Research Article

Genotoxic and Histopathological Effects of Water Pollutants in Three Population Fish (*Oreochromis niloticus*) in Egypt.

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ABSTRACT

In Nile tilapia (*Oreochromis niloticus*), pollution was detected from Lake Qarun, Wadi El-Rayan and Fish Farm at Fayoum Governorate, Egypt using RAPD-PCR and histopathological technique. The effective number of alleles was detected in Wadi El-Rayan population, while the lowest values were found in Lake Qarun population. Lake Qarun and Fish Farm showed closest genetic identity of (0.8775) and farthest genetic distance of (0.4102 and 0.3521, respectively) from Wadi El-Rayan where they are more polluted than Wadi El-Rayan. Clear histopathological changes were appeared in fish gills of Lake Qarun and Fish Farm and slight in Wadi El-Rayan. The liver of Wadi El-Rayan fish showed more or less normal architecture. Brownish hemosiderin granules engulfed by melanomacrophage cells were observed in liver of Lake Qarun and Fish Farm fish. Glomeruli and tubules of the posterior kidney in Wadi El-Rayan fish exhibited less damage in compared to the Fish Farm and Lake Qarun fish.

Keywords: *Oreochromis niloticus*, RAPD-PCR, Genotoxicity, Aquatic pollution, Histopathology.

INTRODUCTION

Tilapias are the main source of protein in many developing countries, which considered as the most eaten fish in Egypt and most important freshwater finfish in world aquaculture[1]. It can persist in a highly polluted habitat and has the potential for the development as a biological monitor of environmental water pollution[2]. Pollutants usually present in natural environments as complex mixtures such as toxic heavy metals, bacteriological contamination and pesticides that provide several biomarkers for a complete diagnosis of environmental degradation[3].

A biomarker based biomonitoring is a promising approach to provide early warning signs to assess the action of these water contaminants on aquatic populations[4]. It can offer complete and relevant information on the potential impact of toxic pollutants on the organism's health[5]. A set of complementary biomarkers might be useful in evaluating responses to a mixture of pollutants of organisms under stress[6]. There are many different biomarkers that occur at many different levels of organization from sub-cellular to whole-organisms. Among the bio-indicators of aquatic ecosystem, fishes were often deemed as the most suitable objects[7], where it is exposed directly to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem[8].

Additionally, fish are sensible to changes in the aquatic environment. Thus, some genetic, biochemical, morphological and behavioral responses measured in exposed fish are useful biomarkers for environmental bio-monitoring[9]. Nile tilapia can be used in bio-monitoring programs and toxicological studies[10]. The impacts of chronic pollution on wildlife populations include increased incidence of disease[11], reduced survivorship and fecundity[12], developmental abnormalities[13], decreased reproductive ability[14] and changes in the genetic structure of populations[15].

The genotoxic potential of aquatic pollution in fish has been investigated by different methodologies like Random amplified polymorphic polymerase chain reaction (RAPD-PCR)[16].

Where, organisms in contaminated environments showed loss of DNA structural or functional integrity[17]. Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish[18]. The present work was performed to address the effects of aquatic contamination on three different populations of the Nile tilapia fish *Oreochromis niloticus* from Lake Qarun, Fish Farm and Wadi El-Rayan, using RAPD-PCR and histopathological technique.

MATERIALS AND METHODS

Total of 180 Nile tilapia fish (*O. niloticus*) were used for studying Genetic diversity and histopathological studies (90 samples for the Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) and 90 samples for histopathological analyses) representing the three locations at Fayoum Governorate, Egypt (Lake Qarun (the southwest side of Lake Qarun at the outlet of El-Wadi drainage canal, which is one of the main drainage canals in El-Fayoum province), Fish Farm at the southern side of Lake Qarun, where it depends on agricultural drainage water as a source of water[19] and Wadi El-Rayan.

Blood sampling and DNA extraction

Blood samples were drawn from the caudal vein under sterile conditions from a total of 90 fish. Detailed numbers of samples were taken as 30 samples from each group (Lake Qarun, Fish Farm and Wadi El-Rayan). The needle was run as deep as possible through the middle line just behind the anal fin in a dorso-cranial direction. DNA was extracted and purified from whole blood collected samples using a QIAamp® DNA blood mini Kit (Qiagen Germany) according to the manufacturer's protocol.

RAPD-PCR

PCR amplification was performed using fifteen commercially available decamer random primers, designed and chosen arbitrarily for these experiments. Primers were obtained from Operon Technologies (Operon, Almeda, CA, USA) and used to initiate PCR amplification. Primers were randomly selected on the basis of GC content (60-70%), primer codes and sequences are presented in table 1.

A total reaction volume of 15 µl contained 5 ng genomic DNA, 0.2 UM of each primer, 1x of *Taq* polymerase buffer, 2 units of *Taq* polymerase (Fermentas). PCR amplification was performed under cycling conditions of 96°C for 4 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 1 min, 72°C for 1 min terminated with elongation at 72°C for 10 min. Length and purity of the PCR products were evaluated by agarose gel electrophoresis. The bands were visualized under UV light and the gels were photographed using digital gel documentation system (Bio-Rad, USA). DNA fragments sizes were estimated by their comparison with standard molecular size marker (ØX174/Hae III).

