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Effect of Water Pollution in El-Rahawy Drainage Canal on Hematology and Organs of Freshwater Fish *Clarias gariepinus*

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Abstract: This study was conducted to assess the effect of the water quality of El-Rahawy drain at El-Rahawy village, Egypt, on the African catfish *Clarias gariepinus* blood, biochemical parameters and histology of digestive tract, testis and ovaries. In addition to that, samples of water and *C. gariepinus* fish were collected from the River Nile at Delta Barrage in front of El-Kanater El-Khayria City as references for comparison. Also, certain heavy metals (Cu, Fe, Pb, Cd, Mn and Zn) in water were detected from the two sites. The present results showed that heavy metals concentrations in water are higher in El-Rahawy drain than in River Nile and this due to sewage and other pollutants discharge. The present results also, indicated that the bad water quality due to pollution, increased blood parameters in fish caught from El-Rahawy site than those of River Nile because the first site receives greater agricultural, industrial and domestic wastes than the second one. Lesions of histological deformations were detected and analyzed to clarify the effect of water pollution on fish organs. The study recommended treatment of the agricultural, industrial and sewage discharges before their entrance into El-Rahawy drain to protect the fish and people from the dangers of pollution.

Key words: *Clarias gariepinus* · El-Rahawy Drain · River Nile · Water Quality · Heavy Metals · Blood · Histopathology

INTRODUCTION

Aquatic ecosystems are major recipients of pollutants, which, over time, can have serious consequences for the biota that might not become apparent until changes occur at the population or ecosystem level, a point at which it may be too late to take effective countermeasures [1]. In Egypt, the problems of the drainage canals have extremely increased in the past years [2], where disposing of partially treated or untreated domestic and industrial wastewater into agricultural drains deteriorates their water quality [3].

El-Rahawy drain is one of the main drains; it starts at El-Rahawy Pump Station on Mansouria Rayah lies at 30 Km, North to Cairo at El-Kanater El-Khayria area, Egypt. El-Rahawy drain lies between latitudes 30°10’ N to 30°12’ N and longitudes 31°2’ E to 31°3’ E. It is about 12.41 km^2 and passes through El-Rahway village and many villages distributed along it receiving agricultural and domestic wastes without purification in addition to sewage of El-Giza governorate and discharging these wastes directly without treatment into Rosetta branch of the River Nile [4]. The drain is surrounded by high density of population area and wide agricultural lands. The surface level of the drain is 12.37 m above sea level. This drain receives waste water from El-Moheet drain that passes by a deep under El- Nassery sub-branch of the River Nile to open into a concrete reservoir of about 20m high at El-Rahawy drain. From this reservoir, the drainage wastewater runs to about 4 km through El-Rahawy village and opens into Rosetta branch. The drain discharges about 2.800000 m^3/day of which about 1.90000 m^3/day sewage effluents. The surface level of the drainage wastewater in the reservoir is about 13 m higher than the water in Rosetta branch.

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The contamination of fresh water with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threats to public water supplies but also the damages caused to the aquatic life [5]. The natural aquatic systems may extensively be contaminated with heavy metals released from domestic and industrial wastes, agricultural activities, physical and chemical weathering of rocks, soil erosions, as well as sewage disposal and atmospheric deposition [6]. Determination of trace metal concentration in natural water system has received increasing attention for monitoring environmental pollution, due to the fact that some metals are not biodegradable and their presence in the food chain through a number of pathways may be accumulated in different organs of human beings or animals [7].

The need to detect and assess the impact of pollutants, particularly at low, sub lethal concentrations, on environmental quality had led to development of a range of biological responses measured in number of different species [8, 9]. Fish are generally considered to be the most feasible organisms for pollution monitoring in aquatic systems. Fish can be found virtually everywhere in the aquatic environment and they play a major ecological role in aquatic food-webs because of their function as carrier of energy from lower to higher trophic levels [9, 10]. Fish live in the closest possible contact with their environment, are extremely dependent upon it and are affected by changes in it. Thus fish could be used as a “warning system” to indicate the presence of pollutants in natural water [11]. Prior to death or overt sickness, fish may respond to stress by changing molecular, physiological, histological or behavioral responses.

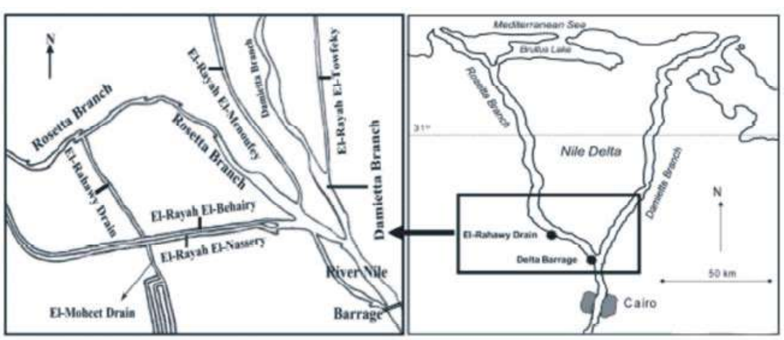
Many studies have shown that hematological parameters can provide satisfactory information on the physiological response of fish to environmental stressors and presence of contaminants; for example exposure to metals; for two major reasons, namely, the close association of the circulatory system with the external environment and the ease of availability of fish blood [12, 13]. Similarly, histopathological investigations have long been recognized to be reliable biomarkers of stress in fish [10, 14]. Histopathological analysis has already been tested and proposed as an efficient and sensitive tool to the monitoring of fish health and environmental pollution in natural water bodies [15].

