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Spatial and Temporal Variations of Saprophytic Bacteria, Faecal Indicators and the Nutrient- Cycle Bacteria in Lake Bardawil, Sinai, Egypt

SHAWKY Z. SABAE
National Institute of Oceanography and Fisheries (NIOF), Inland Waters and Aquaculture Branch, El-Qanater Research Station, Egypt
E-mail: s_sabae@hotmail.com

ABSTRACT

Spatial and temporal variations of saprophytic bacteria and faecal indicators as well as the bacteria associated with nitrogen and carbon cycle in Lake Bardawil waters were investigated during 2004. A total of 144 water samples were collected monthly from 12 sites throughout the Lake. Bacteriological examinations showed that the bacterial counts ranged between 1.2 x 10^6 and 800 x 10^6 cfu/mL for saprophytic bacteria and between 0.9 x 10^6 and 536 x 10^6 cfu/mL for parasitic bacteria. On the other hand, the obtained data revealed that the fecal indicated bacteria [the total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS)] were in acceptable levels in most stations and months according to the Egyptian and European guide standards. However, their distribution throughout the Lake was affected by the water exchange with the Mediterranean Sea waters. The results showed that the bacteria associated with nitrogen cycle in the Lake environment (nitrogen fixers (*Azotobacter* & *Clostridium)*, nitrifying and denitrifying bacteria), in addition to the cellulose- decomposing bacteria were affected by environmental factors e.g. temperature, whereas, their regional variation was related to the presence of sea grass, *Rupia spirales* in the western region of the Lake.

Key Words: Saprophytic bacteria; Faecal indicators; Nitrogen-cycle bacteria; Lake Bardawil

INTRODUCTION

Lake Bardawil is a large coastal lagoon on the Mediterranean Coast of Sinai, Egypt. It is one of the most important Lakes in Egypt as a source of good quality fish and wildlife.

Bacteria in sea-water exhibit a number of interesting properties and additionally play an essential role in cycling of nutrients (Nessim, 1990). Saprophytic bacteria are responsible for remineralisation of particulate and dissolved organic matter in the sea. Under favorable conditions these micro-organisms are able to decompose almost all natural and organic compounds as well as many artificial substances (Rheinheimer & Grocke, 1994).

Microbiological quality is assessed by monitoring the abundance of indicator bacteria that are associated with recent faecal contamination. Several investigators selected coliform bacteria and faecal streptococci to assess the pollution in marine environment (Mancini, 1978; Lara *et al*., 1991; El- Naggar *et al*., 2003; El-Shenawy & Farag, 2005). High levels of faecal indicator bacteria in aquatic environment might indicate the presence of pathogenic micro-organisms. Cholera, typhoid fever, bacterial dysentery and other infectious disease are spread through contaminated water with faecal matter (Rose *et al*., 1996).

Despite the relevance of bacteria for biogeochemical cycling, little is known about their taxonomic composition. Planktonic heterotrophic bacteria are important in aquatic ecosystems as mineralisers of organic matter and used as components of food web. They may affect the amount and the quality of carbon transported by large rivers to oceans (Benner *et al*., 1995). Planktonic bacteria also represent a source of carbon to higher trophic levels because they graze on bacteria and in turn may be consumed by protozoans, crustacean and insects (Wetzel, 1995).

Extensive biogeochemical studies have shown that bacteria constitute a major component of carbon cycling in aquatic systems as the main consumer of dissolved organic matter (Azam, 1998; Ducklow, 2000). Microflora hydrolysing cellulose represented the least numerous physiological groups of micro-organisms isolated from the three estuaries Lakes. They were some-what more abundant in the Lake Lebsko where they constitute from 6 to 15% of the total number of bacteria (Mudryk & Donderski, 1997).

Nitrogen in aquatic ecosystems is an important controlling factor for microbial growth (Barnes & Schelske, 1994). Non–symbiotic nitrogen fixing bacteria have been recognised to be important components of several ecosystems. Dicker and Smith (1980) enumerated three groups of nitrogen fixers (*Azotobacter sp.; Clostridium sp.* & *Desulfribrio sp.*) in Delaware salt marsh. Sabae and Ali (2004) enumerated *Azotboacter* and *Clostridia* in Lake Qarun.

