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ORIGINAL ARTICLES

Histopathological and physiological observations of the kidney and spleen of the Nile catfish *Clarias gariepinus* inhabiting El-Rahawy drain, Egypt

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ABSTRACT

Physico-chemical parameters and heavy metals (Cu, Fe, Pb, Cd, Mn and Zn) concentrations of the water of two sites (El-Rahawy drain and River Nile at El-Kanater El-Khyria as a reference site for comparison) were recorded seasonally from winter 2010 to autumn 2011. Kidney function parameters (creatinine, urea and uric acid) of *Clarias gariepinus* caught from the water of the same two sites were also measured. Moreover, histological changes of the kidney and spleen of the same fish caught from El-Rahawy drain were studied. The results showed depletion in dissolved oxygen and decline in transparency. On the other hand, increase in the levels of ammonia, nitrite, nitrate, creatinine, urea and uric acid in the samples of El-Rahawy drain were found to be higher than that of El-Kanater El-Khyria region. Also, it was found that heavy metals concentrations in water from El-Rahawy drain region were higher than those obtained from River Nile. It was noticed that *Clarias gariepinus* collected from El-Rahawy drain suffer many histopathological changes in the kidney and spleen including degeneration, necrosis, hemorrhage, activation of melanomacrophage centers, hyperplasia and hemosiderosis. All these findings were discussed and referred to the input of agricultural, industrial and sewage water into El-Rahawy drain. Finally, a recommendation is given for treatment of the sewage water and other industrial and agricultural effluents before their entrance into El-Rahawy drain to protect the fish and human from deterioration effects of pollution.

Key words: *Clarias gariepinus*, El-Rahawy drain, River Nile, Physico-chemical parameters, heavy metals, kidney, spleen, histopathology.

Introduction

Water pollution is one of the principal environmental and public health problems, Egypt and the Middle East region are facing (Anwar, 2003). Water pollution does not only greatly damage the aquatic ecosystems but even the terrestrial organisms and ecosystems are severely damaged and threatened (Authman *et al.*, 2013a). The natural resources of water are polluted with a variety of solid and liquid wastes. Every waste is ultimately dumped or emptied in natural water bodies (Garg *et al.*, 2009). Contamination of water can result from both industrial and agricultural sources. Deficiencies in the treatment of wastewater, the disposal of untreated sewage, and inadequate operation and maintenance of treatment plants result in health risks (Anwar, 2003).

In recent years, the problems of sewage pollution in water bodies have become a point of local concern. The disposal of the untreated sewage is harmful concerning its possible hygienic and aesthetical effects and its impact on fauna and flora in the aquatic environment (Bahnasawy *et al.*, 2009). Sewage contains high levels of biochemical oxygen demand (BODs) and nitrogenous compounds. Ammonia and nitrite, in particular, are serious toxicants to fish (Mahamoud and El-Naggar, 2007). High BODs cause the decrease in dissolved oxygen (DO), while low DO lowers the lethal concentration for various toxicants (Kakuta and Murachi, 1997). In addition, the sewage effluents with their microbial and non-organic heavy metal contents represent the most dangerous chemical source of pollution for fish (Mansour and Messeha, 2001).

Heavy metals are toxic at high doses, but some heavy metals such as iron and copper are essential for the growth and well-being of living organisms including man (Ibrahim and Mahmoud, 2005). Other elements such as lead and cadmium are not essential for metabolic activities or growth and exhibit toxic properties and inhibited the photosynthesis, phytoplankton and fish growth at low concentrations (FAO, 1992).

Heavy metals in sewage have been discharged into aquatic environments and have accumulated in sediments, where they have affected the ecology of the environment, with long-term impacts on fishes. 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

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the food chain through a number of pathways may be accumulated in different organs of human beings or animals (Saad *et al*., 2012).

When aquatic ecosystems are constantly suffering the discharges of effluents produced by human activities, fish may be subjected to polluted waters which can lead to different changes ranging from biochemical alterations in single cells up to changes in whole populations (Silva and Martinez, 2007). Fish can be used as a monitoring tool for the quality of the aquatic environment and fish histopathology, with a broad range of causes, is increasingly being used as indicator of environmental stress since it provides a definitive biological end-point of historical exposure (Stentiford *et al*., 2003). Histological changes appear as medium-term responses to sub-lethal stressors, considering their intermediate location with regard to the level of biological organization, and histology represents a rapid method to detect effects of stressors, especially chronic ones, in various tissues and organs (Bernet *et al*., 1999).

