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Impact of the water quality of El-Rahawy Drain on some genetic and histopathological aspects of *Oreochromis niloticus*

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ABSTRACT

The present study aims to evaluate the water quality of two locations in Rosetta Branch of River Nile and its histopathological and genetic adverse effects on Nile tilapia (*Oreochromis niloticus*). Water and fish samples were collected from up and downstream of El-Rahawy Drain discharge point (location I and II, respectively) during the summer and winter seasons. The water quality, liver histopathology and DNA alteration of fish was examined. ISSR-PCR technique was used to assess the genetic variation of fish samples from these locations. The water quality parameters (BOD, COD,……)were increased at location II compared with location I in addition to depletion in dissolved oxygen. Moreover, the fish samples collected from location II showed severe histological and molecular alterations than those collected from location I. It is recommended that the government must increase the awareness of people around the River Nile, and improve the environmental management to reduce the risks of polluted water and consuming the fish exposed to this water on human health.

INTRODUCTION

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).** River Nile water quality has been steadily deteriorating over several decades due to the dumping of anthropogenic inputs and untreated effluents. The Nile runs through a narrow valley (1000 km long). Subsequently, it is bifurcate at a distance of 25 km (north of Cairo) into the Rosetta and Damietta branches forming a delta (**Abdel-Satar *et al.*, 2017**). 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, 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).**

Pollutants include heavy metals can cause an imbalance between the free radical species production and reduction in fish **(Livingstone, 2003; Sevcikova *et al*., 2011; Kamollerd *et al*., 2019).** The free radicals can attack lipid, protein and DNA molecules to induce oxidative stress products as well as cause DNA damage **(Castano and Becerril, 2004; Vilela *et al*., 2018; Kamollerd *et al*., 2019).**

For the estimation of metal pollution concentrations, fishes can be considered as one of the most significant biomonitors in the aquatic system **(Begum, 200**4). As a sequence, *O. niloticus* is the most common freshwater fish used in toxicological studies. They are considered an important food source of protein in Africa and some areas of the world (**Figueiredo-Fernandes *et al.*, 2006; Ahmed, 2007 and Mahmoud and El Naggar, 2007**).

To study the adverse effects of pollution, histopathological and DNA molecular markers have been used. The histopathological study is referring to any adverse effect on fish (**Bayomy and Mahmoud, 2007 and Ahmed et al., 2019).** It allows examining specific target organs, 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).**

On the other hand, inter-simple sequence repeats ISSR analysis by using PCR has been successfully applied in fish gene tagging, genetic diversity analysis and pollutants effects studies **(Archak *et al*., 2003)**. Also, the analysis was widely used for studying the genetic background of fish (**Liu *et al.*, 2006, Rashed *et al.*, 2008 and Saad *et al*., 2009**). ISSR has proven to be outstanding in the analysis of natural population vertebrate species (**Priyanka *et al*.**, **2013**).

So, the present study aims to evaluate the water quality of two locations in Rosetta Branch of River Nile and its histopathological and genetic adverse effects (by using ISSR markers) on Nile tilapia *Oreochromis niloticus.*

MATERIALS AND METHODS

Study area

The River Nile enters Egypt at its southern boundary with Sudan and runs through a narrow valley (1000 km long). Subsequently, it is bifurcate at a distance of 25 km (north of Cairo) into the Rosetta and Damietta branches forming a delta (**Abdel-Satar *et al*.**, **2017**). This study is focused on the two locations, upstream (location I) and downstream (location II) of El-Rahawy Drain discharge point in the River Nile at Rosetta Branch, where the drainage water shows its influence on the branch. Rosetta

River Nile Branch represents the main freshwater stream that extends northwards for about 225 km on the western boundary of the Nile Delta. Fig. 1 shows the study area of Rosetta Branch. Rosetta Branch receives daily huge quantities of polluted waters from many sources including agricultural, industrial in addition to urban sewage containing high amount of organic and inorganic wastes that are causing serious negative impacts on the branch environment **(Abdel-Satar and Elewa, 2001).**

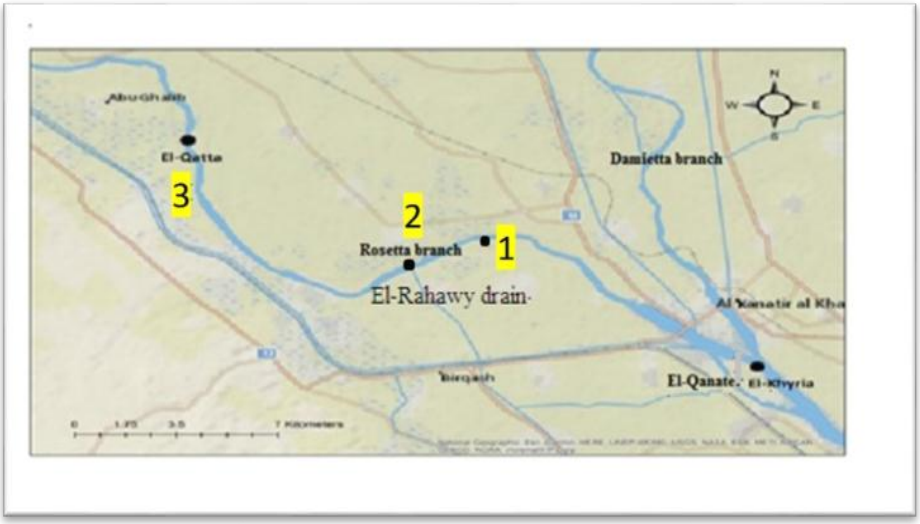

Fig. (1): A map of of **El-Rahawy Drain, showing the sampling locations, 1: Upstream location before 1 km from the drain (location I), 2: discharge point 3: Downstream location after 5 km from the drain (location II).**