Ammonium regeneration in sediment is an important link between the water column and benthic process in shallow water environment (Klump & Marten, 1983). Rematerialised NH_4^+ may be used directly by benthic algae (Sundba *et al.*, 1991) or may be oxidized to nitrate (nitrification) with some fractions of NO_3 subsequently reduced to N_2 (de-nitrification). Microbial nitrification is an important regenerative process in the nitrogen cycle of Lakes, estuaries and marine environments. Nitrification and de-nitrification are controlled primarily by O_2 and NH_4^+ Supply (Caffrey *et al.*, 1993; Blackburn, 1995).

Although the available data dealing with the bacteriological quality of Lake Bardawil are scarce, several studies have been published on the physical and chemical characteristics of the Lake (Siliem, 1989; Shehata, 1989; Lotfy, 2003; Abd El-Satar, 2005; Abdo, 2005). Moreover, Fishar (2005 a, b) studied the ecology of benthic fauna of the Lake. Kimor (1975), Taha (1990) and Toulaibh *et al.* (2002) studied the species composition of phytoplankton in the lake.

This work aims to investigate the monthly variations and distribution of saprophytic bacteria, indicators of faecal pollution and the bacteria associated with nutrient cycle in Lake Bardawil in relation to some environmental factors.

MATERIALS AND METHODS

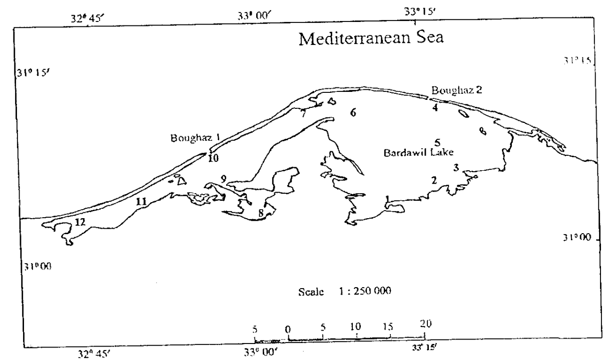
Study area. Lake Bardawil is situated on the Egyptian coast of the Mediterranean Sea northern Sinai, between 32° 40` E, 33° 30` and 31° 03` N and 31° 14` N. It is about 30 km east of Port Said and 20 km west of El-Arish town.

The surface area of the Lake is about 650 km^2, with a maximum length of 90 km and maximum width 22 km. The Lake is shallow and separated from the Mediterranean Sea by a sand bar with only one natural opening "Boughaz El-Zaraniq" at the eastern end. Since 1927 two artificial openings (Boughaz I & II) were established to decrease the salinity through exchange of water (Ben-Tuvia & Herman, 1972). The climate of the Lake region is arid, with high evaporation during summer. Lake Bardawil is the source of an important local fishery, producing over 3, 200 tons annually.

Sampling. A total of 144 water samples were collected from 12 stations during 12 sampling cruises from January 2004 till December, 2004. These sites are shown in Fig. 1. The bacteriological sampling technique was done according to the international standard ISO 5667/9 (ISO, 1992) using 500 mL sterilised bottles. The chemical parameters were estimated by Chemistry Department stuff, NIOF, according to APHA (1995).

Bacteriological analyses. The spread-plate procedure was used to enumerate both saprophytic bacteria (SB) at 22°C and parasitic bacteria (PB) at 37°C (Clark, 1971). Nutrient agar supplemented with glucose (5 g/l) and yeast extract (39/l) was used for the enumeration. The medium supplemented with 3% salt obtained from the Lake (Hassan, 1993).

Fig. 1. Map of Lake Bardawil showing the sampling locations.



Total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) were enumerated using the most probable number (MPN) technique. MacConkey broth medium was used for determination of TC and FC, acid and gas indicated positive reaction. Positive tubes in total coliforms were sub-cultured on fresh single MacConkey broth tubes and incubated in water both 44.5°C for 24 h for fecal coliform bacteria. MPN of faecal streptococci were detected using azid dextrose broth, positive tubes were estimated by dense turbidity after 48 h incubation. Fecal streptococci was confirmed by the formation of purple button at the bottom of the tube (APHA, 1995).

Cellulose–decomposing bacteria were counted using Dubos's medium. The tube contained a strip of filter paper (Whatmann No. 1) as carbon source. After incubation period, the appearance of yellowish brown colour and gradual decay of the filter paper indicated the presence of cellulose–decomposing bacteria (Sabae, 2001).