Histological Preparations

Fishes from each group were rapidly dissected and small specimens from the second gill arch, the left lobe of the liver and the trunk kidney of each fish were taken and fixed directly in neutral buffered formalin and Bouin´s fixatives then dehydrated, cleared and embedded in paraffin wax (m.p.56-58°C). Paraffin sections 4-6 µm thick were then cut with a rotary microtome and stained with Haematoxylin and eosin H & E[20].

Statistical analysis

Statistical analysis of the data was carried out using the population genetic analysis software, POPGENE 1.31 software package[21]. The UPGMA dendrogram and genetic distances of population was constructed based on Nei's genetic identity among the three populations[22]. While overall observed number of alleles and effective number of alleles were calculated according to[23]. Genetic differentiation (Gst) was calculated by using formula: (Genetic dif (Gst) = 1- Hs/Ht). Where, (Hs) is sample gene diversity and (Ht) is total gene diversity[24]. Gene flow was indirectly estimated among the populations by using the formula: Nm =0.5(1 - Gst)/Gst[25]. Shannon's diversity index (I) was calculated to provide a relative estimate of the degree of genetic variation within each population[26].

RESULTS

Random Amplified Polymorphic DNA-Polymerase chain reaction (RAPD-PCR)

The banding patterns generated through RAPD assay were used to assess the effect of pollution on the three different studied populations of *O. niloticus*, where they represent the main aquatic resources in Fayoum province and to deduce genetic diversity among them which in turn will reflect the ecological conditions of these three populations under study. The amplified bands detected were varied in number according to species, primers and individuals. Fifteen different decamer primers considering GC content (60-70%) were screened on a group of twenty five individuals for each of the three populations.

Fifteen selected primers were used to examine the level of polymorphism detectable in the three populations. Clear amplification was produced by only 7 primers (B02, B04, B06, B07, C03, C19, and C20) while 8 primers produced either a smear or no amplification at all. Thus, all examined samples using these seven primers produced the highest number of bands that would be able to differentiate between populations, and the other 8 primers were excluded from further analyses.

Polymorphism

Amplification of **OPC03** was shown in **d** and **e** for Lake Qarun, where **f** for Farm Fish, M: DNA ladder (100 Pb). Detection of PCR products of **OPB07** and **OPC19** primers on 2% agarose gel electrophoresis**,** where **a, b, c** showed the amplification of **OPB07** in Lake Qarun, Fish Farm and Wadi El-Rayan groups respectively, and **d, e, f** presented the amplification of **OPC19** in the three studied populations respectively, M: DNA ladder (100 Pb).

Polymorphism, monomorphism and unique bands among the populations can be illustrated in (Table 2), where El-Rayan population showed the highest number of unique bands, 8 unique bands (718, 704, 592, 580, 547, 498, 448, and 424) through B02 primer, 5 unique bands (1123, 1048, 495, 425, 413) through B04 primer and 3 unique bands (292, 252, 213) through B06 primer (Fig. 1). In Figure 1, Detection of C20 primer represented 7 unique bands (2162, 718, 704, 592, 580, 498, 424) and 4 unique bands (1494, 495, 425, 413) for C03 primer. For B04 primer, Lake Qarun population has only one band (948) as well as the Fish Farm population (1877). In contrast, B07 and C19 bands ranged from (286 to 3132) and (242 to 2643) respectively, Lake Qarun population represented the highest detected markers by scoring 28 bands for B07 and 17 for C19, while the lowest number of bands have scored by Wadi El-Rayan population with 17 and 16 bands for B07 and C19 respectively (Fig. 1).

Percentages of polymorphic RAPD bands were ranged from 0% to 100% in all studied Tilapia species. The polymorphism percentages between the individuals

within population were (23.64%, 27.88% and 29.70%) and the number of the polymorphic loci was (39, 46 and 49) in Lake Qarun group, farm culture group and Wadi El-Rayan, respectively (Table 3).

The observed number of alleles (Na) and the effective number of alleles (Ne) were used as a measure of genetic polymorphism, which were varied among the present studied three tilapia populations. However, they were higher in Wadi El-Rayan population (1.2970±0.4583 & 1.1677±0.2899, respectively) than that in farm culture population (1.2788±0.4498 & 1.1593±0.2798, respectively) and Lake Qarun population (1.2364±0.4261 & 1.1208±0.2401, respectively). The total average number of observed alleles and effective number of alleles among three Tilapia populations were (1.563±0.4974 & 1.4449±0.4185, respectively) (Table 4).