Water Sampling

Water samples were collected during the winter and summer seasons from the two locations (upstream and downstream the discharge point of El-Rahawy drain).

Water analysis

The water temperature was measured by a dry mercury thermometer. The electrical conductivity ($mScm^{-1}$) was estimated 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 samples were kept in one-liter polyethylene bottle in ice box to be analyzed in the laboratory. Concentrations of studied parameters were determined according to the procedures laid down in **APHA (1995).** The dissolved oxygen (DO) content was performed by azide modification, biological oxygen demand (BOD) by incubation 5 days methods and chemical oxygen demand (COD) by using potassium permanganate. Concentrations of nitrite, nitrate and ammonia, were determined using the colorimetric techniques with formation of reddish purple azo-dye, Cd reduction and phenate methods, respectively.

Water samples for metals determination were kept in clean stoppered plastic bottles and preserved with 65% HNO_3 to pH < 2 in the field. After that the water

samples were digested using 65% HNO_3 according to APHA (1995). Analysis of heavy metals concentrations (Fe, Zn, Mn, Pb and Cu) was carried out by using atomic absorption spectrophotometer model (Perkin Elmer 3110 USA) with graphite atomizer HGA-600, according to the method described by APHA (1995) and the reading was compared with a standard curve. All determinations were performed in duplicate and were repeated when added accuracy was needed. The relationships between different studied variables in Nile water were calculated using the Pearson correlation index

Histopathological studies:

Liver samples obtained from *O. niloticus* 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.

ISSR markers

DNA extraction

Fish muscles tissues were used for DNA extraction through genomic DNA extraction kit (G-Spin) from iNtRON Biotechnology, Inc., Korea. DNA extraction of 20 fish samples from the two locations (10 samples from each, 5 males and 5 females) was carried out as described in the manufacturer's protocol.

PCR amplification conditions

Five ISSR markers loci were screened. The ISSR primers sequences are presented in Table ١. PCR conditions were 5 mins at 94^{o}C followed by 60 sec at 94^{o}C, 45 sec at 50 ^{o}C and 60 Sec at 72 ^{o}C for 35 cycles and a final step for 10 mins at 72 ^{o}C. PCR products were tested by using 2% agarose gel electrophoresis. 100 bp DNA ladder plus (iNtRON Biotechnology, Inc. Korea) was used to detect the obtained PCR products. The electrophoresis run was achieved at 75 V in DNA electrophoresis unit (Bio-Rad) for 90 mins.

Table (1): Sequences of ISSR primers

Primer	Primer Sequence (5 ' – 3 ')
1	AGA GAG AGA GAG AGA G
2	GAG AGA GAG AGA GAG AT
3	GAG AGA GAG AGA GAG AC
4	CTC TCT CTC TCT CTC TG
5	CAC ACA CAC ACA CAC AG

RESULTS AND DISCUSSION

Physico-chemical characteristics of Sub-surface water samples along the studied locations of River Nile are recorded in Table (2).

Table (2): Physico-chemical seasonal variations of River Nile water

Parameter	Season				Mean	CCME (2007)
	Summer		Winter			
	Location I	Location II	Location I	Location II		
Temperature (^oC)	26.4	28.2	16	17.4	22	-
Transparency(cm)	112.5	50.5	76	30	67.25	-
EC (µS/cm)	263	532.7	418.7	870	521.1	-
PH	7.4	7.5	7.94	7.04	7.47	6.5 – 9
DO mg/l	7.2	1.2	6.6	0.8	3.95	5.5 – 9.5
BOD mg/l	3.6	46.4	2.5	61.6	28.53	-
COD mg/l	14.7	35.35	9.7	16.55	19.08	-
Ammonia mg/ l	0.22	7.03	0.14	13.3	5.17	0.19 – 0.47*
Nitrite µg/ l	8.45	17.1	11.1	45.15	20.45	60
Nitrate µg/ l	23.1	29.35	45.65	56.1	38.55	2900

*NH_3 concentration depend on pH value

Water temperature

Temperature is an important factor in the aquatic environment since it affects 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 the lowest value (16^{o}C) recorded during winter at location **(I)** and the highest value (28.2^{o}C) during summer at location (**II**). The changes in water temperature may depend on the variations in meteorological conditions, air temperature, latent heat of evaporation and different sampling times and seasons (**Saad *et al*., 2011 and Ahmed, 2012**). On the other hand, bacteria and other microorganisms that affect 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**).

