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Ecophysiological and Histopathological Impacts of Organic Pollution on Two Freshwater Fish Species, Mansoura City, Egypt.

Sherif H. Abdeen, Abeer E. Abdrabouh, Mohamed I. Mashaly, Ahmed E. Hagraas,

Ali A. Al-halany*

Zoology Department, Faculty of Science, Mansoura University, Egypt

*Department of Biology, Faculty of Education, Hajjah University, Yemen

Abstract

Organic pollution is one of environmental hazards, especially in aquatic ecosystems. This study was focusing on levels of total organic carbon (TOC%) in sediment of two freshwater habitats different in quality, as well as tissues including; muscles and gonads of two fish species inhabiting both sites during four seasons of one year of study. Ammar drain as a polluted site showed significantly higher levels of TOC% in both sediment and fish tissues comparing to River Nile as a reference site. Data also illustrated that, fish from polluted site showed remarkable decrease in blood indices; RBCs count, Hb content and Hct%, while WBCs count was mostly elevated, especially during autumn. Accompanying to these results, antioxidant enzymes as superoxide dismutase (SOD) and catalase (CAT) were generally decreased during most seasons in fish samples from Ammar drain. Also, histopathological changes in muscles and gonads were observed, where sever deteriorations in these tissues were more discriminating in fish species from Ammar drain compared to River Nile locality. From the obtained results, polluted water drains affect general health and structure of their inhabiting fish species.

Key words: Organic carbon, Fish, Antioxidants, Blood indices, Histopathology

1. Introduction

Although water is the most important constituent of the ecosystem, it is highly subjected to danger as a result of increasing human population, industrialization and

agricultural activities (Akan *et al.*, 2012). These hazards always discharged legally into drains which are closed systems without self cleaning ability and therefore easily accumulate pollutants such as, heavy metals, organic matters and microbial pollutants. These drains are usually complex and fragile ecosystems with bad aquatic life, especially for fish that unfortunately may be used for human consumption through the food chain (Abida *et al.*,2008). Some of these pollutants may be accumulated in the sediments, as well as benthic fauna or flora, the fundamental diet of different fish species, in a process known as bioaccumulation accompanied by food chain biomagnification (Lu *et al.*,2017). Organic matter plays a fundamental role in controlling the bioavailability and distribution of organic contaminants. The latter mainly comes from domestic wastes and agricultural activities that could seep into water bodies leading to increase in microorganisms which consume water oxygen content and elevated biological oxygen demand (BOD),(Ben Ameer *et al.*,2012; Gwaski *et al.*, 2013). Water or sediment content of organic substances can be expressed by detecting total organic carbon (TOC). The organic carbon content in river water was reported to be correlated with the size of a water region, climate, flora surrounding the watercourse, as well as the season of collecting the sample (Niemi *et al.*, 2006) .

Environmental biomonitoring programs introduced several tools used as biomarkers for assessment of pollution hazards in aquatic ecosystems (Viarengo *et al.*, 2007; Ben Ameer *et al.*, 2012). Fish are sensitive bioindicators in assessing health of aquatic ecosystems (Moharram *et al.*, 2011; Karadag *et al.*, 2014), where general health may be affected including; reproductive capacity and growth rate of different fish species (Moharram *et al.*, 2011; Ben Ameer *et al.*, 2012).

Several studies suggested that oxidative stress biomarkers are very important in evaluation of fish health with different environmental stressors (Monterio *et al.*,2007; Pavlovic *et al.*,2010). Moreover, histological investigations can also represent early diagnostic tools by identification of changes at sub-organismal level (Adeogun *et al.*, 2012). Thus, the present study highlighted on a comparable evaluation of hematological, oxidative stress parameters and histopathological changes in the African catfish (*Clarias gariepinus*) and the white Nile tilapia (*Oreochromis niloticus niloticus*) as the most important edible fish inhabiting two different freshwater habitats at Dakahlia governorate,Egypt, depending on levels of TOC% in sediment samples and different fish tissues (muscle, testis, ovary).

