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Heavy Metals Pollution and Their Effects on Gills and Liver of the Nile Catfish *Clarias gariepinus* Inhabiting El-Rahawy Drain, Egypt

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Abstract: The water quality and heavy metals (Cu, Fe, Pb, Cd, Mn and Zn) concentrations in the water of El-Rahawy drain and River Nile at El- Kanater El-Khyria (as a reference site for comparison) were seasonally estimated. In addition to that, samples of *Clarias gariepinus* fish were collected from the two sites to assess the effects of the water quality and heavy metals concentrations in El-Rahawy drain on the fish liver enzymes and some biochemical parameters. Also, the histological alterations of the gills and liver of the studied fish species were studied. The obtained results showed poor water quality in El-Rahawy drain region compared with those obtained from River Nile at El- Kanater El-Khyria. Also, it was found that the heavy metals concentrations in water from El-Rahawy drain region were higher than those obtained from River Nile. Increase in glutamate-oxaloacetate transaminase (GOT) and glutamate- pyruvate transaminase (GPT) activities and different biochemical parameters of *C. gariepinus* living in El-Rahawy drain were noticed. Also, histological study cleared that gills and liver of *C. gariepinus* living in El-Rahawy drain suffered from several pathological alterations. All these findings were discussed and referred to the input of agricultural, industrial and sewage water into El-Rahawy drain. It could be recommended that treatment of wastewater including sewage and domestic wastes before discharging into water bodies is a necessary process to protect the fish and the public health from the danger of pollution.

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

INTRODUCTION

Due to urban, industrial and agricultural activities, freshwater sources are dumped with different kinds of chemicals that affect the inhabiting biota [1]. In recent years, the problems of sewage pollution of inland waters have become a point of local concern.

The disposal of the untreated sewage may be harmful concerning its possible hygienic and aesthetical effects and its impact on fauna and flora in the aquatic environment [2]. Egyptian drains receive large quantities of partially treated or untreated domestic and industrial wastewater and other human activities, which in turn ultimately discharge into River Nile, canals, lakes, or seas [3]. The amount of waste discharged into receiving water bodies far exceeds the natural ability of these bodies to attenuate the pollutants [3] with dramatic consequences on water quality, sediments and biotic communities [4].

El-Rahawy drain is such a receiving environment; it is located in the southern part of the Nile Delta, Egypt, about 39.5 km north-west of the city of Cairo. It lies between latitudes 30° 10’ N to 30° 12’ N and longitudes 31° 2’ E to 31° 3’ E. El-Rahawy drain is about 12.41 km^2 and passes through El-Rahway village and many villages distributed along it receiving agricultural, industrial and domestic wastes in addition to sewage of El-Giza governorate and discharged these wastes directly without treatment into Rosetta branch of the River Nile at El-Rahway village [5]. This drain receives waste water from El-Moheet drain that passes by a deep under

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El-Nassery sub-branch of the River Nile to open into a concrete reservoir of about 20 m 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. El-Rahawy drain is an ideal ecosystem for studying aquatic organism responses to these different contaminants.

The domestic wastewaters contain fairly high concentrations of metals [6], where these metals are derived from household products such as cleaning materials (detergents), toothpaste, cosmetic and human feces [7]. Also, there are additional quantities introduced from industrial wastes [8] and washing of herbicides and pesticides of the agricultural lands [9].

Due to their toxicity, long persistence, bioaccumulative and nonbiodegradable properties in the food chain, metals constitute a core group of aquatic pollutants [6]. Thus, in view of the quality of public food supplies, their levels in aquatic environment should be monitored regularly to check water quality and animal health [10].

In order to evaluate the adverse effects of the pollutants on aquatic organism, there is a worldwide trend to complement chemical and physical parameters with biomarkers in aquatic pollution monitoring [1]. Fish are used as excellent indicator of aquatic pollution due to their high sensitivity to environmental contaminants which may damage certain physiological and biochemical processes when contact with the organs of fishes [11].

