



Impacts of Blooming Phenomenon on Water Quality and Fishes in Qarun Lake, Egypt

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ABSTRACT

Blooming phenomenon was recorded in Qarun Lake in the last three years (2008, 2009 and 2010). The present work investigates the effect of this phenomenon on water quality and its impact on the fish *Solea vulgaris* and *Mugil* species in Qarun Lake. The physico-chemical parameters and trace metals in the lake water and fish tissues were found to be higher than the permissible limits. Fish samples revealed some external clinical signs as well as histopathological changes in their organs, according to infectious causes. Gram-negative bacteria represented the higher natural infectious causes than gram-positive bacteria in Qarun Lake. The study revealed that there are three factors causing massive mortalities of fishes, namely blooming phenomenon, poor water quality (trace metals & physico-chemical parameters) and microbial pathogens in the aquatic environment.

1. INTRODUCTION

Algal bloom contributes to the natural "aging" process of a lake, and in some lakes can provide important benefits by boosting primary productivity (Oberemm, 1999). However, recurrent excessive blooms can cause dissolved oxygen depletion as a result of dead algae decay. In highly eutrophic (enriched) lakes, algal blooms were reported to lead to anoxia with consequent fish mortality during the summer (Oberemm, 1999). Similarly, heavy metals concentration causes a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnification in the food chain (Yilmaz *et al.*, 2007).

Fishes are considered as one of the most significant biomonitors in an aquatic system for the estimation of metal pollution concentration (Begum *et al.*, 2005). They offer several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes observed in habitats (Lamas *et al.*, 2007). In addition, fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through consumption causing chronic or acute diseases (Al-Yousuf *et al.*, 2000).

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The heavy metals concentration in fish tissues reflects earlier exposure to water and/or food. Birungi *et al.*, (2007), studies from the field and laboratory experiments showed that accumulation of heavy metals in a tissue is mainly dependent upon concentrations of metals and in water and exposure period; besides other environmental factors such as salinity, pH, hardness and temperature. The ecological damage of the aquatic environment are mainly related to anthropogenic factors as well as the presence of hazardous, infections and parasitizing agents (Silveira *et al.*, 2004). Histological analysis is used in fisheries-related sciences to compare normal and pathological signs- related to diseases, toxicant exposures (Pieterse, 2004). Bacterial diseases are also, considered as one of the main cause of high mortalities among wild fish and fish farms (Austin and Austin, 2007).

Clinical signs, whether external and/or internal caused by each pathogen are dependent on the host species, age and stage of the disease (acute, chronic, subclinical carrier). However, in some cases, there is no correlation between external and internal signs. In fact, systemic diseases (i.e. pasteurellosis, piscirickettsiosis) with high mortality rates cause, internal signs in the affected fish but often present a healthy external appearance. On the contrary, other diseases with relatively lower mortality rates (flexibacteriosis, winter ulcer syndrome some streptococcosis) cause significant external lesions, including ulcers, necrosis and exophthalmia (Alicia *et al.*, 2005).

The objective of this study is to evaluate the environmental changes (physico-chemical parameters and trace metals), microbial status and histopathological changes among some marine fishes (*S. vulgaris* and *Mugil* spp.) during blooming phenomenon (Red-tide), february 2010 in Qarun Lake.

2. MATERIALS AND METHODS

2.1 Blooming phenomenon:

Qarun Lake is exposed to a recurrent blooming phenomenon revealed since the year (2008-2010) during the winter or autumn seasons. This phenomenon was observed to be

accompanied by massive fish mortalities and discoloration of the lake's water, revealing a red-tide. The most common reported type of harmful algal blooms (HABs) is referred to a (Red-tide) since they produce potent hepatotoxins or neurotoxins that can be transferred through the food web which may kill other life forms such as zooplankton, shellfish, fish, birds, marine mammals and even humans (through feed, either directly or indirectly). Also, harmful algal blooms can block sunlight from phytoplankton under the water's surface leading to decreased food and oxygen levels (NOAA, 2009) and (Ibrahim, 2007).

2.2. Study area:

Lake Qarun is the only enclosed saline lake in Egypt. It is located in the western desert part of Fayoum depression and lies 83 km southwest of Cairo. The lake is located between longitudes of 30° 24' & 30° 49' E and latitude of 29° 24' & 29° 33' N. It is bordered from its northern side by the desert and by cultivated land from its south and southeastern side (Abdel-Satar *et al.*, 2010). The lake receives the agricultural drainage water from the surrounding cultivated land. The drainage water reaches the lake by two huge drains, El-Batts drain (at the northeast corner) and El-Wadi drain (near mid-point of the southern shore).