MPN of non-symbiotic nitrogen-fixing bacteria, *Azotobacter* and *Clostridium*, were estimated using modified Ashby's and Winogradeskey's media, respectively. *Azotobacter* positive tubes were distinguished by a characteristic pellicle and turbidity. For Clostridial spore counts, the water samples were pasteurised at 80°C for 15 min. The presence of Clostridia was detected by accumulation of gases in the tube (Sabae, 1996).

The counts of nitrifying bacteria were detected using Stephenson ammonium sulphate with $CaCO_3$ medium. The presence of nitrifying bacteria was de detected by the presence of nitrate or nitrite, which produce blue colour with diphenylamine (Belser, 1979). Whereas, denitrifying bacteria wee determined using nitrate–peptone medium, their presence was recorded by gas formation (Sabae, 1996).

Statistical analysis. The correlation coefficients (r) between physico-chemical parameters and the bacteria were tested using computer programme EXCEL (ver. 97).

RESULTS AND DISCUSSION

Lake Bardawil lies in an arid area. Its climate has a high amplitude of water temperature fluctuating between (29.7°C) hot summer and (17.4°C) cold winter.

With respect to the water transparency, Secchi-disc readings reached the Lake bottom at most of the stations. The increase in water transparency may be affected by the nature of the Lake bottom, which is composed of sand and silt (Levy, 1971). The transparency values decrease in front of Boughaz I and II at the mixing point of the sea water with the Lake. pH values were always on the slight alkaline side. It showed variations from 7.94 and 8.8, this range is favorable for bacterial activity and growth.

Dissolved oxygen is essential to most aquatic organisms and is greatly affected by their metabolism. The dissolved oxygen concentration in the Lake waters ranged between 4 and 10.4 mg O_2/L. The highest value was recorded during January (winter), which explained the effect of complete mixing of water by wind action. On the other hand, the decrease of oxygen during hot months may reflect the decrease in oxygen solubility with the increase in water temperature and salinity (Abd El –Sater, 2005). Also, the shallowness of the Lake reduces the likelihood of stratification and anoxia from the decay of organic matter (Siliem, 1989; Taha, 1990).

Ammonium concentration in Lake Bardawil ranged between 33 µg/L at site 3 during January and 276 µg/L at site 1 during September (Table I). Huck and Kirchman (1995) found that the decrease in ammonium in estuaries might be due to nitrification by nitrifying bacteria and volatilisation as well.

The concentration of nitrate and nitrite in the lake waters were much lower than ammonium. The lack of nitrite during June (summer) at most stations might be due to the effect of denitrifying bacteria. Nitrate concentration in the lake water varied from 13.63 and 89.2 µg/L (Table I). The maximum concentration of nitrate was recoded during January (winter) might be attributed to the low counts of denitrifying bacteria.

The bacteriological examinations of the twelve sites in the lake showed that the saprophytic bacteria (at 22°C) during the course of study ranged between 12 x 10^5 and 80 x 10^7 cfu mL^{-1}, recording their maximum number in July (summer) and minimum one during January (winter) (Fig. 2). On the other hand, the counts of parasitic bacteria (at 37°C) varied form 8 x 10^5 to 71 x 10^7 cfu mL^{-1}. Also, the maximum and minimum numbers were recorded during July (summer) and February (winter), respectively, which is mainly attributed to the effect of temperature. The temperature values have a direct effect upon the number of bacteria (r = 0.68) within limits, increase of temperature facilitates the growth and multiplication of bacteria (Salle, 1988). The results revealed that the differences between the counts obtained at both incubation temperatures (at 22°C & 37°C) were non-significant (r = 0.91).

The regional variations of the bacterial counts throughout the Lake showed that their minimum numbers were recorded at Station 3 (El-Zaraniq), whereas the maximum count was recorded at Station 4 (Boughaz I). This might be explained by the effect of Mediterranean Sea water, which is loaded with organic matter (Shabana, 1999).

It is clearly shown that the bacterial count is directly related to sea grass production, because of enhanced in put of organic matter by sea grass. The results revealed that the western region of the lake (stations 10 to 12), which is dominated by the sea grass (*Rupia spirales)*, recorded the highest bacterial numbers. These high counts of bacteria was due to the effect of labile organic nutrients produced by sea grass in addition to organic nutrients and carbon derived from plant detritus (Moriarty *et al*., 1990; Chin Leo & Benner, 1991).

Bacterial number in oligotrophic lakes is in the range between 5 x 10^3–340 x 10^3 cfu mL^{-1}, while mesotrphic lakes characterised with bacterial counts between 450 x 10^3 and 14 x 10^5 cfu mL^{-1} (Rheinheimer, 1980). Therefore, Lake Bardawil is considered a mesotrophic Lake.

