Histopathological studies on the hematopoietic organs of *Clarias gariepinus* in relation to water quality criteria at different localities in the River Nile.

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Abstract: The physico-chemical parameters (water temperature, electrical conductivity, transparency, pH value, oxygen studies and nutrient salts) were carried out at El Kanater El-Khayria and discharge point of El-Rahawy drain at Rosetta branch of the River Nile seasonally from spring 2011 to winter 2012. In addition, liver, spleen and kidney samples of *Clarias gariepinus* fish were examined histopathologicaly. The results showed decrease in transparency and nitrite as well as depletion of dissolved oxygen at discharge point of El Rahawy drain. On the other hand, the increase in BOD, COD, ammonia and nitrate at discharge point of El-Rahawy drain was observed. The liver was characterized by vacuolar degeneration, necrosis, hemorrhage, hemolysis, hemosidrin and parasite cysts. The kidney alterations were necrosis, degeneration, dilation of capillaries in the glomerulus and reduction of Bowman´s space. Hemorrhage, hemosidrinosis, degeneration, hemolysis, parasitic forms and necrosis were the most changes in spleen tissue. The collected samples of fish from discharge point of El-Rahawy area were suffered from more pathological changes than those collected from El-Kanater El-Khayria. It was concluded that the discharge of different types of wastes deteriorates the water quality in the River Nile and consequently effect on fauna and fish production. It is recommended to treat the different wastes before discharging to the River Nile stream.

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Key words: River Nile, Rosetta branch, *Clarias gariepinus*, liver, kidney, spleen, necrosis, El-Rahawy.

1. Introduction

Egypt can be considered as one of the immense countries in Africa and represents the heart of the Arabic world (**Mahmoud**, **2002**). The River Nile is the donor of life to Egypt and represents the principle freshwater resource that meets nearly all demands for drinking water and irrigation (**Korium and Toufeek, 2008 and Ali *et al.*, 2008).** Consequently, Rosetta branch passes cutting six governorates; EL-Kalubia, EL-Menofiya, EL-Giza, EL-Gharbia, Kafr El-Shiekh and EL-Boheira `over a length of about 236 km on the western boundary of the Nile Delta from Egypt's Delta Barrage with an average width of 180 m and depth from 2 to 4 m (**El Gammal and El Shazely, 2008).**

There are three main sources of pollution which potentially affect and deteriorate the water quality of Rosetta branch: 1) El-Rahawy drain that receives all sewage of El-Giza governorate in addition to agricultural and domestic wastes of El-Rahway village and discharge these wastes directly without treatment into the branch **(Tayel *et al.*, 2008).**

2) Kafr El-Zayat industrial area, which include the industrial effluents from the factories of super phosphate and sulfur compounds, oil and soap industries and pesticides factories **(Daboor, 2006).** 3) Several small agricultural drains that discharge their waters into the branch in addition to sewage discharged from several cities and its neighboring villages that are distributed along the two banks of the Rosetta branch **(Sayed, 2003)**.

In addition, the physico-chemical parameters are considered as the most important principles in the identification of the nature, quality and type of the water (fresh, brackish or saline) for any aquatic ecosystem **(Abdo, 2005).** The increase of industry, agriculture urbanization and tourism, human activities are responsible for chemical pollution sources for the environment and aquatic ecosystems.

In general, fishes can be considered as one of the most significant biomonitors in aquatic system for the estimation of metal pollution concentration **(Begum, 200**4). As sequence, *Clarias gariepinus* is the most common freshwater fish used in the toxicological studies. They are considered as important food source of protein in Africa and some areas of the world (**Figueiredo-Fernandes *et al.*, 2006a; Ahmed, 2007 and Mahmoud and El-Naggar, 2007**). While, the histopathological study is considered as direct evidence referring to any adverse effect on fish (**Bayomy and Mahmoud, 2007**). It allows examining specific target organs, including spleen, kidney and liver, that are responsible for vital functions, such as producing antibody against blood born antigens, excretion, the accumulation and biotransformation of xenobiotics in the fish

(Thophon *et al.,* 2003; Tayel *et al.*, 2008 and El-Naggar *et al.,* 2009).

The aim of the present study is to evaluate the spatial and temporal distribution of physical variables (water temperature, EC and Transparency), chemical variables (pH, Oxygen studies and Nutrient salts) in addition to histopathological changes in liver, kidney and spleen of *Clarias gariepinus* fish collected from El-Kanater El-Khayria and at discharge point of El-Rahawy drain at Rosetta branch, River Nile, Egypt.

2. Material and methods

Sampling stations:

Water samples were collected seasonally from two stations along Rosetta branch during the period from spring 2011 to winter 2012 covering the different environmental conditions of the River Nile. These stations were El-Kanater El-Khayria (**site I**) and at discharge point of El-Rahawy drain (**site II**). The experimental procedure of the studied parameters was carried out according to (**APHA, 1995)** as follows:

Water analysis:

The water temperature was measured by a dry mercury thermometer. The electrical conductivity (mScm-1) was estimated by using conductivity meter model (S.C.T.33 YSI) and transparency (cm) was recorded in the field using Secchi-disc (diameter 25cm). pH was measured on the spot by using pH-meter model (Janway 3150).