In teleosts, the kidney, together with the gills and intestine, are responsible for excretion and the maintenance of the homeostasis of the body fluids and, besides producing urine, act as an excretory route for the metabolites of a variety of xenobiotics to which the fish may be exposed (Hinton *et al*., 1992). Since a large volume of blood flows through the kidney, so, the kidney of fish is a suitable organ for histological examination (Kadry *et al*., 2003) where lesions found in this organ can be useful as signs of environmental pollution. The effects of pollutants on fish kidneys have been studied in some species and the severity of damage seen depends on the sensitivity of the species to the substances released into the environment (Silva and Martinez, 2007). Many authors reported histopathological abnormalities in kidney of different fish species that were exposed to several pollutants (agricultural, industrial and sewage) (lliopoulou-Georgudaki and Kotsanis, 2001).

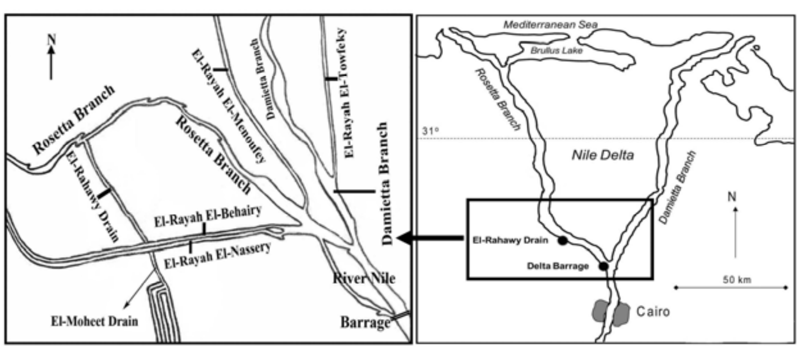
Spleen is an important member of the body's immune and lymphatic system as well as it is a haematopoeitic organ in which blood cells synthesized and stored (Tayel *et al*., 2008; Roberts, 2012), also it acts as a filter and purifier of the blood (Garcia-Abiado *et al*., 2004). The histological investigations of the spleen in teleosts could be used as an indicator of health in fish. Garcia-Abiado *et al.* (2004) mentioned that the presence of different toxicants in the environment caused alterations in fish spleen. Hoeger *et al.* (2004) reported that the changes in water quality with sewage lead to changes in spleen histology (Tayel *et al*., 2007).

Clarias gariepinus is a bottom feeder and is economically important species of the family Siluridae that is well represented in the River Nile and Egyptian inland waters where it is the most abundant fish next to tilapias and is considered as an important food source of protein in Africa and some areas of the world (Mahamoud and El-Naggar, 2007).

So, the objective of this study was to monitor the seasonal variations in water quality and the levels of heavy metals pollution in El-Rahawy drain, and to determine the effects of water pollution in this drain on kidney functions and the histological changes in *Clarias gariepinus* kidney and spleen.

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 carryout 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.



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

El-Rahawy drain is one of the main drains, which is far from El-Kanater (The Barrage) by about 15 km. It starts at 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 30º 10’ N to 30º 12’ N and longitudes 31º 2’ E to 31º 3’ E. It is about 12.41 km^2. It 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 discharged these wastes directly without treatment into Rosetta branch of the River Nile (El Bourie *et al.*, 2011). 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 wastewater 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.

I- 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 (APHA, 1998).

a) Field observations:

In situ, air and surface water temperatures (ºC) 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.

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 (APHA, 1998). 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.

b) 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. 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. All previous analyses were carried out according to the standard methods for examination of water and wastewater (APHA, 1998).

II-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.

a) Kidney function parameters:

Blood samples were taken from the caudal vein of the fish using a heparinized syringe and collected into small sterilized plastic tubes. The 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 colorimetrically to determine serum creatinine and urea (mg/dl) by the methods described by Henry *et al.* (1974) and uric acid (mg/dl) by using enzymatic determination method according to Barham and Trinder (1972).

b) Histopathological examination:

After dissecting the fish, spleen and kidney were removed and small pieces were fixed in 10% formalin fluid for 24 hr. The fixed samples were dehydrated in ascending series of ethanol, cleared in methyl benzoate 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 (2012) by a complex Olympus light microscopy and photographed by a built in camera.