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 (**Mahmoud *et al*., 2008**). In the present study, the transparency values fluctuated between 112.5 cm recorded at location **(I)** during the summer season and 30 cm recorded at location **(II)** during the winter season. The obtained result showed a remarkable decrease in transparency values at location **(II)** which was attributed to the discharge of heavily polluted effluent loaded with domestic, industrial, and agriculture 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 **(Ahmed, 2012)**. On the other hand, the high values of transparency may be attributed to the increase in the uptake of

suspended matter by phytoplankton and increased solar radiation penetrating the surface water as well as for settling out of suspended particles to the bottom sediments especially during summer **(Abdel-Satar and Elewa 2001; Abdel-Satar, 2005 and Saad *et al.*, 2011).**

Electrical conductivity (EC)

Electrical conductivity is a measure of the ability of an 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. The more abundant of the ions in aqueous solution lead to increase the electrical conductivity and vice versa **(APHA, 1995)**. The obtained data showed that the lowest value of EC (263 µS/cm) measured at location **(I)** during the summer season may be attributed to the increase of water level during flood period and the uptake of dissolved salts by phytoplankton **(Ghallab, 2000 and Saad *et al.*, 2011).** On the other hand, the increase in EC (1160.2 µS/cm) at location **(II)** during winter may be attributed to the intrusion of the drain's effluent into the lowered level water in the branch causing elevation of dissolved and suspended particles which increase the ability to convey electrical current **(El-Sayed, 2011 and Ahmed, 2012)**. Generally, the high values of EC may be attributed to domestic and agricultural wastes that contain a high amount of organic and inorganic constituents (**Al-Afify and Abdel-Satar, 2020**).

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 wastewater temperature, e.g. acid-base neutralization, water softening, precipitation, coagulation, disinfection and corrosion control is pH-dependent **(Abdel-Satar, 2005)**. The principle system regulating the pH of water is the carbonate system which includes CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}**.** 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 the geology of the under-laying sediment **(Tayel, 2003).**

The obtained values of pH were on the alkaline side (7.04-7.94). The increase in pH values at location **(I)** in the winter season may be attributed to the dense 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 location **(II)** in the same season 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 the formation of organic acids **(Ahmed, 2012)**

Dissolved Oxygen (DO)

Dissolved oxygen is considered as an important parameter in the assessment of the degree of pollution in natural water **(Mahmoud *et al.*, 2008)**. There are many factors affect 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 **(Das and Acharya, 2003).**

In the present study, dissolved oxygen values varied from 6.6 mg/l to 7.2 mg/l at location **(I)** and 0.8 mg/l to1.2 mg/l at location **(II),** respectively (Fig. 2). Downstream the decrease of DO was recorded, where the domestic wastes discharged contains a high load of the organic pollutants that consume the dissolved oxygen during oxidation processes **(Mahmoud *et al.*, 2008, Abdo *et al.*, 2010 and Ahmed, 2012)**. On the other hand, the increase of DO at location **(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 the increase of photosynthetic activity by phytoplankton **(Mahmoud *et al.*, 2008 and Saad *et al.*, 2011).** Finally**,** DO concentrations at site II were lower than the **CCME (2007)** threshold limits established to protect water quality for aquatic life, Table 2.

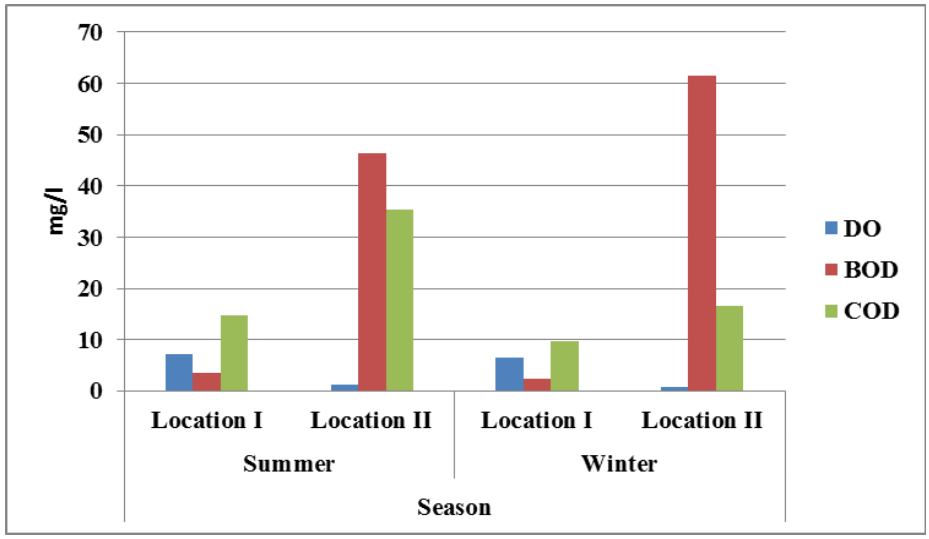

Fig. (2): The variation of DO, BOD and COD in the River Nile at Rosetta Branch water

Biological Oxygen Demand (BOD)

The BOD is the amount of DO consumed to decompose the organic matter in water by microorganisms. It depends on several factors such as temperature, the concentration of organic matter and the density of phytoplankton. The BOD test is the mostly useful method in estimating the amount of biodegradable organic matter present in the aquatic environment (**El-Sayed, 2011)**.