2.3. Water sampling and analysis:

Three water samples were collected for physical, chemical and trace metals evaluations during blooming phenomenon (February 2010). The first sample (I) was collected at northeast region of the Lake. The second sample (II) was collected at Middle East region of the Lake. The third sample (III) at southwest area of the Lake. The physical-chemical and trace metals analysis of water were measured according to APHA, (2002). Three parallel subsurface water samples were collected from the same stations in sterile glass tubes to be used in bacteriological analysis.

2.4. Fish sampling:

2.4.1 Fishes collected for trace metals residual analysis in muscle tissues:

Five samples from both of *S. vulgaris* and *Mugil* spp. were collected from Qarun Lake during the same period. Total body length (cm) and weight (g) for *S. vulgaris* and *Mullet Sp.*

were 16.0 - 28.5 and 19.5 - 30.0 cm, 37.5 - 150.0 and 167.0 - 378.0 g respectively. A block of edible portion (muscle), devoid of skin and bone, was taken and stored in a deep freezer (-20 °C) until processing for metal analysis. Trace metals were extracted from fish muscle samples according to AOAC, (1995). Total concentrations of the heavy metals, namely Fe, Mn, Zn, Cu, Pb and Cd in fish muscle samples were determined using a flame atomic absorption spectrophotometer (Model 2380, Perkin Elmer, USA).

2.4.2 Fish samples collected for microbiological examination:

Ninety moribund and freshly dead fishes of *Solea vulgaris* and *Mugil* spp. were collected from Qarun Lake. Water and Fish samples were placed in strong aseptic bags and put in an ice box and transported to the laboratory of Shakshouk Research Station (NIOF) for examination. All fish samples were subjected directly to clinical, bacteriological and postmortem examination according to Buller, (2004). Signs and lesions were recorded. In addition, liver and gills were carefully removed and prepared for histological studies.

2.5. Culture media:

The following media (general and specific) were used for bacterial and fungal growth according to Popoff, (1984). Nutrient agar, nutrient broth (oxoid), MacConky agar, Brain heart infusion agar (oxoid), Tryptic soy agar and tryptic soy broth (Difco), blood agar, Triple sugar iron agar (oxoid), thiosulfate-citrate bile salt agar (TCBS), Rimler-Shots (R-S) media, (oxoid and difco), Salmonella-Shigella agar (SSA), Pseudomonas agar and Sabouraud dextrose agar (oxoid) were used. All media were supplemented with 1.5 % (w/v) NaCl and incubated at 25 ± 1 °C for 48 hours.

2.6. Bacterial isolation and identification:

Aseptic swabs from skin, lesions, gills, spleen, liver, kidney and water were inoculated onto general and specific media. The inoculated plates and broth were incubated aerobically at 25 ± 1 °C for 48 hours and 35 ± 2 °C at aerobic and anaerobic conditions. The growing colonies were subjected to culture and morphological characters, motility and gram reaction. In addition, microbial isolates were identified

using biochemical characterization according to APHA, (1985) and Murray *et al.*, (2003). Pure bacterial isolates were confirmed using the analytical profile index of API20-E system (Buller, 2004).

2.7. Fungal isolation and identification:

Under aseptic condition tissue, samples measuring approximately 5 - 10 mm were taken from dorsal fin, caudal peduncle and gills before inoculated onto sabouraud dextrose agar plates at 22 ± 1 °C for 2 - 7 days. Pure cultures were identified using single spore isolation method (Booth, 1971). It was based on culture characteristics, namely colony color, type of mycelium, shape and septation of conidia. The spores were stained with Lacto phenol cotton blue and microscopically examined.

2.8. Histopathological studies:

Fish tissue specimens collected were fixed in 10 % formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned into five micrometers thick, and then stained with hematoxylin and eosin method. Slide sections 3-5µ thickness were examined under light microscope and photographed by using a microscopic camera (Bemet *et al.*, 1999). Control group was collected from Shakshok Research Station, fish farm of (NIOF) (raised under optimal conditions).

3. RESULTS AND DISCUSSION

3.1. Physico-chemical characteristics of lake water quality:

The distributions of the most physical parameters of water quality e.g. Electrical Conductivity (EC) salinity, Total Solids (TS), Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) were found higher than the permissible limits of (WHO, 1993) as shown in (Table 1). This may be attributed to the evaporation rate, the intrusion of drainage water and consumption of lake salts by EMISAL Company as mentioned by Abdel-Satar *et al.* (2010).