Table 1: Codes, sequences and CG content used in RAPD analysis

Primers	Sequence	CG%	Primers	Sequence	CG%
A02	TGCCGAGCTG	70	C05	GATGACCGCC	70
A19	CAAACGTCGG	60	C07	GTCCCGACGA	70
B02	TGATCCCTGG	60	C10	TGTCTGGGTG	60
B04	GGACTGGAGT	60	C17	TTCCCCCCAG	70
B06	TGCTCTGCCC	70	C18	TGAGTGGGTG	60
B07	GGTGACGCAG	70	C19	GTTGCCAGCC	70
C03	GGGGGTCTTT	60	C20	ACTTCGCCAC	60
C04	CCGCATCTAC	60			

Table 2: Polymorphic, monomorphic and unique bands among the populations

Primer	Polymorphic bands	Monomorphic bands	Unique bands	Description of unique /population specific bands (bp)		
				Lake Qarun	Fish Farm	Wadi El-Rayan
OPB02	6	6	9		1 (2852)	8 (718, 704, 592, 580, 547, 498, 448, 424)
OPB04	6	5	7	1 (948)	1 (1877)	5 (1123, 1048, 495, 425, 413)
OPB06	4	20	6		3 (2128, 1952, 1397)	3 (292, 252, 213)
OPB07	10	16	3	3 (3132, 2792, 2627)		
OPC03	8	3	7	3 (1048, 967, 948)		4 (1494, 495, 425, 413)
OPC19	6	15	4	4 (2643, 2432, 2269, 1837)		
OPC20	8	5	8		1 (448)	7 (2162, 718, 704, 592, 580, 498, 424)

Table 3: The number and the percentage of polymorphic loci in the three populations.

POP ID	The number of polymorphic loci	% The percentage of polymorphic loci
Lake Qarun	39	23.64%
Fish Farm	46	27.88%
Wadi El-Rayan	49	29.70%
Total	93	56.36%

Table 4: Population wise genetic analyses among the three populations

Population	Na	Ne	H	I
Lake Qarun	1.2364±0.4261	1.1208±0.2401	0.0770±0.1460	0.1186±0.2204
Fish Farm	1.2788±0.4498	1.1593±0.2798	0.0982±0.1650	0.1486±0.2455
Wadi El-Rayan	1.2970±0.4583	1.1677±0.2899	0.1023±0.1687	0.1547±0.2494
Overall	1.563±0.4974	1.4449±0.4185	0.2448±0.2213	0.3518±0.3147

Na = Observed number of alleles; Ne = Effective number of alleles [Kimura and Crow (1964); H = Nei's (1973) gene diversity; I = Shannon's Information index [Lewontin (1972)]