Statistical analysis:

The basic statistics (means, standard deviations and standard errors) of the measured parameters were estimated. Comparison of the kidney functions 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:

A) 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.

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

Site	Season	Air Temperature (ºC)	Water Temperature (ºC)	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 El-Khyria	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
	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 (1982)	NA	NA	>5	<6-10	NA	6.5-9	<0.5	NA	40	NA

NA = not available.

B) 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.

Table 2: Heavy metals concentrations (mean ± standard deviation) in water and the African catfish *Clarias gariepinus* muscle and the accumulation factor (AF) 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 (1982)	1000	1000	50	10	500	1000
Permissible limits (µg/l)	U.S.EPA (2009)	9.0	1000	2.5	0.25	NA	120.0

NA = not available.

C) Kidney Functions:

The mean values of kidney functions of *C. gariepinus* collected from River Nile and El-Rahawy drain waters are shown in table (3). On comparing the present results of kidney functions 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 some investigated water parameters and heavy metals with kidney functions of *C. gariepinus* collected from El-Rahawy drain (Table 4), demonstrated some significant positive and negative correlations.

Table 3: Kidney functions (mean ± standard error) of the African catfish *Clarias gariepinus* at various sampling sites.

Site	Season	Creatinine (mg/dl)	Urea (mg/dl)	Uric Acid (mg/dl)
River Nile at El-Kanater El-Khyria	Winter	0.53±0.04[ab]	34.39±0.58[b]	3.60±0.04[c]
	Spring	0.51±0.01[a]	54.19±1.10[d]	1.60±0.02[a]
	Summer	0.60±0.02[b]	44.87±0.30[c]	1.89±0.02[b]
	Autumn	0.60±0.02[b]	25.28±0.29[a]	1.67±0.02[a]
	Mean	0.56±0.01	39.68±2.51	2.19±0.19
	F-ratio	3.280	367.229	1381.072
	Sig.	(0.059)	(0.000)**	(0.000)**
El-Rahawy Drain	Winter	0.77±0.05[a]	79.85±0.40[d]	5.79±0.12[d]
	Spring	0.84±0.03[a]	66.35±0.64[b]	1.78±0.03[a]
	Summer	0.80±0.02[a]	75.32±0.29[c]	4.04±0.06[c]
	Autumn	0.87±0.08[a]	37.20±0.15[a]	3.00±0.09[b]
	Mean	0.82±0.02	64.68±3.81	3.65±0.34
	F-ratio	0.684	2166.662	445.260
	Sig.	(0.579)	(0.000)**	(0.000)**

Means with the same letter 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 kidney functions of the African catfish *Clarias gariepinus* and some physico-chemical parameters and heavy metals concentrations at El-Rahawy drain.

	Creatinine	Urea	Uric Acid
Ammonia	0.09	-0.13	-0.47*
NO_2	-0.28	0.87**	0.62**
NO_3	0.36	-0.88**	-0.71**
Copper	0.60**	-0.16	0.87**
Iron	0.03	0.09	0.45*
Lead	0.75**	0.71**	0.37
Cadmium	0.19	0.50*	0.64**
Manganese	0.81*	0.76**	-0.42
Zinc	-0.06	0.38	-0.38

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

D) Histopathological findings:

Spleen:

The main elements of the spleen parenchyma, of normal *C. gariepinus* fish, are white and red pulp, a distinction between them is possible, although demarcation between red and white pulp is poorly defined. The white pulp is composed of lymphoid tissue, surrounding small arteries and diffusely intermeshing with the red pulp. The red pulp is composed of a reticular cell network and supporting blood-filled sinusoids (S) that hold diverse cell populations, including macrophages and lymphocytes. Scattered through the parenchyma are numerous accumulations of the pigmented macrophages, i.e. melanomacrophage cells (MMC) were found (Fig. 1). Histopathological examination of spleen of *C. gariepinus* fish, collected from El-Rahawy drain, revealed edema lead to severe vacuolation (Fig. 2), hemorrhages, early granuloma around artery (Fig. 2). Also, activation of MMCs can be seen (Fig.3). Multifocal lymphocytic cell necrosis (Fig. 4) and hemosidrine (Fig. 5) were common.