The decrease in Dissolved Oxygen (DO) concentration reaching 5 mg/l may be due to the fall in water temperature and phytoplankton blooming (Konsowa, 2007). During algal and

phytoplankton blooming, the DO concentration decrease and CO₂ increase, leading to decrease in pH (6.80) and CO₃²⁻ not detected as well as HCO₃⁻ concentration increase reaching 544.3 mg/l. Cl⁻, SO₄²⁻, Ca²⁺ and Mg²⁺ concentrations in Qarun Lake water were found to be higher than the permissible limits of (WHO, 1993), (Table 1). This return to the intrusion of drainage and agricultural wastewater together with modifications observed in environment and climate (Edwards and Withers, 2008).

Natural water has small concentrations of nitrates and phosphorus. However, these nutrients increase with runoff from agricultural lands (especially intensively cultivated lands with large inputs of synthetic fertilizers) and urban wastewater, creating eutrophication (Liu *et al.*, 2009). The present results declared that ammonia accounted for the major proportion of total soluble inorganic nitrogen. The ammonia concentrations ranged between (850 – 1450 µg/l) higher than the permissible limits of (WHO, 1993) (50 – 500 µg/l). Nitrite showed very low levels (2.18 – 3.26 µg/l) than the corresponding values of nitrate (37.6 – 61.2 µg/l) due to the fast conversion of NO₂⁻ to NO₃⁻ ions by nitrifying bacteria (Abdel-Satar *et al.*,

2010). NO₂⁻ and NO₃⁻ are within permissible limits of (WHO, 1993).

Phosphorus that enters the aquatic system through anthropogenic sources, e.g. fertilizer-runoff, potentially could be incorporated into either inorganic or organic fraction. Once phosphorus accumulates within a lake, it can cycle through the water column and promote algal blooms indefinitely (Edwards and Withers, 2008). The PO₄³⁻ and total phosphorus (TP) concentrations, (Table 1) showed lower rates than that measured in the same lake by Abdel-Satar *et al.*, (2010) and ranged between (235 – 1074 and 743 – 2925 µg/l) respectively. This reflects the indirect negative effect of algal blooming on the food web by decreasing the amount of edible phytoplankton that zooplankton and other primary consumer need to survive on (NOAA, 2009). In addition, the higher values of silicates (10.11 – 10.5 mg/l) was concurrently revealed with diatoms abundance and algal blooming in Qarun Lake. This is supported by (Konsowa, 2009) who reported abundance of diatoms (75 %) of total phytoplankton density from July to the end of autumn at the same lake. SiO₃ concentrations were higher than permissible limits of (WHO, 1993), (Table 1).

Table1: Physico-chemical analysis of Qarun Lake water during blooming phenomenon, February 2010.

stations parameters	I	II	III	Permissible limits WHO, (1993)
Air Temp. °C	20	20	22	
Water Temp. °C	18	18	20	25 – 35
Transp. cm	40	50	45	
EC µS/cm	21900	43400	40700	400 - 1400
Salinity	13.1	27.8	25.6	-
TS mg/l	18414	36040	33300	500 – 1500
TDS mg/l	15200	31940	30000	400 – 1200
TSS mg/l	3216	4100	3300	None
DO mg/l	5	5	4.5	6 – 14
BOD mg/l	4	4	3.5	Up to 6
COD mg/l	40	30	35	Up to 10
pH	7.35	6.80	7.1	7 – 8
CO ₃ ²⁻ mg/l	Nil	Nil	Nil	-
HCO ₃ ⁻ mg/l	544.3	428	486	20 – 150
Cl ⁻ mg/l	11700	14180	13160	200 – 600
SO ₄ ²⁻ mg/l	1859	2606	2440	200 - 400
Ca ²⁺ mg/l	241	480	388	75 – 200
Mg ²⁺ mg/l	610	1269	1085	30 – 150
NO ₂ ⁻ µg/l	2.18	3.26	2.25	None
NO ₃ ⁻ µg/l	37.6	61.2	51.3	2500 – 5000
NH ₃ µg/l	1450	850	990	50 – 500
SiO ₂ mg/l	10.5	10.11	10.30	1 - 10
PO ₄ ³⁻ µg/l	168	101	120	400-500
TP µg/l	504	303	310	-

3.2. Heavy metals concentration in water and fish muscles:

The obtained results of the average concentration levels of Fe, Cu and Cd (235, 13.3 and 9.33 $\mu\text{g/l}$) in water (Table 2) were lower than a previous study reported by Abdel-Satar *et al.*, (2010) (average: 400, 30 and 20 $\mu\text{g/l}$) respectively in the same lake. However, Mn, Zn and Pb values in the present study demonstrated higher

concentrations (average: 70, 40 and 86 $\mu\text{g/l}$). This results reflect that the anthropogenic influences rather than natural environment of the water may be the main reasons (Wasim Aktar *et al.*, 2008) where Cd is present as an impurity in several products, including phosphate fertilizers and detergents. Fe, Zn and Cu are within permissible limits of (U.S.EPA, 2006), but Mn, Pb and Cd were higher than permissible limits, (Table 2).