Another water sample was kept in one liter polyethylene bottle in ice box to be analyzed in the laboratory. The dissolved oxygen content was performed by azide modification, biological oxygen demand by incubation 5 days methods and chemical oxygen demand by using potassium permanganate. Concentrations of nutrient salts were determined using the colorimetric techniques according to the method described by **(APHA, 1995).**

Fish analysis:

Histopathological studies:

Liver, spleen and kidney samples obtained from *C. gariepinus* fish were carefully removed then fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned at 5 microns by using Euromex Holland microtome. Sections were stained according to Harris Hematoxylin and Eosin method **(Bernet *et al.,* 1999)**, examined microscopically and photographed by using a microscopic camera.

3. Results and Discussions:

Water temperature:

Temperature is an important factor in the aquatic environment since it effects directly or indirectly not only on the survival and distribution of the aquatic organisms at any stage of life, but also on their growth rate, development, activity, activation of reproduction processes and susceptibility to diseases **(Moustafa *et al.*, 2010 and Abdo *et al.*, 2010).**

In the present study, water temperature showed noticeable seasonal trends with a lowest value (18.5^{o}C) recorded during winter at site **(I)** and a highest value (38.5^{o}C) during summer at sites (**I and II**). The changes of water temperature may depend on the variations in meteorological conditions, air temperature, latent heat of evaporation and different sampling times and seasons (**Abdel-Satar, 2005; Mahmoud *et al.*, 2008; Saad *et al.*, 2011 and Ahmed, 2012**).

On the other hand, bacteria and other microorganisms which affect on the breakdown of organic matter at El-Rahawy drain are very much influenced by temperature changes. Consequently, they are more active during summer than winter. This observation was agreed with that reported by **(Mahmoud and El-Naggar, 2007 and Tayel *et al.*, 2008**).

Table (1): Seasonal variation of physico-chemical parameters in the studied areas during the seasons from spring 2011 to winter 2012:

Seasons	Spring		Summer		Autumn		Winter		±SE
Stations	Mean		Mean		Mean		Mean		
Parameters	I	II	I	II	I	II	I	II	
Temperature (^{o}C)	23.1	21.5	38.5	38.5	26.0	26.5	18.5	18.9	2.82
Transparency(cm)	110.0	20.0	120.0	15.0	80.0	15.0	65.0	10.0	16.04
EC (µmohs)	280.0	790.0	290.0	802.0	390.0	980.0	456.2	1340.5	133.49
pH	8.0	7.3	7.65	7.6	8.3	7.5	8.2	7.3	0.13
DO mg/l	7.9	0.3	6.4	0.0	6.9	0.0	12.0	0.0	1.66
BOD mg/l	4.5	25.5	3.5	35.5	5.6	18.5	4.7	16.5	4.178
COD mg/l	15.5	50.0	15.2	48.4	9.5	30.2	8.9	25.0	5.80
Ammonia mg/ l	0.210	8.120	0.153	8.343	0.374	5.105	0.158	4.207	19.32
Nitrite µg/ l	4.5	68.0	10.5	75.0	14.4	39.2	9.6	68.8	10.72
Nitrate µg/ l	26.9	210.5	25.2	205.0	54.0	68.4	46.8	62.6	26.81

I: El-Kanater El-Khayria II: At discharge point of El-Rahawy drain

Transparency:

Transparency means the penetration of the light into water layers. It is controlled by depth and turbidity of the water and affected by particulate contents of water from suspended matter and floating substances (**Talling, 1976 and Mahmoud *et al.,* 2008**).

In the present study, the transparency values fluctuated between 110 cm at site **(I)** during both summer and spring and 10 cm at site **(II)** during winter, respectively. The obtained result showed a remarkable decrease in transparency values at site **(II)** which was attributed to the discharge of heavily polluted effluent loaded with agriculture, industrial, and domestic wastes **(Saad *et al.,* 2011)**. Also, the decrease in transparency during winter was attributed to the effect of the prevailing wind which helps in mixing water and stirring up the bottom sediments **(Saad, 1987, Siliem, 1995; Ali, 1998; Mahmoud, 2002 and Ahmed, 2012)**. On the other hand, the high values of transparency may be attributed to the increase in the uptake of suspended mater by phytoplankton and increased solar radiation penetrating the surface water as well as settling out of suspended particles to the bottom sediments especially during summer **(Abdel-Satar and Elewa, 2001; Abdo, 2002; Abdel-Satar, 2005 and Saad *et al.*, 2011).**

In general, the seasonal average values showed that, transparency values increased during hot season (spring and summer) more than cold seasons (winter and autumn). However, increase of water level in addition to the decrease of the concentration of total suspended matter lead to the transparency increase **(Elewa and Ali, 1999 and Abdo, 2010).**

Electrical conductivity (EC):

Electrical conductivity is a measure of the ability of aqueous solution to carry an electric current. This ability depends on the presence of ions, their total concentration, mobility, valance and temperature of the medium. Thus, the more abundant of the ions, the higher in the conductivity and vice versa **(APHA, 1995)**.