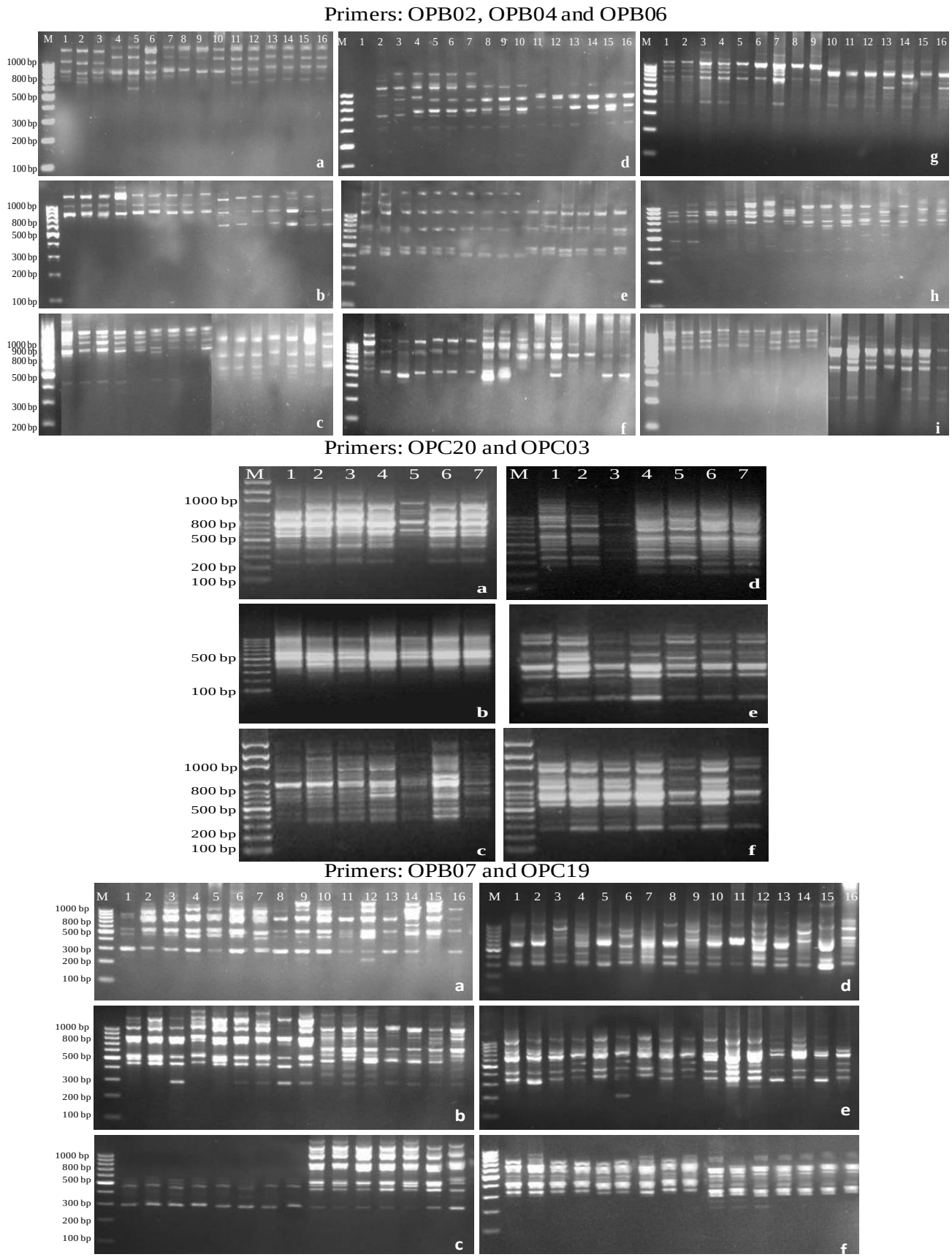

Figure 1: Detection of PCR products of **OPB02, OPB04** and **OPB06** primers on 2% agarose gel electrophoresis, where **a, b, c** described **OPB02** in Lake Qarun, Farm Fish and Wadi El-Rayan respectively, **d, e, f** the PCR products of **OPB04** and **g, h, i** the PCR products of **OPB06** in three groups respectively, M: DNA ladder (100 Pb). Detection of PCR products of **OPC20** and **OPC03** primers on 2% agarose gel electrophoresis**,** where **a, b, c** showed the amplification of **OPC20** in Lake Qarun, Fish Farm and Wadi El-Rayan respectively.

Genetic variability parameters

The highest values of Nei's gene diversity (H=0.1023±0.1687) and Shannon's information index (I=0.1547±0.2494) were observed in El-Rayan valley population while the lowest values were found in Lake Qarun population (H=0.0770±0.1460 and I=0.1186±0.2204) (Table 5). The genetic diversity was calculated according to heterozygosity (Ht) for the total populations, sample heterozygosity (Hs), the genetic differentiation (Gst), gene flow (Nm), genetic distance (D) and genetic identity. The overall estimated total diversity (Ht) was (0.2448±0.0490); sample diversity (Hs) was (0.0925±0.0115). Genetic differentiation (Gst) between all loci in three tilapia populations was (0.6221) and the gene flow value was (0.3037) (Table 5).

The genetic identity and distance between three tilapia populations were presented in (Table 6). Lake Qarun population was closest to Fish farm population with a genetic identity of (0.8775), whereas Wadi El-Rayan population was farthest to Fish farm population & Lake Qarun population with a genetic distance of (0.3521 and 0.4102, respectively). The UPGMA-tree dendrogram (Fig. 2) indicates the relationship among the three tilapia populations taking in consideration the close relationship between populations of Lake Qarun and Fish Farm.

Table 5: Overall Nei's (1987) analysis of gene diversity in subdivided populations.

Population genetic parameters	Obtained values
Intra-population (Hs)	0.0925±0.0115
Total heterozygosity (Ht)	0.2448±0.0490
Relative differentiation (Gst)	0.6221
Estimate gene flow (Nm)*	0.3037

* Nm = estimate of gene flow from Gst. E.g., Nm = 0.5(1-Gst)/Gst.

Table 6: Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA Tree (100 replications) generated from RAPD data of the three Tilapia populations.

Pop ID	Lake Qarun	Fish Farm	Wadi El-Rayan
Lake Qarun	****	0.8775	0.6635
Fish Farm	0.1307	****	0.7032
Wadi El-Rayan	0.4102	0.3521	****

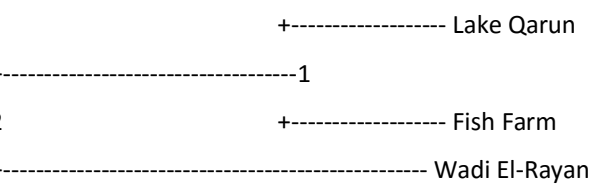

Figure 2: Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA Tree (100 replications) generated from RAPD data of the three Tilapia populations

Histopathological observations

Gill Histopathology

The gill tissues of *O. niloticus* of Lake Qarun fish showed many histopathological alterations in the secondary lamellae (Fig. 3) including drooping (IA_1), shortening of the secondary lamellae and hyperplasia of the interlamellar epithelial cells of the gill filaments starting at the bases of the secondary lamellae and extending towards their tips (IA_2) causing complete fusion (IA_3) and may even meet with neighboring interlamellar cells above the lamellar tips (IA_3 & IA_4). The filaments separated from the deformed cartilaginous skeleton (IA_4). Also, vascular congestion was shown in the marginal blood channels of most of the lamellae and telangiectasis (IA_5).

In Fish Farm, the filaments were separated from the deformed cartilaginous skeleton (IB_2), and drooping of some secondary lamellae was observed (IB_2). In some areas of the examined sections the filaments were shortened (IB_2). In addition, epithelial cell hyperplasia was observed (IB_1) and this may cause rupture of the secondary lamellae (IB_4). In contrast, the gill filaments of fish collected from Wadi El-Rayan were similar to the healthy fish. The gill filaments showed slight drooping ($IC_{1\&3}$) and absence of alteration signs that observed in fish of the other two studied populations. A slight separation of the epithelium of few numbers of the secondary lamellae was observed ($IC_{1\&2\&3}$).