The obtained results showed a remarkable increase in biological oxygen demand values downstream of untreated wastes (sewage, agriculture, domestic) discharged from El-Rahawy Drain to Rosetta Branch that reach to the maximum value (61.6 mg/l) during winter season (Fig. 2); this may be attributed to decomposition of a high amount of organic matter by microorganisms. These results are in agreement with that obtained by **Al-Afify (2010) and El-Sayed (2011).** While the minimum value (2.5mg/l) was

recorded at location **(I)** 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 to H_2O and CO_2 (**Sincero and Sincero, 2003**). The high values of COD that recorded at location **(II),** (Fig. 2) may be due to the effect of pollution by sewage and agriculture wastes discharged from El-Rahawy Drain as well as the 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 increase in COD value during summer season could be attributed to the increase in water temperature which accelerates the oxidation of organic matter (**Abdo, 2010).** As classified by **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, the reduction of COD in the present study at location I may be due to the algal biomass which is capable of consuming organic material as recorded by **Ghallab (2000).**

Ammonia (NH_3-N)

Ammonia and nitrogen concentrations more than 1 mg/l have been given as an indicator of organic pollution and can be toxic to aquatic species if they are higher than 2.5 mg/l **(Ahmed, 2012).** In the present study, the values of ammonia ranged from 0.14mg/l to 0.22 mg/l at location **(I)** and 7.03 mg/l to 13.3 mg/l at location **(II), Table (2)**. The increase of ammonia values at location **(II)** during all seasons may be due to a large amount of organic matter outfalls and their decomposition of the organic matter exhausting dissolved oxygen and produce a high level of ammonia (**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 location **(I) (Saad *et al.*, 2011).** The present results declared that ammonia levels in water samples at location II were exceeded the **CCME (2007)** guidelines for the protection of aquatic life, Table 2.

Nitrite (NO_2^--N)

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 natural water. Resistance to toxic effect of nitrite ion is enhanced by the presence of chloride or increased water hardness (**APHA, 1995**).

The values of nitrite ranged between 8.45 and 11.1µg/l at location **(I)** during summer season while, the values fluctuated from 17.1 to 45.15µg/l at location **(II)** during the winter season, Fig. 3. 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 wastewater where *nitrosomonas* bacteria oxidize ammonia to nitrite **(Saad *et al.*, 2011).**

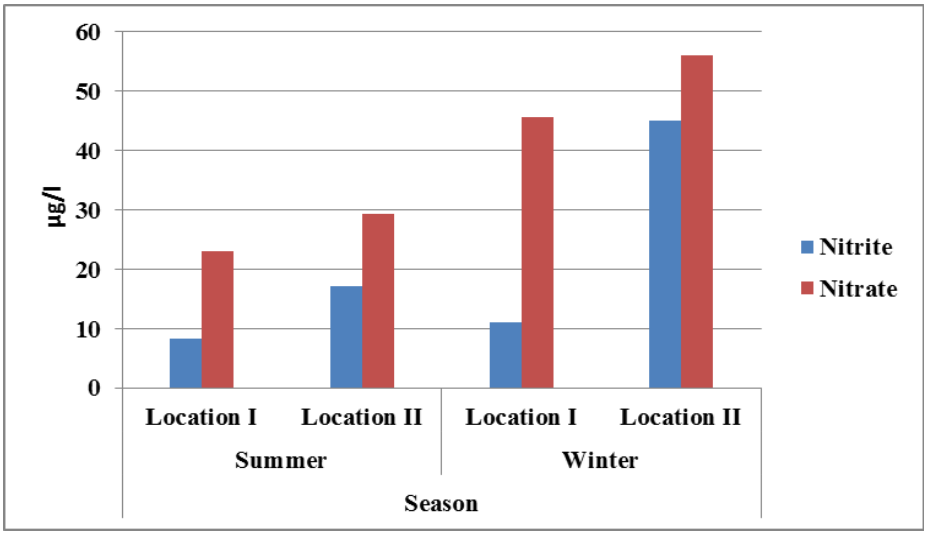

Fig. (3): The variation of nitrite and nitrate levels in the River Nile at Rosetta Branch water

Nitrate (NO_3^--N)

The reduction of nitrate can be brought about by certain nitrate reducing bacteria especially in the presence of organic matter and only limited amounts of oxygen **(Abdel-Satar, 2005).** 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 was reduced to nitrite and finally to ammonia, under certain circumstances nitrous oxidation (NO_2^-) and nitrogen are also produced **(Abdel-Satar *et al.*, 2017).**

The values of nitrate fluctuated within a wide range between 23.1 and 56.1 mg/l, Fig. 3. 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; 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 **(Abdo, 2010 and Saad *et al.*, 2011).**