Table 2: Trace metals concentration ($\mu\text{g/l}$) in water samples of Qarun Lake during blooming phenomenon, February 2010.

Water samples Elements	I	II	III	Permissible limits U.S.EPA, (2006)
Fe	300	150	255	1000
Mn	150	100	135	100
Zn	24	84	60	120
Cu	18	10	12	90
Pb	170	128	150	100
Cd	6	11	11	10

On the other hand, the concentration levels of Fe, Mn, Zn, Cu, Pb and Cd in fish muscles of *S. vulgaris* and *Mugil* in the present study were found to be (543, 7.75, 225, 37.5, 202, 18) and (440, 7.25, 230, 38.1, 200, 18.5 $\mu\text{g/l}$) respectively, higher than the permissible limits of WHO, (1993), except for Mn which was within normal limits (Table 3). Our results are higher than that previously reported in the same lake (Ali and Fishar, 2005) and in Bardawil Lagoon (Abdo and Yacoub, 2005).

The interference between several factors such as surrounding environment, closed basin, climatic conditions and algal blooming effects may suggest the irregular distribution pattern of trace metals in Qarun Lake water and fish muscles.

Consequently, heavy metals may be regarded as potential hazards that can endanger both animal and human health. The obtained results of this study revealed that concentration levels of heavy metals in water (Table 2) and bioaccumulation in fish tissue (Table 3) were higher than the permissible limits and may be regarded as the second main cause for the massive mortalities and pathological changes observed in Qarun Lake fishes. However, bioaccumulation of trace metals does not only depend on the structure of the organ, but also on the interaction between metals and the target organs. Fish could accumulate trace metals and act as pollution indicators (Mersch *et al.*, 1993).

Table 3: Trace metals concentration $\mu\text{g/g}$ in fish muscles tissue of Qarun Lake during blooming phenomenon, February 2010.

Fish species Elements	<i>S. vulgaris</i>	<i>Mugil sp</i>	WHO, (1993)
Fe	543	440	5
Mn	7.75	7.25	100
Zn	225	230	40
Cu	37.5	38.1	5
Pb	202	200	2
Cd	18	18.5	0.5

3. Microbial infections and diseases:

In the present study the infection rate among fish recorded 81.1%.The prevalence of microbial infection among fish were related to Gram-negative bacteria identified as, (67.1) % *V. anguillarum*, (10.95 %) *Ps. fluorescens*, (8.21 %) *Y. ruckeri* (2.73 %) *A. hydrophila*, (2.73 %) *A. sobria* and Gram-positive bacteria represented in (8.21 %) *S. pyogenes*, (1.37 %) *E. faecalis*, and (1.37 %)

Staph. Aureus. Also fungus infection presented (1.37 %) and is represented by *Asperagillus niger* (Table 4). However, stress plays a major role in susceptibility of fish to infectious diseases. Consequently, massive mortalities and pathological changes in fishes accompanied blooming phenomenon.

Table 4: Natural infection among water and marine fishes in Qarun Lake (Feb. 2010) during blooming phenomena.

Microbial isolates Fish & Water samples	Examined Samples		Aeromonas spp.				Streptococcus spp.				<i>Y. ruckeri</i>	<i>V. anguillarum</i>	<i>P. fluorescens</i>	Staph. aureus	Other organisms							
	Examined No.	Infected	A. hydrophila		A. sobria		S. pyogenes		E. Faecalis													
			No.	%	No.	%	No.	%	No.	%												
<i>S. vulgaris</i>	50	45	90	2	4.44	1	2.22	4	8.88	1	2.22	-	-	35	77.7	2	4.44	1+ (A*)	2.22	1 (A*)	2.22	
<i>Mugil</i>	40	28	70	-	-	1	3.57	2	7.14	-	-	6+(As*)	21.42	14	50	6	21.42	-	-	-	-	
Total	90	73	81.1	2	2.73	2	2.73	6	8.21	1	1.37	6	8.21	49	67.1	8	10.94	1	1.37	1(A*)	1.37	
North east	3	3			+(E*)									+(Staph*)								A/A.niger
Middle	3	3			(+*)									+								C/ Candida albicans
South west	3	2												+(L*)								L/ Lactococcus

(+*) = Some fish have more than one microbial infection.
 Plus to: A=*Asperigilles niger*. C = *Candida albicans*. L = *Lactococcus lactis*. B = *Lactococcus Sp.*
 Staph. = *Staph.aureus*.
 Ps = *Pseudomonas fluorescens*. E. *Enterococcus faecalis*. A.s. = *Aeromonas sobria*.