Liver histopathology

The studied liver sections of Lake Qarun possessed hypertrophied hepatocytes with abnormal nuclei (IIA_1) (Fig.3). Many hepatocytes contained vacuolated cytoplasm (fatty degeneration) with pyknotic deeply stained nuclei ($IIA_{1\&2\&3}$). Cloudy swelling of hepatocytes as well as dilation of both blood sinusoids and central vein was observed ($IIA_{1\&3}$). Brownish hemosiderin granules were present in between hepatocyte and adjacent to pancreatic acini engulfed by melanomacrophage cells ($IIA_{1\&2}$). Large lysed areas (liquefied necrosis) with leucocyte infiltration were noted (IIA_4). Similarly, liver sections of *O. niloticus* from Fish Farm showed hypertrophied hepatocytes with irregular deeply stained nuclei ($IIB_{1\&2}$), vacuolar and degenerated cytoplasm (IIB_2), congested, dilated central vein ($IIB_{1\&2}$) and blood sinusoids ($IIB_{1\&2\&3}$). Degenerative pancreatic nodules around dilated portal vein (IIB_3) were surrounded by large lysed necrotic areas with mononuclear white cell infiltration and brownish hemosiderin granules ($IIB_{2\&3}$). In contrast, Wadi El-Rayan fish showed more or less normal liver architecture, where moderate dilation of portal veins ($IIC_{1\&2\&3}$), congestion of blood vessels ($IIC_{2\&3}$) and slight lymphocyte infiltration were detected (IIC_3).

Kidney Histopathology

Severe damage was observed in fish exposed to pollutant in different studied groups affecting both glomeruli and tubular cells. The posterior kidney of the fish collected

from Lake Qarun and Fish Farm showed necrotic foci represented both glomerular and tubular degeneration in-between tissue parenchyma with mononuclear leucocyte infiltration ($IIIA_{1\&2}$ and $IIIB_{1\&2}$). As well, different degrees of glomerular shrinkage with complete degeneration were observed ($IIIA_2$ and $IIIB_{1\&2}$). Some tubular epithelial cells showed less degree of vacuolation and separated from the basement membrane resulting from edema, this lesion implied the loss of tubule shape, reduction of lumen diameter and, frequently, tubule disappearance ($IIIA_2$ and $IIIB_2$). Wadi El-Rayan fish sustained less renal damage when compared to the two previous populations, which depicted more or less normal kidneys, similar to healthy fish. The glomeruli were found to be in groups or clusters with normal size. Also, there was a somewhat moderate shrinkage in some glomerulus ($IIIC_{1\&2}$).

