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Distribution of bacteria in Lake Qarun, AL Fayoum, Egypt (2014 -2015) in relation to its physical and hydrochemical characterization

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Abstract

The bacteriological monitoring of Lake Qarun water and sediment (aerobic heterotrophs, *Staphylococcus* sp., *Vibrio* sp. *Aeromonas* sp., *S. feacalis*, *E. coli*, and total coliform sp.) through the period of study (2014-2015) was carried out. Six common bacterial isolates were fully identified as; *Bacillus firmus*, *Bacillus stratosphericus*, *Exiguobacterium mexicanum*, *Stenotrophomonas rhizophila*, *Halomonas stevensii*, and *Halomonas korensis* based on partial sequencing of 16Sr DNA. In addition, physical and chemical analyses of Lake Qarun water and sediment (pH, temperature, salinity, dissolved oxygen, BOD, COD, and nutrients) were estimated. The pH varied between 7.08 at sit I and 8.73 at sit V. The temperature varied between 15.6°C at sit VIII and 32.2°C at sit I. The salinity ranged between 3.18‰ at sit I and 51.72‰ at sit III. The dissolved oxygen fluctuated between 3.89 mg/l at sit VI and 8.96 mg/l at sit IV. The BOD ranged between 1.08 mg/l at sit VIII and 6.47 mg/l at sit IV. The COD varied between 11.36 mg/l at sit I and 53.64 mg/l at sit VIII. The phosphate in lake water varied between 1.453.4 µg/l at sit VIII and 498.4 µg/l at sit I. The ammonia ranged between 14.73 µg/l at sit II and 1485 µg/l at sit I. The nitrite varied between 4.10 µg/l at sit IV and 646.5 µg/l at sit I. The nitrate varied between 14.79 µg/l at sit III and 2138 µg/l at sit I. The silicate fluctuated between 894.6 µg/l at sit VI and 4682 µg/l at sit VII.

Keywords: Distribution of bacteria, physical characterization, hydrochemical characterization, Lake Qarun.

1 Introduction

Lake Qarun is the only enclosed, saline, highly eutrophied lake among the inland lakes of Egypt, snuggling

forty-five meters below sea level into the lowest, northern section of El- Fayoum Depression, Egypt. Although Lake Qarun designated as protected area back in 1989, the Lake has hardly been protected from various polluting elements. It suffers from a serious water pollution problem which is due to uncontrolled solid and liquid domestic and industrial waste disposal practices, in addition to agrochemical contamination and lack of sustainable wastewater management. Many fish farms were established around this Lake (Mansour and Sidky, 2003). The Lake suffered drastic chemical changes during the last years where it is used as a general reservoir for agricultural wastewaters drainage of El-Fayoum Governorate, as well as for the drainage of the fish farms. Therefore, its salinity increases progressively which affects greatly the Lake biota. In addition, the exacerbation of eutrophication of the Lake's water is caused by the nutrient load from the agricultural drainage water (Sabae and Ali, 2004). Moreover, groundwater appears to be continuously seeping from a number of sub-surface springs at the Lake bottom. The Egyptian Company for Salts and Mineral (EMISAL) located on the southern coast of the Lake, where a part, known as Batnat Abo Ksah, was cutoff from the Lake and divided into number of concentrating ponds, to concentrate the Lake water as much as 10 times the original salinity. The effluents of EMISAL brine water discharged also into the Lake and caused increasing in the most parameters (Shaltout *et al.*, 2015). Lake monitoring may provide early warning signs of ecosystem degradation resulting for example from contaminant inputs, nutrient addition, sediment runoff. By monitoring the physical, chemical, and biological status of a Lake, changes for many aspects of the ecosystem can be

detected quickly, and hopefully, harmful impacts can be eliminated before their consequences become unmanageable (Leiser *et al.*, 2015). Therefore, the present study was aimed for the assessment of bacterial distribution in Lake Qarun (2014-2015) in relation to study the seasonal changes at the physical and chemical properties of water and sediments.

2 Materials and Methods

Study area

In the current study, water and sediment samples were collected from the Qarun Lake during summer 2014 and spring 2015. Lake Qarun (Figure 1), located about 27 kilometers north of Al Fayoum City and 80 kilometers southwest of Cairo. Al Fayoum is not far from the Nile Valley, and it is one of Egypt's most treasured natural landmarks and a resource that has helped support human culture for some 8,000 years. It is the only natural contemporary lake of any size in Middle Egypt. It is therefore rich in both natural and archaeological resources. All samples were collected from eight stations that are distributed along Lake as shown on the map (Figure 1). However, they are included as follows:

- ✓ Station I: From the front of the Bank of Bats,
- ✓ Station II: From the front of machines, agricultural drainage (in front of Treasures village),
- ✓ Station III: From the front of EL-Auberge,
- ✓ Station IV: From far northeast Qarun Lake,
- ✓ Station V: From the front of the Lesan Abunimah,
- ✓ Station VI: From the front of the village Shakshuk,
- ✓ Station VII: From the front of Valley Bank,
- ✓ Station VIII: From the front of the village of Egypt for reconstruction station.

Methods

Sample collection for bacterial isolation

Water samples were collected in 500 ml sterile screw-capped bottles as previously described by Austin (1988). The central portion of the top 2 cm sediment samples was taken out with the help of a sterile spatula. The samples were then transferred to a sterile polythene bag.

Isolation of bacteria from water and sediment samples

Serial dilutions from 10^{-2} to 10^{-6} were made using filtered sterilized distilled water. A portion (0.1 ml) from each appropriately diluted sample was used to inoculate plates prepared with nutrient agar for counting aerobic heterotrophs. The sediment samples were collected by sediment sampler (Peterson grab), it was sterilized with alcohol before sampling at each station.

Plates were incubated at 30-37°C for 24-48 h. Plates of seven selective media used were inoculated with 1 ml of appropriately dilution sample for counting the different bacterial groups: *Staphylococci*, *Vibrios*, *Aeromonas*, *Salmonella*, *Shigella*, while total coliforms, faecal *Streptococci* and *Escherichia coli* were estimated according to ISO (SO 9308/1; 1990 and ISO 7899/2; 1984).