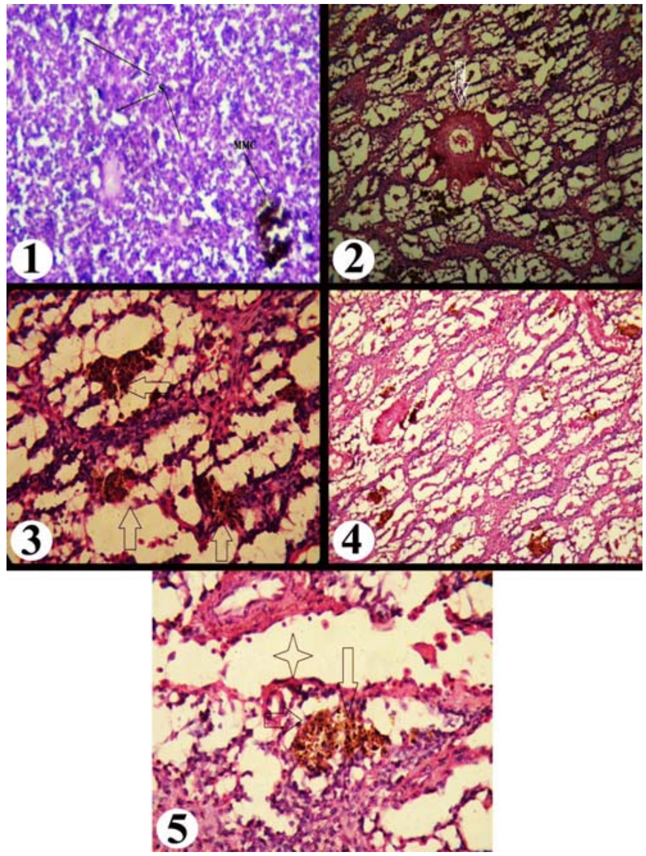


Fig. 1: Section in spleen of normal *C. gariepinus* fish (X100).
Fig. 2: Section in spleen of *C. gariepinus* showing severe vacuolar degeneration and granuloma (arrow) (X100).
Fig. 3: Section in spleen of *C. gariepinus* showing hemorrhages, dark pigment derived from hemoglobin, nucleated erythrocytes, large fat vacuole and hemosidrine (arrows) are visible (X400).
Fig. 4: Section in spleen of *C. gariepinus* showing necrotic tissues (X100).
Fig. 5: Section in spleen of *C. gariepinus* showing edema (star) and hemosidrosis (arrows) (X400).

Kidney:

The functional units of the kidney, of normal *C. gariepinus* fish, are nephrons which are composed of renal corpuscles (rc) and renal tubules (rt); these structures are surrounded by haemopoietic tissue (sh). The shape of the renal corpuscle is roughly spherical consisting of a double membrane capsule (Bowman's capsule) enclosing a tuft of blood capillaries (glomerulus) (g). Bowman's space; a space between the glomerulus and the capsule is present (Fig. 7). Examination of kidney sections of *C. gariepinus* fish, collected from El-Rahawy drain, revealed edema in the epithelium lining of renal tubules and aggregate of MMC cells (Fig. 6), degeneration and vacuolation of Bowman's capsule. Moreover, necrosis and pyknotic nuclei were observed in renal tubules (Figs. 7, 8 and 9).