Fish in Qarun Lake exposed suddenly to environmental fluctuations including blooming phenomenon revealed some clinical signs as superficial irregular red ulceration with hemorrhagic lesion on the skin and base of fins (Figs.1&2). Also, exophthalmia, abdominal distension and vent inflammation were observed as well as eye cataracts in *Mugil* sp. (Fig. 2) All clinical

signs of bacterial septicemia were observed in Qarun Lake fishes. Similar, clinical signs and pathological lesions were reported by (Abou El-gheit, 2005); Austin and Austin, 2007). The infection incidence of pseudomonas septicemia in our study among Qarun Lake Fishes recorded 10.95 % while Eissa *et al.*, (2010) in January, 2009 recorded 17.77 % in *O. niloticus* collected from farms around Qarun and El-Rayan Lakes.

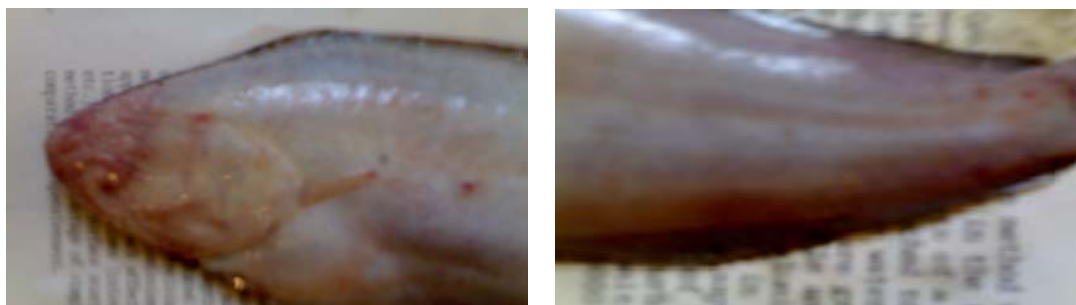


Fig.1: *S. vulgaris* showing sever and clear hemorrhagic lesions on the skin, caudal peduncle, around the mouth and operculum.



Fig.2: Mullet fish showing Sever Hemorrhagic lesions on the skin and base of fins, exophthalmia, vent inflammation and abdominal distension.

Moustafa *et al.* (2010), reported that Qarun Lake and Suez Gulf are among the most vulnerable areas suffering from continuous depletion due to devastating environmental changes and prevalence of bacterial infection among marine fishes which recorded in winter season (15.91 %). Moreover, their results were lower than ours during the same period, which recorded 81.1 %. This may be attributed to fish immune suppression and bacterial virulence during the event of blooming phenomenon. Eissa *et al.* (2010) recorded that the infected fishes, *S. vulgaris* and *Mugil* sp. in Qarun Lake recorded 80 % and 50%, respectively. In addition general septicemic lesions, some cutaneous ulceration, frayed fins have been noticed in fish infected with vibriosis and streptococcosis while reddening around the mouth and ascities (yersiniosis) in *mullet*. The main causative agent of sever mortality among Qarun Lake fish was vibrio species.

The present study revealed that infection with Gram-negative bacteria was higher than the infection with Gram-positive bacteria. These results agreed with Zorrilla *et al.* (2003) who declared that the main microorganisms isolated from diseased gilt-head seabream in the marine water at south western Spain were; *Vibrio* spp, *Pseudomonas* spp, *P. piscici*, *Flavobacteria maritimus*, *Aeromonas* spp. and Gram positive bacteria were also isolated but in

low percentage. Moustafa *et al.* (2010) recorded that, Gram-negative bacteria prevailed the Gram-positive isolates with *Vibrio anguillarum*, *Vibrio alginolyticus*, *Pasteurella piscicida* (*photobacterium damsella subspp piscicida*), *Pseudomonas fluorescens*, *Streptococcus fecalis*, *Aeromonas hydrophila*, *Aeromonas sobria* and *Staphylococcus aureus* being the most common isolated spp. from Qarun Lake and Suez Gulf marine fishes. Alicia *et al.* (2005) reported that bacterial infections such as *Vibriosis*, *Aeromonads*, *Pseudomonads*, *Photobacteria*, *Streptococci* and *Staphylococci* were recorded in several fingerlings, juveniles, adults and brood stocks of some marine fish species

The present study showed that the incidence of infection of *A. hydrophila* in winter season was 2.73 %. Moustafa *et al.* (2010) reported the highest prevalence of *A. hydrophila* in winter season (5.71 %). Similarly, Pathak *et al.*, (1988) recorded that the highest isolation rates of *A. hydrophila* occurred during late winter followed by a progressive decline in density during summer and monsoon seasons. Popovic *et al.* (2000) mentioned that there was clear seasonality in the prevalence of *A. hydrophila* as there were no isolates recovered in the summer months.