2. Materials and Methods

2.1. Sampling Areas

Sediment and water samples were collected seasonally from two freshwater ecosystems differed in quality, both at Dakahlia Governorate, Egypt. The first site (as a polluted environment) was Drain No.2, commonly known as Ammar drain that begins at Kafr Demerah Al-Gadeeda, located in the vicinity of Belquas city, 50 km north Mansoura city and about 36 km from Gamasa city. The other site, acted as a reference site was a part of the Damietta branch of the River Nile located at Kafr Al-Tawilah, Talkha city (Figure 1).

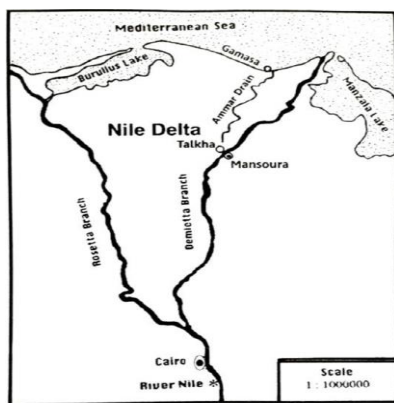


Figure 1. Map of the Nile Delta illustrating the Two Investigated sites

2.2. Sediment Sampling

During the period from March 2015 to February 2016 sediment samples were obtained monthly by using hand trowel help in collecting the top layer sediments, which were filtered from excess water and allowed to dry in air,

then crushed into fine granules in which total organic carbon (TOC) was detected using Winkler Black titration method described by (Hesse,1971).

2.3. Fish Sampling

A number of about (10-15) samples were collected seasonally from each of the studied fish species; *Clarias gariepinus* and *Oreochromis niloticus niloticus* from the two investigated sites during the study period. All fish were hunted by the same fisherman to prove regular fishing method. Fish were transferred in containers filled with its water environment to the laboratory and then aerated in aquarium using air pumps.

2.4. Fish Preparation

2.4.1. Blood and Serum investigations

From both investigated fish species; *C. gariepinus* and *O. n. niloticus*, blood samples were collected from the caudal vein. Each sample was divided into two portions; few drops on EDTA tube for the same day assessment of hematological parameters; red blood cells (RBCs) count, hemoglobin (Hb) content, hematocrite percentage (Hct%) and white blood cells (WBCs) count, using the fully automatic hematological analyzer (Sysmax XE-2100, Japan) according to Dacie and Lewis (2001).

The rest of obtained blood sample was collected in centrifuge tube without anticoagulant, centrifuged at 3000 rpm for 10 minutes. Clear serum samples were preserved at -80°C till analysis for levels of superoxide dismutase (SOD) and catalase (CAT) using the method described by Candan and Tuzmen (2008).

2.4.2. Tissue Investigations

Following blood withdrawal, fish were dissected to obtain gonads (testes and ovaries), as well as a part of the dorsal muscle. Each tissue was divided into two portions; some tissues were left to dry overnight in electric oven at 75°C until complete dryness for detection of TOC % according to (Hesse,1971) as mentioned above, while other tissues were fixed in 10% formaldehyde for histological investigations using hematoxyline and eosin stain according to Alagappan *et al.*(2009).

3.Statistical Analysis

The present data were analyzed using SPSS package (version 20), in which analysis of variance (one-way ANOVA) and Pearson's correlation coefficient tests were utilized to clarify the difference between the two studied localities and fish parameters, where p -value ≤ 0.05 was considered significant (Snedecor and Cochran,1982) .

4.Results

The present work was conducted to evaluate seasonal variation of TOC% in sediment and fish tissues including; muscle and gonads (testis & ovary) from two different freshwater localities; Ammar drain, as a polluted site and River Nile at Altawela village , as a reference site.

4.1.TOC % in sediment and tissues

Table (1) showed seasonal fluctuations of TOC % in sediment samples from the two investigated sites recording significant elevation in Ammar drain compared to River Nile site, through the four seasons of the study year, that was maximally observed during autumn. Moreover, TOC% in male and female tissues (muscle, testis, ovary) either of *C. gariepinus* or *O. n. niloticus* was positively correlated with sediment TOC% and maximally observed also during autumn, but non-significant for all tissue samples at both investigated sites during different seasons of the study year, Table 2 (a,b).