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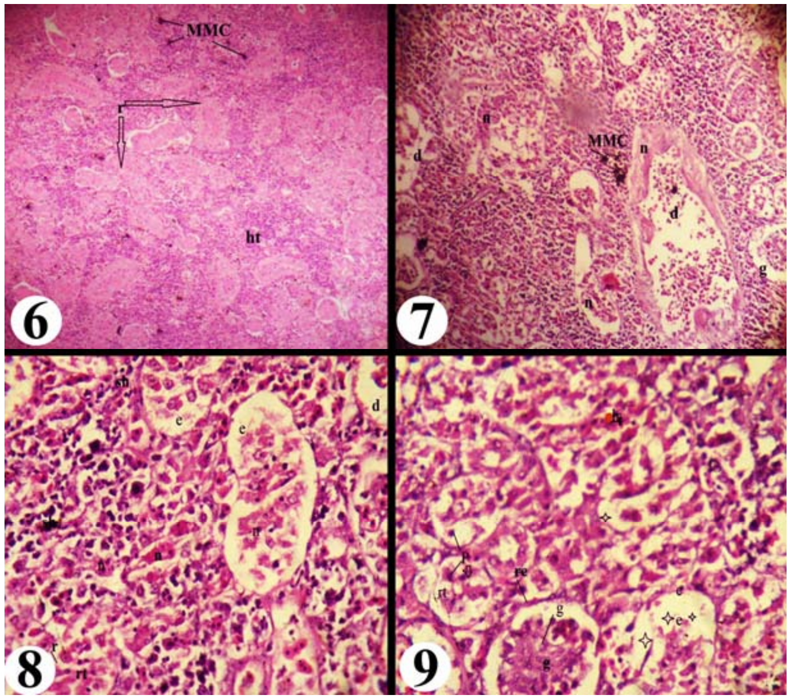

Fig. 6: Section in the kidney of normal *C. gareipinus* fish showing normal renal tubules (r and arrows) with epithelial lining, lumen, haemopoitic tissue (ht) and melanomacrophage cells (MMC) (X400).

Fig. 7: Section in the kidney of *C. gareipinus* showing edema in the epithelium lining of renal tubules, degeneration and vacuolation of glomeruli (X100).

Fig. 8: Section in kidney of *C. gareipinus* showing necrotic cells (n) and inflammatory cells infiltration (X100).

Fig. 9: Section in kidney of *C. gareipinus* showing edema (e, star), damage epithelial cells of renal tubule (rt), necrosis (n) and dilated glomerulus (g) (X400).

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. In some cases the pollution has been extensive enough to lead to environmental disasters and ecosystem shutdown (Adeogun, 2012).

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 (Abdo, 2005). 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 (UNEP, 2006) as increases in temperature affects the levels of dissolved oxygen in the water column where DO is inversely proportional to temperature (Adeogun, 2012). In addition, Veado *et al.* (2000) 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 (Tayel *et al.*, 2007). Prolonged exposure to low dissolved oxygen level (<5-6 mg/L) will increase organisms susceptibility to other environmental stress (Osman *et al.*, 2010) 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 (Adeogun, 2012).

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 (APHA, 1998; Elewa *et al.*, 2007). 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 (Abdel-Hamid *et al.*, 1992).

On the other hand, the increase of turbidity (low transparency) may be due to the disposal of domestic and industrial effluent in this drain (Osman *et al.*, 2010).

The pH value is considered to be an important factor in the chemical and biological system of aquatic environment (Osman *et al.*, 2010). The relatively highest pH of El-Rahawy drain water can 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 (Adeogun, 2012).

Dissolved inorganic nitrogen is the summation of the ammonia, nitrate and nitrite (Tayel *et al.*, 2007; Osman *et al.*, 2010). 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.* (2007). 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 (Osman *et al.*, 2010). 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 (Elghobashy *et al.*, 2001). 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 (Osman *et al.*, 2010). Although mean ammonia value in El-Rahawy drain water exceeded acceptable limits by the Egyptian governmental law No. 48 (1982), 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 (UNESCO-WHO-UNEP, 1996) of conductivity being a direct measure of anthropogenic impact.

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 (1982).

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 (Rajeshkumar and Munuswamy, 2011).

Present results showed that, most of the heavy metal 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 (1982) and U.S.EPA (2009). These results are in agreement with El Bouraie *et al.* (2010) 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.* (2012) 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.

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 (Authman *et al.*, 2013b). So, analyses of serum constituents have been proved to be useful in the detection and diagnosis of metabolic disturbance and disease processes (Elghobashy *et al.*, 2001). Heavy metals are some of the most-active polluting substances as they can cause serious impairment to circulatory, metabolic, physiological, and even structural systems when high concentrations are present in aquatic ecosystems (Yang and Chen, 2003).