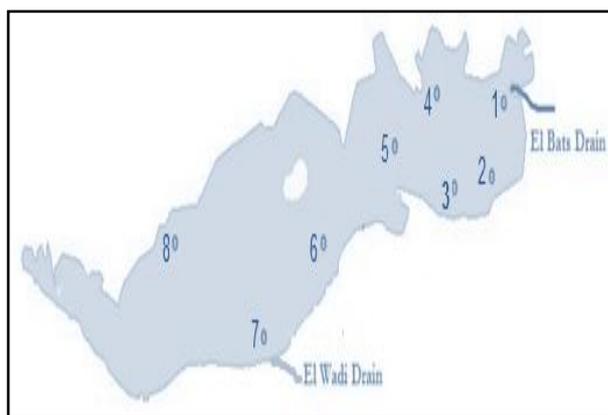
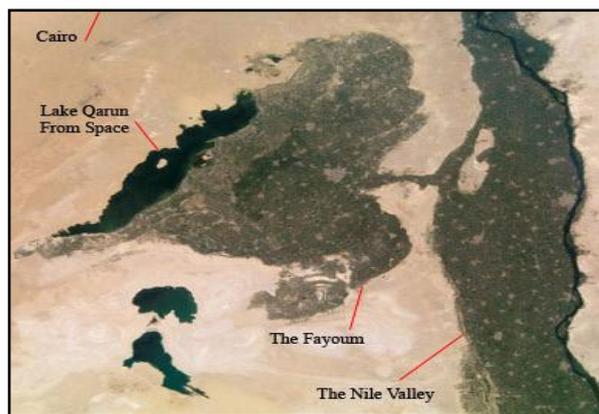


Figure 1: The location of Lake Qarun in the southwest of Cairo and from the Nile Valley (<http://www.touregypt.net>) and distribution of sampling sites on the Lake

Characterization of the selected bacteria

Genotype characterization

The common bacterial isolates were cultured in NB medium for 2 days and genomic DNAs were extracted with genomic DNA extraction protocol of GeneJet genomic DNA purification Kit (Fermentas). PCR using Maxima Hot Start PCR Master Mix (Fermentas) and PCR clean up to the PCR product using GeneJET™ PCR Purification Kit (fermentas) were determined at Sigma Scientific Services Co. Egypt. The sequencing to the PCR product on GATC Company was made by using ABI 3730xl DNA Sequencer by using universal primaries (16S 27F; 5' AGAGTTTGATCCTGGCTCAG 3' and 16S 1492R; 3' GGTTACCTTGTTACGACTT 5'). Bacterial isolates were submitted to genotypic characterization through 16S rRNA gene technique. The sequences were compared with known sequences in the Gene Bank nucleotide database and identified as the nearest phylogenetic neighbor with the highest similarity percent (Hentschel *et al.*, 2001).

Phenotype characterization

Phenotypic characteristics such as Gram's staining, motility, cultural characteristics, catalase, oxidase and IMViC test of all the marine bacterial isolate was studied by adopting standard procedures. Effect of sodium chloride, pH level, and temperature on growth was tested. All the tests were performed by adopting standard procedures (Garrity *et al.*, 2005).

Physical analyses of lake water

The following operations (pH, temperature and salinity) were performed for each water sample, some of which were performed in the field. Salinity was measured using Beckman (No. R.S.7C) salinometer calibrated using standard sea water. The conductivity ratio was measured to the nearest 7×10^{-5} (Wooster *et al.*, 1969).

Hydrochemical analyses of lake water and sediment

Dissolved oxygen (DO)

Dissolved oxygen was determined by Winkler's method (Strickland and Parsons, 1972). Titration was carried out using 200 ml of the sample against 0.025 N solution of sodium thiosulfate. However, the thiosulfate solution was standardized against potassium bi-iodate solution 0.025 N. The dissolved O_2 expressed in mgO_2l^{-1} was calculated by the following equation: $[DO (mg l^{-1}) = (V_{titrant} \times N_{titrant} \times 8000) / V_{sample}]$.

Biological (BOD) and chemical oxygen demand (COD)

Water samples for BOD determination were collected in parallel with DO samples in 300 ml BOD bottles and avoided formation of air bubbles. The bottles were then incubated in the dark for five days at $27^\circ C$. After that, dissolved oxygen was determined by Winkler's method and expressed in $mg O_2l^{-1}$. The BOD was calculated through the following equation: $[BOD (mgO_2l^{-1}) = D1 - D2]$ (APHA, 1992); Where: D1= initial dissolved oxygen concentration (mgO_2l^{-1}) and D2= sample DO (mgO_2l^{-1}) after 5 days. On the other side, the chemical oxygen demand is the quantity of oxygen required to oxidize the organic matter in water, under specific conditions of oxidizing agents, temperature and time. The organic and oxidizable inorganic substance in the water sample were oxidized by potassium dichromate and then titrated against standard solution of ferrous ammonium sulphate using orthophenanthroline ferrous complex (ferroin) as an indicator (Medalla, 1951).

Nutrients in lake water

Samples for nutrient analysis were collected in polyethylene bottles of 1 liter capacity (except for inorganic phosphate) where it should be stored in hard glass bottle. Few drops of chloroform were then added to prevent biological activities. The water samples were directly deep frozen at $-25^\circ C$ before carrying out chemical analysis (Grasshoff, 1976). The samples were filtered as soon as possible through $0.45 \mu m$ membrane filter. Immediate analysis was recommended but if it wasn't possible, samples was directly deep frozen till carrying out chemical analysis. Several methods employed for the determination of dissolved phosphate Grasshoff (1976), dissolved nitrate (Strickland and Parsons, 1965), dissolved nitrite

(Grasshoff, 1976), dissolved ammonia Koroleff (1969), and dissolved silicate (Grasshoff *et al.*, 1999) were suggested.

3 Results

The current study is one of the many investigations that focus on state of the lake from the bacteriological and chemical point of view. All these attempts aim to draw the attention of the government to make more of an effort to stop or control the different pollution features of the lake. The present study presented through the results the bacteriological monitoring of Lake Qarun water and sediment (aerobic heterotrophs, *Staphylococcus* sp., *Vibrio* sp. *Aeromonas* sp., *S. feacalis*, *E. coli*, and total coliform sp.), beside the physical and chemical parameters of Lake Qarun water and sediment (pH, temperature, salinity, dissolved oxygen, BOD, COD, and nutrients) that affect the distribution of different bacterial groups.