4.2.Hematological Parameters

From Table 3 (a,b), collected blood samples of the two investigated fish species from Ammar drain showed general decrease in RBCs count, Hb content and Hct% compared to River

Nile locality during the period of study that was obviously observed during autumn. However, WBCs count showed remarkable elevation in both species at the polluted site (Ammar drain).

4.3.Oxidative stress enzymes

Detected oxidative stress enzymes (SOD and CAT) levels in serum exhibited different patterns between both fish species at Ammar

drain, where serum levels of SOD and CAT were remarkably decreased during all seasons, except spring for *C. gariepinus*, while *O. niloticus* showed the same reduction in both antioxidant enzymes during all seasons of investigation, except during spring and winter for SOD levels compared to the reference site as seen in Table 4 (a,b).

Table1. Seasonal changes in total organic carbon (TOC %) (Mean±SD) of sediment samples from the two investigated sites.

Season	River Nile	Ammar Drain
Spring	10.56 ± 7.48	15.59 ±9.27
Summer	13.16 ±5.43	24.10 ±14.94
Autumn	4.46 ±4.58	29.90 ±3.14
Winter	4.04 ±1.18	6.71 ±1.66

Table 2(a). Seasonal changes in the total organic carbon (TOC %) (Mean \pm SD) in tissues of *C. gariepinus* from the two investigated sites.

Season	River Nile				Ammar drain			
	Male <i>C. gariepinus</i>		Female <i>C. gariepinus</i>		Male <i>C. gariepinus</i>		Female <i>C. gariepinus</i>	
	Muscle	Testis	Muscle	Ovary	Muscle	Testis	Muscle	Ovary
Spring	24.56 ± 5.37	20.60 ± 1.55	22.79 ± 4.67	22.38 ± 0.95	28.34 ± 1.66	28.17 ± 1.43	28.19 ± 2.24	27.41 ± 1.01
Summer	19.44 ± 9.06	20.12 ± 6.64	16.74 ± 9.20	16.77 ± 6.04	21.79 ± 6.39	21.37 ± 2.34	20.97 ± 4.65	21.51 ± 1.52
Autumn	30.55 ± 6.20	34.10 ± 6.08	26.63 ± 9.24	31.99 ± 13.86	38.21 ± 2.84	38.41 ± 2.28	29.91 ± 13.67	34.28 ± 6.35
Winter	15.47 ± 3.30	14.63 ± 1.95	16.48 ± 2.78	17.13 ± 3.03	18.62 ± 0.93	19.28 ± 0.06	19.21 ± 0.10	18.66 ± 0.96

Table 2(b). Seasonal changes of the total organic carbon (TOC %) (Mean \pm SD) in tissues of *O. n. niloticus* from the two investigated sites.

Season	River Nile				Ammar Drain			
	Male <i>O. n. niloticus</i>		Female <i>O. n. niloticus</i>		Male <i>O. n. niloticus</i>		Female <i>O. n. niloticus</i>	
	Muscle	Testis	Muscle	Ovary	Muscle	Testis	Muscle	Ovary
Spring	24.86 ± 4.85	21.11 ± 1.17	21.27 ± 1.02	24.80 ± 4.82	25.90 ± 4.01	28.23 ± 2.03	25.55 ± 1.67	26.00 ± 1.46
Summer	19.76 ± 8.87	17.88 ± 2.98	13.36 ± 5.06	17.69 ± 2.58	25.84 ± 1.95	20.64 ± 4.91	21.64 ± 4.12	21.76 ± 0.86
Autumn	34.50 ± 4.61	28.36 ± 9.87	34.06 ± 3.07	31.49 ± 1.20	39.99 ± 2.51	37.70 ± 3.14	37.67 ± 6.45	34.68 ± 6.10
Winter	14.69 ± 5.35	16.45 ± 2.73	15.15 ± 3.47	13.00 ± 3.94	18.72 ± 0.76	18.72 ± 0.76	19.14 ± 0.05	17.88 ± 1.13

Table 3(a). Seasonal changes in the blood parameters (Mean \pm SD) of *C. gariepinus* from the two investigated sites.

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

RBCs: Red blood cells, Hb:Hemoglobine, Hct: hematocrite, WBCs:White blood cells

Table 3(b). Seasonal changes in the blood parameters (Mean \pm SD) of *O. n. niloticus* from the two investigated sites.