The aim of the present study was to analyze the water quality of El-Rahawy drain, Nile delta, Egypt. Also, a set of important heavy metals concentrations were measured. In addition, considering the significance of hematological and histopathological parameters as indicators of fish health, the present study aimed at investigating the effects of water quality and metal pollution of El-Rahawy drain on the freshwater African catfish *Clarias gariepinus* inhabiting this drain to assess the impact of pollution.

MATERIALS AND METHODS

The present study was extended from winter 2010 to autumn 2011 during four successive seasons. Two sites (Map 1) were chosen to carry out present study; the first one was located in River Nile at Delta Barrage in front of El-Kanater El-Khayria City (used as the reference point) and the second was selected in El-Rahawy drain at El-Rahawy village. Samples were collected from River Nile at Delta Barrage and different locations of El-Rahawy drain to represent the drain ecosystem.

Sampling, preservation and experimental procedure of the water samples were carried out according to the standard methods for examination of water and wastewater [16].



Map 1: The study area at El-Rahawy drain and River Nile at Barrage.

Water Samples Collection: Water samples were collected at 60 cm depth from different sites (10 samples/ season); using polyvinyl chloride Van Dorn plastic bottles (1.5 liter capacity). For trace elements analysis, water samples were collected in one-liter plastic bottles and preserved with 5 ml concentrated nitric acid on the spot and stored in refrigerator [16]. One-liter plastic bottles were also filled with water samples for undertaking the rest of chemical analysis. The samples were preserved in an icebox and sent immediately to the laboratory.

Field Observations: In situ, air and surface water temperatures (°C) were measured by a dry mercury thermometer, transparency (cm) by Secchi disc, electrical conductivity (EC)(µmohs/cm) by using conductivity meter (model S.C.T. 33 YSI) and hydrogen ion concentration (pH) by Orion Research Ion Analyzer 399A pH meter.

Laboratory Analysis: Dissolved oxygen was measured using the modified Winkler method and biochemical oxygen demand (BOD) was determined with the 5-days incubation method. Concentration of ammonia, nitrite and nitrate were determined by using the colorimetric techniques. All previous analyses were carried out according to the standard methods for examination of water and wastewater [16].

Heavy metals (copper, iron, lead, cadmium, manganese and zinc) in water samples were determined using atomic absorption spectrometry (Perkin-Elmer 3110, USA) with graphite atomizer HGA-600, after using the digestion technique by nitric acid [16].

Fish Samples Collection and Analysis: Samples of African catfish *Clarias gariepinus* were collected seasonally (30 fish/ season) from each site. The fishes were transposed alive back after catching to the laboratory for subsequent analysis. In the laboratory, for each fish, the total length and total weight were recorded. Fish total length and total weight were from 250 to 440 mm and from 290 to 500 g, respectively.

Hematological Analysis: Blood samples were taken from the caudal vein of the fish using a heparinized syringe and collected into small sterilized plastic tubes containing heparin solution. The whole blood was immediately used for the estimation of RBCs count, total WBCs count, platelets count, hemoglobin concentration (Hb) and hematocrit value (Ht).

Some other blood samples were collected and left to coagulate for 15–20 min at room temperature and then centrifuged at 3000 rpm for 10 min to separate serum and serum samples were stored at -20°C until serum analysis. The serum was subjected to biochemical analysis; serum cholesterol, total lipids and triglycerides were determined colorimetrically by kits supplied by Spectrum Diagnostics, Egypt.

Histopathological Examination: After dissecting the fish, stomach, intestine and gonads (testis and ovaries) were removed. The organs were fixed in Bouin's solution for 24 hr. The tissues were routinely dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 4-6 µm thick were cut, processed and stained with hematoxylin and eosin (H&E). They were examined according to Roberts [17] under a complex Olympus light microscope and photographed by a built in camera.

Statistical Analysis: The basic statistics, means and standard deviations of the measured parameters were estimated. Pearson's correlation coefficients matrixes among the different parameters were computed as well. All statistical analyses were done, using the computer program of SPSS Inc. (version 17.0 for Windows) at the 0.05 level of significance.

RESULTS

Physico-Chemical Parameters: Table (1) shows the mean values of physico-chemical parameters of the sampling sites. It is obvious that, the mean values of the different parameters of the water collected from El-Rahawy drain were very high as compared to the reference site of the River Nile, with the exception of DO and transparency. The present results cleared depletion in oxygen content and transparency and increasing in ammonia, nitrate and nitrite concentrations at El-Rahawy drain.