Heavy metals

The average concentrations of heavy metals in Nile water were in the decreasing order of: Fe > Zn > Mn > Cu > Pb. The High levels of all studied metals were recorded at location II during different seasons reflecting the overwhelming influence of wastewater on metals distribution in the Nile water, Table 3. Statistically, Fe, Mn, Pb and Cu concentrations shows negative correlations with pH (r= (-0.65) – (-0.76), n=4, $p < 0.01$), during summer and winter seasons, which suggest that, the pH have been acclaimed to responsible for mobilization of heavy metals in Rosetta Branch (**Abdel-**

Satar and Elewa 2001). Also, the levels of Fe Zn, Pb and Cu often surpassed the **CCME (2007)** guidelines for aquatic-life. These elevated concentrations are almost certainly a result of anthropogenic sources (**Abdel-Satar *et al*., 2017**). The significant correlations ($r = 0.5–0.99$; $n = 4$, $p < 0.01$) among heavy metals pairs indicated common sources and association of metals (**Abdel-Satar, 2005**).

Table (3): Seasonal variations of heavy metals concentrations (mg/l) in the River Nile water

	Seasons				
	Summer		Winter		CCME
Parameter	Location I	Location II	Location I	Location II	(2007)
Fe	**0.140**	**0.720**	**0.250**	**0.940**	**0.3**
Zn	**0.085**	**0.154**	**0.098**	**0.112**	**0.03**
Mn	**0.025**	**0.085**	**0.032**	**0.150**	**-**
Pb	**0.022**	**0.041**	**0.025**	**0.058**	**0.002**
Cu	**0.012**	**0.034**	**0.015**	**0.045**	**0.002**

Histopathological studies

Histopathology is used as a sub-lethal test for evaluating toxic effect of water pollutants on fish (**Tayel *et al*., 2020).** The liver is the principal organ of detoxification in vertebrates and particularly in fish. The normal structure of the liver shows that, it is made up of hepatocytes which arranged in the branched lamina. They are polygonal cells with a central spherical nucleus that separated from each other by blood sinusoids. Blood flows from branches of the hepatic portal vein and hepatic artery through the sinusoids to central veins which empty into the hepatic vein **(Fayed, 2004).**

The liver of *O. niloticus* fish collected from upstream (A&C) and downstream (B&D) locations (Fig. 4), suffered from many pathological alterations**.** These alterations were necrosis, and fatty degeneration in hepatocytes as well as the blood vessel showed congestion, destruction in its wall. Severe hemorrhage was accompanied and congestion in blood sinusoids. These findings were in agreement with those reported by (**Yacoub and Abdel-Satar, 2003; Ibrahim and Mahmoud, 2005; El- Naggar *et al.*, 2009 and Saad *et al.*, 2011**, **Ismail *et al.*, 2017; Tayel *et al.*, 2018).** The fatty degeneration changes in studied liver may be due to the decrease in the rate of utilization of energy reserve or pathological enhance synthesis while the abnormal accumulation of fats in an experimental animal could be due to induced imbalance between fat production and utilization **(El-Naggar *et al.*, 2009 and Tayel *et al.*, 2018).** The same investigation was regarded by **(Tayel, 2003)** on the tilapia fish. As a sequence, the fish collected from location **(II)** showed more histopathological alteration than those collected from location **(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 **(Abu-Elala *et al.* (2016), Bayomy *et al.*, 2017 and Ahmed *et al*., 2019).**