The occurrence of faecal pollution bacteria (total coliform, faecal coliforms & faecal streptococci) is used as sanitary parameters for the evaluation of water quality. It is also known that these indicators are associated with disease – causing species (Hall & Rodriguers, 1992).

The ranges of regional variations of faecal indicator bacteria are shown in Table I and Fig. 3. The obtained

Table I. The range and average of some physico-chemical characteristics in Lake Bardawil

Stations	Temp.		pH		Do (mg/l)		NH_3 (µg/l)		NO_2 (µg/l)		NO_3 (µg/l)	
	range	average	range	average	range	average	range	average	range	average	range	average
1	19.4-27.9	23.60	8.01-8.72	8.72	4.8-9.6	7.31	87-276	177.25	0-19.2	7.81	13.63-83.12	42.83
2	20.0- 27.5	23.7	8.0-8.66	8.35	4-8.8	6.76	59.4-223.5	104.81	0.74-18.6	5.59	13.65-83.41	47.66
3	17.4-28.4	23.0	7.94-8.57	8.37	5.6-10.4	7.45	33-171	98.38	0-18.9	4.61	18.64-69.78	41.29
4	20.4-29.1	24.7	8.03-8.5	8.30	5.6-10.4	7.6	33-168	94.63	0-14.8	4.36	16.55-81.46	42.02
5	21.6-28.8	25.1	8.11-8.55	8.40	5.2-8.8	7.07	36-129	87.68	0-15.6	5.27	18.88-63.3	36.15
6	21.5-28.8	25	8.14-8.58	8.34	4.8-9.6	6.87	42-139	94.88	0.3-7.03	2.96	18.82-72.16	41.84
7	20.5-29.7	25.1	8.07-8.40	8.39	5.6-8.8	7.93	39-139.5	88.88	0.6-14.4	4.43	16.82-72.16	37.68
8	20.8-28.3	24.9	8.07-8.63	8.22	5.6-8.8	6.84	39-144	89.38	0-12.58	4.50	14.89-73.10	39.2
9	19.1-27.4	23.2	8.1-8.53	8.35	5.6-8.8	7.57	39-136	85.00	0-11.47	3.17	14.4-75.58	38.20
10	20.1-28.1	24.1	8.18-8.80	8.34	5.6-8.8	7.5	33-165	79.10	0.74-12.58	4.37	17.04-89.2	43.79
11	20.9-29.5	25.6	8.21-8.79	8.45	5.6-8.8	6.87	18-129	74.65	0-13.32	3.85	14.09-64.86	42.20
12	21.1-28.3	24.3	8.23-8.72	8.47	5.6-8.8	7.23	51-144	79.65	0-9.99	2.68	12.92-78.39	42.29

Fig. 2. Seasonal average distributions of saprophytic bacteria (22°C) and parasitic Bacteria (37°C)(cfu/ml water) throughout Lake Bardawil