Season	River Nile				Ammar Drain			
	RBCs ($\times 10^6/\mu\text{L}$)	Hb (g/dl)	Hct (%)	WBCs ($\times 10^3/\mu\text{L}$)	RBCs ($\times 10^6/\mu\text{L}$)	Hb (g/dl)	Hct (%)	WBCs ($\times 10^3/\mu\text{L}$)
Spring	1.61 ± 0.07	9.87 ± 0.51	27.40 ± 1.23	191.00 ± 10.95	1.60 ± 0.02	8.07 ± 0.45	25.87 ± 1.06	210.57 ± 4.67
Summer	2.34 ± 0.15	9.80 ± 0.10	32.13 ± 2.23	201.23 ± 6.05	1.84 ± 0.45	7.80 ± 2.86	30.27 ± 6.70	201.63 ± 13.68
Autumn	2.09 ± 0.62	8.27 ± 1.29	30.07 ± 4.39	192.10 ± 12.44	1.81 ± 0.30	7.47 ± 1.19	29.47 ± 5.35	201.87 ± 20.80
Winter	1.66 ± 0.24	11.43 ± 0.51	26.11 ± 1.36	126.27 ± 2.65	1.46 ± 0.27	8.33 ± 1.82	24.67 ± 4.58	199.33 ± 22.50

RBCs: Red blood cells, Hb:Hemoglobine, Hct: hematocrite, WBCs:White blood cells

Table 4 (a). Seasonal changes in the serum antioxidants (Mean \pm SD) of *C. gariepinus* from the two investigated sites.

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

SOD: Superoxide dismutase, CAT: Catalase enzymes

Table 4(b). Seasonal changes in the serum antioxidants (Mean \pm SD) of *O. n. niloticus* from the two investigated sites.

Season	River Nile		Ammar Drain	
	SOD (u/ml)	CAT (u/l)	SOD (u/ml)	CAT (u/l)
Spring	175.00 \pm 78.06	869.10 \pm 137.04	225.00 \pm 75.00	605.00 \pm 180.90
Summer	249.93 \pm 62.45	750.13 \pm 192.20	145.77 \pm 36.14	472.43 \pm 111.60
Autumn	246.08 \pm 34.18	917.16 \pm 80.28	126.73 \pm 35.20	669.31 \pm 147.13
Winter	299.87 \pm 54.37	324.77 \pm 391.15	337.37 \pm 12.56	231.91 \pm 125.36

SOD: Superoxide dismutase, CAT: Catalase enzymes

4.4.Histopathological changes

4.4.1.Muscles

Histological investigation in muscles of the two studied fish species collected from the River Nile showed polygonal muscle bundles formed of well organized closely-packed units for *C. gariepinus* and *O. n. niloticus* (Figures 2 & 2¹ A,B) during winter and summer, respectively. However, at Ammar drain,

marked deformation of muscle bundles represented by loosened and widely-spaced muscle fibers with clumps of inflammatory cells (granulomas) and encysted parasitic form was enveloped by loosened fibrous tissue, as shown in Figures 2 & 2¹ (C, D) for *C.*

gariiepinus and *O.n. niloticus* during winter and summer , respectively at both localities.

4.4.2.Testes

Fish species collected from the River Nile showed testicular lobules surrounded by interstitium showing high density of centrally located spermatozoa and tubules were also surrounded by blood vessels, during winter and summer, Figures 3 & 3¹ (A,B). On contrast, sections of testes from fish species obtained from Ammar drain Figures 3 & 3¹ (C, D) were characterized by distended testicular lobules that sometimes empty from spermatozoa, where the later were seen scattered in the center of lobules, especially during winter, Figures 3 & 3¹ (C) in both species.

4.4.3.Ovaries

The normal ovarian histology of both freshwater teleosts; african sharptooth catfish

(*C. gariiepinus*) and Nile white tilapia (*O.n. niloticus*) was seen at the River Nile locality, where fully developed oocytes loaded with vitellioe cells were evident in addition to previtellogenic and postvitellogenic forms that occupied most of the cytoplasm, Figures 4 & 4¹ (A,B). Regarding to fish samples from Ammar drain, Figures 4 & 4¹ (C,D) sections in ovaries showed remarkable degeneration and resorption of oocytes, vaculation of cytoplasmic matrix, disordered oogenesis and collapse of germinal epithelium were evident, where this was more obvious during summer for both species, Figures 4 & 4¹ (D).