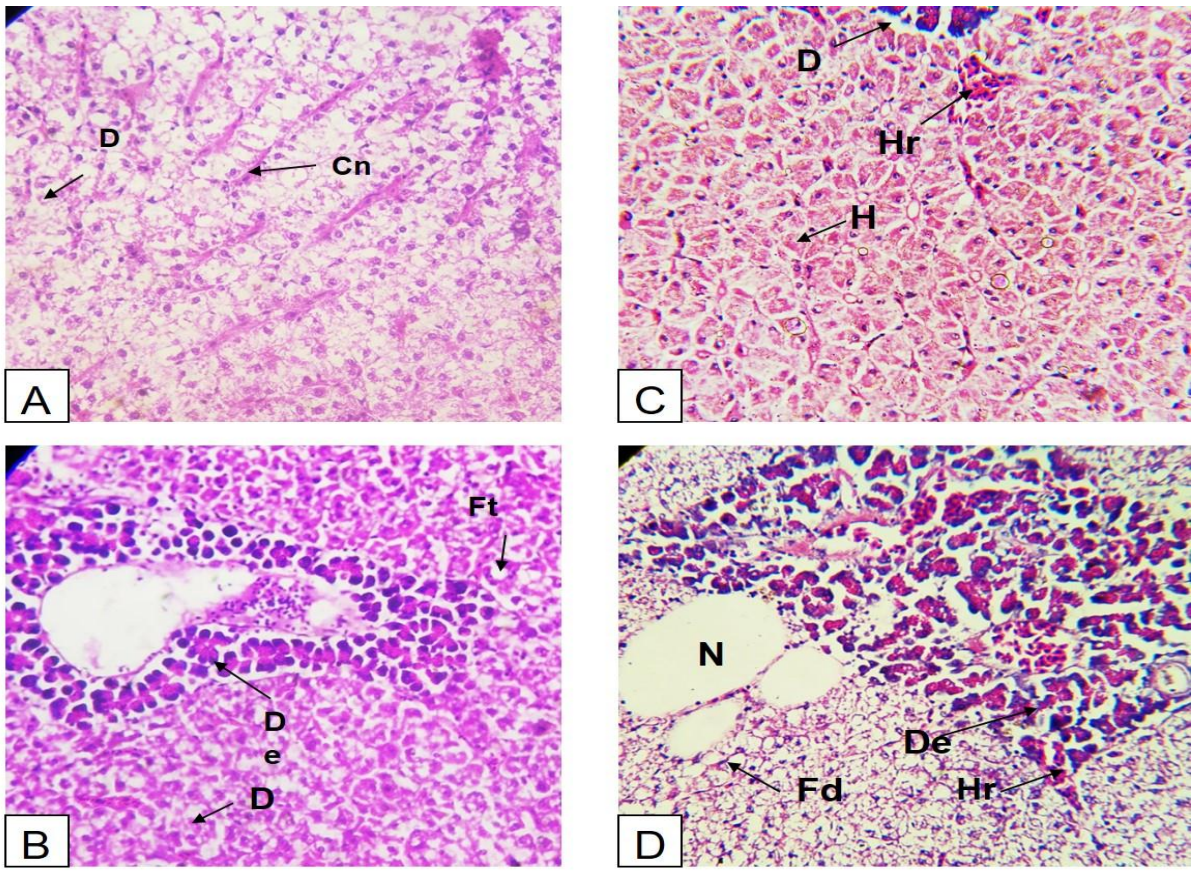

Fig. (4): Liver sections of *O niloticus* collected from up and downstream locations of El-Rahawy Drain discharge point at Rosetta Branch (formalin 10 –H&E) showing:

a) Congestion (Cn) in blood sinusoid and Degeneration (D) in hepatocytes.
b) Fatty degeneration (Fd), Degeneration (D) in hepatocytes and Destruction (De) in blood vessel
c) Degeneration (D) in blood vessel Hemorrhages (Hr) and hepatocytes appear normal shape (H)
d) Necrotic area (N), Degeneration (D) & fatty degeneration (Fd) in hepatocytes and Destruction (De) in blood vessel.

ISSR markers

Pollutants include heavy metals can cause an imbalance between the free radical species production and reduction in fish **(Livingstone, 2003; Sevcikova *et al*., 2011; Kamollerd *et al*., 2019).** The free radicals can attack lipid, protein and DNA molecules to induce oxidative stress products as well as cause DNA damage **(Castano and Becerril, 2004; Vilela *et al*., 2018; Kamollerd *et al*., 2019).** Genetic markers such as ISSR markers are vital tools for monitoring fish populations **(Rashed *et al*., 2008)** and fish species genetic variability **(Saad *et al*., 2009).**

In the present study, five primers of ISSR were used to study the effects of water pollution on tilapia nilotica fish from the two locations. The DNA fragments generated by the five primers of ISSR (P1-P5) were separated using 2% agarose gel electrophoresis and are shown in Fig. 5.