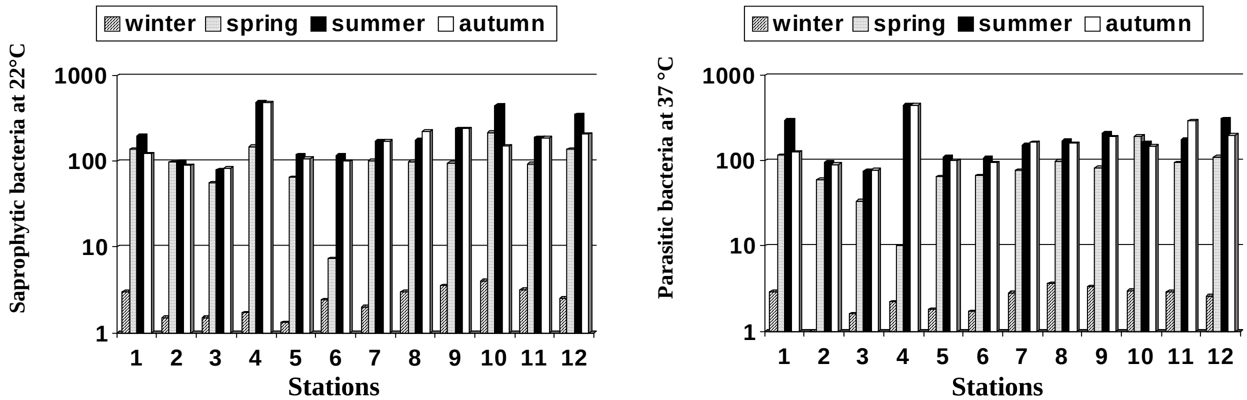
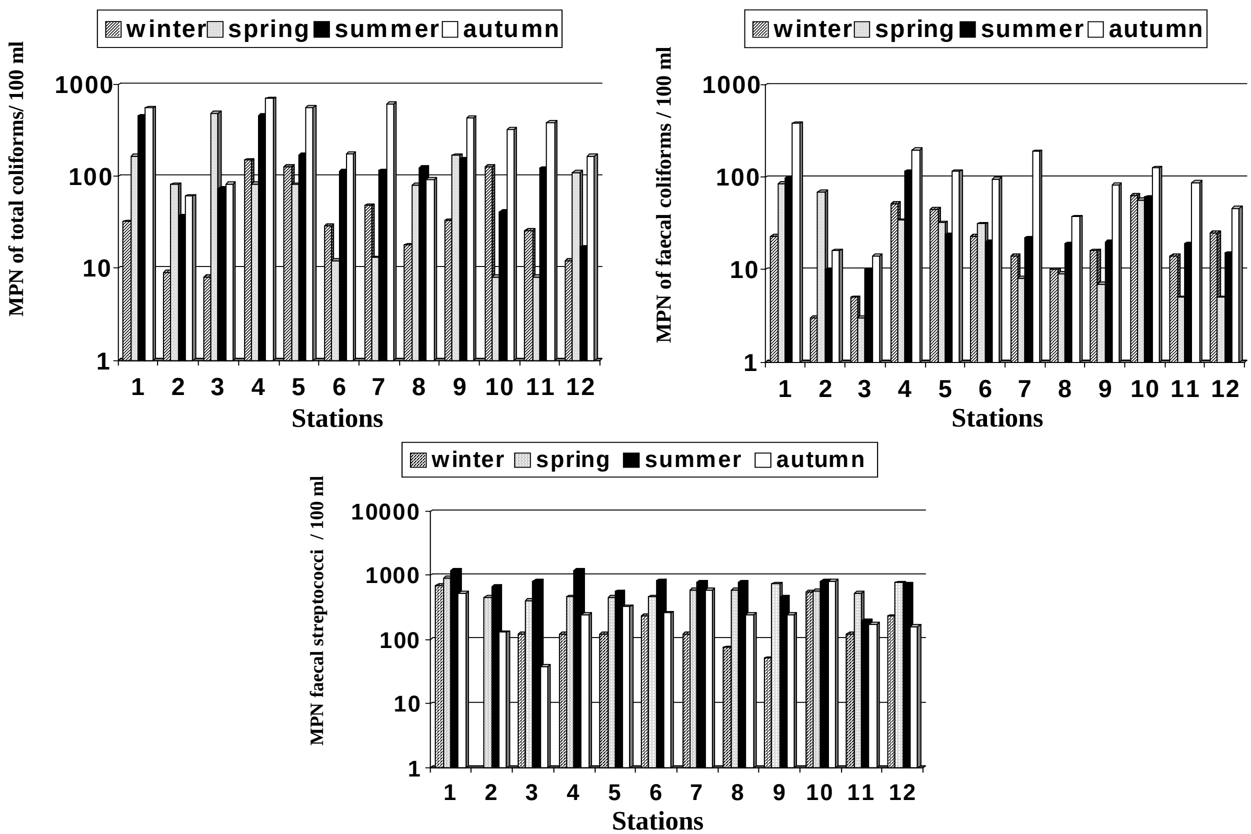


Fig. 3. The most probable number (MPN) of indicator bacteria [total coliforms (TC) Faecal coliforms (FC) and faecal streptococci (FS)/100 ml water] of Lake Bardawil.



results showed that MPN of faecal indicators ranged between 0 and 15 x 10^2/100 mL for total coliforms, 0 and 11 x 10^2/100 mL for fecal coliforms and between 0 and 16 x 10^2/100 mL water for faecal streptococci. In accordance with earlier findings of Rabeh (1999), the maximum and minimum counts wee recorded during July (summer) and February (winter), respectively, which is mainly related to the temperature (r = 0.74) and human activities.