Heavy Metals in Water: Data reported in Table (2) indicated that the values of the detected heavy metals in El-Rahawy drain are appreciably higher than those in the River Nile water. The mean values of the elements at different sites showed Fe to be the most abundant element in water whereas Cd got the least concentration.

Table 1: Physico-chemical parameters (mean ± standard deviation) of water at various sampling sites.

Site	Season	Air Temperature (°C)	Water Temperature (°C)	DO (mg/L)	BOD (mg/L)	Transparency (cm)	pH	Ammonia (mg/L)	Nitrite (NO_2) (µg/L)	Nitrate (NO_3) (µg/L)	Conductivity (µmohs/cm)
River Nile at	Winter	19.25±0.53	17.36±0.39	8.36±0.34	3.18±0.26	90.05±2.17	7.32±0.06	0.59±0.02	11.80±1.71	32.28±2.92	457.65±5.41
El-Kanater	Spring	27.30±0.32	25.13±0.28	7.88±0.17	5.77±0.17	107.63±5.28	7.48±0.18	0.57±0.01	14.70±1.67	35.18±3.55	346.95±8.41
El-Khyria	Summer	32.45±0.58	30.48±0.40	6.98±0.17	1.93±0.06	118.93±2.05	7.68±0.16	0.65±0.06	17.85±1.52	27.55±2.34	382.68±6.01
	Autumn	26.98±0.38	24.95±0.34	8.15±0.26	4.10±0.21	79.85±1.55	7.36±0.17	0.52±0.04	18.28±2.86	41.23±1.98	375.08±6.82
	Mean	26.49±4.88	24.48±4.83	7.84±0.59	3.75±1.46	99.11±15.90	7.46±0.20	0.58±0.06	15.66±3.25	34.06±5.68	390.59±42.71
El-Rahawy Drain	Winter	18.98±0.35	16.96±0.21	4.18±0.47	14.10±0.29	29.24±1.02	8.16±0.32	10.08±0.29	40.08±0.92	39.43±1.46	930.20±11.45
	Spring	27.80±0.19	25.86±0.29	4.88±0.18	13.92±0.26	21.62±2.01	8.11±0.28	12.50±0.56	36.04±0.92	60.26±1.07	537.60±23.97
	Summer	32.38±0.53	30.16±0.21	3.38±0.08	12.66±0.21	20.78±0.90	8.35±0.31	7.14±0.23	41.80±1.16	59.20±1.11	587.58±14.85
	Autumn	26.90±0.43	25.10±0.34	3.66±0.11	13.04±0.24	24.98±0.41	7.87±0.23	9.70±0.25	33.23±0.96	76.25±1.39	724.74±17.09
	Mean	26.52±4.96	24.52±4.90	4.03±0.63	13.43±0.66	24.16±3.60	8.12±0.31	9.86±1.98	37.79±3.56	58.79±13.45	695.03±156.85
Permissible limits (mg/l)	Egyptian law No. 48 [28]	NA	NA	>5	<6-10	NA	6.5-9	<0.5	NA	40	NA

NA = not available.

Table 2: Heavy metals concentrations (mean ± standard deviation) in water at various sampling sites

	Heavy metals concentrations in water (µg/L)						
Site	Season	Copper (Cu)	Iron (Fe)	Lead (Pb)	Cadmium (Cd)	Manganese (Mn)	Zinc (Zn)
River Nile at	Winter	11.65±0.52	298.20±8.64	29.18±1.13	7.02±0.32	145.50±4.16	15.80±0.73
El-Kanater	Spring	14.12±0.53	804.14±15.67	33.15±1.87	7.98±0.20	155.82±4.26	12.94±0.36
El-Khyria	Summer	15.88±0.28	942.24±83.48	35.67±0.83	11.31±1.00	120.68±1.99	15.16±0.44
	Autumn	13.77±0.39	718.44±20.46	31.47±1.41	7.15±0.26	170.32±2.95	19.66±0.75
	Mean	13.86±1.60	690.76±249.81	32.37±2.74	8.37±1.85	148.08±18.85	15.89±2.54
El-Rahawy	Winter	20.16±0.94	851.90±32.57	71.37±1.84	7.14±0.33	79.10±0.93	61.72±2.44
Drain	Spring	29.82±0.90	951.80±33.60	63.18±3.26	8.54±0.15	176.87±4.88	80.44±1.36
	Summer	26.92±0.98	1002.92±7.57	49.12±1.47	9.08±0.28	169.44±2.70	51.00±1.84
	Autumn	24.77±0.54	904.50±7.34	42.58±1.49	8.95±0.16	181.66±1.89	46.80±1.01
	Mean	25.42±3.70	927.78±61.48	56.56±11.80	8.43±0.82	151.77±43.36	59.99±13.43
Permissible limits (µg/l)	Egyptian law No. 48 [28]	1000	1000	50	10	500	1000
Permissible limits (µg/l)	U.S.EPA [31]	9.0	1000	2.5	0.25	NA	120.0

NA = not available.