The fish liver plays an important role in vital functions in basic metabolism and it is the major organ of accumulation, biotransformation and excretion of contaminants in fish [12]. The measurement of suitable biomarkers in liver becomes useful and can give an idea about the health state of fish. Toxicological studies have shown that the impact of contaminants on aquatic ecosystems can be evaluated by measuring biochemical parameters in the liver of the fish that respond specifically to the degree and type of contamination [13]. Also, the liver histology is used as biomarker for the environmental pollution [14] and there have been numerous reports of histopathological changes in livers of fish exposed to a wide range of organic compounds and heavy metals [1, 15].

On the other hand, fish gill is a multifunctional organ responsible for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion. Fish gills are exposed directly to aquatic media and sensitive to any change of water components, since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water [14, 15]. Thus, changes in fish gills are among the most commonly recognized responses to environmental stressors and are indicative of physical and chemical stress [1, 15, 16]. Therefore, gill histology is used extensively as indication of environmental pollution [14]. Gill histopathological lesions as indicators of exposure to a wide range of contaminants including heavy metals have previously been used in numerous laboratory and field studies around the world [15, 17, 18].

African catfish (*Clarias gariepinus*) is one of the most important tropical fish due to high growth rate, high consumer acceptability and high resistance to poor water quality and oxygen depletion [19]. Moreover, it has been used in fundamental research and considered as an excellent model for toxicological studies [20].

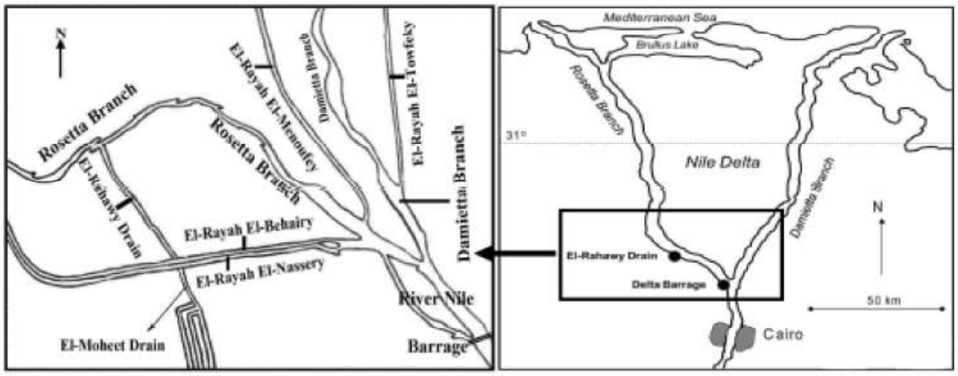
So, the overall objective of this study is to evaluate the water pollution in an effluents-dominated drain in Egypt (El-Rahawy drain) and effects of this pollution and heavy metals on wild *Clarias gariepinus* fish. The impacts on some biochemical parameters, including liver enzymes assays and histopathology of liver and gills were evaluated.

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.

Water Samples Collection and Analysis: Sampling, preservation and experimental procedure of the water samples were carried out according to the standard methods for examination of water and wastewater [21].

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.



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

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 [21]. 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 returned immediately to the laboratory.

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 [21].

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 [21].

Fish Samples Collection and Analysis: Samples of African catfish *Clarias gariepinus* were collected seasonally (30 fish/ season) from each site. The fishes were transposed a live 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.

Liver Enzymes and Biochemical Analyses: Blood samples were taken from the caudal vein of the fish using a heparinized syringe and collected into small sterilized plastic tubes. Blood samples were 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 in polyethylene Eppendorf test tubes at -20°C until serum analysis. Serum samples were used to estimate, colorimetrically, the liver enzymes and biochemical parameters. GOT, GPT, glucose and albumin were determined using Stanbio kit as described by Reitman and Frankel [22], Trinder [23] and Dumas and Biggs [24], respectively. Protein content was determined using the Boehringer Mannheim Kits according to Gornall *et al.* [25]. The content of globulin is obtained by subtraction of protein content with albumin content as follows: Globulin content = protein content - Albumin content. Albumin/ Globulin ratio is obtained as follows: A/G Ratio = Albumin content / Globulin content.