Distribution of bacterial groups in the water of lake

Aerobic heterotrophs as well as different bacterial groups recovered from water samples obtained from eight stations in lake during the period of study (2014 -2015) were isolated and counted on selective media previously described. Data are shown in Table 1. The total viable count of heterotrophic bacteria (TBVC) in lake water varied between 0.31×10^4 CFUml⁻¹ at sit VIII in winter and 200.4×10^4 CFUml⁻¹ at sit I in summer. The relative high values of TBVC were observed in spring season (120.1×10^4 CFUml⁻¹) in station I and 106.4×10^4 CFUml⁻¹ in the station I in autumn, while the relative low values of TBVC were observed in many stations especially during winter season. The total viable count of *Staphylococcus* sp. (SC) in lake water fluctuated between 0.05×10^4 CFUml⁻¹ at station V in winter and 9.55×10^4 CFUml⁻¹ at station I in summer. The relative high values of SC were observed in spring season (6.1×10^4) in station I and 5.48×10^4 CFUml⁻¹ in the station I in autumn, while the relative low values of SC were observed in many stations especially during winter season. The total viable count of *Vibrio* sp. (VC) in lake water ranged between 0.03×10^3 CFUml⁻¹ at station VII in winter and 75.3×10^3 CFUml⁻¹ at station VII in summer. The relative high values of VC were observed in summer season (30.1×10^3 CFUml⁻¹) in station I in summer, while the relative low values of VC were observed in many stations especially during winter season. The total viable count of *Aeromonas* sp. (AC) in lake water varied between 5.54×10^2 CFUml⁻¹ at station II in winter and 60.32×10^2 CFUml⁻¹ at station VII in summer. The relative high values of AC were observed in spring season (30.24×10^5 CFUml⁻¹) in station VIII, while the relative low values of AC were observed in many stations especially during winter season. The total viable count of *S. feacalis* (SFC) in lake water fluctuated between 0.30×10^5 CFUml⁻¹ at station III in winter and 56.13×10^5 CFUml⁻¹ at station I in summer. The relative high values of SFC were observed in summer season (50.8×10^5 CFUml⁻¹) in station VII, while the relative low values of SFC were observed in many stations especially during winter season. The total viable count of *E. coli* (EC) in lake water ranged between 0.10×10^5

CFUml⁻¹ at station VI in winter and 90.22x10⁵ CFUml⁻¹ at station I in summer. The relative high values of EC were observed in summer season (32.93x10⁵ CFUml⁻¹) in station III, while the relative low values of EC were observed in many stations especially during winter season. The total viable count of total coliform sp. (TC) in lake water fluctuated between 1.32x10⁵ CFUml⁻¹ at station IV in autumn and 126.0x10⁵ CFUml⁻¹ at station VII in summer. The relative high values of TC were observed in summer season (100.1x10⁵ CFUml⁻¹) and in spring (89.17x10⁵ CFUml⁻¹) in station I, while the relative low values of TC were observed in many stations especially during winter season.

Distribution of bacterial groups in the sediment of lake

Aerobic heterotrophs as well as different bacterial groups recovered from sediment samples obtained from eight stations in lake during the period of study (2014-2015) were isolated and counted on selective media. Data are shown in Table 2. The total viable count of TBVC in lake sediment ranged between 0.15x10⁵ CFUg⁻¹ at sit VII in winter and 60.49x10⁵ CFUg⁻¹ at sit I in summer. The relative high values of TBVC were observed in summer season (40.04x10⁴ CFUg⁻¹) in station VI, while the relative low values of TBVC were observed in many stations especially during winter season. The total viable count of SC in lake sediment varied between 1.74x10⁴ CFUg⁻¹ at station III in winter and 640.3x10⁴ CFUg⁻¹ at station I in summer. The relative high values of SC were observed in summer season (80.38x10⁴ CFUg⁻¹) in station VI, in spring 78.33x10⁴ CFUg⁻¹ in station I and others. Moreover, the relative low values of SC were observed in many stations especially during winter season. The total viable count of VC in lake water fluctuated between 0.13x10³ CFUg⁻¹ at station VII in winter and 35.24x10³ CFUg⁻¹ at station I in summer. The relative high values of VC were observed in summer season (27.33x10³ CFUg⁻¹) in station I in summer, while the relative low values of VC were observed in many stations especially during winter season. The total viable count of AC in lake water ranged between 2.86x10² CFUg⁻¹ at station VI in winter and 60.53x10² CFUg⁻¹ at station V in summer. The relative high values of AC were observed in spring season (44.73x10² CFUg⁻¹) in station V and 40.66x10² CFUg⁻¹ in station VI in summer. Moreover, the relative low values of AC were observed in many stations especially during winter.

Genotype and phenotype characterization of common isolates habited in Lake Qarun

Sequencing data were aligned against the 16S rRNA sequences of (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Data are shown in Table 3. It has been found that the bacterial isolate 19T showed 97% sequence homology to *Bacillus firmus*. It was observed that the sequence of the isolate 22T showed 98% sequence homology to *Bacillus stratosphericus*. It has been conducted that the isolate 14P showed 97% sequence homology to *Exiguobacterium mexicanum*. It has been observed that the isolate 11S

showed 97% sequence homology to *Stenotrophomonas rhizophila*. It has been found that the isolate 2V showed 97% sequence homology to *Halomonas stevensii*. It has been detected that the isolate 9S showed 89% sequence homology to *Halomonas korlensis*. On the other side, the isolates (19T, 22T, 14P, 11S, 2V, and 9S) were activated on a nutrient agar plate. Moreover, Table 4 shows some phenotypic characteristics of the selected isolates. These included colony and cell morphology, Gram reaction, catalase and oxidase test in addition to some physiological and biochemical experiments.

Physical analysis of lake water

Physical analyses (pH, temperature, and salinity) were determined in subsurface water. Data are shown in Table 5. The pH varied between 7.08 at sit I and 8.73 at sit V. The relative high values of pH (8.73) were observed in winter in the station V, while the relative low values of pH were observed in the station I during summer. The temperature varied between 15.6°C at sit VIII and 32.2°C at sit I. The relative high values of temperature (32.2°C) were observed in summer in the station I, while the relative low values of temperature were observed in the station VIII during winter. The salinity varied between 3.18% at sit I and 51.72% at sit III. The relative high values of salinity (51.72%) were observed in winter in the station III, while the relative low values of salinity were observed in the station I during summer.

Hydrochemical characterization of lake water

Some chemical analyses (DO, BOD and COD) were determined in subsurface water of lake. Data are given in Table 6. The dissolved oxygen varied between 3.89 md/l at sit VI and 8.96 md/l at sit IV. The relative high values of DO (8.96 md/l) were observed in winter in the station IV, while the relative low values of DO were observed in the station VI during autumn. The BOD varied between 1.08 mg/l at sit VIII and 6.47 mg/l at sit IV. The relative high values of BOD (6.47 mg/l) were observed in winter in the station VI, while the relative low values of BOD were observed in the station V during autumn. The COD varied between 11.36 mg/l at sit I and 53.64 mg/l at sit VIII. The relative high values of COD (53.64 mg/l) were observed in spring in the station VIII, while the relative low values of COD were observed in the station V during winter.