Table 3: Hematological and biochemical parameters (mean ± standard deviation) of the African catfish *Clarias gariepinus* at various sampling sites.

Site	Season	Erythrocytes Counts (RBCs) (10^6/mm^3)	Hemoglobin Concentration (Hb) (gm/100ml)	Hematocrit value (Ht) (%)	Total Leucocyte Counts (WBCs) (10^3/mm^3)	Platelets Counts (PL)(10^3/m3)	Total Lipids (TLp) (gm/100 ml blood)	Cholesterol (Ch) (gm/dl)	Triglycerides (TrG) (mg/dl)
River Nile at	Winter	2.07±0.38	10.58±2.73	32.70±5.15	68.45±13.05	11.08±1.63	932.75±103.41	309.75±34.74	75.00±22.95
El-Kanater	Spring	2.61±0.16	12.83±1.58	44.30±3.36	69.25±11.39	11.63±4.28	1074.75±128.90	375.25±114.83	121.75±28.27
El-Khyria	Summer	2.72±0.11	13.48±1.41	38.90±3.27	64.25±4.38	12.33±2.51	1007.00±28.40	429.00±69.90	100.75±19.79
	Autumn	2.61±0.10	13.78±0.88	39.10±2.82	57.05±3.98	14.45±3.67	899.25±129.64	355.50±44.40	95.25±45.51
	Mean	2.50±0.32	12.66±2.05	38.75±5.41	64.75±9.59	12.37±3.15	978.44±117.92	367.38±78.76	98.19±32.45
El-Rahawy Drain	Winter	2.57±0.11	13.78±0.80	44.32±1.58	83.66±17.31	18.68±4.96	1243.80±49.48	341.00±56.61	104.25±22.46
	Spring	3.10±0.30	12.24±1.53	38.20±2.68	67.82±5.16	17.93±2.87	1067.60±63.61	390.00±44.18	142.50±12.45
	Summer	3.26±0.22	12.06±2.00	35.30±4.84	68.02±11.34	24.78±7.80	1317.60±53.95	385.25±90.55	141.25±13.30
	Autumn	3.30±0.32	12.94±1.87	41.90±4.46	72.54±8.83	15.13±0.71	1018.00±83.80	406.25±74.42	127.25±35.96
	Mean	3.06±0.38	12.76±1.64	39.93±4.87	73.01±12.48	19.13±5.66	1161.75±139.75	380.63±66.35	128.81±26.05

Hematological Parameters: The mean hematological indices and biochemical parameters of *C. gariepinus* collected from River Nile and El-Rahawy drain waters were shown in Table (3). The different parameters of fish collected from El-Rahawy drain were very high as compared to the reference site fish.

On the other hand, the correlation coefficient matrix (r) of the investigated water parameters and heavy metals with blood and biochemical parameters of *C. gariepinus* collected from El-Rahawy drain (Table 4), demonstrated some significant positive and negative correlations.

Table 4: Pearson's correlation coefficient matrix between different hematological and biochemical parameters of the African catfish *Clarias gariepinus* and different physico-chemical parameters and heavy metals concentrations at El-Rahawy drain

	RBCs	Hb	Ht	WBCs	PL	TLp	Ch	TrG
Air Temperature	0.76**	0.80**	0.67**	0.64**	-0.12	-0.06	0.13	0.24
Water Temperature	0.77**	0.81**	0.69**	0.65**	-0.09	-0.01	0.14	0.27
DO	-0.24	-0.30	-0.12	-0.34	0.42	0.56*	0.33	0.14
BOD	-0.56*	-0.64**	-0.51*	-0.57*	0.26	0.31	0.09	0.03
Transparency	-0.73**	-0.81**	-0.67**	-0.61*	0.17	-0.02	-0.09	-0.42
pH	-0.17	-0.18	-0.36	-0.36	0.31	-0.29	-0.10	-0.39
Ammonia	-0.17	-0.30	-0.21	-0.37	0.36	0.54*	0.36	0.32
NO_2	-0.26	-0.11	-0.18	0.03	-0.21	-0.43	-0.71**	-0.60*
NO_3	0.87**	0.81**	0.67**	0.56*	-0.07	0.09	0.51*	0.62*
Conductivity	-0.11	0.001	-0.04	-0.21	0.08	0.32	0.24	0.27
Copper	0.66**	0.66**	0.60*	0.41	0.20	0.31	0.40	0.35
Iron	0.70**	0.75**	0.68**	0.62**	0.04	0.12	0.06	0.16
Lead	-0.75**	-0.78**	-0.56*	-0.59*	0.38	0.37	-0.12	-0.34
Cadmium	0.84**	0.85**	0.72**	0.69**	-0.09	0.07	0.24	0.44
Manganese	-0.13	-0.29	-0.26	-0.44	0.37	0.41	0.59*	0.32
Zinc	-0.14	-0.21	-0.07	-0.23	0.45	0.64**	0.26	0.18

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Histopathological Findings

Stomach: The sub-mucosal tissues were fully vacuolated with degeneration of the serosal layer, edema, degeneration of columnar epithelium (cracked clay), goblet cell and basement membrane as well as the secretory cells were damaged and fully distorted (Figs. 1 and 2)

Intestine: In cross section, anterior intestine was made up of four distinct layers: outer serosa, muscularis, submucosa and mucosal epithelium. The mucosa forms numerous extremely long finger-like folds called villi. These villi possess tapering luminal ends, parallel sides and narrow crypts (Fig. 3). The mucosa is composed of simple columnar epithelium containing scattered goblet cells.