Histopathological Examination: After dissecting the fish, liver and gills were removed and 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 heamatoxylin and eosin (H&E). They were examined according to Roberts [26] by a complex Olympus light microscopy and photographed by a built in camera.

Statistical Analysis: The basic statistics, means and standard deviations and errors of the measured parameters were estimated. Data were statistically analyzed using one way analysis of variance (ANOVA) test. Pearson's correlation coefficients matrix among the different parameters was 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

Physicochemical Parameters: Table (1) shows the mean values of physicochemical 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 were 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.

Liver Enzymes and Biochemical Parameters: The mean values of liver enzymes and different biochemical parameters of *C. gariepinus* collected from River Nile and El-Rahawy drain waters are shown in Table (3). On comparing the present results of liver enzymes and different biochemical parameters with those collected from the River Nile at El-Kanater El-Khyria (as a control fish), it is clear that, 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 liver enzymes and different biochemical parameters of *C. gariepinus* collected from El-Rahawy drain (Table 4), demonstrated some significant positive and negative correlations.

Histopathological Findings

Gills: The histopathological alterations of the gill of *C. gariepinus* fish collected from El-Rahawy drainage canal include alterations such as dilation and congestion in blood vessel of primary gill filaments (Fig. 1). Hyperplasia of epithelial cells between secondary lamellae led to fusion and separated from pillar system were detected (Fig. 2). Also increase in intracellular vacuolation (Figs. 2 & 3) was observed. Most of gill filaments were sloughed (Fig. 4).

Liver: The histopathological changes found in the liver of *C. gariepinus* fish, collected from El-Rahawy drain, include loss of cellular architecture of liver (Fig. 5), vacuolar degeneration, pycnotic nuclei and focal areas of necrosis

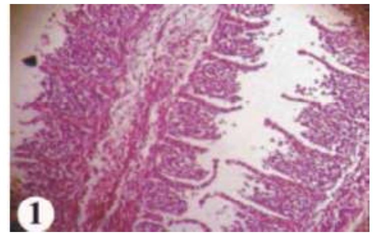


Fig. 1: Section in the gill of *C. gariepinus* showing dilated and congested primary lamellae (H&E, X100)

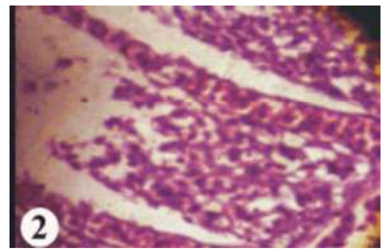


Fig. 2: Section in the gill of *C. gariepinus* showing hyperplasia of epithelial cells and separated from pillar system (H&E, X 400)

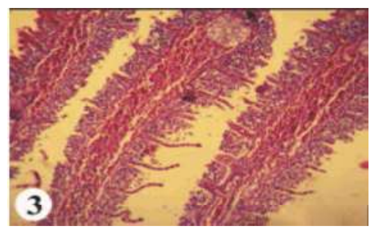


Fig. 3: Section in the gill of *C. gariepinus* showing dilated and congested primary lamella (H&E, X100)

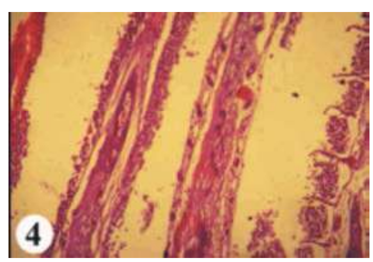


Fig. 4: Section in the gill of *C. gariepinus* showing sloughing of secondary lamellae (H&E, X 100)