Nutrients profile of lake water

The phosphate (PO₄-P) in lake water during the period of study (2014 -2015) varied between 1.453.4 µg/l at sit VIII and 498.4 µg/l at sit I. The relative high values of PO₄-P (498.4 µg/l) were observed in summer in the station VIII, while the relative low values of PO₄-P were observed in the station I during autumn. The ammonia (NH₄-N) in lake water varied between 14.73 µg/l at sit II and 1485 µg/l at sit I. The relative high values of NH₄-N (498.4 µg/l) were observed in summer in the station I, while the relative low values of NH₄-N were observed in the station II during summer. The nitrite (NO₂-N) in lake water varied between 4.10 µg/l at sit IV and 646.5 µg/l at sit I. The relative high

values of NO₂-N (646.5 µg/l) were observed in autumn in relative low values of NO₃-N were observed in the station the station I, while the relative low values of NO₂-N were III during winter. The silicate (SiO₄) in lake water varied observed in the station IV during winter. The nitrate (NO₃- between 894.6 µg/l at sit VI and 4682 µg/l at sit VII. The N) in lake water varied between 14.79 µg/l at sit III and relative high values of SiO₄ (4682 µg/l) were observed in 2138 µg/l at sit I. The relative high values of NO₃-N (2138 winter in the station I, while the relative low values of SiO₄ µg/l) were observed in summer in the station I, while the were observed in the station III during autumn.

Table 1: Regional and seasonal of different bacterial groups in subsurface water samples of Lake Qarun during the period of study (2014 -2015)

Season	TBVCx10 ⁴ CFUml ⁻¹ /Station									
	I	II	III	IV	V	VI	VII	VIII	Min.	Max.
Autumn	106.4	47.3	22.3	1.43	41.5	5.10	6.36	1.09	1.09	106.4
Winter	40.3	38.3	2.05	0.45	0.33	0.60	0.34	0.31	0.31	40.30
Spring	120.1	52.0	28.3	2.10	43.1	5.40	6.50	24.4	2.10	120.1
Summer	200.4	55.1	30.4	60.5	56.4	6.36	7.83	25.3	6.36	200.4
SCx10 ⁴ CFUml ⁻¹ /Station										
Autumn	5.48	1.04	1.12	0.41	2.1	1.12	1.14	2.17	0.41	5.48
Winter	0.40	0.18	0.31	0.25	0.05	0.22	0.27	0.12	0.05	0.40
Spring	6.10	2.6	1.30	1.03	2.53	1.73	1.84	3.93	1.03	6.10
Summer	9.55	4.63	2.52	1.80	8.15	2.12	1.91	4.66	1.8	9.55
VCx10 ³ CFUml ⁻¹ /Station										
Autumn	5.10	0.12	1.07	0.13	0.20	0.19	0.11	0.81	0.11	5.1
Winter	0.13	0.10	0.2	0.04	0.18	0.14	0.03	0.51	0.03	0.51
Spring	7.10	1.40	1.50	0.14	0.25	0.22	0.123	1.00	0.123	7.1
Summer	30.1	1.40	8.04	4.80	1.61	1.29	75.3	2.40	1.29	75.3
ACx10 ² CFUml ⁻¹ /Station										
Autumn	14.47	7.29	24.27	20.29	10.63	17.37	9.59	22.5	7.29	24.27
Winter	12.33	5.54	21.77	17.43	8.38	14.16	7.63	20.41	5.54	21.77
Spring	16.52	13.29	26.43	22.30	12.16	20.27	10.23	30.13	10.23	30.13
Summer	22.48	18.25	45.76	30.45	15.23	36.29	12.5	60.32	12.5	60.32
SFCx10 ⁵ CFUml ⁻¹ /Station										
Autumn	12.18	2.23	2.10	1.10	2.05	3.22	8.32	1.29	1.10	12.18
Winter	1.07	1.80	0.30	0.60	0.40	1.50	0.72	0.40	0.30	1.80
Spring	13.21	3.21	3.93	1.12	2.23	3.41	12.55	1.32	1.12	13.21
Summer	56.13	3.22	29.13	3.23	20.23	18.13	50.8	1.61	1.61	56.13
ECx10 ⁵ CFUml ⁻¹ /Station										
Autumn	8.21	0.70	2.02	1.03	0.83	0.31	7.74	0.82	0.31	8.21
Winter	0.50	0.30	1.19	0.51	0.21	0.10	2.21	0.61	0.10	2.21
Spring	23.9	1.32	3.32	1.13	1.14	0.72	7.83	1.54	0.72	23.90
Summer	90.22	2.92	32.93	1.40	3.25	0.82	12.24	1.91	0.82	90.22
TCx10 ⁵ CFUml ⁻¹ /Station										
Autumn	24.63	4.21	4.33	1.32	2.81	4.12	9.21	2.33	1.32	24.63
Winter	1.59	3.31	1.91	1.72	1.41	2.31	5.54	2.12	1.41	5.54
Spring	89.17	5.12	5.42	3.21	2.91	4.12	16.63	3.32	2.91	89.17
Summer	100.1	6.63	49.9	3.83	3.83	5.04	126.0	4.50	3.83	126.0

Table 2: Regional and seasonal of different bacterial groups in sediment samples of Lake Qarun during the period of study (2014 -2015)

Season	TBVCx10 ⁵ CFUg ⁻¹ /Station								Min.	Max.
	I	II	III	IV	V	VI	VII	VIII		
Autumn	3.72	1.82	11.05	14.06	2.80	3.81	3.22	4.11	1.82	14.06
Winter	3.62	0.82	2.43	1.63	1.13	1.83	0.15	3.81	0.15	3.81
Spring	10.03	2.33	11.20	14.16	3.33	5.53	8.27	4.51	2.33	14.16
Summer	60.49	3.82	12.83	14.53	8.41	40.04	11.89	24.09	3.82	60.49
SCx10 ⁴ CFUg ⁻¹ /Station										
Autumn	50.14	7.52	8.11	3.14	15.7	8.21	10.15	3.32	3.14	50.14
Winter	40.13	2.73	1.74	4.05	11.31	7.51	8.62	2.84	1.74	40.13
Spring	78.33	40.14	20.42	6.24	21.36	38.28	14.46	4.06	4.06	78.33
Summer	640.3	63.25	33.06	18.3	40.54	80.38	27.34	44.03	18.3	640.3
VCx10 ³ CFUg ⁻¹ /Station										
Autumn	0.82	0.40	8.09	0.55	0.38	0.53	0.16	0.18	0.16	8.09
Winter	0.73	0.32	7.41	0.42	0.26	0.43	0.13	0.15	0.13	7.41
Spring	27.33	6.02	8.26	0.71	1.63	3.36	0.63	19.16	0.63	27.33
Summer	35.24	15.05	9.42	0.83	1.82	3.53	0.84	20.02	0.83	35.24
ACx10 ² CFUg ⁻¹ /Station										
Autumn	11.32	9.33	14.12	8.40	30.62	21.10	17.50	38.13	8.40	38.13
Winter	9.17	4.21	10.86	2.86	16.31	10.21	8.27	19.47	2.86	19.47
Spring	13.6	10.4	15.8	15.9	44.73	25.6	20.3	39.3	10.4	44.73
Summer	17.57	21.28	18.21	20.4	60.53	40.66	34.3	51.76	17.57	60.53