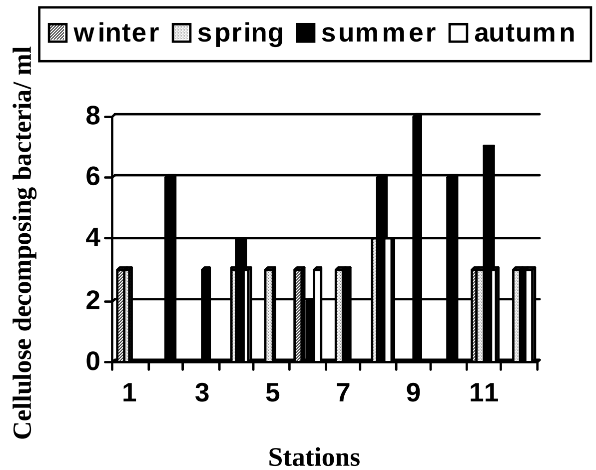
The distribution of faecal indicator bacteria throughout the Lake showed remarkable variations. These bacteria were not detected during February (winter) at Stations 3 (El-Zaraniq), whereas, their highest counts were estimated at Station 1 (El–Telool). This is mainly attributed to the effect of fishermen activities, this agrees with Abdo (2005) who concluded the presence of high organic mater content (OM %) in this Station. Also, higher counts of indicator bacteria were recorded in Boughazes I and II, which is explained by the exchange of the lake waters with the contaminated sea water. Statistically, there is a positive correlation (r = 0.74) between the two types of enteric bacteria, faecal coliforms and faecal streptococci, which supports the view that, they are useful as indicators for the presence of enteropathogens.

Interpretation of the detected bacterial indicators in the lake waters were done according to the European Commission (EC) Guide Standard (EC, 1997), which meet with the Egyptian Guide Standard (Ministry of Health, Egypt, 1996). They accept the guide values of the investigated bacteria up to 500/100 mL of marine water for coliforms and 100/100 mL for both faecal coliforms (*E. coli*) and faecal streptococci. The combination of the three indicators is likely to offer a global picture of water quality (Seyfried *et al*., 1985).

In general as seen from the obtained results of investigated faecal indicators at most of Stations throughout the Lake are found to be within acceptable level. On the other hand, the average numbers of streptococci exceeded the standard level since faecal streptococci have been found to be more persist ant than faecal coliforms to natural conditions e.g. high salinity of the lake waters (Geldreich, 1976).

Cellulose–decomposing bacteria play a significant role in decomposition of organic matter (cellulose) influencing the productivity of aquatic environment. The counts of cellulose–decomposing bacteria are shown in Fig. 4. Their numbers ranging between 0 and 8/mL water. The results showed that the bacteria recorded their maximum counts during June (summer) and February (winter), respectively. This might be explained by the effect of temperature, since the temperature is known to have strong influence on bacterial activity (Hall *et al*., 1989). It is clearly shown that the count of cellulose decomposers were higher in the western region of the lake than the eastern one. The predominance of the sea grass, *Rupia spirales*, in the western region supports efficient bacterial growth providing organic nutrients and carbon derived from plant detritus

Fig. 4. Seasonal average distributions of aerobic cellulose- decomposing bacteria/mL water of Lake Bardawil



which is essential for the bacterial growth (Shabana, 1999).

Since, bacteria play an important role in the transformations of nitrogen, particularly in coastal regions; there is need for estimation of spatial and temporal variations of nitrogen–cycle bacteria in relation to physico-chemical parameters.

Spatial and temporal variations of non-symbiotic nitrogen-fixing bacteria (*Azotobacter* & *Clostridum*) are shown in Table II, III and Fig. 5. The counts of *Azotobacter* ranged between 0 and 460/mL, with maximum number during April (spring). This could be explained by the effect of high temperature and dissolved oxygen content. This agrees with earlier findings of Sabae and Abd El-Satar (2001). On the other hand, the data showed that the clostridial spore count varied from 0 to 93/mL water of the lake, recording maximum number in July (summer). This may be due to the anaerobic nature of *Clostridia*, which failed to survive at high oxygen content of the Lake water during winter. However, the number of *Clostridia* were

Table II. The range and average of saprophytic bacteria (SB) at 22°C and parasitic bacteria (PB) at 37°C. (cfu x 10^6/mL) and the most probable number (MPN) of indicator bacteria (Total coliforms, fecal coliforms and, fecal streptococci/100 ml) in Lake Bardawil waters