The obtained data showed that the lowest value of EC (280.0 μ mohs/cm) at site **(I)** during spring and summer may be attributed to the increase of water level during flood period and the uptake of dissolved salts by phytoplankton **(Nour El-Din, 1985; Ghallab, 2000 and Saad *et al.,* 2011).** On the other hand, the high values of EC (1340.5μ mohs/cm) at site **(II)** during winter may be attributed to the intrusion of the drain's effluents into the lowered level water in the branch causing elevation of dissolved and suspended particles which increase the ability to convey electrical current **(El-Bordiny, 2001; El-Sayed, 2011 and Ahmed, 2012)**.

Generally, the high values of EC may be attributed to domestic and agricultural wastes that contain high amount of organic and inorganic constituents.

Hydrogen ion concentration (pH):

Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically, every phase of water supply and waste water temperature, e.g. acid base neutralization, water softening, precipitation, coagulation, disinfection and corrosion control, is a pH dependent **(Abdel-Satar, 2005)**. The principle system regulating pH of water is carbonate system which include CO_2, H_2CO_3, HCO_3^- and CO_3^{-2} **(Stumm and Morgan, 1970).** The pH value of water is controlled by dissolved oxygen, algal photosynthetic activity, temperature, sewage discharge, decomposition of organic matter and complex factors related to geology of the under-laying sediment **(Rashad, 1994; Abdel Satar, 1994 and Tayel, 2003).**

The obtained values of pH were in the alkaline side (7.3-8.3). Small local differences were observed with no clear seasonal variations. The increase in pH values at site **(I)** may be attributed to the dense of vegetation and phytoplankton, which were accompanied by photosynthetic activity and consumption of CO_2 with expected pH elevation **(Sabae, 2004 and Abdel- Satar, 2005).** On the other hand, the relative decrease of pH values at site **(II)** may be attributed to lower activities of phytoplankton as well as to bacterial and fungal action in the sediment; these activities liberate methane and hydrogen sulphide that lead to formation of organic acids **(Lenz 1977and Ahmed, 2012).**

Dissolved Oxygen (DO):

The principle source of dissolved oxygen in water is directly from the atmosphere through the exposed surface and from the photosynthesis of chlorophyll-bearing plants **(Abdo, 2010).** Absorption of oxygen from air is accomplished in two ways: by direct diffusion at the surface and through the various forms of surface water agitation, such as wave action, waterfalls and turbulence due to obstructions (**El-Sayed, 2011**).

Also, dissolved oxygen is considered as an important parameter in assessment of the degree of pollution in natural water **(Mahmoud *et al.,* 2008)**. It is controlled to susceptibility of fish to toxicity by chemicals which increase at low oxygen concentration (**Erez *et al.*, 1990**). There are many factors effect on the amount of oxygen in natural water such as temperature, salinity, amount of mixing between air and water, pH, photosynthetic activity of phytoplankton, submerged plant and aeration by living organisms as well as decomposition of organic matter **(Saad, 1978 and Das and Acharya, 2003).**

In the present study, dissolved oxygen values varied from 6.4 mg/l to 12.0 mg/l at site **(I)** and 0.00 mg/l to 0.3 mg/l at site **(II),** respectively. The decrease of DO during summer might be due to the elevation of water temperature and increase in oxidative processes of organic matter (**Abdel-Satar and Elewa, 2001 and Abdel- Satar, 2005**). At discharge point of El-Rahawy drain, the depletion of DO as expected for domestic drain during different seasons may be due to the presence of high load of the organic pollutants that consumes the dissolved oxygen during oxidation processes especially during hot seasons as observed by **(Mahmoud *et al.,* 2008, Abdo *et al.,* 2010 and Ahmed, 2012)**. On the other hand, the increase of DO at site **(I)** may be due to the high solubility of oxygen at low water temperature, the activities of wind action and air movement which allow more transfer of oxygen across the air-water interface as well as increase of photosynthetic activity by phytoplankton **(Ahmed, 2007; Mahmoud *et al.,* 2008 and Saad *et al.,* 2011).**

Biological Oxygen Demand (BOD):

The BOD is the amount of DO which is used to decompose the organic matter in water by microorganisms. It depends on several factors such as: temperature, concentration of organic matter and density of phytoplankton. The BOD test is the mostly useful method in estimating the amount of biodegradable organic matter present in the aquatic environment (**Siliem, 1984, Mahmoud, 2002 and Tayel, 2003**). Also, BOD rapidly deplete DO content of polluted water with sewage, so it is important to estimate the amount of these pollutants in the given water body **(El-Sayed, 2011)**.