Yersinia could be spread through water and carrier fish serve as important

source of infection under stress conditions (Alicia *et al.*, 2005). Abou El-gheit, (2005) mentioned that facultative microbial fish pathogens such as *Yersinia*, *Aeromonas*, *Pseudomonas*, *Vibrio*, *Streptococcus*, etc., are continuously present in water and carrier fish in Qaroun Lake fishes. These reports agreed with our results, which recorded 8.1 % *Y. ruckeri* from natural infected fish.

Yanong and Francis-Floyd (2002), mentioned that most infectious diseases of fish are opportunistic. This means that the simple presence of the pathogen in the environment of the fish is insufficient to cause a disease outbreak. Stress often plays a significant trigger in outbreaks of opportunistic infectious disease in fish populations some stressors that have been associated with streptococcosis outbreaks include high water temperatures, high stocking densities, harvesting or handling and poor water quality such as high ammonia or nitrite concentrations.

V. anguillarum infections in the present study was 67.1 % among Qarun Lake fishes in winter Season. However, Moustafa *et al.* (2010) recorded (0.4 %) *V. anguillarum* infections in the same area and season. *Streptococcosis* affects fish of any size or age, and therefore cautions must be taken during all stages of the production cycle. In regards to the seasonal incidence of streptococcal infection, there is a general acceptance for the division of *Streptococcosis* into two forms according to the virulence of the agents involved at high or low temperatures (Ghittino, 1999). "Warm-water" *Streptococcosis*, causing mortalities at temperatures higher than 15°C, typically involves species such as *Lactococcus garvieae* (formerly *Enterococcus seriolicida*), *Streptococcus iniae*, *S. agalactiae* or *S. parauberis*. On the other hand, cold-water" *Streptococcosis* caused by *Vagococcus salmoninarum* and *Lactococcus piscium*, occurs at temperatures

below 15°C. These study agree with our results, which recorded 9.58 % of streptococcal infections, 8.21% of *St. pyogens* and 1.34% of *E. faecales* among different size and age of Qarun Lake fish in winter season .

Only one case of *Lactococcus* sp. (1.37 %) was recorded from total infected fish. *Lactococcus* sp. may play a role in mortality rate among fish as mentioned by Romalde and Toranzo (2002), who recorded that *L. garvieae* infects salt water fish species, such as yellowtail in Japan and fresh water species like rainbow trout (mainly in Italy, Spain, France and to a lesser extent in UK and Australia)

On the other hand, the natural infection rate of *Staphylococcus aureus* among Qarun Lake fish in winter season recorded (1.37 %). These results were lower than results recorded by Athanassopoulou *et al.* (1999) which showed total prevalence of Staph. Epidermidis among diseased *Puntazzo puntazzo* in marine aquaculture systems in Greece was (10 %). However, Moustafa *et al.* (2010) has not recorded any *Staphylococcus* infection among infected Qarun Lake fish in winter season, whereas, *Aspergillus niger* recorded one case (1.37 %) among infected fish. Ogbanna and Alabi, (1991) mentioned that few species such as *A. flavus* and *A. niger* have been implicated in disease of fresh water fish and their fertilized eggs. Massive mortalities in Qarun Lake fishes may be attributed to the third factor (bacterial virulence and immune suppression).

4. Histopathological changes in fish:

The results of the present study revealed that *S. vulgaris* and *Mugil* sp. from Qarun Lake, manifested histopathological changes in liver and gills. Qarun Lake fishes are exposed to blooming phenomenon, heavy metals, poor water quality and microbial pathogens. Liver of *Solea vulgaris*, exhibited balloon necrosis in hepatocytes, fatty degeneration, focal areas of necrosis, severe degeneration and pyknotic in hepatocytes (Fig. 3). The liver of *Mugil* sp. exhibited hemorrhages, necrosis and degeneration in (Fig. 4).