Table 3: Accession number of the experimental 16S rRNA sequence and similarity percentage to the closest known species

Isolate code	Accession no.	Most related Species	Similarity ¹ (%)
19T	<u>NR_112615-1</u>	<i>Bacillus firmus</i> strain NBRC 15306	97
22T	<u>NR_118441-1</u>	<i>Bacillus stratosphericus</i> strain 41KF2a	98
14P	<u>NR_042424-1</u>	<i>Exiguobacterium mexicanum</i> strain 8N	97
11S	<u>NR_028930-1</u>	<i>Stenotrophomonas rhizophila</i> strain e-p10	97
2V	<u>NR_115088-1</u>	<i>Halomonas stevensii</i> strain S18214	97
9S	<u>NR_044397-1</u>	<i>Halomonas koralensis</i> strain XK1	89

¹Aligned ribosomal DNA fragments were obtained by PCR using primers F27 and R149.

Table 5: Phenotypic characterization of the most common bacterial isolates

Test	Result					
	2V	9S	19T	22T	14P	11S
Gram reaction	-ve	-ve	+ve	+ve	+ve	+ve
Colony color	Cream	Orange	Cream	White	Orange	Yellow
Motile	Motile	Non-motile	Motile	Motile	Motile	Motile
Temperature	30-35°C	4.0-43°C	25-42°C	8-37°C	10-30°C	4-37°C
pH	8 - 9	8.5-9.0	4-10	6-10	6-10	6-12
NaCl %	3-7.5%	6-10%	2-7%	0-10%	1-7%	Up to 4.5
Catalase	+ve	+ve	+ve	+ve	+ve	-ve
Oxidase	+ve	+ve	+ve	+ve	-ve	+ve
Nitrate reduction	-ve	-ve	+ve	+ve	+ve	-ve
Methyl red test	-ve	+ve	+ve	-ve	-ve	+ve
Voges proskaur test	-ve	-ve	-ve	+ve	+ve	-ve
Citrate test	+ve	-ve	-ve	-ve	+ve	+ve
H ₂ S production test	-ve	-ve	-ve	-ve	-ve	+ve
Glucose utilization	+ve	+ve	-ve	+ve	+ve	+ve
Lysinedecarboxylase test	-ve	-ve	-ve	-ve	+ve	-ve
Urease	+ve	+ve	-ve	+ve	-ve	-ve

Table 6: Regional and seasonal of physical parameters in Lake Qarun water during the period of study (2014-2015)

Season	pH/Station								Min.	Max.
	I	II	III	IV	VI	VII	VIII			
Autumn	7.97	8.19	8.29	8.20	8.29	8.30	8.30	8.41	7.97	8.41
Winter	7.79	8.35	8.41	8.51	8.73	8.49	8.20	8.05	7.79	8.73
Spring	7.29	8.32	8.21	8.17	8.28	8.35	8.19	8.29	7.29	8.35
Summer	7.08	8.35	8.13	8.23	8.28	8.37	8.23	8.32	7.08	8.37
Temperature (°C)/Station										
Autumn	21.5	21.4	21.1	21.1	21.1	21.2	21.4	21.0	21.0	21.5
Winter	16.8	16.2	15.7	15.8	15.7	15.8	16.1	15.6	15.6	16.8
Spring	26.5	26.3	26.1	26.1	26.1	26.1	26.2	26.0	26.0	26.5
Summer	32.2	32.0	31.5	31.6	31.6	31.8	31.9	31.5	31.5	32.2
Salinity (‰)/Station										
Autumn	5.27	15.04	23.84	28.85	29.22	29.35	24.62	30.04	5.27	30.04
Winter	3.97	49.80	51.72	32.04	40.52	35.64	35.97	37.65	3.97	51.72
Spring	3.18	27.18	26.22	29.13	34.13	32.32	34.57	34.88	3.18	34.88
Summer	2.26	28.51	29.30	30.71	33.60	32.27	33.26	34.51	2.26	34.51

Table 7: Regional and seasonal of some chemical parameters in Lake Qarun water during the period of study (2014-2015)

Season	DO (mg/l)/Station								Min.	Max.
	I	II	III	IV	V	VI	VII	VIII		
Autumn	4.53	8.74	5.18	6.15	3.91	3.89	7.13	3.90	3.89	8.74
Winter	4.97	5.89	6.56	8.96	8.42	7.98	7.45	8.62	4.97	8.96
Spring	4.17	6.51	6.74	7.80	7.35	6.04	5.84	6.09	4.17	7.80
Summer	4.36	6.30	6.68	7.63	6.81	6.22	5.98	6.18	4.36	7.63
BOD (mg/l)/Station										
Autumn	1.77	3.32	2.61	3.29	1.08	1.17	3.50	1.40	1.08	3.50
Winter	4.2	3.23	5.24	5.84	6.11	6.47	5.17	6.34	3.23	6.47
Spring	3.24	2.16	3.86	4.89	3.83	3.94	2.51	3.36	2.16	4.89
Summer	2.15	2.12	3.53	4.35	3.66	3.81	2.32	3.23	2.12	4.35
COD (mg/l)/Station										
Autumn	25.65	30.16	32.91	35.42	33.83	47.88	50.00	52.19	25.65	52.19
Winter	11.36	21.02	18.48	17.66	20.68	21.18	19.83	16.87	11.36	21.18
Spring	30.48	37.33	40.56	38.63	36.65	51.66	49.39	53.64	30.48	53.64
Summer	36.36	39.29	45.67	42.82	40.27	42.11	43.58	41.25	36.36	45.67