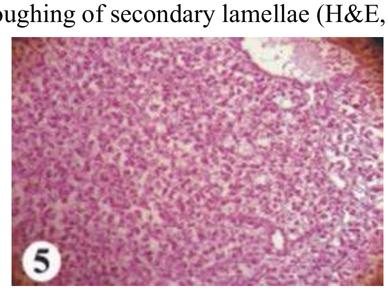


Fig. 5: Section in the liver of C. *gariepinus* showing loss of cellular architecture (H&E, X100)

Table 1: Physicochemical parameters (mean ± standard deviation) 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 El-Kanater	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-Khyria	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
	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 [37]	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 El-Kanater El-Khyria	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
	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
	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 Drain	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
	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 [37]	1000	1000	50	10	500	1000
Permissible limits (µg/l)	U.S.EPA [40]	9.0	1000	2.5	0.25	NA	120.0

NA = not available

Table 3: Liver enzymes and biochemical parameters (mean ± standard error) of the African catfish *Clarias gariepinus* at various sampling sites

Site	Season	GOT (Iu/ml)	GPT (Iu/ml)	Albumin (g/100 ml)	Globulin (g/100 ml)	A/G ratio	Protein (g/100 ml)	Glucose (mg/100 ml)
River Nile at El-Kanater El-Khyria	Winter	24.68±0.31^{a}	81.36±0.63^{a}	1.80±0.02^{a}	2.50±0.02^{a}	0.72±0.01^{c}	5.55±0.06^{a}	99.58±1.32^{ab}
	Spring	24.35±0.27^{a}	101.12±1.12^{c}	2.20±0.01^{c}	4.18±0.08^{c}	0.52±0.01^{b}	6.41±0.04^{c}	102.88±1.10^{c}
	Summer	34.93±0.44^{b}	133.46±1.29^{d}	2.51±0.02^{d}	3.59±0.02^{b}	0.70±0.01^{c}	5.95±0.07^{b}	108.56±1.34^{d}
	Autumn	25.42±0.33^{a}	90.79±1.88^{b}	1.99±0.04^{b}	4.03±0.08^{c}	0.50±0.01^{a}	5.85±0.08^{b}	97.90±0.74^{a}
	Mean	27.35±1.02	101.68±4.54	2.12±0.06	3.57±0.15	0.61±0.02	5.94±.08	102.23±1.07
	F-ratio	218.985	299.223	142.471	171.514	158.253	31.005	16.691
	Sig.	(0.000)**	(0.000)**	(0.000)**	(0.000)**	(0.000)**	(0.000)**	(0.000)**
El-Rahawy Drain	Winter	42.11±0.84^{b}	164.37±2.64^{b}	2.63±0.03^{b}	3.37±0.03^{a}	0.78±0.01^{d}	7.24±0.09^{a}	62.02±0.96^{a}
	Spring	40.54±0.65^{b}	152.08±0.81^{a}	2.78±0.01^{c}	4.76±0.11^{b}	0.58±0.01^{b}	7.53±0.03^{bc}	124.71±2.13^{b}
	Summer	61.92±0.90^{c}	181.23±1.09^{d}	3.16±0.07^{d}	4.57±0.03^{b}	0.69±0.02^{c}	7.60±0.03^{c}	139.47±1.20^{c}
	Autumn	36.97±0.45^{a}	169.75±0.77^{c}	2.24±0.03^{a}	5.00±0.08^{c}	0.45±0.01^{a}	7.40±0.05^{ab}	152.26±1.22^{d}
	Mean	45.39±2.26	166.86±2.51	2.71±0.08	4.42±0.15	0.63±0.03	7.44±0.04	119.61±7.98
	F-ratio	235.996	62.344	77.921	104.436	92.263	8.380	764.963
	Sig.	(0.000)**	(0.000)**	(0.000)**	(0.000)**	(0.000)**	(0.000)**	(0.000)**

Means with the same superscript letters at the same column are not significantly different (P>0.05). *F*-ratio = ANOVA's *F*-test. (Sig.) = significance level.
**Highly significant (P<0.01)