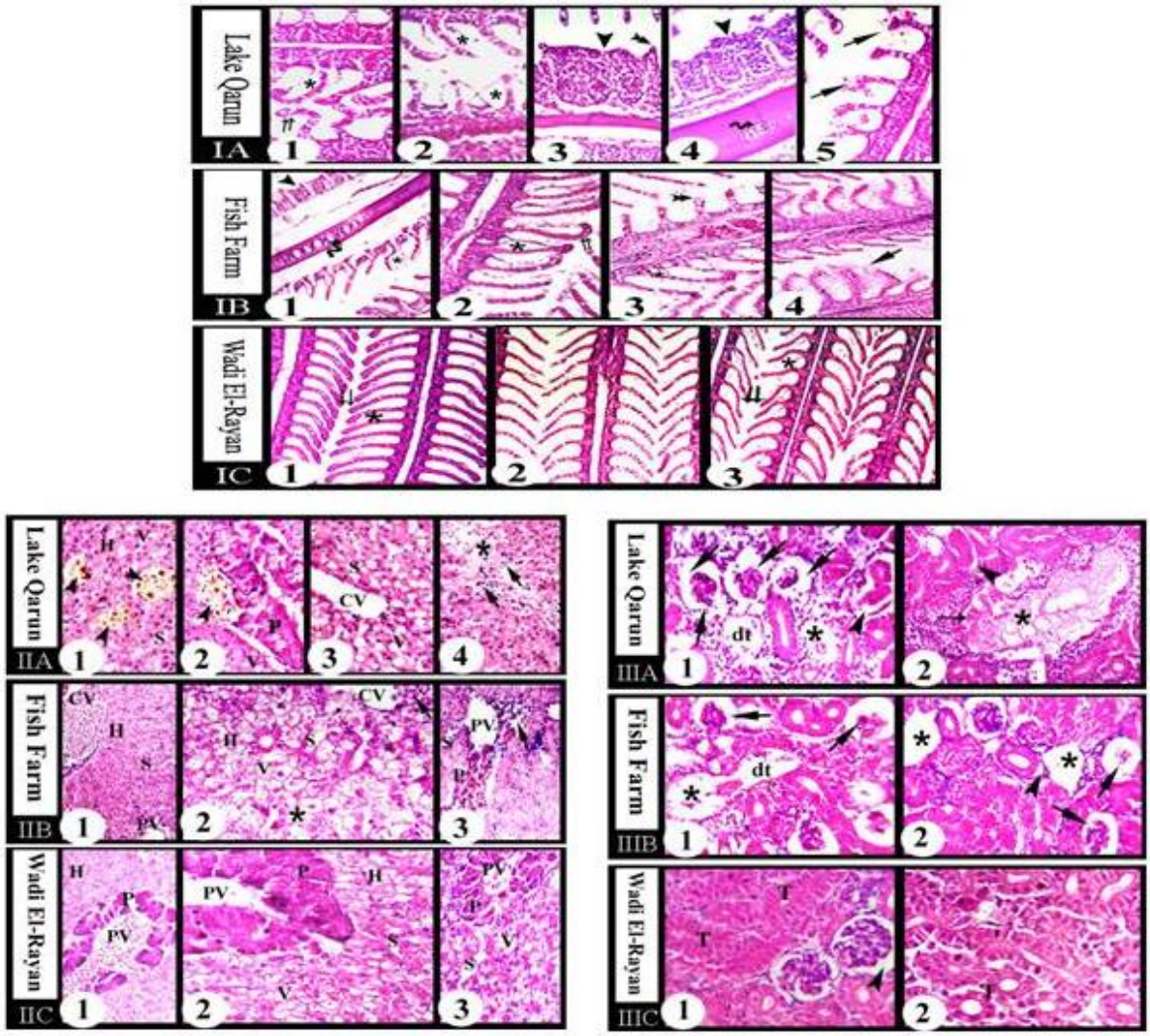

Figure 3: I**A)** Lake Qarun fish showing: 1. drooping of the 2ry lamellae (double arrow) and separation at both sides with edema (asterisk). 2. desquamation of secondary lamellae and edema (asterisk). 3&4. Hyperplasia starting at the bases of the filaments and extending towards their tips with complete fusion of the secondary lamellae (arrowhead) with shortening (double arrowhead) and deformed cartilaginous skeleton (bent arrow). 5. telangiectasis with vascular congestion and rupture (thin arrow). X 400 HE. I**B)** Fish Farm fish showing: 1. Desquamation of secondary lamellae (asterisk), hyperplasia (arrowhead), deformed cartilaginous skeleton (bent arrow). X 200 HE. 2. Separation of epithelial cells at the base of 2ry lamellae forming edema (asterisk) and some of the 2ry lamellae showing drooping (double arrow). 3. Shortening of secondary lamellae (double arrowhead). 4. Hyperplasia leads to rupture of the secondary lamellae (thin arrow). X 400 HE. I**C)** Wadi El-Rayan fish showing: 1. Slight desquamation forming edema (asterisk) in few numbers of 2ry lamellae and slight dropping (thick arrow). 2. desquamation of secondary lamellae and edema (asterisk). 2. Separation of epithelial cells forming edema (asterisk) and some of the 2ry lamellae showing drooping (double arrow). X 400 HE). II**A)** Lake Qarun fish showing: 1. Swollen hepatocytes with pyknotic deeply stained nuclei and vacolated cytoplasm (V). 2. Proliferated and degenerated acinar cells surrounded by brownish hemosiderin granules (arrowhead) and fatty change (V). 3. Hepatocytes with pyknotic deeply stained nuclei and fatty infiltration raddiating from dialated central vein (CV) and surround dilated sinusoids (S) 3. Showing (arrowhead), dilated blood sinusoids (S) and central vein (CV). 4. Large lysed areas (liquefied necrosis) (asterisk) with leucocyte infiltration (arrow). X 400 HE. II**B)** Fish Farm fish liver showing. 1. Hypertrophied hepatocytes (H), congested and dilated central vein (CV) and sinusoids (S). X 200 HE. 2. Hepatocytes (H) with vacuolar and degenerated cytoplasm (V), irregular nuclei, dilated congested central vein (CV) and blood sinusoids

(S) and necrotic area (asterisk). X 400 HE. 3. Degenerated pancreatic tissue (P) with hemosiderin granules (arrowhead) surround dilated portal vein (PV) and lymphocyte infiltration (L). X 200 HE. II**C)** Wadi El-Rayan fish liver showing: 1. hexagonal hepatocytes (H) and hepatopancreas that composed of strongly basophilic, large columnar pseudostratified cells (P) surround portal vein filled with blood cells (PV). X 200 HE. 2. Hexagonal hepatocytes (H) with vacuolated cytoplasm (v), hepatopancreas (P) and slightly dilated blood sinusoids (S). 3. Swollen hepatocytes (H) with vacuolated cytoplasm (v) surround dilated blood sinusoids (S). X 400 HE. **IIIA)** Posterior kidney of Lake Qarun showing. 1. Degrees of glomerular shrinkage (arrow), necrotic foci represented both glomerular (asterisk) and tubular degeneration (dT), some tubular epithelial cells are vacuolated and separated from the basement membrane resulting from edema (arrowhead). Mononuclear leucocyte infiltration was clearly visible (L). 2. Showing large necrotic foci (asterisk) surrounded by mononuclear leucocyte infiltration (L). Tubular epithelial cells are separated from the basement membrane resulting from edema (arrowhead) or degenerated (dT). X 400 HE. **IIIB)** Posterior kidney of Fish Farm showing:. 1. Glomerular shrinkage with complete degeneration (arrow), necrotic foci (asterisk) surrounded by mononuclear leucocyte infiltration. 2. Showing glomerular shrinkage (arrow) or complete disappearance (asterisk). Tubular epithelial cells showed less degree of vacuolation and separated from the basement membrane resulting from edema (arrowhead). X 400 HE. **IIIC)** Posterior kidney of Wadi El-Rayan showing: 1&2) Somewhat moderate shrinkage in some glomerulus (arrowhead), normal kidney tubules hematopoietic interstitial (intertubular) tissue surrounding the tubules (T). X 400 HE.