Table 8: Regional and seasonal of nutrients ($\mu\text{g/l}$) in Lake Qarun water during the period of study (2014-2015)

Season	PO ₄ -P ($\mu\text{g/l}$)/Station								Min.	Max.
	I	II	III	IV	V	VI	VII	VIII		
Autumn	396.8	112.7	29.65	15.5	16.84	5.166	11.7	1.453	1.453	396.8
Winter	398.6	96.72	124.4	107.9	99.93	97.76	162.6	113.3	96.72	398.6
Spring	415.3	152.7	132.0	136.5	105.8	207.2	172.7	122.6	105.8	415.3
Summer	498.4	146.4	141.6	157.8	134.4	144.4	143.2	148.5	134.4	498.4
	NH ₄ -N ($\mu\text{g/l}$)/Station									
Autumn	460.2	15.02	26.08	32.29	214.1	98.56	174.7	123.6	15.02	460.2
Winter	954.3	40.46	23.1	21.74	25.62	58.1	42.42	43.26	21.74	954.3
Spring	1349	23.47	22.04	19.49	200.4	116.1	203.0	114.2	19.49	1349
Summer	1485	14.73	26.22	32.82	218.6	96.57	178.6	124	14.73	1485
	NO ₂ -N ($\mu\text{g/l}$)/Station									
Autumn	646.5	139.2	52.93	29.77	5.55	13.62	41.67	18.85	5.553	646.5
Winter	101.0	5.92	8.4	4.10	9.19	17.5	29.02	19.74	4.10	101.0
Spring	114.2	76.42	35.51	40.70	30.78	57.59	63.88	39.05	30.78	114.2
Summer	127.7	56.56	45.22	44.29	43.2	43.61	47.06	46.62	43.2	127.7
	NO ₃ -N ($\mu\text{g/l}$)/Station									
Autumn	1271	1122	317.4	335.1	71.2	74.18	647.8	139.6	71.2	1271
Winter	165.8	31.36	14.79	16.05	30.8	34.26	86.19	108.9	14.79	165.8
Spring	2038	161.8	73.10	85.42	62.70	78.71	96.97	74.85	62.70	2038
Summer	2138	139.7	83.77	93.10	68.40	72.55	85.33	80.37	68.40	2138
	SiO ₄ ($\mu\text{g/l}$)/Station									
Autumn	1121	1086	1078	1012	1317	894.6	1502	917.0	894.6	1502
Winter	4191	2810	2379	2707	3213	2743	4682	2052	2052	4682
Spring	2246	3981	3549	2317	3081	3568	2319	1980	1980	3981
Summer	2754	4289	3415	2218	3623	3444	2484	3483	2218	4289

4 Discussion

Bacteriological characteristics of Lake Qarun

Microbial pollution is one of the most dangerous types of water pollutants, as it leads to the spread of many diseases such as cholera, typhoid and severe diarrhea and hepatitis. Therefore, attention must be paid to monitoring microbial contamination in the Egyptian lakes, especially Lake Qarun, where is considered the key to development in the province of Fayoum, to a source of fisheries and salts. On the other side, international standards and health risk criteria of water resources based on indicator bacteria. European Commission Guide Standard 1998 and Ministry of Health, Egypt (1996) accepted the guide values of 500/100 ml water for coliform bacteria and 100/100 ml water for each fecal coliform and fecal *Streptococci*. In

lake fisheries, the Egyptian standard accepted 70/100 ml water of the lake for total coliform bacteria and 5000/100 ml for drainage water. Interpretation of the indicator bacteria during the period of study (2014-2015) in Lake Qarun was objected to the Egyptian Guide Standard (Ministry of Health, Egypt, 1996). The use of fecal indicator bacteria of sewage (total coliform and fecal *S. feacalis* and *E. coli*), which are used locally and internationally as a measure of the quality of water in order to protect the water from pollution and the preservation of human health, as well as, fisheries. This may because the existence of these bacteria are considered excellent index on the possibility of the presence of other bacteria causing serious diseases.

By judging the hygienic water quality, the indicator bacteria in Lake Qarun subsurface waters exceeded the acceptable values in the following stations along the period of study:

1. The lowest count of *S. feacalis* was detected in station III in winter, while the highest was determined in station I in summer. The relative high values were observed in summer season in station VII, while the relative low values were observed in many stations especially during winter season.
2. The lowest count of *E. coli* was recorded in station VI in winter, while the highest was estimated in station I in summer. The relative high values were observed in summer season in station III, while the relative low values were observed in many stations especially during winter season.
3. The lowest count of total coliform sp. was detected in station IV in autumn, while the highest count was conducted in station VII in summer. The relative high values were observed in summer season and in spring in station I, while the relative low values were observed in many stations especially during winter season.
4. The values of these indicators were not exceeded than the acceptable values according to Egyptian Guide Standard & European Commission Guide Standard.

As well as, the relative low values were observed in many stations especially during winter season. The lowest count of bacteria during the period of study was of *Aeromonas* sp. in sediment in winter in station IV. On the other side, the highest count of bacteria during the same period was of TBVC in sediment in summer in station I. The most affected station was I (front of the Bank of Bats), and the most affected season was summer.

However, some studies have been conducted on the microbiological properties of Lake Qarun included; Sabae (1993) studied the microbiological properties of the waters of Lake Qarun by studying the total number of bacteria at a temperature of 22°C (saprophytic bacteria) and 37°C (pathogenic bacteria), as well as, fecal indicator bacteria of sewage (total coliform and fecal *S. feacalis* and *E. coli*). The study also included the distribution of bacteria own cycle of nitrogen and bacteria degrading cellulose. The study showed that the lake has not yet reached dangerous phase of the pollution affecting the biodiversity. In addition, Sabae (1996) monitored the microbiological properties of the layers of the bottom of the lake by studying the total bacterial number at a temperature of

22°C (saprophytic bacteria) and 37°C (pathogenic bacteria), as well as, fecal indicator bacteria of sewage (total coliform and fecal *S. feacalis* and *E. coli*), as well as the distribution of bacteria reducing sulphate bacteria degrading cellulose. The study showed that the Lake Qarun from the rich lakes in the numbers of bacteria up to 10^{12} CFU/ml, as the bacteria are used as food for plankton which is the main food for the fish. Sabae and Rabeih (2000) declared that the total number of bacteria in the Lake Qarun water ranges between 0.3×10^{10} and 93.9×10^{10} CFUml⁻¹ at a temperature of 22°C and between 0.1×10^{11} and 71×10^{11} CFUml⁻¹ at 37°C.