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

	GOT	GPT	Albumin	Globulin	A/G	Protein	Glucose
Air Temperature	0.60**	0.39	0.48**	0.76**	-0.36	0.75**	0.85**
Water Temperature	0.54*	0.37	0.42	0.80**	-0.42	0.76**	0.87**
DO	-0.51*	-0.90**	-0.08	-0.13	0.05	-0.08	-0.35
BOD	-0.55*	-0.75**	-0.25	-0.55*	0.29	-0.52*	-0.73**
Transparency	-0.47*	-0.14	-0.50*	-0.66**	0.29	-0.76**	-0.69**
pH	0.51*	0.15	0.41	-0.13	0.30	0.34	-0.16
Ammonia	-0.75**	-0.93**	-0.37	0.03	-0.22	-0.17	-0.22
NO_2	0.77**	0.43	0.76**	-0.57**	0.84**	0.06	-0.43
NO_3	-0.16	0.16	-0.36	0.93**	-0.92**	0.37	0.93**
Conductivity	-0.70**	-0.68**	-0.42	0.24	-0.40	-0.10	0.10
Copper	0.73**	0.89**	0.95**	0.64**	-0.13	0.53*	0.62*
Iron	0.59**	0.80**	0.88**	0.79**	-0.34	0.65**	0.54*
Lead	0.73**	0.88**	0.89**	0.47	0.004	0.52*	0.57*
Cadmium	0.93**	0.94**	0.90**	0.16	0.39	0.25	0.75**
Manganese	0.26	0.51*	0.62**	0.95**	-0.70**	0.67**	0.24
Zinc	-0.09	-0.31	-0.35	0.02	-0.25	-0.50*	-0.48*

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

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

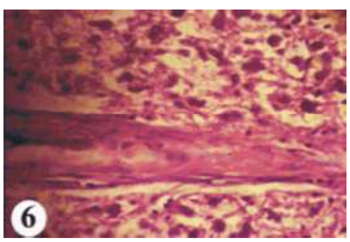


Fig. 6: Section in the liver of *C. gariepinus* showing pycnotic nuclei and hepatic necrosis (H&E, X 400)

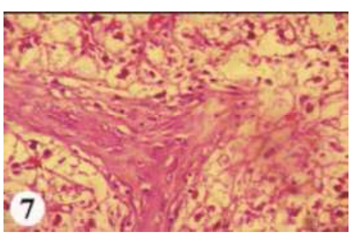


Fig. 7: Section in the liver of *C. gariepinus* showing hyaline degeneration and focal areas of necrosis (H&E, X100)

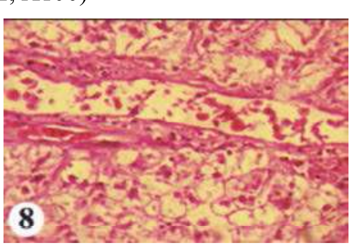


Fig. 8: Section in the liver of *C. gariepinus* showing dilated vein, congested and leukocyte infiltration (H&E, X 400)

of the hepatocytes (Fig. 6). Leukocyte infiltration and hyaline degeneration were also detected in the hepatic tissues of fish (Figs. 7 & 8). Dilation of the central vein accompanied by blood congestion was detected (Fig. 8).

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 [27]. In some cases the pollution has been extensive enough to lead to environmental disasters and ecosystem shutdown [28].

The elevated levels in physicochemical 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 physicochemical 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 [29]. 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 has potential implications on the oxygen retention capacity of the water [30] as increases in temperature affects the levels of dissolved oxygen in the water column where DO is inversely proportional to temperature [28]. In addition, Veado *et al.* [31] 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 [32]. Prolonged exposure to low dissolved oxygen level (<5 mg/l) will increase organisms susceptibility to other environmental stress [33] 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 [28].

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 [21, 34]. 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 [35].

On the other hand, the increase of turbidity (low transparency) may be due to the disposal of domestic and industrial effluent in this drain [33].