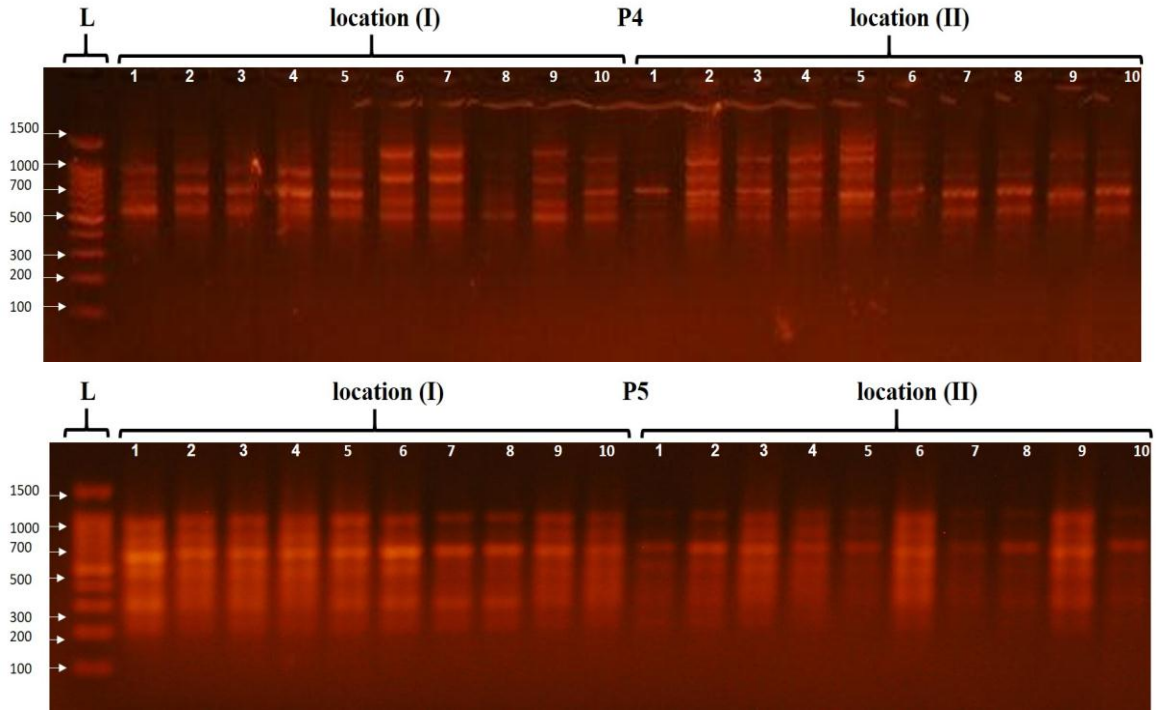

Fig. (5): Examples of Agarose gel electrophoresis for amplified ISSR fragments using P4 and P5 primers with samples of the two different locations. Lines 1-5: males, Lines 6-10: Female and L: DNA ladder.

A total of 29 reproducible fragments were obtained for each sex samples with the used primers. Tables 4 and 5 displayed the obtaining fragments that were scored as 1 or 0 (appearance/disappearance, respectively). The ISSR results of different samples from the two locations were compared based on the sex (males or females) to eliminate any effects of the sex. The five ISSR primers produced 3 - 10 fragments with an average of 5.8 fragments per primer. The highest number of fragments was obtained with P1, while P3 gave the lowest number. The obtained fragments were primer dependent and were in the size range of 300 to 2000 bp. As observed from Table 4, in males' samples, there were a number of 6 unique fragments (1 with P1, 2 with P3 and 4 with P4) between the two locations, while 8 unique fragments were obtained in females' samples (2 with each P1 and P2, 3 with P3 and 1 with P4), Table 5.

These unique fragments indicate to mutation occurrence. Compared with location I samples; five mutations were occurred in the template DNA of location II samples at DNA sites that previously were complementary to the primer. So, the fragments were not produced as fragments 2 and 3 with P3 and 5 and 9 with P4 in males while in females, there was only one produced fragment (fragment 2 with P3). While, eight mutations were induced in sites were not previously complementary to the primer and new fragments were produced such as fragments 4 and 6 with P4 in males and 4 and 5 with P1, 1 and 2 with P2 and 4 and 10 with P4 in females' samples.

Table (4): The obtained fragments and 0/1 table of the five ISSR primers that were used with males' samples from the two locations.

Primer	Band	Male samples									
		Location (I)					Location (II)				
		Male1	Male2	Male3	Male4	Male5	Male1	Male2	Male3	Male4	Male5
P1	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	0	0	0	1	1
	3	1	1	1	1	1	0	0	1	1	1
	4	1	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	1
P2	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1	1
P3	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	0	0	0	0	0
	3	1	1	1	1	1	0	0	0	0	0
P4	1	0	1	1	1	1	1	0	0	1	1
	2	0	1	1	1	1	1	1	1	1	1
	3	0	1	1	1	1	0	1	1	1	1
	4	0	0	0	0	0	1	1	1	1	1
	5	1	1	1	1	1	0	0	0	0	0
	6	0	0	0	0	0	0	1	1	1	1
	7	1	1	1	1	1	0	1	1	1	1
	8	1	1	1	1	1	1	1	1	1	1
	9	1	1	1	1	1	0	0	0	0	0
	10	1	1	1	1	1	1	1	1	1	1
P5	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	1

In addition, in males, fragment number 3 that was obtained with P1 was disappearance in males 1 and 2 of location II samples. As well as, fragment 3 produced with P1 was not exhibited in females 3, 4 and 5 of location II samples compare with other samples. This indicates that, the mutations occurred in some individuals only.

On the other hand, the dendrogram of males and females' samples were shown in Figs. 6 and 7, respectively. The dendrogram separated the studied samples into two clusters corresponding to studied location. The location I samples were in a separated cluster from those samples of location II in the two sexes.

Table (5): The obtained fragments and 0/1 table of the five ISSR primers that were used with females' samples from the two locations.

Primer	Band	Female samples									
		Location (I)					Location (II)				
		Female1	Female2	Female3	Female4	Female5	Female1	Female2	Female3	Female4	Female5
P1	1	1	0	0	1	1	1	1	0	0	1
	2	0	0	1	1	0	1	1	0	1	1
	3	1	1	1	1	1	1	1	0	0	0
	4	0	0	0	0	0	1	1	1	1	1
	5	0	0	0	0	0	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	1
P2	1	0	0	0	0	0	1	1	1	1	1
	2	0	0	0	0	0	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	1
P3	1	0	1	0	0	0	0	0	0	0	0
	2	1	1	1	1	1	0	0	0	0	0
	3	1	1	1	1	1	0	0	1	1	0
	4	0	0	0	0	0	0	0	0	0	1
P4	1	0	1	0	0	0	0	0	0	0	0
	2	1	1	0	0	0	1	0	0	0	0
	3	0	1	0	1	0	1	0	0	0	0
	4	1	0	1	0	0	0	0	0	0	0
	5	0	1	0	0	0	0	0	0	0	0
	6	1	0	0	1	1	1	1	1	1	1
	7	1	1	1	0	1	1	1	1	1	1
	8	1	0	1	1	1	1	0	0	1	0
	9	1	0	1	1	1	1	1	1	1	1
	10	0	0	0	0	0	0	1	1	1	1
P5	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	1