The kidney has been proposed as one of the major target organs for environmental contaminants such as heavy metals, and they are important organs for metabolic waste excretion and heavy metal elimination in fish (Yang and Chen, 2003). Kidney functions (serum creatinine, uric acid and urea) can be used as a rough index of the glomerular filtration rate where low values of creatinine, uric acid and urea have no significance but increasing values indicate the presence of disturbances in the kidney (Elghobashy *et al.*, 2001). In the present investigation, *C. gariepinus* fish collected from El-Rahawy drain showed an elevation in serum creatinine, uric acid and urea. This may be attributed to the action of heavy metals and other pollutants on the glomerular filtration rate which causes pathological changes of the kidney (Oikari and Soivio, 1977). These recorded results are in agreement with that of Elghobashy *et al.* (2001) who observed increase in kidney functions in *Oreochromis niloticus* fish collected from the River Nile and some Egyptian Lakes due to heavy metals pollution. Yang and Chen (2003) found significantly higher concentrations of urea and creatinine in serum of intoxicated carp after 28 days of gallium (intermetallic elements) exposure. Zaki *et al.* (2009) observed a pronounced elevation of urea and creatinine levels in grey mullet after three weeks of exposure to 0.25 ppm of cadmium chloride and attributed this to kidney injury. The highly significant positive correlations between some heavy metals values and kidney functions values (Table 4) confirm this.

Hadi *et al.* (2009) reported that, creatinine and uric acid are biomarkers for muscle and purine metabolism, liver damage and kidney acid. The rise in the creatinine of *C. gariepinus* fish collected from El-Rahawy drain may be attributed to heavy metals and other pollutants effects on muscle metabolism whereas the increase in uric acid may be due the effect of these pollutants on purine metabolism and renal tubules may also be damaged (Sayed *et al.*, 2011). Hadi *et al.* (2009) reported that the increase of creatinine level might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrates metabolism.

Histopathological changes in kidney of *C. gariepinus* fish collected from El-Rahawy drain may be due to pathogens and metals pollutions as recorded by Tayel *et al.* (2007, 2008) and Authman *et al.* (2012). Lesions such as degeneration and activation of melanomacrophage centers observed in the kidney suggested that pollution with heavy metals is highly to affect them (Oshode *et al.*, 2008). Melanomacrophage centers are quite known as an unusual sequel to infection or irritation in fish belonging to fish immune response (Roberts, 2012). Moreover, hyaline degenerations in kidney tubules were detected in some fish species following exposure to sublethal levels of lead (Sipple *et al.,* 1983). Also, histopathological changes were seen in the kidneys of *Heteropnettstes fossilis* exposed to mercury (Bano and Hasan, 1990), *Cyprinus carpio* exposed to cadmium (Singhal and Jain, 1997), *Sarotherodon galilaeus* exposed to copper and zinc (Al-Zahaby *et al.*, 1998) and rainbow trout exposed to $CdCl_2$ (lliopoulou-Georgudaki and Kotsanis, 2001). Palavi and Srivastava (2006) have found that sublethal zinc concentrations lead to inconvertible histological changes in the kidneys of experimental fish specimens. Abdel-Tawwab *et al.* (2007) reported that kidney of Nile tilapia, *Oreochromis niloticus* when exposed to copper concentrations for 6 weeks, showed focal coagulative necrosis and hyaline droplets degeneration in renal tubular epithelium. Zaki *et al.* (2009) observed necrobiotic changes of epithelial lining of renal tubules especially the proximal convoluted tubules in grey mullet after three weeks of exposure to 0.25 ppm of cadmium chloride. Similar results of histopathological alterations in kidney of *Oreochromis niloticus* fish due to heavy metals pollution were reported by Authman *et al.* (2012).

Moreover, the histopathological alterations in the kidney of fish induced by exposure to different toxicants have been reported by several authors. Histopathoiogical changes in the kidneys were observed in European eels, *Anguilla anguilla,* infected with the parasite *Myxidium giardi* Cépède (Ventura and Paperna, 1984), in *Tilapia nilotica* exposed to fluorine and sulphur emitting from factory of fertilizer (Aly *et al,* 1992), in *Oreochromis niloticus* infected by *Streptococcus* sp. (Chang and Plumb, 1996), in carp (*Cyprinus carpio*) exposed to 20 and 50% sewage (Kakuta and Murachi, 1997), in trout exposed to wastewater containing high levels of un-ionized ammonia (Schmidt *et al.*, 1999), in *Oreochromis niloticus*, *Tilapia zillii* and *Synodontis schall* collected from El-Salam canal, Egypt, due to agricultural, sewage and industrial pollutants (Mohamed, 2003) and in rainbow trout (*Oncorhynchus mykiss*) exposed to chloride (Mahjoor and Loh, 2008).