The pH value is considered to be an important factor in the chemical and biological system of aquatic environment [33]. The relatively highest pH of El-Rahawy drain water can be attributed to the large amounts of different pollution sources discharged into 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 [28].

Dissolved inorganic nitrogen is the summation of ammonia, nitrate and nitrite [32, 33]. 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.* [32]. 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 [33]. 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 [36]. 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 [33]. Although mean ammonia value in El-Rahawy drain water exceeded acceptable limits by the Egyptian governmental law No. 48 [37], the value in River Nile water was considerably elevated.

The high conductivity values observed in El-Rahawy drain water suggests 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 [38].

It was found that the values of water samples collected from El-Rahawy drain were higher than that 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 [37].

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 [39].

Present results showed that, most of the heavy metals concentrations in surface water of El-Rahawy drain and River Nile water were found within the permissible limits of both the Egyptian governmental law No. 48 [37] and U.S.EPA [40]. These results are in agreement with El Bouraie *et al.* [41] 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.* [42] 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 prove 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.

Chemical analyses alone may not suffice to describe the adverse effects of the complex mixtures of chemicals present at contaminated sites. Therefore, the use of a set of biomarkers for assessment of environmental quality has been recommended by many researchers [1, 43, 44]. Generally, the presence of pollutants in aquatic environment exerts its effect at cellular or molecular level which results a significant changes in biochemical responses and for monitoring of aquatic environment analysis of biochemical methods offer as important biomarkers [45].

Among the biochemical profiles, monitoring of liver enzymes leakage into the blood has proved to be a very useful tool in liver toxicological studies [46]. The transaminases, GOT and GPT are two key enzymes considered as a sensitive measure to evaluate hepatocellular damage and some hepatic diseases [47]. So, in the present study, the increase in GOT and GPT transaminases might be attributed to tissue damage, particularly liver [48]. In different fish species including *C. gariepinus*, GOT and GPT enzymes activity were found to increase in response to heavy metals [49]. An increase in plasma GOT and GPT activities due to metals (Zn, Cu and Cd) was also found in experimental conditions [50], as well as in fish chronically exposed to metals [51]. The highly significantly positive correlations between heavy metals values and GOT and GPT values (Table 4) confirm this. In addition, the present findings coincide with the reported histopathoiogical lesions, which revealed a marked degeneration and necrosis of hepatocytes as the elevation in transaminases activities may be attributed to liver injury [52].

Glucose is one of the most important sources of energy for the animals and glucose has been studied as an indicator of stress caused by physical factors [53] in particular pollutants [54]. In the present study the increase in plasma glucose level of *C. gariepinus* collected from El-Rahawy drain may due to gluconeogenesis to provide energy for the increased metabolic demands imposed by the poor water quality and heavy metals. Previous investigations proved that, heavy metals such as cadmium modulate the metabolism of carbohydrates, causing hyperglycemia by stimulating the glycogenolysis in some marine and freshwater fish species [51]. Present results confirm this where there are significant positive correlations between glucose levels in blood of *C. gariepinus* collected from El-Rahawy drain and some heavy metals (Cu, Fe, Pb and Cd) concentrations (Table 4).

Serum albumin and globulin have been used as indicators of healthy status of fish and considered as important indicators for the effect of pollutants in fish [32]. Protein is also one of the important biochemical parameters which have been used to understand the general state of health and biological mechanism of metabolism under pollutant stress [11]. During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. So, due to this, proteins in liver degrade and the serum protein level increase. Reddy *et al.* [55] and Singh and Sharma [56] reported decline in protein constituent in liver and increase in serum in different fish under stress of pollutants. Results of the present study revealed increased values of protein, albumin, globulin and A/G ratio in blood of *C. gariepinus* collected from El-Rahawy drain compared to the reference site fish. In the present study the increase of these parameters goes in parallel with the elevation in the levels of water parameters studied and heavy metals concentrations as a result of pollution stress [32]. The statistical analysis (Table 4) showed that, these parameters had significant positive correlations with heavy metals concentrations. This is an evidence of the response of fish to the environmental pollution.

