathological changes of fish muscles may be due to uptake of pollutants coming from sediment and its surrounding environment (Jeffree et al., 2006). Thophon et al. (2003) and Kaoud and El-Dahshan (2010) found that fish exposed to pollutants may undergo histological alterations in the form of degeneration of muscles bundles with certain focal areas of necrosis. Similar results were described by Kaoud and El-Dahshan (2010) who observed that fish exposed to pollutants show degeneration of muscles bundles with atrophy and splitting of muscles fibers.

In the present study, histological preparations indicated alterations in the oogenesis or spermatogenesis of fish exposed to pollutants where the development cycles of gametes were incomplete and some stages were malformed. Histopathological changes in testis and ovary of African catfish collected from Ammar Drain may be due to their exposure to sewage, domestic, and agricultural wastes as recorded by Tayel et al. (2007). Similar histopathological changes (lesions) have been reported in fish exposed to pollutants (Gaber et al., 2013).

In female fish, the mean oocytes number is considered as an important criterion for assessment of the reproductive performance in fish (Gaber, 2000& Gaber et al., 2013). In the present study, the mean oocytes number of fish was affected by the polluted environment. Mohamed (2001&2003) recognized histopathological alterations in fish testis and ovary suggesting that this may reduce the ability of fish to reproduce. It is well known that water pollution has a serious inhibitory effect on fish reproduction (Mohamed, 2001&2003) resulting in a decrease in their abundance and, consequently, a decline in fish species diversity. In the present study, pollution effects appeared as

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disruption in gonadal development. It comes in agreement with other studies for fish inhabiting polluted water (Balch et al., 2000). Also, oogenesis and spermatogenesis were influenced by exposure to sewage effluents (Kiparissis et al., 2003).

V. Conclusion

The bad water quality of Ammar Drain has induced changes or modification in the blood profile and alterations in the muscles, gonads and skin histological features of African catfish, C. gariepinus. The present findings indicate also that Ammar Drain represents a potential environmental disaster threatening the human health, deteriorating aquatic life and degrading the ecosystem as a whole. The bad water quality of Ammar Drain may cause massive fish kills and induce chronic health problems such as renal failure and hepatic dysfunction, in addition to its deleterious impacts on the soil.

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