Stations	Saprophytic bacteria (22°C)		Parasitic bacteria (37°C)		Total Coliforms		Fecal coliforms		Fecal streptococci	
	range	average	range	average	range	average	range	average	range	average
1	1.9-520	129.7	2-250	150.5	3-460	307.9	3-240	148.9	0-1600	910.1
2	1.5-140	81.4	1-100	71.2	0-240	61.9	0-210	36.7	0-1100	414.1
3	1.2-110	61.1	8-9.5	53.8	0-240	45.2	0-35	9.8	0-1100	352.7
4	1.5-800	313.4	2.2-710	288	0-1100	428.2	0-240	115.1	3-1500	621.2
5	1.2-140	79.5	1.7-135	76.1	4-1100	228.9	0-210	52.2	0-1100	445.8
6	2.3-120	80.6	0.9-115	74.9	0-360	100.4	0-240	40.4	3-1100	463.8
7	1.5-230	134.4	2-210	116.4	0-1400	294.1	0-1100	148.6	3-1400	633.6
8	3-368	132.5	2.8-200	110.9	0-240	64.5	0-75	20.2	0-1100	457.6
9	2.9-320	215.1	2.8-240	148.1	0-1200	174	0-190	34.7	3-1100	388.6
10	2.8-760	215.9	1.6-400	137.1	15-1500	372.2	7-1100	162.7	0-1600	771.7
11	2.5-210	130.6	1.7-536	151.9	0-1100	135.4	0-240	30.6	0-1100	335
12	2.2-240	188.2	2.6-220	169.3	0-360	73.4	0-93	19	0-1100	492.2

Table III. The range and average of nutrient- cycle bacteria/mL water of Lake Bardawil waters

Stations	Azotobacter		Clostrium		Nitrifying bacteria		Denitrifying bact.		Aerobic cellulose-decomposing bact.	
	range	average	range	average	range	average	range	average	range	average
1	0-360	122	0-93	43	0-24	7	0-6	3	0-3	2
2	0-240	65	0-46	25	0-36	10	0-14	4	0-6	2
3	0-160	49	0-75	25	0	0	0-15	7	0-3	1
4	3-290	136	0-75	39	0-11	5	0-6	3	0-4	3
5	0-290	113	0-36	17	0-15	4	0-7	3	0-3	1
6	0-43	18	0-42	21	0-4	1	0	0	0-3	2
7	0-360	98	0-43	25	0-6	3	0-20	5	0-3	2
8	7-110	80	13-64	35	0-9	4	0-29	10	0-6	4
9	0-240	30	7-43	19	0-7	2	0-24	6	0-8	2
10	0-460	145	10-75	38	0-36	8	0-15	4	0-8	2
11	0-240	66	0-24	18	0-20	7	0-24	7	0-7	4
12	0-120	41	0-75	30	0-3	4	0-23	6	0-3	2

Fig. 5. Seasonal average distributions of non-symbiotic nitrogen- fixing bacteria (*Azotobacter* and *Clostridia*/ml water) thoughout Lake Bardawil