Histological biomarkers of toxicity in fish organs are a useful indicator of environmental pollution [57]. Several histopathological changes have been reported in the gills, liver, kidneys and gonads of fish in response to agricultural, sewage and industrial pollutants [58].

In the present study, a wide spectrum of histopathologies was revealed in the gills and liver of *C. gariepinus* fish collected from El-Rahawy drain. In the present study the pathological changes in gills of fish *C. gariepinus* may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants through the gill surface [58]. Specific classes of toxicants have been linked to a number of histological alterations in gills. Several investigators have reported histopathological changes in the gills of different fish species exposed to heavy metals [59, 60]. These included several alterations similar to those of *C. gariepinus* in the present study. Histopathological changes in the gills were observed in *Fundulus heteroclitus* exposed to cadmium [61], in *Tilapia nilotica* exposed to lead acetate, mercuric chloride and cadmium chloride [62], in *Salmo trutta* exposed to iron sulphate [63], in Asian freshwater catfish, *Saccobranchus fossilis* exposed to mercury [64], in *Clarias gariepinus* exposed to 0.08 mg/l lead for nine days [65] and in turbot (*Scophthalmus maximus*) exposed to Cu, Cd and Zn [66]. Also many investigators have reported histopathological changes in the gills of different fish species exposed to changing water quality as in steelhead trout, *Salmo gairdneri,* exposed to nitrite [67], in carp, *Cyprinus carpio,* exposed to 20 and 50% sewage [68], in *Ictalurus punctatus* exposed to a combination of ammonia and low levels of monochloramine [69].

Since gills are the respiratory and osmoregulatory organ of the fish, the histopathological changes of the gills might impair the respiratory function of the gills by reducing respiratory surface area, resulted in hypoxia, respiratory failure problems [70, 71] and this badly affects the physiology and may lead to the death of fish [58].

The liver is particularly susceptible to damage from a variety of toxicants. One of the most important functions of liver is to clean pollutants from the blood, so it is considered as indicator of aquatic environmental pollution [72].

Liver changes in the *C. gariepinus* fish samples were more severe and in some cases irreparable, reflecting the poor water quality of El-Rahawy drain water system. These changes may be attributed to the direct toxic effects of pollutants on hepatocytes, since the liver is the principal organ responsible for detoxification in vertebrates generally and in fish particularly [73]. The present study suggests a strong link between heavy metals and lesions in the liver. Sorensen [74] cited that heavy metals in Elbe were might cause liver damage. Aly *et al.* [52] obtained similar results after exposure of *Clarias gariepinus* to lead pollution. Similar alterations in the liver of *Tilapia zillii* and *Clarias gariepinus* were observed in fishes living in Nile water polluted with heavy metals [47, 75]. Yacoub and Abdel Sater [76] observed histopathological effects of heavy metals on some fishes inhabiting Bardawil lagoon. Ptashynski *et al*. [77] reported histopathological alteration after exposure of the lake whitefish (*Coregonus clupeaformis*) to nickel. Olojo *et al.* [65] observed liver histopathological lesions after exposure of *Clarias gariepinus* to Pb for 9 days. Several histopathological changes in the liver were also observed in *Oreochromis niloticus* and *Tilapia zillii* collected from the southern region of Lake Manzalah contaminated with domestic, industrial and agricultural pollutants [78].

Finally, the histopathological alterations observed in the gills and liver of the studied fish may be attributed to the effects of the agricultural, industrial and sewage wastes discharge into El-Rahawy drain.

CONCLUSION

This study confirms previous reports on the altered water quality of El-Rahawy drain as a result of discharging the industrial, agricultural and sewage effluents, which affect directly water quality and fish health and human health indirectly and some of the measured parameters, exceeded the permissible limits. Furthermore the use of enzymatic and biochemical parameters as biomarker together with histological parameters are very realistic approaches. It is recommended to treat different wastes before discharging to the natural water sources to avoid the negative effects of pollutants.

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