The genetic variations in fish samples from the same sex in different locations indicated a positive correlation with metals concentrations in the water. These results suggested that the heavy metals accumulation in the water causes DNA damage. These finding support the study of **Kamollerd *et al*. (2019). Wood *et al*., 2001; Monserrat *et al*., 2007; Vilela *et al*., 2018** reported that, in the polluted water, heavy metal exposure could cause DNA damage in fish as double and strand single breakages, alterations in the mechanism of DNA repair and DNA-protein crosslinks. Heavy metals can induce

oxidative stress, DNA damage, point mutations and several other indirect genotoxic effects **(Waalkes, 2003; Castano and Becerril, 2004; Suhartono *et al*., 2013 and Kamollerd *et al*., 2019).**

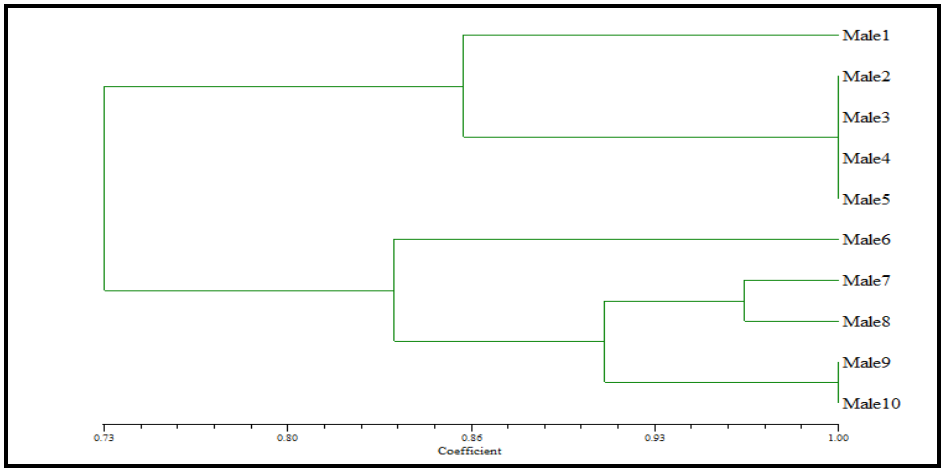

Fig. (6): The dendrogram constructed from five primers of ISSR showing the tilapia males samples genetic relationships from location I (Males 1-5) and location II (males 6-10).

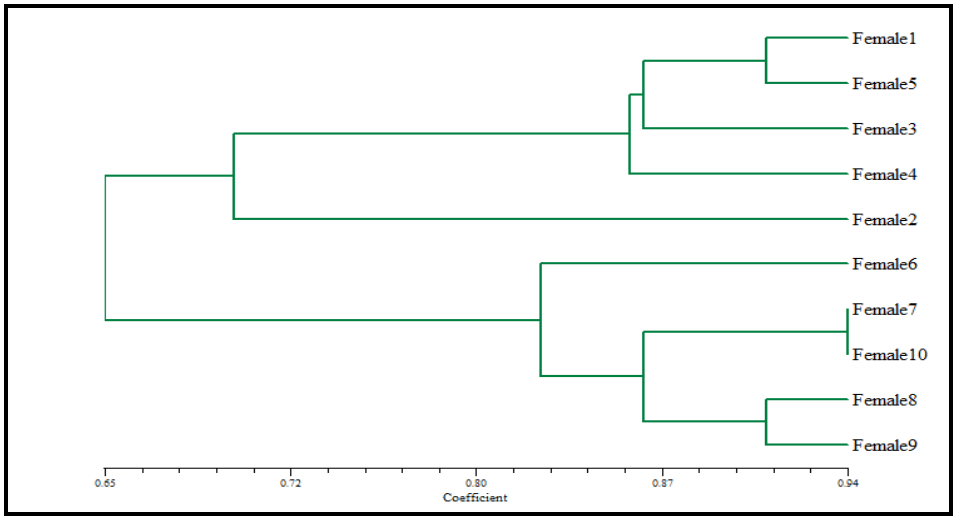

Fig. (7): The dendrogram constructed from the five primers of ISSR showing tilapia females samples genetic relationships from location I (females 1-5) and location II (females 6-10).

CONCLUSION

From the previous discussion, it can be concluded that, the water quality parameters were increased at location II in addition to depletion in dissolved oxygen. Also, the fish samples collected from this location showed severe histological and molecular alterations than those collected from location I. It is recommended that the government must increase the awareness of people around the River Nile, and improve the environmental management to reduce the risks of polluted water and consuming the fish exposed to this water on human health.

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