BOD values fluctuated between 3.5 mg/l and 5.6 mg/l at site **(I)** during summer and autumn respectively. While, at site **(II)**, the results ranged from 16.5 mg/l to 35.5 mg/l during winter and summer respectively. As a sequence, the obtained results showed a remarkable increase in biological oxygen demand values at the discharge point of untreated wastes (sewage, agriculture, domestic) discharged from El-Rahawy drain to Rosetta branch that reach to the maximum value (35.5mg/l) during summer; this may be attributed to decomposition of high amount of organic matter by microorganisms which increased by elevation of water temperature. These results are in agreement with that obtained by **(Tayel, 2003; Al-Afify, 2010 and El-Sayed, 2011).** While, the minimum value (3.5 mg/l) was recorded at El-Kanater El-Khayria which may be attributed to low photosynthetic activity and no abundance of phytoplankton at this area **(Ahmed, 2007).**

Chemical Oxygen Demand (COD):

The chemical oxygen demand is the total amount of oxygen required to oxidize all the organic matter completely in a site to CO_2 and H_2O. Similarly, the COD test has been used to measure the oxygen equivalent content of a given waste by using a chemical to oxidize the organic content of that waste (**Sincero and Sincero, 2003**).

Organic substances in the investigated area are mainly produced by the decomposition of domestic wastes, planktonic organisms, particulate organic matter, and industrial wastes discharged through several drains into the stream.

In the present study, chemical oxygen demand values varied from 8.9 mg/l to 15.2 mg/l at site **(I)** during winter and summer respectively. While, at site **(II),** the values ranged from 25.0 mg/l to 50.0 mg/l during winter and spring respectively. The high values of COD may be due to the effect of pollution by sewage and agriculture wastes discharged from El-Rahawy drain as well as high load of organic matter and the low capacity of its water for self purification **(Abdel- Satar, 2005; Abdo, 2010; Saad *et al.,* 2011**). Also, the higher value of COD during hot seasons (summer) could be attributed to the increase in water temperature which accelerates the oxidation of organic matter and decrease in oxygen content (**Abdo, 2004 and 2010).** As classified by **Beger (1974) and Saad *et al.* (2011),** the water to be of good quality when it contains not more than 12 mg/l of organic matter expressed as oxygen consumed by permanganate.

On the other hand, reduction of COD in the present study may be due to the algal biomass which is capable of consuming organic material as recorded by **Ghallab (2000).**

Ammonia (NH_3):

Ammonia and nitrogen concentrations more than 1 mg/l have been given as indicator of organic pollution and can be toxic to aquatic species if they are higher than 2.5 mg/l **(Reid, 1961and Siliem, 1984).** In the present study, the values of ammonia ranged from 0.153mg/l to 0.374 mg/l at site **(I)** during summer and autumn and 4.207 mg/l to 8.343 mg/l at site **(II)** during winter and summer respectively. The increase of ammonia values at site **(II)** during all seasons may be due to the large amount of organic matter outfalls and their decomposition of the organic matter exhausting dissolved oxygen and produce high level of ammonia (**Sayed, 1998; Abdel-Satar, 2005 and Saad *et al*., 2011)**. Also, the increase of ammonia concentration may be attributed to the activity of denitrifying bacteria which are much higher under anaerobic conditions as mentioned by **Gallab (2000).** On the other hand, the decrease in the ammonia concentrations was related to the decrease in biological activities of aquatic organisms and nitrification in the water column as investigated at site **(I) (Saad *et al*., 2011).**

Nitrite (NO_2^-):

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate, such oxidation and reduction occur in waste water treatment plants, water distribution systems, and natural water (**APHA, 1995**). Resistance to toxic effect of nitrite ion is enhanced by the presence of chloride or increased water hardness.

The values of nitrite ranged between 4.5 and 14.4µg/l at site **(I)** during spring and autumn respectively while, the values fluctuated from 39.2 to 75.0µg/l at site **(II)** during autumn and summer. The low values of nitrite might be attributed to the fast conversion of NO_2^- by nitrobacteria to NO_3^- **(Abdo, 2004 and Tayel, 2007).** On the other hand, the high nitrite level may be attributed to the decomposition of organic matter present in the waste water where, *nitrosomonas* bacteria oxidize ammonia to nitrite by denitrification process **(Tayel, 2003 and Saad *et al.*, 2011).**

Nitrate (NO_3^-):

Nitrate ion is the final oxidation product of nitrogen compounds in the aquatic environment, at the same time nitrate is considered the only thermodynamically stable form of nitrogen in the absence of oxygen **(Ahmed, 2007).**

The reduction of nitrate or nitrification can be brought about by certain nitrate reducing bacteria especially in the presence of organic matter and only limited amounts of oxygen **(Tayel, 2003).** This happens, for instances in heavily polluted, streams and in sewage percolating filters that have become pended or clogged **(Bayomy and Mahmoud, 2007).** During denitrification, nitrate were reduced to nitrite and finally to ammonia, under certain circumstances nitrous oxidation (NO^-_2) and nitrogen are also produced **(Abdel-Satar, 2005).**