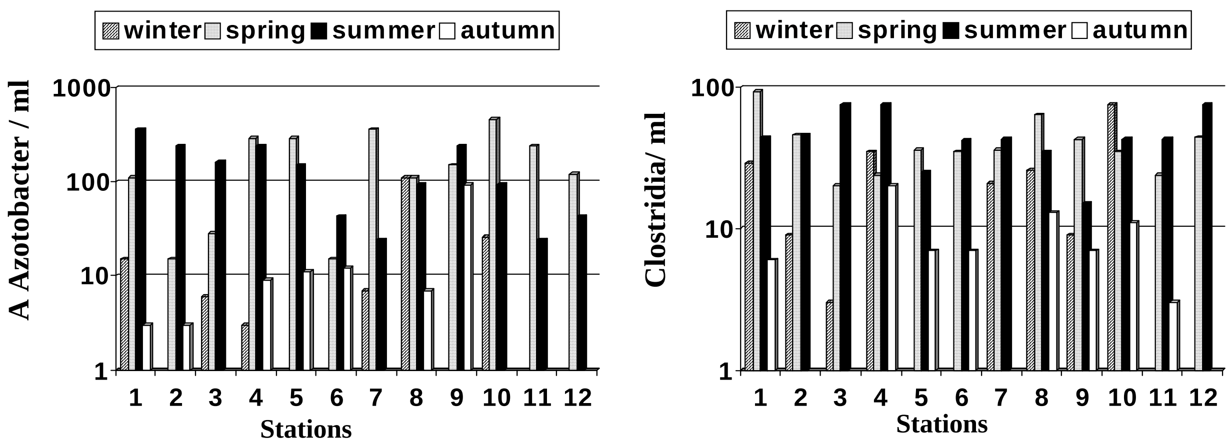
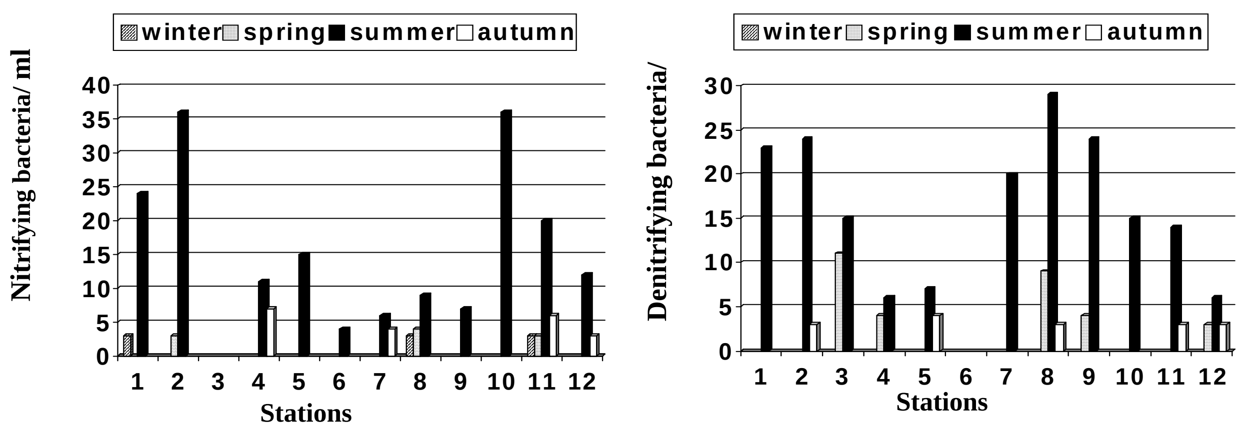


Fig. 6. Seasonal average distributions of nitrifying bacteria and denitrifying bacteria/ml water) thoughout Lake Bardawil



negatively correlated with dissolved oxygen (r = - 0.89).

Microbial nitrification is an important regenerative process in the nitrogen cycle of Lakes, estuaries and marine environments. Nitrification process is often the crucial link between mineralisation of organic nitrogen and loss of N_2 via denitrification (Caffrey & Miller, 1995).

The pattern of monthly variations of nitrifying and denitrifying bacteria throughout the Lake is presented in Table II and Fig. 6. Nitrifying and denitrifying bacteria were not recorded in the Lake waters during cold months; this is likely due to low nutrients. The maximum number of nitrifying bacteria was detected during June (summer). Statistically, nitrifying bacteria were positively correlated with the nitrate content in the waters (r = 0.26). Its number ranged between 0 and 36/mL. On the other side, the number of denitrifying bacteria varied form 0 to 29/mL, with maximum counts during August (summer) and the minimum one in January (winter). Denitrifying bacteria recorded higher numbers in the western region compared to the eastern one. Large areas of the western region of Lake Bardawil are densely covered with aquatic macrophyte, *Rupia spirales*, which supports the denitrifying bacteria through: (i) supplying organic carbon and organic matter for denitrifying bacteria (ii) lowering the redox potential, which is essential for denitrifying bacteria (Weisner *et al*., 1994).

The counts of nitrogen cycle bacteria in Lake Bardawil with other Egyptian lakes (e.g. Lake Qarun & Lake Manzalah) were compared. In Lake Manzalah, the counts were in the ranges of 1.8 x10^2 – 11 x 10^2/mL (*Azotobacter)*, 0 - 3.5 x 10^2 mL (*Clostridium*), (0.9 x 10^2 – 17 x 10^2/mL) (nitrifying bacteria) and 0 - 4 x 10^2/mL (denitrifying bacteria) (Rabeh, 2001). However, in Lake Qarun, 30 - 64 x 10^3/mL *(Azotobacter)*, 1 - 44 mL (*Clostridium*), 0 x 54/mL (nitrifying bacteria) and 0 - 49/mL (denitrifying bacteria) (Sabae & Ali, 2004). However, the abundance of nitrogen cycle bacteria in Lake Bardawil was considerably less than in the Lake Manzalah and Lake Qarun.

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