N.B. For all investigated tissues, River Nile locality represented by A & B during winter and summer, respectively. However, Ammar drain demonstrated by Figures C & D during winter and summer, respectively.

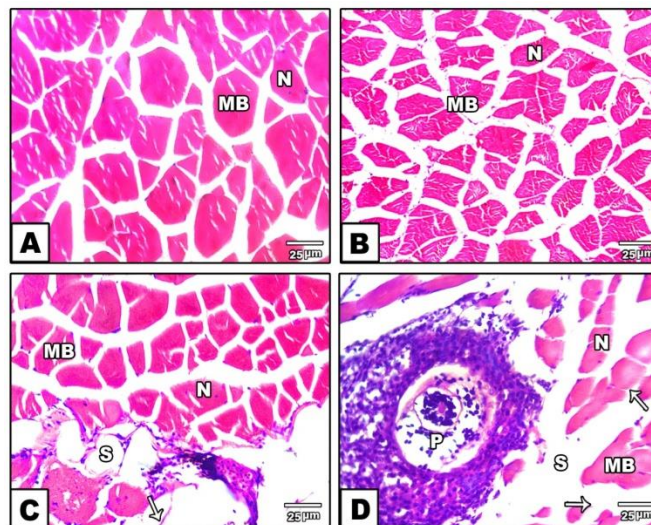


Figure (2). Photomicrograph showing the histological features of the muscle fibers of the African sharptooth catfish, *Clarias gariiepinus* inhabiting the River Nile (A and B) and Ammar Drain (C and

D). Scale bar = 25 μ m. MB, muscle bundle; S, spaces between muscle bundles; N, nucleus of muscle bundle; P, parasitic cyst; arrow, degenerated fibers.

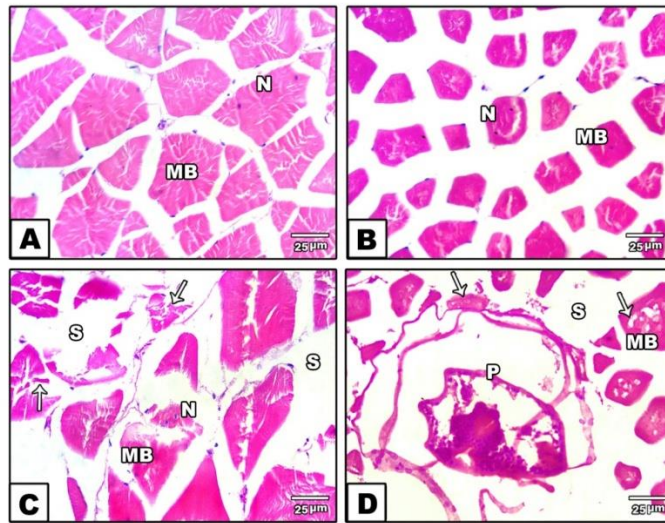


Figure (2). Photomicrograph showing the histological features of the muscle fibers of the White Nile tilapia, *Oreochromis niloticus niloticus* dwelling the River Nile (A and B) and Ammar Drain (C and D), during winter and summer, respectively. Scale bar = 25 μ m. MB, muscle bundle; S, spaces between muscle bundles; N, nucleus of the muscle bundle; P, parasitic cyst; arrow, disintegrated fibers.