The values of nitrate fluctuated within a wide range between 25.2 and 54.0 mg/l at site **(I)** during summer and autumn respectively. While, the values ranged from 62.6 and 210.5mg/l at site **(II)** during winter and spring respectively. The low values of nitrate might be attributed to the uptake of nitrate by natural phytoplankton and its reduction by denitrifying bacteria and biological denitrification **(Sabae and Abdel- Satar, 2001; Abdo, 2002; Bayomy and Mahmoud, 2007 and Saad *et al*., 2011).** On the other hand, the increase of nitrate levels might be attributed to sewage wastes at El-Rahawy drain and low consumption of phytoplankton as well as the oxidation of ammonia by nitrosomonas bacteria and biological nitrification **(Macdonald *et al.,* 1995; Tayel, 2003; Abdo, 2010 and Saad *et al*., 2011).**

Histopathological studies:

Histopathology is used as a sub-lethal test for evaluating toxic effect of water pollutants on fish **(EIFAC, 1983 and Murty, 1986).**

Liver:

The liver is the principle organ of detoxification in vertebrates and particularly in fish. The normal structure of liver shows that it is made up of Hepatocytes which arranged in branched lamina, two cells thicks. They are polygonal cells with a central spherical nucleus that separated from each other by blood sinusoids. Blood flows from branches of hepatic portal vein and hepatic artery through the sinusoids to central veins which empty into the hepatic vein **(Fayed, 2004).**

The liver of *C. gariepinus* fish collected from El-Kanater El-Khayria (Figs Ia, Ib, Ic & Ig) and down stream of El-Rahawy (Figs. Id, Ie, If and Ih) suffered from many pathological alterations**.** These alterations were necrosis, vacuolar and fatty degeneration in hepatocytes as well as the blood vessel showed dilation, congestion, parasitic forms and fibrosis in its wall. Severe hemorrhage was accompanied by deposition of hemosidrin pigments and congestion in blood sinusoids. Hemolysis and hemosidrin pigments may result from rapid and continuous destruction of erythrocytes by breakdown of hemoglobin and convert it into hemosidrin. These findings were in agreement with those reported by (**Yacoub and Abdel-Satar, 2003; Tayel, 2003; Ibrahim and Mahmoud, 2005; El- Naggar *et al.,* 2009 and Saad *et al.,* 2011**). The fatty degeneration changes in studied liver may be due to a decrease in the rate of utilization of energy reserve or pathological enhance synthesis while the abnormal accumulation of fats in experimental animal could be due to induced imbalance between fat production and utilization **(El- Naggar *et al.,* 2009).** The same investigation was regarded by **(Tayel, 2003)** on the same fish. As a sequence, the fish collected from site **(II)** showed more histopathological alteration than those collected from site **(I);** this may be attributed to heavy metals accumulation **(Sitohy *et al*., 2006; Ibrahim, 2007 and Yacoub *et al.,* 2008)**, parasitic infection **(Mahmoud and El-Naggar, 2007 and El-Naggar *et al.,* 2009)** and changes in water quality **(Ahmed, 2007; Saad *et al*., 2011 and Ahmed, 2012).**

Kidney:

The teleostean kidney is the important organ for the maintenance of a stable environment with respect to water and salt, excretion and partially for the metabolism of xenobiotics (**Thophon *et al.,* 2003**). Normal kidney is composed of identical nephrons. Each nephron contains renal corpuscles that lead to renal tubules.