A degenerative effect was evident in the mucosal lining and villi of the intestine. Widening of lamina propria of villi and infiltration of inflammatory cells in the lamina propria and cracked clay of outline of villi (Figs. 3 and 4) were observed. Flattening of microvilli and a cracked clay appearance of the tissue were likewise apparent, flattening of intestinal folds, rupture of muscular layer, reduction of villi and necrosis were prominent (Figs. 5 and 6). Like degeneration of peritoneal lining, the loss of longitudinal, vacuolization, histolysis of columnar cells were the other marked changes (Fig. 6).

Duodenum: It had nearly the same lesions of intestine, damage of longitudinal muscle fibers, circular muscle fibers and blood vessels. The lamina propria of the digestive tube, especially in the foremost part, consisted of considerable solitary lymphoid tissue and inflamed eosinophilic cells (Figs. 7 and 8).

Testis: *C. gariepinus* testis showed significant changes, extensive cytotoxic damage, general inflammatory response and other histological abnormalities were quite prominent. Although, inter-tubular vacuoles were clearly visible (Figs. 9 and 10), the extent of histological damage was evident by the presence of large number of both inter and intra-tubular vacuoles. Gross condensation of spermatogenic cells, which was evident by clump formations (Figs. 11 and 12) and appearance of inflammatory lesions were also quite prominent. The extent of vacuolation in tubular epithelium increased.

Ovary: *C. gariepinus* ovary showed increased oocytes atresia, an increase in degradation and resorption of oocytes at any point in development. Atresia was characterized by clumping and perforation of the chorion, fragmentation of the nucleus, disorganization of the ooplasm and/or the uptake of yolk materials by perifollicular cells (Figs. 13 and 14). Decrease in the amount of vitellogenic/yolk material that was deposited in the developing oocytes. Decreased vitellogenesis was characterized by the presence of oocytes in which yolk material was not present despite their relatively large size. Coagulative necrosis of zona radiata of large oocytes and liquefaction of the yolk sphere with large vacuoles of ripe stage were noticed (Figs. 15 and 16).

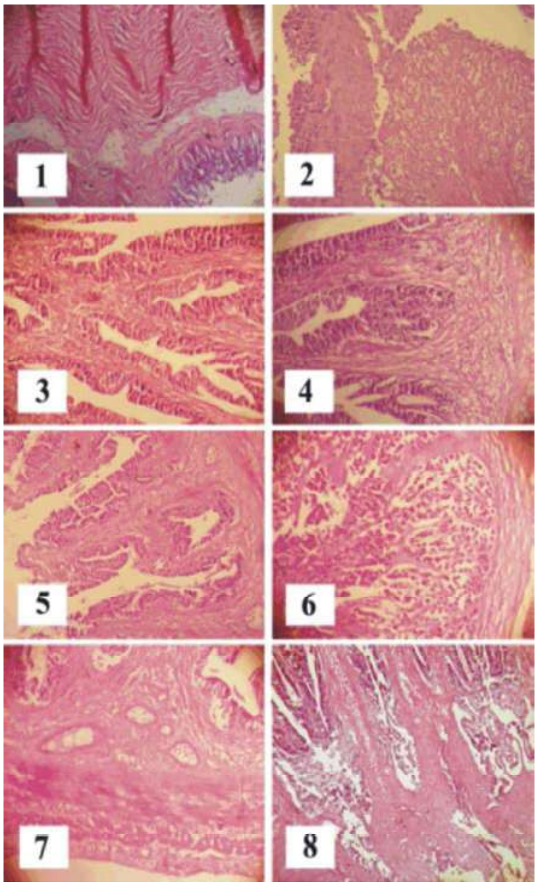


Fig. 1: Section in stomach of *C. gariepinus* showing circular muscle fiber and submucosa (H&E, X100).

Fig. 2: Section in stomach of *C. gariepinus* showing edema in mucosal layer and a cracked clay (H&E, X400).

Fig. 3: Section in medium intestine of *C. gariepinus* showing inflammatory cells in the lamina propria (H&E, X100).

Fig. 4: Section in the medium intestine of *C. gariepinus* showing cracked clay of villi (H&E, X100).

Fig. 5: Section in the intestine of *C. gariepinus* showing hyaline degeneration of villi and necrosis (H&E, X400).

Fig. 6: Section in the intestine of *C. gariepinus* showing cracked clay of tissue and necrosed epithelia of villi and intraepithelial lymphocytes (H&E, X400).