DISCUSSION

RAPD amplification with fifteen different decamer primers for three populations was showed 44 specific DNA bands (unique bands). Wadi El-Rayan population had 27 unique bands, where both Fish Farm and Lake Qarun populations had 17 unique bands, which indicated that genetic variation had the highest level in Wadi El-Rayan population. According to **Bhat**[27]. The detected polymorphic and unique bands can be used as genetic markers to monitor the level of DNA variability.

The percentage of polymorphism among the three populations was 56.36%, these results indicated that there are clear variations among the studied genetic variants in response to the environmental stresses, which coincides with previous results of **El-Wakil**.[28]. Moreover the percentages of polymorphism between the individuals within population were (23.64%, 27.88% and 29.70%) in Lake Qarun, Fish Farm and Wadi El-Rayan populations, and the number of the polymorphic loci was as follow (39, 46 and 49) in Lake Qarun, Fish Farm and Wadi El-Rayan, respectively. Wadi El-Rayan population had a higher polymorphisms in comparison to other two populations. These results in agreement with **Das**[29], who observed the varied range of 42.6, 31.7, 30, 19.2, 16.8 and 14.3% polymorphic loci in six *Labeo* species carp species from Odisha. Similarly[30] in *Prochilodus marggravii* from three collecting sites of San Francisco River (Brasil), Dergam[31] in fresh water fish *Hoplias malabaricus* (trahira) from Rio Doce Lake and Macacu river basin (Brazil). Whereas, **Faddagh**[32] observed a high proportion of polymorphisms among eight cyprinid fish species of Iraqi inland water. In the current investigation, the data obtained demonstrated that the higher value of Nei's gene diversity (H=0.1023±0.1687) and Shannon's information index (I=0.1547±0.2494) were observed in Wadi El-Rayan than in Lake Qarun population (H=0.0770±0.1460 and I=0.1186±0.2204). Similar results obtained[33] who stated that genetic diversity of prawn population near smelter discharging site was lower than that of the uncontaminated site. These results also coincide with previous results of **Grzywacz**[34] on *Tetrix tenuicornis* who stated that insects in a population from metal polluted areas have reduced genetic variability in contrast to other populations located in unpolluted areas.

Genetic diversity evolutionarily helps to adapt to environmental changes and stressors within a population. According to our findings, Lake Qarun is more polluted than Wadi El-Rayan, where it scored less genetic diversity as mentioned earlier. These results in line with several studies on aquatic organisms which have shown higher genetic variability in populations originating from an environment with a low contamination level in comparison with populations living in a highly contaminated environment[35].

According to[36] marine pollutants have a negative induction which can be determined using RAPD markers by determining the alteration in the genomics. Also our results corroborated those of **Omar**[37] and **Osman**[16] who reported that the genetic diversity is altered in fish populations living in polluted sites in comparison to non-polluted areas. In addition, loss in the intra or inter species genetic diversity and heritable mutations can be caused by exposure to genotoxic contaminants[38]. Reduced genetic diversity has been observed in wild and experimental populations of invertebrates[39,40] and fish[35] as a result of exposure to pollutants. A decrease of polymorphism may occur as a result of selection for toxicant resistance depending on the species. In turn, heavy metals have mutagenic effects on DNA and may indirectly decrease growth and reproduction and increase mortality in populations[41]. Changes in genetic variability may also be a consequence of adaptation to a contaminated environment[42]. Reduction in species diversity have been previously reported at the metal contaminated study site[43], which confirmed our results of reduced genetic diversity in Lake Qarun population.

The estimated total genetic diversity (Ht) was (0.2448±0.0490); sample diversity (Hs) was (0.0925±0.0115). Genetic differentiation (Gst) between all loci in three tilapia populations was (0.6221) and the gene flow value was (0.3037), these data suggested that the gene flow rate in the three populations seemed to be very low and indicative of little migration among these populations and this is because the three studied populations are considered a closed separated aquatic

systems. Similarly, **Shanmughavalli**[44] observed limited migration rate with levels of genetic variation within four species of Indian major carps and molly fish species.