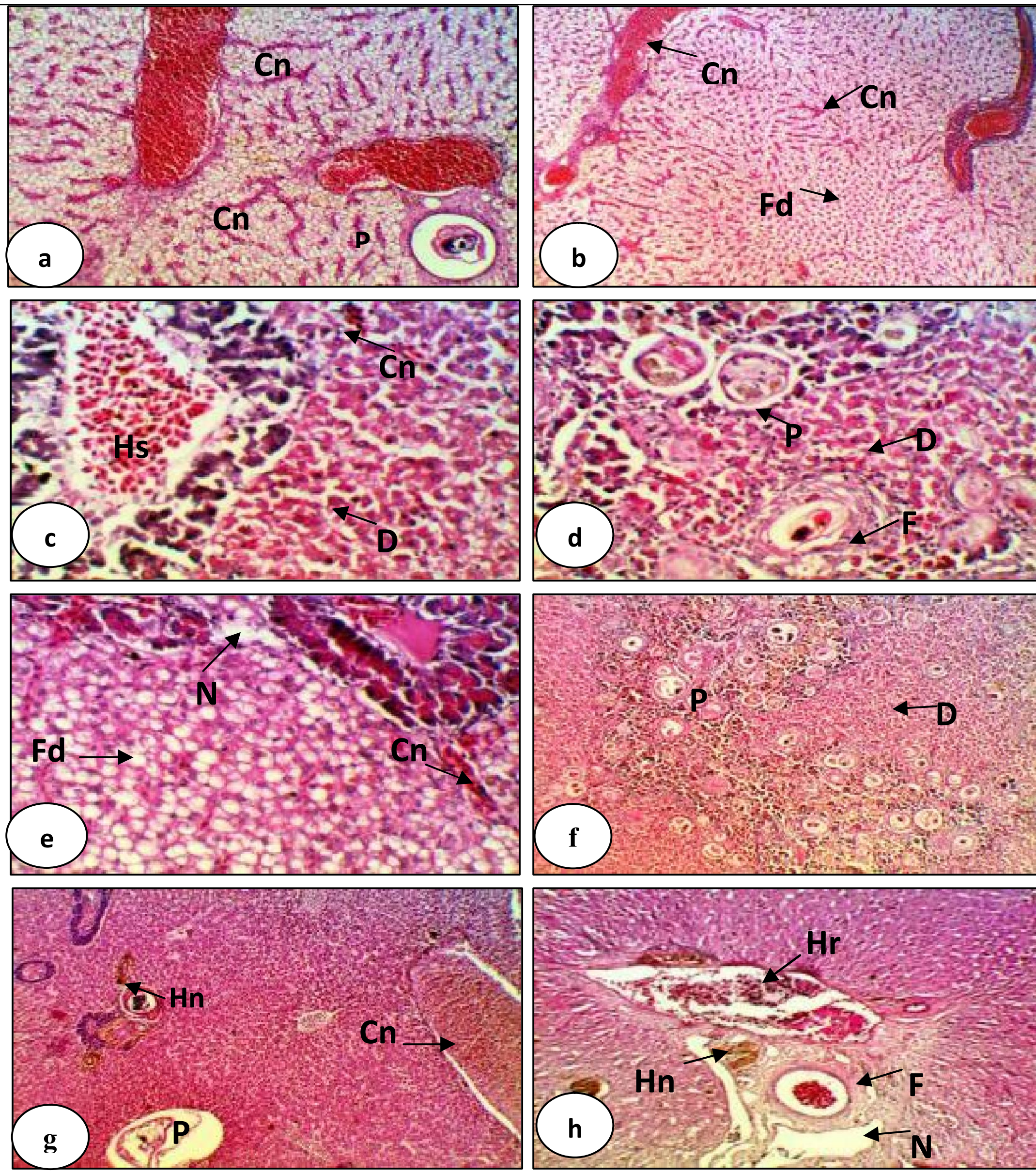

Figure (I): liver section of *Clarias gariepinus* obtained from El-Kanater El-Khayria and downstream of El-Rahawy regions (Bouin's –H&E) showing:
(**a**): Congestion (Cn) in blood vessel & blood sinusoid and parasite form (P) in hepatocytes.
(**b**): Fatty degeneration (Fd) in hepatocytes and congestion (Cn) in blood vessel & blood sinusoid.
(**c**): Degeneration (D) in hepatocytes, hemolysis (Hs) and congestion (Cn) in blood sinusoid.
(**d**): Degeneration (D) in hepatocytes, parasite form (P) and fibrosis (F) around blood vessel.
(**e**): Necrosis (N) & fatty degeneration (Fd) in hepatocytes and congestion (Cn) in blood sinusoid.
(**f**): Aggregation of parasite forms (P) in blood vessel and degeneration (D) in hepatocytes.
(g): Hemosidrin (Hn) & parasite form (P) in hepatocytes and congestion (Cn) in blood sinusoid.
(**h**): Hemorrhage (Hr) in blood vessel, fibrosis (F), necrosis (N), and hemosidrin (Hn) in hepatocytes

The renal corpuscle contains vascular capillary glomerulus that is enclosed by Bowman's corpuscle. Within the nephron, the kidney contains hematopoietic tissue and blood vessels.

The most common alterations found in the kidney of *C. gariepinus* fishes posed to water contamination in El Kanater El-Khayria (Figs. IIi - III) and down stream of El-Rahawy (Figs. IIm - IIp) were vacuolation and necrosis of the proximal tubule epithelium, tubule degeneration as well as dilation of capillaries in the glomerulus and reduction of Bowman´s space in addition to parasite form as shown in **(Fig. II).** The kidney of the fish collected from site **(II)** showed more histopathological changes than that obtained from site **(I).** These findings were in agreement with those reported by (**Mabrouk, 2004; Ayas *et al.*, 2007 and Ahmed, 2012)** for the same areas. These changes may be attributed to impaired blood supply due to toxic action of different pollutants in the branch, including heavy metals, especially cadmium which is known as renal toxicity **(Ahmed, 2012).**