Fig.7: Section in duodenum of *C. gariepinus* showing edema (H&E, X100).

Fig. 8: Section in duodenum of *C. gariepinus* showing edema and necrotic tissue (H&E, X400).

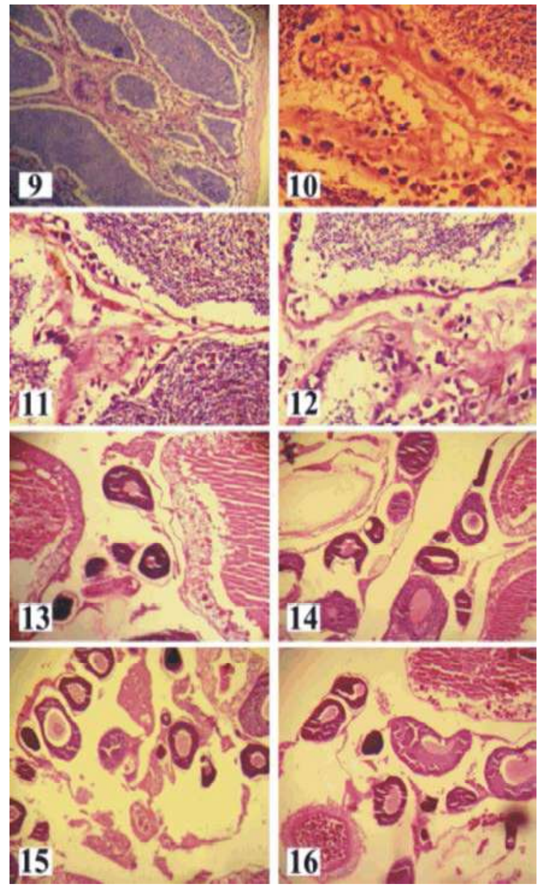


Fig. 9: Section in testes of *C. gariepinus* showing lumen of seminiferous tubules filled with spermatozoa (H&E, X100).

Fig. 10: Section in testes of *C. gariepinus* showing the wall of seminiferous tubules vacuolated, with fibrotic and necrotic spermatocytes (H&E, X400).

Fig. 11: Section in testes of *C. gariepinus* showing degenerates of spermatogenic cells (H&E, X400).

Fig. 12: Section in testes of *C. gariepinus* showing clump of spermatogenic cells (H&E, X400).

Fig. 13: Section in the ovary of *C. gariepinus* showing coagulative necrosis of zona radiata and liquefaction of the yolk sphere with large vacuoles of ripe stage (H&E, X100).

Fig. 14: Section in the ovary of *C. gariepinus* showing atretic oocytes with irregular membrane (H&E, X100).

Fig. 15: Section in the ovary of *C. gariepinus* showing atretic young oocytes (H&E, X100).

Fig. 16: Section in the ovary of *C. gariepinus* showing atretic yolky stage oocytes (H&E, X100).

DISCUSSION

Anthropogenic sources such as agriculture run-off, industrial and sewage have created both localized and regional pollution problems in nearly every country around the world [18]. In some cases the pollution has been extensive enough to lead to environmental disasters and ecosystem shutdown [19].

The elevated levels in physico-chemical properties observed in El-Rahawy drain implicate pollution as the source of alteration in water quality. The negative impact of different sources of pollutants discharged into this drain was further confirmed by the highest values in all physico-chemical parameters with a concomitant decrease in DO.

Temperature is a factor of great important for aquatic ecosystem, as it affects the organisms, as well as the chemical and physical characteristics of water [20]. As expected the water temperature of the studied points followed more or less that of the air.

The relative increase in temperature of El-Rahawy drain water had potential implications on the oxygen retention capacity of the water [21] as increases in temperature affects the levels of dissolved oxygen in the water column where DO is inversely proportional to temperature [19]. In addition, Veado *et al*. [22] reported that the introduction of excess of organic matter may result in a depletion of oxygen from an aquatic system mainly during warm stagnant condition.

Similarly, the reduction in dissolved oxygen content may be due to decomposition of suspended organic matter of sewage in this drain [23]. Prolonged exposure to low dissolved oxygen level (<5-6 mg/L) will increase organisms susceptibility to other environmental stress [24] and has dire consequences for the survival of fish and other aquatic animals as reduced DO will elicit physiological regulatory mechanisms involved in the maintenance of oxygen gradient from water to tissues which is essential to maintain the metabolic aerobic pathways [19].

BOD measures the dissolved oxygen consumed by microorganisms present in the studied samples to stabilize any biodegradable organic matter, as well as the quantity of oxygen used in its respiration [16, 25]. Increase in BOD values monitored in El-Rahawy drain environment being affected by quantity and quality of discharges, as well as seasonal and spatial effects [26].

On the other hand, the increase of turbidity (low transparency) may be due to the disposal of domestic and industrial effluent in this drain [24].