Spleen of fish is an important member of the body's immune and lymphatic system (Tayel *et al.*, 2008). Its functions in haematopoiesis, storage of blood, erythrocyte disintegration and hemoglobin release, as a filter and purifier of the blood, and as part of immunological defence mechanisms (Garcia-Abiado *et al.*, 2004). The spleen being a haemopoietic and important immune organ is found to be affected by chemical pollutants absorbed into the blood stream. The histological investigations of the spleen in teleosts have been mainly focused on the compartments that are important for the defense systems of the fishes; the lymphocytes and the macrophages (Fournie *et al.*, 2001), and/or alterations in this organ caused by the presence of different toxicants in the environment (Garcia-Abiado *et al.*, 2004). In the present study the histopathologic examination of spleen of *C. gariepinus* fish, collected from El-Rahawy drain, may be attributed to the effects of different kinds of pollutants discharged in El-Rahawy drain that led to deteriorate its water quality and increase heavy metals concentrations. These findings were in agreement with Agius and Roberts (2003) who mentioned that melanomacrophages were increased in size or frequency in condition of environmental stress and have been suggested as reliable biomarkers for water quality. In addition, this agrees with findings by Fournie *et al.* (2001) who associated the density of splenic macrophage aggregates in estuarine fishes to exposure to degraded environments and contaminants. These findings further agree with Kerambrun *et al.* (2012) who reported that the density of melanomacrophage centers values in the spleen of juvenile turbot, *Scophthalmus maximus*, were higher after 21 days of exposure to sediments contaminated with metals and PAHs. Comparative toxicity of trivalent and hexavalent chromium compounds was assayed in the African mouth breeder *Oreochromis mossambicus* (Peters) by Arunkumar *et al.* (2000) and they found out that chromium caused reduction in spleen weight, splenocytes number and percentage of blood lymphocytes. Zaki *et al.* (2009) concluded that after three weeks of exposure, cadmium chloride at 0.25 ppm induced deleterious effects in grey mullet such as damage of spleen. Oliveira Ribeiro *et al.* (2005) and Tomova *et al.* (2008) observed toxic changes caused ruptures or even the formation of tumors in the spleen of *Anguilla anguilla* and *Carassius gibelio*, respectively, exposed to zinc. Mahjoor and Loh (2008) mentioned that chlorine toxicity in rainbow trout (*Oncorhynchus mykiss*) induced prominent erythrophagocytosis and depletion of haematopoietic tissue. Tayel *et al.* (2007, 2008) observed similar histopathological lesions, of the present study, in spleen of *Oreochromis niloticus* and *Clarias* *gariepinus* fishes captured from a polluted drainage canal in Al-Minufiya Province and El-Rahawy drain at El-Rahawy village, respectively.

Macrophages have been reported to digest phagocytosed erythrocytes leading to the deposition of hemosiderin with melanomacrophages (Naigaga, 2002). Under normal physiological conditions, the amount of hemosiderin is usually small but when large quantities are deposited they lead to a condition known as hemosiderosis (Takashima, 1982). The results of the present study of spleen hemosiderosis are in accordance with the findings of Khan *et al.* (1994) who reported a hemosiderosis in the spleen of *Pleuronectes americanus* in waters with a chronic content of heavy metals. The hemosidrin formation may be attributed to the increase in iron content of water as reported by Mohamed *et al.* (1993). The present results of higher iron concentrations in El-Rahawy drain water confirm this.

Conclusion And Recommendations:

The present findings indicate that El-Rahawy drain water; due to the continuous discharge of different pollutants into it; contained toxic constituents that caused changes in kidney functions and kidney and spleen histopathological profiles in *C. gariepinus*. The drain at the study site is therefore of prime health concern because there is no known containment or treatment system for it, and the contaminants possibly transferred from polluted drain waters by fish to the consumers. So, to protect the drain from pollution and reduce environmental risk, it could be recommended that treatment of the agricultural, industrial and sewage effluents should be carried out before their discharge to the drain. Regular evaluation of pollutants in the drain is also very important.

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