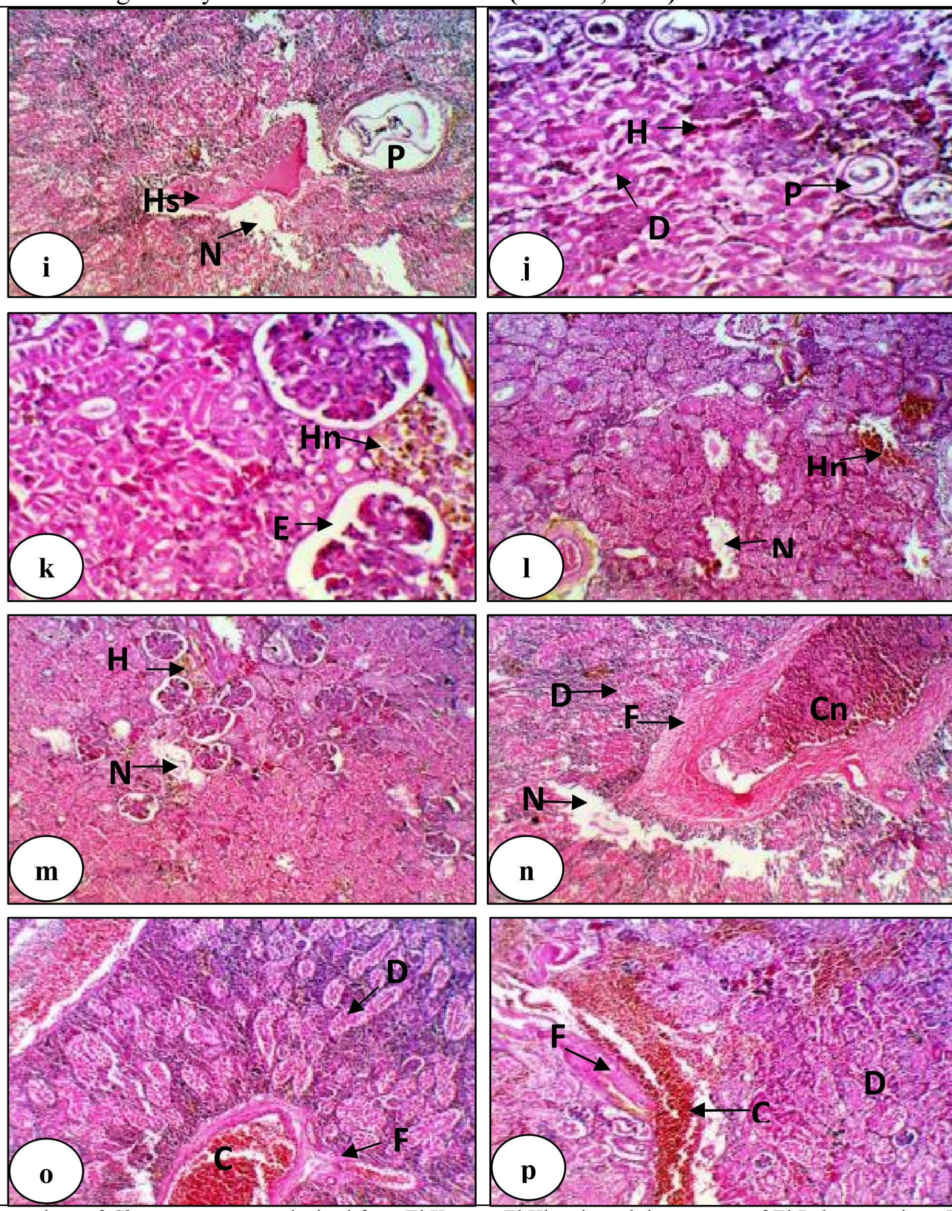

Figure (II): kidney sections of *Clarias gariepinus* obtained from El-Kanater El-Khayria and downstream of El-Rahawy regions (Bouin's –H&E) showing:
(i): Necrosis (N in renal tubule, hemolysis (Hs), parasite form (P) in hematopoietic tissue.
(**j**): Degeneration (D) in renal tubule, hemorrhage (Hr) in hematopoietic tissue and parasite form (P) in blood vessel.
(**k**): Edema (E) in renal corpuscle and hemosidrin (Hn) in hematopoietic tissue.
(**l**): Necrosis (N) in renal tubule, and hemosidrin (Hn) in hematopoietic tissue.
(**m**): Necrosis (N) in renal tubule and hemosidrin (Hn) in hematopoietic tissue.
(**n**): Degeneration (D) & necrosis (N) in renal tubule and fibrosis (F) & congestion (Cn) in hematopoietic tissue.
(**o**): Degeneration (D) in renal tubule and fibrosis (F) &congestion (Cn) in blood vessel.
(**p**): Degeneration (D) in renal tubule and fibrosis (F) & congestion (Cn) in blood vessel.

Spleen:

Spleen is a small red organ and important member of the body's immune and lymphatic system. Its functions are filtration of blood, producing & storage of red blood cells, removing old & abnormal erythrocytes and producing antibody against blood borne antigen **(Tayel *et al.*, 2008).**

The normal spleen of fish is enclosed with a capsule and consists of lymphatic laden area, white pulp surrounded by pink areas, the red pulp which contains large numbers of blood cells and sinusoids. Endothelial cells of the sinusoids are not in contact but are separated by slit like spaces. Red blood cells are broken in the red pulp **(Groman, 1982).**

The spleen of *C. gariepinus* fish inhabiting the water of El Kanater El-Khayria (Figs. IIIq - IIIt) and downstream of El-Rahawy (Figs. IIIu - IIIx) were represented by fibrosis around blood vessels, increase in white pulp and decrease in red pulp, degeneration between white and red pulp, hemorrhage and hemolysis in spleen tissue. These changes may be due to viral or parasitic infection and trace metals which were accumulated in the spleen from polluted water. Such explanations were recorded by **(Mazher *et al.*, 1986; Tayel, 2003; Tayel *et al.*, 2008 and Saad *et al.*, 2011).** The hemosidrin formation (accumulation) may be attributed to the increase in iron content as reported by **(Haggag *et al.*, 1993; Tayel *et al.*, 2007; Saad *et al.*, 2011 and Ahmed, 2012).**