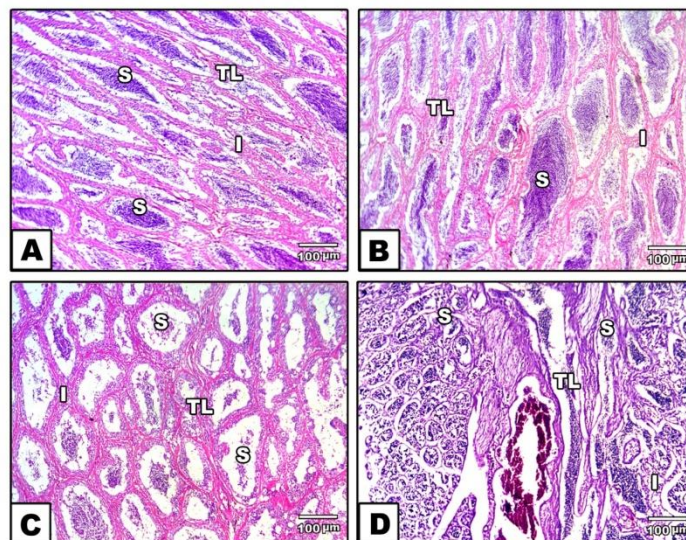


Figure (3). Photomicrograph showing the histological features of the testis of catfish, *Clarias gariepinus* dwelling the River Nile during winter (A) and summer (B), and the histopathological features of the some fish from Ammar Drain during winter (C) and summer (D). Scale bar = 100 μ m. TL, testicular lobule; S, spermatozoa; I, interstitium.

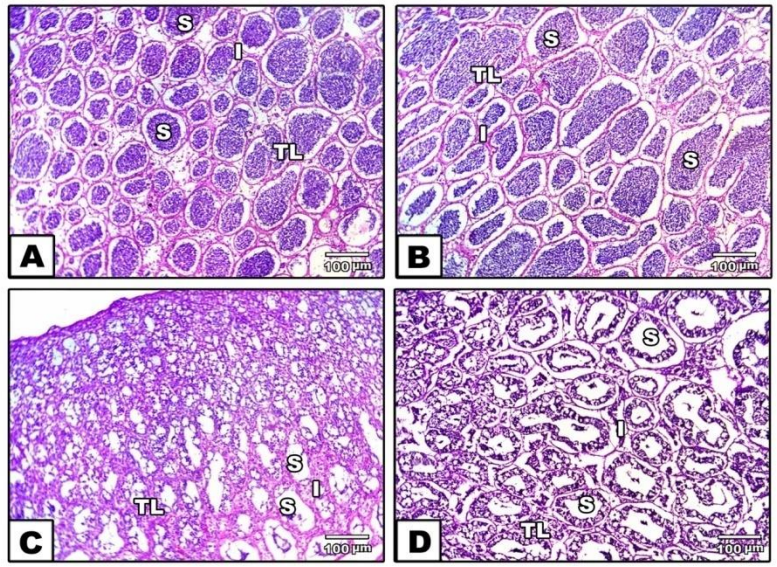


Figure (3). Photomicrograph showing the histological features of the testis of Nile tilapia, *Oreochromis niloticus niloticus* from the River Nile during winter (A) and summer (B), and the histopathological features of the same fish from Ammar Drain during winter (C) and summer (D). Scale bar = 100 µm. TL, testicular lobule; S, spermatozoa; I, interstitium.

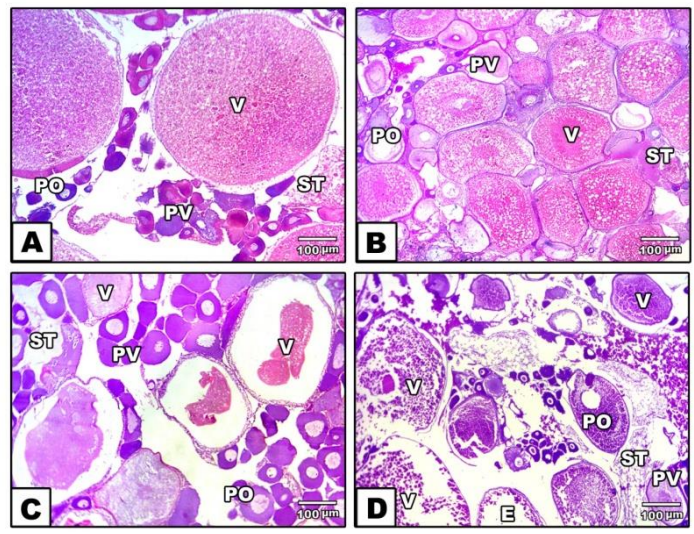


Figure (4). Photomicrograph showing the histological features of the ovary of catfish, *Clarias gariepinus* dwelling the River Nile during winter (A) and summer (B), and the histopathological features of the some fish from Ammar Drain during winter (C) and summer (D). Scale bar = 100 µm. E, empty follicle;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