The pH value is considered to be an important factor in the chemical and biological system of aquatic environment [24]. The relatively high pH of El-Rahawy drain water could be attributed to the large amounts of different pollution sources discharged in this drain. pH has profound effects on water quality affecting the ability of bacteria which require slightly acidic pH to degrade toxic substances to less harmful forms [19].

Dissolved inorganic nitrogen is the summation of the ammonia, nitrate and nitrite [23, 24]. These parameters were found in high concentrations in El-Rahawy drain water which may be due to sewage outfalls, as recorded by Tayel *et al*. [23]. The higher contents of nitrite in El-Rahawy drain water are indication of the microbial activity. The recorded increase in NO_3 comparing to in River Nile water might be attributed to the fast conversion of NO_2-NO_3-ions by nitrifying bacteria [24]. The increase in ammonia level in water samples collected from El-Rahawy drain water is indicator of the presence of pollutants of high activity viz.: sewage discharge, industrial effluents and agriculture-runoff and could be attributed to the increase in the oxygen consumption of the decomposing organic matter and oxidation of chemical constituents [27]. The presence of large concentration of NO_2 and NO_3 in water can create a large oxygen demand. High concentration of nitrate and nitrite can cause algae to grow in large quantity. Dead algae can cause oxygen depletion problems which in turn can kill fishes and other aquatic organisms [24]. Although mean ammonia value in El-Rahawy drain water exceeded acceptable limits by the Egyptian governmental law No. 48 [28], the value in River Nile water was considerably elevated.

The high conductivity values observed in El-Rahawy drain water suggested possible sources of run-off from adjacent land and strongly implicates industrial and sewage sources. This agrees with reports of conductivity being a direct measure of anthropogenic impact [29].

It was found that the values of the most of physico-chemical parameters of water samples collected from El-Rahawy drain are higher than those collected from Nile water but generally the detected values of the water samples from both sites are in the permissible levels set by the Egyptian governmental law No. 48 [28].

Heavy metals may enter an aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills, shipping and harbor activities and atmospheric deposits [30].

Present results showed that, most of the heavy metal concentrations in surface water of El-Rahawy drain and River Nile water are found within the permissible limits of both the Egyptian governmental law No. 48 [28] and U.S.EPA [31]. These results are in agreement with El Bouraie *et al*. [32] who studied heavy metals in five drain outfalls and found that the level of metals is within the permissible limits of Egyptian law 48/1982. Also, Lasheen *et al*. [33] stated that the average concentrations of heavy metals in El-Moheet drain; which discharge in El-Rahawy drain; are within the permissible range according to the Egyptian law 48/1982.

Generally, lower mean value of DO and higher mean values of turbidity, conductivity, BOD, NO_2, NO_3 and trace metals in El-Rahawy drain comparing to the Nile water proved the presence of large quantities of organic and inorganic pollutants in El-Rahawy drain. This was expected due to the fact that the water of such drain receives large quantities of domestic, agricultural and industrial effluents.

Hematological and biochemical profiles of blood can provide important information about the internal environment of the organism. The evaluation of hematological and biochemical characteristics in fish has become an important health indicators and means of understanding normal and pathological processes and toxicological impacts [34]. Short-term exposures to low concentrations of heavy metals mostly induce an increase in the hematological indices [35].

Our results showed that all hematological and biochemical parameters of *C. gariepinus* collected from El-Rahawy drain were higher than those collected from River Nile. This is an evidence of the response of fish to the environmental pollution [23]. The increase of these parameters goes in parallel with the elevation in the levels of chemical parameters studied such as ammonia, depletion in dissolved oxygen content and increase in heavy metals levels as a results of pollution stress in that area [23]. The significant positive correlations (Pearson's correlation coefficients) observed between the hematological and biochemical parameters and different physico-chemical parameters and heavy metals concentrations at El-Rahawy drain (Table 4) suggested that the detected blood parameters are influenced by water quality and metals concentrations.

The hematological parameters changes can be interpreted as compensatory responses that improve the O_2 carrying capacity to maintain the gas transfer and a change in water blood barrier for gas exchange in gill lamellae, which were also reported in previous results [36]. The negative correlation between DO values and RBCs, Hb, Ht values (Table 4) confirmed this. In hypoxia condition, spleen and may be the liver may reactivate the erythropoiesis to compensate the demand by increased oxygen transport to peripheral tissues [37]. Chowdhury *et al*. [38] noted an increase of blood hematocrit and hemoglobin during environmental hypoxia and chronic or acute exposure to waterborne metals (Cd, Zn, Cu, Al and Ni) to increase blood oxygen carrying capacity when impairment of gas exchange occurs. Oliveira Ribeiro *et al*. [39] observed increase in red blood cells counts in *H. malabaricus* exposed to MeHg and attributed this to an increase in the blood O_2 carrying capacity. Increase in RBCs number and hematocrit level was reported in *Mystus vittatus* exposed to sub-lethal and lethal concentrations of copper and zinc [40]. The RBCs may also be affected by other metals as reported by Allin and Wilson [41] in *O. mykiss* after an acute exposure to aluminum. The white blood cells are the regulators of the immune system and they are increased in the blood of *C. gariepinus* collected from El-Rahawy drain and this attributed to expose to chronic sewage. This is in agreement with that of Hoeger *et al*. [42]. The increase in WBCs count in the present study may be due to generalized immune response and a protective response to metals stress [43]. In general, increased WBCs count in fish exposed to copper indicates leucocytosis [44]. Stimulation of lymphopoiesis and/or enhanced release of lymphocytes from lymphomyeloid tissue under toxic stress may lead to an increase in WBCs number [45].