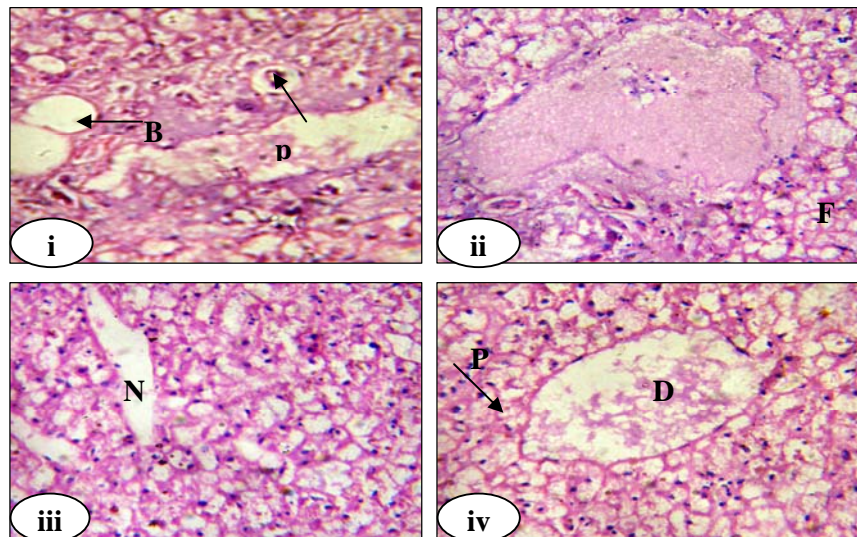


Fig 3 : Liver of *Solea vulgaris* showing : (i) balloon necrosis (B) and pyknosis (P) in hepatocytes ,(ii) Fatty degeneration (F) , (iii) focal areas of necrosis(N) and (iv) severe degeneration (D) and pyknosis in hepatocytes (P) .

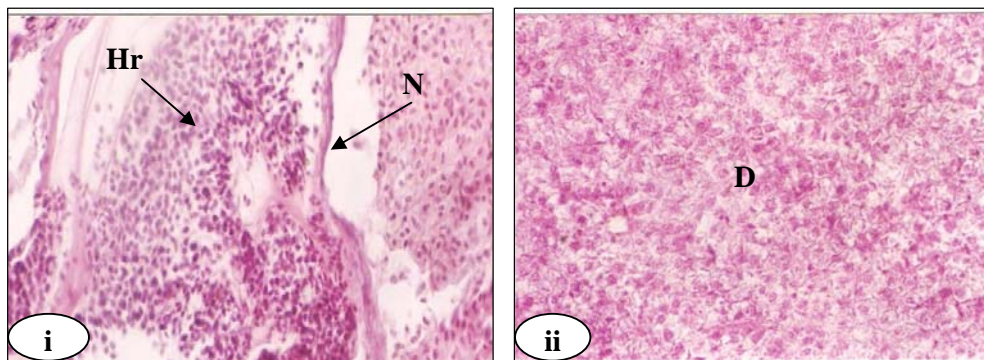


Fig. 4: Liver of *Mullet* H&E, formalin (10%) showing: (i) hemorrhages (Hr) and necrosis in hepatocytes (N) and (ii) degeneration in the hepatocytes (D).

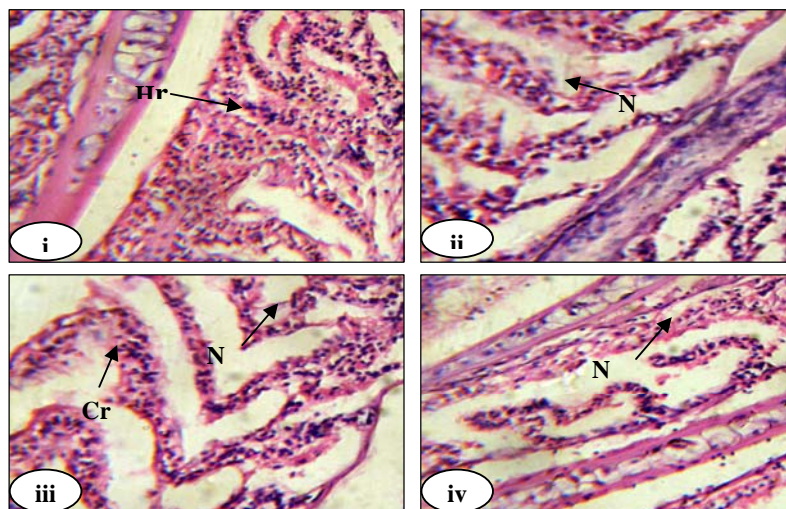


Fig. 5: Gill lamellae of *Solea vulgaris*, formalin 10% Showing: (i) hemorrhage (Hr) between gill filaments and (ii-iv) curling (Cr) and necrotic changes (N) in the epithelium of gill filaments and secondary lamellae (H&E).