As well as, the study showed that fecal indicator bacteria decreased as we head west of the lake away from the banks. Sabae and Ali (2004) explained that the total number of bacteria in the Lake Qarun water ranges from 2×10^{11} and 115×10^{11} CFUml⁻¹ at a temperature of 22°C and between 2×10^{11} and 110×10^{11} CFUml⁻¹ at 37°C. On the other side, the study has shown that distribution of bacteria responsible for cycle nitrogen in the lake is changing through the different seasons, depending on the changing physical and chemical characteristics of the waters of the lake. Ali *et al.* (2008) monitored that the total number of bacteria in the waters of Lake Qarun ranges from 10^3 CFUml⁻¹ in the center of the lake and between 10^7 cfu/ml near the beach. They, also, pointed to fecal indicator bacteria of sewage increased as we head hand lake beach. Abou El-Gheit *et al.* (2012) have studied the physical and chemical properties and in Lake Qarun and the impact on pathogenic bacteria in fish, as well as, histopathological changes associated with this bacterial diseases in the lake fish. Sabae and Mohamed (2015) have studied the environmental pollution in the Lake Qarun. The study also addressed such impact on tilapia fish from the physiological and histopathological. They found that there were some histopathological changes in the liver, muscle and kidney, in addition to an increase in liver enzyme activity and an increase in glucose, protein and cholesterol level in the blood. Also, a severe shortage at the level of protein in muscles was observed.

On the other hand, the characterization of common bacterial isolates habited in Lake Qarun was achieved using genotypic and phenotypic means. Six bacterial isolates coded; 19T, 22T, 14P, 11S, 2V, and 9S were identical counterpart with respect to its 16S rRNA sequence. They were identified as; *Bacillus firmus* strain NBRC 15306, *Bacillus stratosphericus* strain 41KF2a, *Exiguobacterium mexicanum* strain 8N, *Stenotrophomonas rhizophila* strain e-p10,

Halomonas stevensii strain S18214, and *Halomonas korensis* strain XK1, respectively. *Bacillus firmus* is a species of bacteria within the genus *Bacillus*. Some strains of this species are very alkaline-tolerant and may grow in environments with pH as high as 11 (Guffanti *et al.*, 1980). *Bacillus stratosphericus* is a gram-positive, motile, rod-shaped bacteria. Based on characteristics such as being endospore-forming, catalase-positive bacteria. López-Cortés *et al.* (2006) isolated *Exiguobacterium mexicanum* Gram-positive strain from cysts of the brine shrimp *Artemia franciscana*. It was subjected to a polyphasic taxonomic analysis. Based on 16S rRNA gene sequence comparison and composition of isoprenoid quinones, peptidoglycan and fatty acids, these organisms are members of the genus *Exiguobacterium*. Both strains showed 95.9% 16S rRNA gene sequence similarity to one another.

In addition, Chaturvedi *et al.* (2005) isolated a novel psychrophilic bacterium, designated strain DVS 3YT, from a moraine sample from the McMurdo Dry Valleys, Antarctica. Phenotypic and chemotaxonomic characteristics and data from a phylogenetic analysis based on 16S rRNA gene sequences indicated that strain DVS 3YT was related to the genus *Exiguobacterium*. Wolf *et al.* (2002) studied a polyphasic taxonomic 16 *Stenotrophomonas* strains from environmental sources. The defining characteristics of the new species were as follows: growth at 4 degrees C and the absence of growth at 40°C; the utilization of xylose as a carbon source; lower osmolytic tolerance (< 4.5% NaCl, w/v), although the isolates can produce trehalose and glucosylglycerol as osmoprotective substances; the absence of lipase and beta-glucosidase production; and antifungal activity against plant-pathogenic fungi. They showed a mean similarity of 99.5% within the cluster. The family Halomonadaceae of the class Gammaproteobacteria currently comprises ten genera, Carnimonas, Chromohalobacter, Cobetia, Halomonas, Halotalea, Kushneria, Modicisalibacter, Salicola, Salinicola and Zymobacter, which accommodate halophilic/halotolerant and non-halophilic bacteria. Since the family Halomonadaceae was first described (Franzmann *et al.*, 1988), its members typically have occurred in saline lakes, solar salt facilities, saline soils and marine environments. Since some of its members are also alkaliphilic, they are found in soda lakes and alkaline soils. The exceptions to the above are *Carnimonas nigrificans*, *Chromohalobacter beijerinckii*, *Chromohalobacter canadensis*, *Chromohalobacter japonicus*, *Halomonas alimentaria*, *Halomonas alkaliphila*, *Halomonas*

campaniensis, *Halomonas daqingensis*, *Halomonas desiderata*, *Halomonas halodenitrificans*, *Halomonas muralis*, *Halotalea alkalilenta*, *Kushneria aurantia*, *Kushneria avicenniae*, *Modicisalibacter tunisiensis* and *Zymobacter palmae* (Arahal and Ventosa, 2006; Ben Ali Gam *et al.*, 2007; Ntougias *et al.*, 2007; Peconek *et al.*, 2006; Romano *et al.*, 2005, Sanchez-Porro *et al.*, 2009; Wu *et al.*, 2008). Lim *et al.* (2004) isolated a moderately halophilic bacterium, strain SS20(T), capable of growing at salinities of 1-20% (w/v) NaCl from a solar saltern of the Dangjin area in Korea and characterized taxonomically. Kim *et al.* (2013) confirmed that *Halomonas* has been organized as a genus since 1980, and comprises halophilic and/or halotolerant Gram-negative aerobic bacteria, typically found in saline environments.

Hydrochemical characteristics of Lake Qarun

The values of pH measured are still lying on the alkaline side. In the present study the pH in Lake Qarun water varied between 7.08 at sit I and 8.73 at sit V with general average in the lake 8.45. However, the ideal pH level for fish growth lies in the range of 6.5-8.0, while the level lower than 4 and higher than 11 is toxic for most of fishes (Delince, 1992). Generally, the pH values were on the alkaline side and no significant difference was observed between the surface and near bottom water layer. Changes in pH values are mainly attributed to photosynthesis activities of phytoplankton, aquatic plants, respiration and variations in temperature. Hydrogen ion concentration plays an important role in many of the life processes. Living organisms are very dependent on, and sensitive to the pH. Not only is the hydrogen ion a potential pollution in itself, but it is also related intimately to the concentration of many other substances, particularly the weakly dissociated acids and bases (Delince, 1992). The temperature varied between 15.6°C in station VIII and 32.2°C in station I. The relative high values of temperature (32.2°C) were observed in (summer season), while the relative low values of temperature were observed in winter. This is in agreement with the results obtained by Rabeh, (2012). The air temperatures ranged on averages between 15.4°C during winter and 31°C during summer with an annual amplitude of 15.6°C. The life process of all aquatic microorganisms is affected by water temperature which enhances their active proliferations, so 15.6°C amplitude in water temperature of Lake Qarun might be translated into high TVBCs during summer. Temperature is an important physical factor influencing the growth of microorganisms, the annual seawater temperature