In the present investigation, the blood cholesterol level was increased in *C. gariepinus* collected from El-Rahawy drain as compared with those collected from River Nile. Triglycerides and cholesterol are known to participate in the rise of total lipid [46]. The rise of these energy reserves in response to pollution could be due to the fact that excess energy reserves (as glucose, triglycerides and cholesterol) are required by organisms to mediate the effects of stress [47]. Since homeostasis of lipids is one of the principal liver functions, any change in serum triglyceride concentration is used as an indicator of liver dysfunction [48]. According to Shalaby [49], Hg-induced increase in serum total lipids of *O. niloticus* may be due to disturbance in the metabolism of lipids.

The employment of histopathological biomarkers to determine the effects of environmental contamination has been perceived as a highly relevant methodology since they reflect the true health state of the organism [50].

With respect to aquatic environments, the fish gonads and digestive tract with the liver, gills and kidneys being the major targets of assessment due to their functions in xenobiotic storage and, even, elimination [51].

In the present study, histopathological changes in digestive tract, testis and ovaries of *C. gariepinus* collected from El-Rahawy drain may be due to pathogens and metals pollutions as recorded by Tayel *et al*. [23].

The presence of necrosis is in fact one of the most visible damages in tissues affected by a pollutant [52]. According to Manahan [53] the occurrence of necrosis is also a consequence of enzymatic inhibition, damages in the cellular membrane integrity and disturbances in the synthesis of proteins and carbohydrate metabolism.

In the present study, the histopathological findings in intestine of *C. gariepinus* collected from El-Rahawy drain are in agreement with Gardner and Yevich [54], Newman and MacLean [55] and Gutierrez *et al*. [56] who reported nearly same lesions in intestine of different fish species exposed to cadmium chloride ($CdCl_2$). Establier *et al*. [57] reported intestinal toxic lesions in intestine of *Mugil auratus* exposed to inorganic and organic mercury. Sastry and Gupta [58] reported toxic lesions in intestine of *Channa punctatus* exposed to mercuric chloride. Smith and Piper [59] reported toxic lesions in the intestine of different fishes chronically exposed to ammonia.

Histopathological damage in testis of *C. gariepinus* can be visualized in the form of shrinkage of interstitial cells and vacuolation of tubular cells, which has resulted in peculiar starry sky appearance of the testicular tissue. Testicular inflammation was documented as one of the common responses in both aquatic and terrestrial animals exposed to environmental toxicants [60]. A relationship between heavy metals and the presence of tubules damages has been reported in other study [61], in fact heavy metals are recently reported by Dyer [62] as endocrine disturbing chemicals. Histopathological changes were seen in the testis of *Salmo gairdneri* exposed to cyanide (HCN) [63] and in the testis of catfish, *Clarias batrachus* L., exposed to mercuric chloride and methyl mercuric chloride [64].

In females, mean number of yolky oocytes is considered as an important criterion for the assessment of reproductive performance in fish. In the present study, mean number of yolky oocytes of *C. gariepinus* was affected by the polluted environment. The same ovary lesions similar to those found in the present study have been reported in fish exposed to nickel [65]. Histopathological changes were seen in the ovary of rainbow trout exposed to cyanide (HCN) [66], in the ovary of *Puntius conchonius* exposed to zinc, copper and lead [67] and in the ovary of *Oreochromis niloticus* infected by *Streptococcus* sp. [68].

The observed histopathological alterations in the testis and ovaries of the studied fish may reduce the ability of fish to reproduce. It is well known that water pollution has a serious inhibitory effect on fish reproduction [69, 70] resulting in a decrease in their abundance and, consequently, a decline in fish species diversity. The pollutants discharges in this study were presented as disruption in gonads development. It comes in agreement with other studies for fish inhabiting water impacted by domestic sewage [71]. Also oogenesis and spermatogenesis were inhibited in medaka exposed to sewage effluents [72]; and on exposure of *Siganus rivulatus* to copper works effluent [73].

Our results are supported by previous research work that various heavy metals and toxins enter into the aquatic system exerted a specific toxic effect on fish blood and tissues [74].

Results of the present study clearly demonstrated that El-Rahawy drain is contaminated with heavy metals due to the continuous discharge of different pollutants into it. It was found that these metals induce changes in the blood and cause several histopathological changes in the digestive tract and gonads of *C. gariepinus*. Consequently, great efforts and co-operation between different authorities are needed to protect the drain from pollution and reduce environmental risk. This can be achieved by treatment of the agricultural, industrial and sewage discharges that fulfill their safety.

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