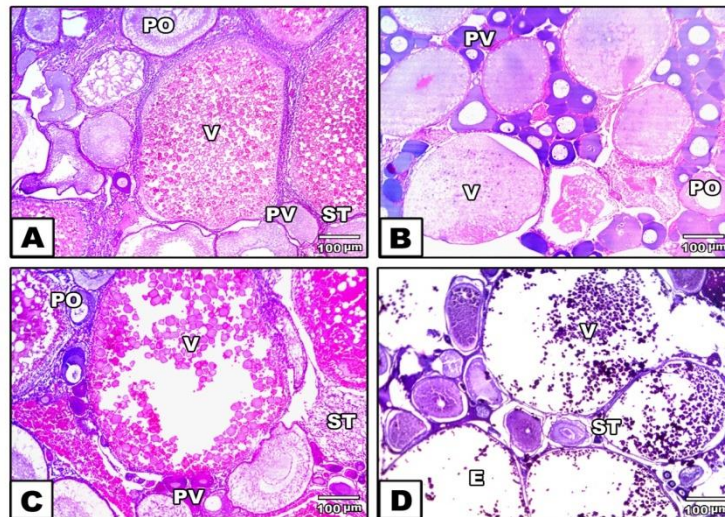


Figure (4). Photomicrograph showing the histological features of the ovary of Nile tilapia, *Oreochromis niloticus niloticus* from the River Nile during winter (A) and summer (B), and the histopathological features of the some fish from Ammar Drain during winter (C) and summer (D). Scale bar = 100 µm. E, empty follicle; PO, postvitellogenic stage; PV, previtellogenic stages; ST, stroma; V, vitellogenic stages.

5. Discussion

Human activities induce several environmental health hazards, that may affect hardly on aquatic organisms. Freshwater pollution by organic contaminants is one of these hazards. Total organic carbon (TOC) is a very important indicator in monitoring this problem (Abida *et al.*,2008; Akan *et al.*,2012). The present data showed that TOC% in the sediment samples from Ammar drain were greater than that recorded of the River Nile locality. This could be attributed to the fact that Ammar drain received several agricultural and sewage wastes as a main source of organic carbon as recorded by (Donia,2005 and El-Amier *et al.*,2015). To follow this pollutant in fish body, the present study was interested in studying TOC% in fish tissues including; muscle, testis and ovary of the two studied

teleosts which were generally recorded higher levels in those inhabiting Ammar drain compared to the River Nile, confirming the higher levels detected in sediment samples. Accumulation of these pollutants may come through nutrition, where *C.gariepinus* and *O. n. niloticus* are known as omnivorous and herbivorous fish, respectively. In this concern, infaunal organisms and phytoplanktons, as first steps in the trophic levels were considered as important reservoirs for organic contaminants as reported by (Gunnarsson and Sköld,1999).

For more biomonitoring, some hematological, biochemical and histopathological investigations were taken in consideration. Blood is a pathophysiological mirror of the health status of living organisms exposed to pollutants (Cazenave,2005;

Li,2011).The potential oxygen-carrying capacity of the fish blood generally fluctuate with the biological life cycle, habitats and physicochemical characters of the environment (Singh and Tandon,2009). The present work is complementary to former paper published by El-Naggar *et al.* (2016), where significant decrease in water oxygen content at Ammar drain was observed compared to River Nile locality accompanied by elevation of biological oxygen demand (BOD), as important indicators of increasing organic contamination in Ammar drain. In this concern,the present study showed depletion in RBCs count, Hb content and Hct% in blood samples of both fish species at Ammar drain compared to River Nile. Rifkind *et al.*(1980) explained that as these blood parameters are positively correlated with water oxygen content, where under oxygen depletion, liver probably revive erythropoiesis to recompense the need for oxygen transportation to marginal tissues. Besides this, several studies revealed that the decrease in RBCs indices is due to RBCs lysis as a result of pollution by textile dyes on *Prussian carp* (Al-Sabti,2000), diazinon on *Cyprinus carpio* L. (Svoboda *et al.*,2001), cassave effluents on *Oreochromis niloticus* and *Clarias gariepinus* (Adekunle *et al.*,2007) and trace metals on *Siganus rivulatus* fish (Moharram *et al.*,2011).