cycle is directly affected by solar radiation and seasonal change in air temperature. The maximum water temperature in summer is a result of the solar heating with a relatively longer day-time in summer than that in winter. The rapid decrease and increase of water temperature occurs in autumn and spring. Water temperature are subjected to considerable variations, due to several factors, such as air temperature, latitudes, sun, seasons, wind action, depth of water, waves and gain or loss of heat in shallow waters close to lands (Delince, 1992). The effect of domestic sewage throughout the year mainly affects the upper 5 m layer where the surface water salinity may decrease specially in summer and autumn. The minimum surface salinity (3.18‰) was observed during summer at station I and the maximum one (51.72‰) was found in winter season at station III. The increased of human activities, action of winds and the discharge of sewage wastes and their distribution into the sea water is mostly responsible for the decrease in surface water salinity in spring and summer seasons (APAH, 1995).

Dissolved oxygen (DO)

The presence of oxygen dissolved in water is influenced by photosynthesis and respiration and gas exchange between the air and the surface of the water rates (Krom *et al.*, 1989; Erez *et al.*, 1990). Oxygen content of water depends on a number of physical, chemical, biological and microbiological processes. The deficiency in DO is an important indicator of pollution in a natural water body, describing its biological state, the predominant processes occurring in it, the destruction of organic substances and the intensity of self-purification, Grasshoff (1975) and Dyrssen and Wedborg (1980). The amount of DO was fluctuated between 3.89 mg/l at sit VI and 8.96 mg/l at sit IV. Generally, The deficiency of DO values in summer are related mainly to the increase in the rate of oxygen consumption through decomposition of organic matter supplemented by the rise of water temperature Abdel-Halim and Khairy (2007).

Biochemical oxygen demand (BOD)

It is the amount of dissolved oxygen needed by aerobic biological organisms to break down organic material present in a given water sample at certain temperature over a specific time period. The BOD value is most commonly expressed in milligrams of oxygen consumed per liter of sample during 5 days of incubation at 20°C and is often used as a surrogate of the degree of organic pollution of water. BOD can be used as a gauge of the effectiveness of wastewater treatment plants. It is listed as a conventional pollutant in the U.S. Clean Water Act. BOD is similar

in function to COD, in that both measure the amount of organic compounds in water. However, COD is less specific, since it measures everything that can be chemically oxidized, rather than just levels of biodegradable organic matter. The BOD in lake water varied between 1.08 mg/l at sit VIII and 6.47 mg/l at sit IV. The relative high values of BOD were observed in winter in the station VI, while the relative low values of BOD were observed in the station V during autumn season. Anon, (1975) pointed to the greater the value of the consumer oxygen was vital evidence of water contamination. He explained that the water containing the values of the oxygen consumed vital less than (1) is the water completely pure and (1-3) are considered pure to some extent and when this value up to (5) water pollution begins. APAH, (1995) flats water containing a quantity of vital oxygen consumed less than (1) the pure waters ; the containing of (2-8) as a moderate water pollution.

Chemical oxygen demand (COD)

Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers) or wastewater, making COD a useful measure of water quality. It is expressed in milligrams per liter (mg/l), which indicates the mass of oxygen consumed per liter of solution (Tayel *et al.*, 1996). Many governments impose strict regulations regarding the maximum chemical oxygen demand allowed in waste water before they can be returned to the environment. For example, in Switzerland, a maximum oxygen demand between 200 and 1000 mg/l must be reached before waste water or industrial water can be returned to the environment. Value of consumed oxygen explains chemically the amount of oxygen necessary to oxidize the organic matter present in the water and turn it into carbon dioxide and water (Tayel *et al.*, 1996). Water containing chemically oxygen consumed less than 12 mg/L is considered high quality water (Berg, 1942). The COD in lake water varied between 11.36 mg/l at sit I and 53.64 mg/l at sit VIII. The relative high values of COD (53.64 mg/l) were observed in spring.

Nutrients in lake water

Nitrogenous salts are; ammonia, nitrite, nitrate and total nitrogen. Bacteria produce ammonia as a result of oxidation of organic matter for energy, hydrolyzed protein and biological processes for some organisms that reduce the nitrogen and transform into ammonia; a toxic gas for the aquatic environment (Faragallah, 2009).

Nitrites are formed by the oxidation of bacteria to the inorganic materials for energy. This compound is unstable; it is oxidized by certain bacteria and

converted into nitrate or reduced by other bacteria, the opposite of the first converts to ammonia. Nitrite is one of toxic compounds which combine with hemoglobin in the blood and prevents it from carrying oxygen to the body, especially in children and cause serious diseases in the blood. Nitrates are result in oxidation nitrites and urea by oxidizing bacterial species. Also it is produced from the nitrogen gas in the air and coming off in the rain water in the form of nitrate. Ammonia is the major nitrogenous product of the bacterial decomposition of organic matter containing nitrogen, and is an important excretory product of invertebrates and vertebrates. As for the utilization of nitrogenous materials, ammonia is the preferred inorganic source because of its ease uptake and incorporation into amino acids (N-assimilation) (Faragallah, 2009).

In present study, concentration of ammonia ranged between 14.73 and 1485 $\mu\text{g/l}$. Ammonia contents in Lake Qarun showed high fluctuations, the values showing an increase at the stations facing the outlet of the two drains (Station 1 and 7), and it varied between a minimum value of 14.73 $\mu\text{g/l}$ recorded at station II in summer and maximum one of 1485 $\mu\text{g/l}$ recorded at station 1 in summer also. On the other hand, the concentration of ammonia in the drains fluctuated between 42.42 – 1485 $\mu\text{g/l}$. The $\text{PO}_4\text{-P}$ in Lake Qarun water varied between 1.453.4 $\mu\text{g/l}$ at sit VIII and 498.4 $\mu\text{g/l}$ at sit I. The relative high values of $\text{PO}_4\text{-P}$ (498.4 $\mu\text{g/l}$) were observed in (summer season) in the station VIII. The PO_4^{3-} and total phosphorus (TP) concentrations showed lower rates than that measured in the same lake by Abdel-Satar *et al.* (2010) and ranged between (0.235–1.074 mg/l). 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).

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