The genetic identity and distance between three tilapia populations showed that Lake Qarun population was closest to Fish Farm population with a genetic identity of (0.8775), whereas Wadi El-Rayan population was farthest to Fish farm population and Lake Qarun population with a genetic distance of (0.3521, 0.4102, respectively). The dendrogram showed two clusters formation, one consisting of Lake Qarun and Fish Farm and the other was Wadi El-Rayan population. The principal aspect of UPGMA dendrogram was the striking separation of Wadi El-Rayan population from the other two, which were closely grouped. Similar to the present study, Baradakci[45] found that this technique would be able to distinguish among three species of tilapia genus *Oreochromis* and subspecies of *O. niloticus*.

Histopathological assessment of fish tissue describes the effect of toxic substances on fish health in polluted aquatic ecosystems that helps for early warning signs of disease in tissues or organ[46].

The present study showed that the gills of *O. niloticus* collected from Wadi El-Rayan had well-structured primary filaments which more or less similar to those of healthy fish except from a slight drooping and separation. On the other hand, clear histopathological changes such as lifting, shortening, drooping, vascular congestion or telangiectasis and proliferative changes of the secondary lamellae were appeared in the fish gills of both Lake Qarun and Fish Farm populations but with less degree in the later one. The lifting of the lamellar epithelial cells may lead to the increase of diffusion distance and decrease of respiratory gas exchange[47]. Besides, it may be the first signs after exposure to hazardous chemicals, or physical agents[48]. Similar findings as fusion of the adjacent lamellae can be caused by different pollutants[49]. Injured pillar cells can cause an increased blood flow inside the lamellae that led to dilation of the marginal channel, blood congestion or even an aneurysm[50]. **Giari**[51] recorded telangiectasia in the gills of European sea bass (*Dicentrarchus labrax L*.1758) exposed to sublethal concentration of mercury. Differences and severity of changes may be due to the pollutant type and concentration as mentioned by[52].

Sections of the liver of Wadi El-Rayan fish showed more or less normal architecture. By contrast, many histopathological alterations were observed in liver sections of fish from Lake Qarun and Fish Farm such as deshaped hepatocytes, vacuolization, cloudy swelling, pyknosis, and necrosis of parenchyma, infiltration of leucocytes and dilation of both blood sinusoids and central vein. Similar results have been reported in liver of different fishes, as of *Gambusia affinis* exposed to deltamethrin insecticide[53], *Oreochromis mossambicus* exposed to cadmium and zinc[54], *Clarias gariepinus* exposed to fuel oil for 14 days[55], *Channa punctatus* exposed to hexavalent chromium[56], *Cyprinus carpio* exposed to lethal concentrations of Cr[57], *Tilapia zilli* exposed to Al[58] and *Clarias gariepinus* exposed to sewage/domestic wastewater containing Cu, Fe, Pb, Cd, Mn and Zn[59].

The large lysed necrotic areas with leucocyte infiltration noted the liver sections also observed in tilapia liver by **Miranda**[60] as a typical response against hydrophobic toxins and metals, and in liver of the African catfish *Clarias gariepinus*[61] after acute exposure to glyphosate. According to **Mela**[62] this infiltration reflects a set of disorders such as disturbances of enzyme activities, loss of cell membrane integrity, alteration in protein synthetic machinery and carbohydrate metabolism, or strongly associated with oxidative stress.

Fish collected from Wadi El-Rayan group exhibited less renal damage. In contrast, fish collected from Lake Qarun and Fish Farm showed different degrees of glomerular shrinkage. Tubular epithelial cell showed hypertrophy, vacuolation and separation from the basement membrane resulting from edema. Also, leucocyte infiltration was clearly visible in kidney sections.

Similar degenerative changes were also reported in the kidney tissue of various fishes such as arctic charr (*Salvelinus alpinus*) exposed to heavy-metal (inorganic mercury and methylmercury)[63], white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure[64], *Liza ramada* fish obtained from water polluted with industrial and agricultural wastes in Lake Manzalah[65], *Anabas testudineus* that exposed to unused lignite mine[66], *Solea aegyptiaca* collected from Lake Qarun and Qarun Fish Farms[67], *Cyprinus carpio* under Sublethal effects of chromium[57] and *Oreochromis niloticus* and *Mugil cephalus* collected from Lake Qarun and Qarun Fish Farms[19]. Therefore, this study strongly recommends the coordination of different efforts to rescue these three polluted habitats (Lake Qarun, its neighboring Fish Farms and Wadi El-Rayan) in Fayoum province from serious ecological problems using proper management and scientifically specialized research.

CONCLUSION

Histopathological studies and RAPD-PCR assay on *O. niloticus* represented effective tools for reflecting adverse environmental conditions for fish health and for identifying ecosystems perturbation. The histopathological changes reflect clearly that three studied aquatic populations suffer from water contamination, which found to be severe in Lake Qarun, moderate in Fish Farm and with less impact on Wadi El-Rayan fish. Moreover, DNA polymorphisms and genetic diversity can be applied as a suitable biomarker assay for detection of genotoxic effects of water pollutants on *O. niloticus*, where Lake Qarun fish showed less genetic diversity and polymorphism than Wadi El-Rayan fish.

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