On contrast, the present study also recorded remarkable elevation in WBCs in fish species collected from Ammar drain compared to

those from the River Nile in a process known as leukocytosis, where leucocytes increased to protect the fish body against infection that may be increased with pollution as reported by Mazon *et al.* (2002) in case of *Prochilodus scrofa* exposed to copper.

On the other hand, water pollutants were considered as the main source of physiological disorders in fish (Adams *et al.*,2001; Viarengo *et al.*,2007). Water pollutants may lead to oxidative stress condition, where reactive oxygen species (ROS) which result in activation of antioxidant enzymes (Winston and Di Giulio,1991) or inhibited due to toxicity that may handicap the creation of reactive oxygen species (Cossu *et al.*,1997). Concerning antioxidant enzymes, the principal protection against undesirable ROS comprises SOD enzyme, which stimulates the change of ROS, such as superoxide anions to oxygen and hydrogen peroxide that converted by CAT into water (Ben Ameer *et al.*,2012). The enzyme response to pollutants usually displays an early elevation in activity as a result of enzyme induction, followed by a decline in action as a result of improved catabolic pathways and/or inhibition by contaminants (Viarengo *et al.*,2007) as seen in the present study,where general decrease in serum SOD and CAT was recorded in both fish samples from Ammar drain compared to the River Nile site.

Reduction in SOD and CAT serum levels of both fish species at Ammar drain during most

seasons of the study year, especially summer and autumn may be correlated with increased TOC%, especially during autumn in sediment and tissue samples. These goes in accordance with several authors who connected between environmental pollutants and variations in levels of antioxidant enzymes (Adeogun *et al.*, 2012; Ben Ameer *et al.*, 2012; Karadag *et al.*, 2014).

For more evaluation of fish health status, the present study was interested also in investigating histopathological changes in different fish' tissues including; muscles, testes and ovaries. Muscles as the most edible part of the fish were widely affected in both investigated fish; *C. gariepinus* and *O.n. niloticus* from the polluted site (Ammar drain) compared to those from the reference site (River Nile). As shown in Figures 2(A-D) for *C. gariepinus* & 2¹ (A-D) for *O.n. niloticus* during winter and summer at both localities, where degeneration, atrophy and splitting of muscle fibers of both fish species collected from Ammar drain were observed comparing with River Nile fish species. This was in agreement with the results of (Patnaik *et al.*, 2011) who explained that fish' muscles are directly contacted with pollutants through water and sediments which may induce hyperactivity and excitability in fish species leading to lactic acid release that result in muscular fatigue.

Moreover, the histological structures of testes and ovaries of the two studied fish species; *C.*

gariepinus , Figures 3 & 4 (A- D) & *O.n. niloticus*, Figures 3¹ & 4¹ (A-D) during winter and summer showed detrimental development of gonads that was more obvious at Ammar drain compared to River Nile , due to the discharge of organic pollutants including, sewage and agricultural activities. Testes and ovaries are pairs of compact bodies .They are not directly contacted with pollutants, but they can be affected indirectly through their contact with blood that was highly influenced by pollution as mentioned above in reduction of RBCs and Hb content . The affected fish blood goes to Sertoli cell that are responsible for nutrition of the developing oocytes result in incomplete oogenesis. Parallel to that spermatogenesis is also affected by pollution as reported by (Pugazhvendan *et al.*, 2009; Moharram *et al.*, 2011). These degradations in gonads' histopathology supported our results (El-Naggar *et al.*, 2016) that recorded significant low levels of sex steroid hormones and gonadosomatic index in both fish species collected from Ammar drain, where this may directly result due to disruptions of the pituitary-gonadal axis or gonadal damage with exposure to contaminants, as mentioned by (Ebrahimi and Taherianfard, 2011).

Conclusion

Ammar drain was enriched in organic pollutants represented by the increased the levels of organic carbon in water sediments in different seasons that subsequently accumulated

in fish tissues through the food chain and affect general health of inhabiting fish species represented by reduction in blood indices and antioxidant enzymes accompanied by deteriorations in structures of different tissues in both investigated fish species compared to the River Nile, as a reference site.

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