Section of gill lamellae of *Solea vulgaris* revealed hemorrhage between gill filaments, curling, severe necrosis, hyperplasia and hemorrhages in the epithelium of gill filaments and secondary lamellae (Fig. 5). Sections of gill lamellae of *Mugil* showed, severe necrosis in the gill filaments of secondary and primary lamellae, curling in secondary lamellae and hypertrophy changes in the chloride cell of gill filament and hemorrhage in primary lamellae (Fig. 6). The results of histopathological changes in liver and gills may be attributed to toxins of algal

blooming, heavy metals and microbial pathogens. These results are supported by Soufy *et al.*, (2007) who reported vacuolar atrophy of degeneration in the hepatocytes, focal areas of necrosis, thrombosis formation in central veins, dilation in fish liver. Changes may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals. Olojo *et al.* (2005) observed degeneration of the hepatocytes and focal necrosis in the liver of *Clarias gariepinus* exposed to lead.

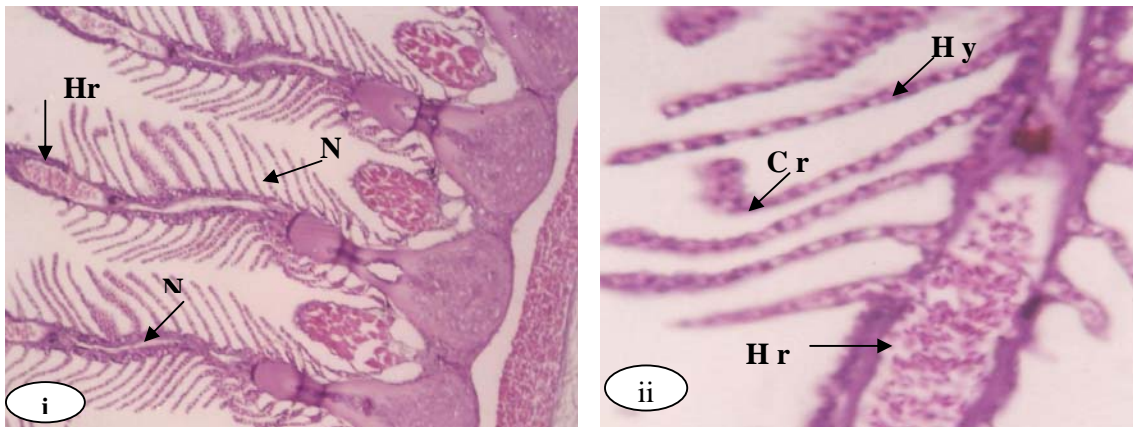


Fig. 6: Gill lamellae of *Mullet*, formalin (10%), showing: (i) severe necrosis (N) and hemorrhage (Hr) in the gill filaments of secondary & primary lamellae and (ii) curling (Cr) in secondary lamellae and hypertrophy (H) changes in the chloride cell of gill filament and hemorrhage (Hr) in primary lamellae, H&E.

Exposure of *Oncorhynchus mykiss* to copper sulphate was found to induce degeneration of hepatocytes, sinusoidal dilation and congestion in the blood vessels of the liver (Atamanalp *et al.*, 2008). Also, Mohamed (2008) showed that several histopathological alterations in the liver of *Oreochromis niloticus* and *Lates niloticus* from Nasser Lake contaminated with some heavy metals. The histopathological alterations in the livers of both fish from Nasser Lake were more or less similar. Moreover, haemosiderin was seen around central veins and hepatoportal blood vessels (Eder and Gedigk, 1986). While,

histopathological changes of gills supported by Tribskorn *et al.*, (2007) noticed epithelial lifting, proliferation of epithelial cells of primary and secondary lamellae, hyperplasia of mucous cells and necrosis of epithelial cells in the gills of *C. nasus* and *L. cephalus* from River Mures, Western Romania, polluted by heavy metals, faecal coliforms and streptococci bacteria. In this respect, Fernandes and Mazon, (2003) suggested that the bacteria produce an extracellular hyperplasia-inducing factor, which can reproduce typical Bacterial Gill Disease (BGD) lesions.

The gills of both studied fish showed degenerative, necrotic and proliferative changes in gill filaments and secondary lamellae, edema in gill filaments and secondary lamellae and congestion in blood vessels of gill filaments. These pathological changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants thorough the gill surface (Fernandes and Mazon, 2003).

The present results are in agreement with those observed in other fish species under the influence of different pollutants.

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