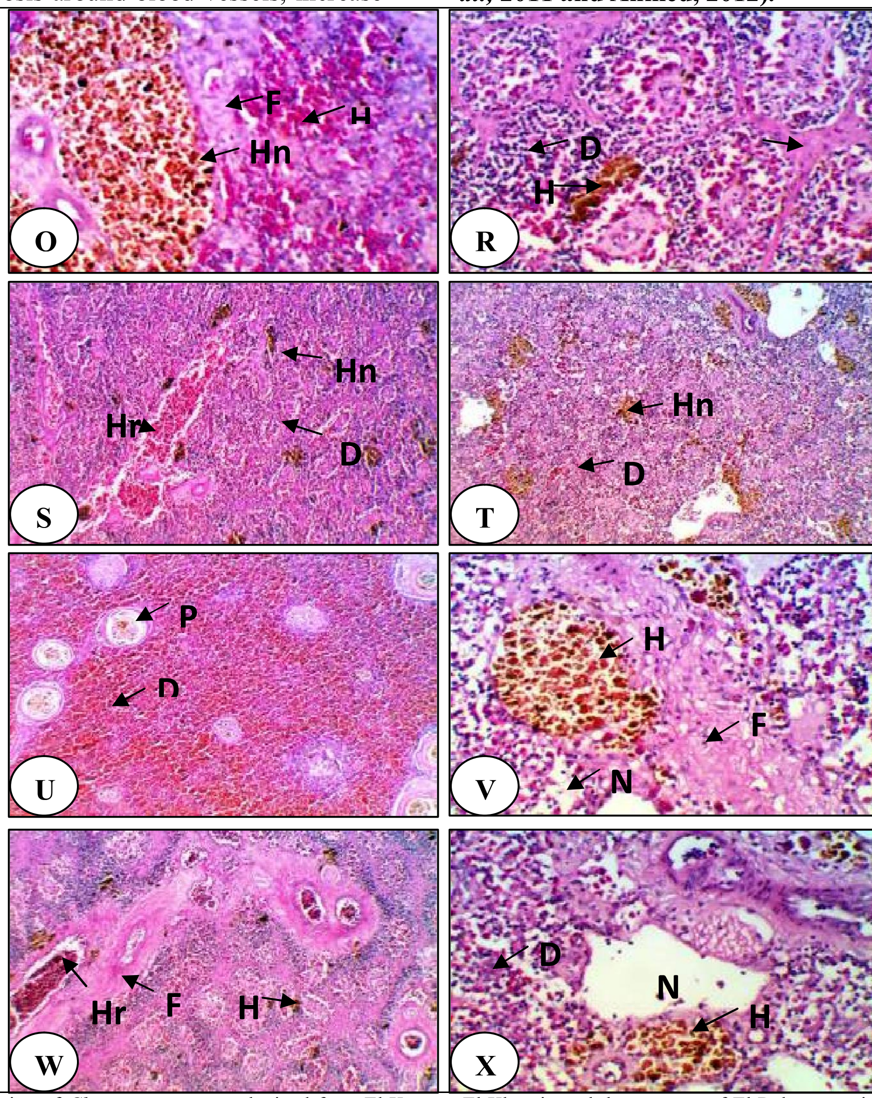

Figure (III): spleen section of *Clarias gariepinus* obtained from El-Kanater El-Khayria and downstream of El-Rahawy regions (Bouin's –H&E) showing:
(**q**): Fibrosis (F), hemosidrin (Hn) and hemorrhage (Hr) in splenic tissue.
(**r**): Degeneration (D), fibrosis (F) and hemosidrin (Hn) in splenic tissue.
(**s**): Degeneration (D) in white pulp, hemosidrinosis (Hn) in red pulp and hemorrhage (Hr) in blood vessel.
(t): Degeneration (D) in white pulp and hemosidrinosis (Hn) in red pulp.
(**u**): Degeneration (D) between red and white pulp and aggregation of parasite form (P).
(**v**): Necrosis (N), hemosidrinosis (Hn) and fibrosis (F) in splenic tissue.
(**w**): Hemosidrinosis (Hn) in red pulp, hemorrhage (Hr) and fibrosis (F) in splenic tissue.
(**x**): Necrotic area (N) & hemosidrinosis (Hn) in red pulp and degeneration (D) in white pulp.

Conclusion

From the previous discussion, it can be concluded that, the water quality parameters were slightly increased especially at discharge point of El Rahawy drain in addition to depletion in dissolved oxygen. Also, the fish samples collected from this station showed severe alterations than that collected from El Kanater El-Khayria**.** It is recommended to make a master plan for sewage treatment with identified target in rural areas.

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