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ECOLOGICAL STUDIES ON NITROGEN CYCLE BACTERIA IN LAKE MANZALA, EGYPT

Saleh A. Rabeh

National Institute of Oceanography and Fisheries, Inland Waters Branch, Lab. of Microbiology, El-Qanater Research Station.

Key words : Lake Manzala, nitrogen cycle bacteria.

ABSTRACT

Six stations were chosen to represent the different micro-habitats along the southern part of Lake Manzala. In addition, two main drains, Bahr El-Baqar and Hadous were represented. Water and Bottom sediments were collected through four successive seasons (Autumn 1999 - Summer 2000) for physico-chemical and bacteriological analyses. The results showed that water temperature recorded the lowest value during Winter, while it recorded the highest during Summer with an annual amplitude 14.8°C. The Lake is shallow with a depth ranging from 0.85 - 1.31 m. The pH values were in the alkaline side. The water transparency fluctuated from 19.5 - 32.5 cm . The organic matter and organic carbon ranged from 5.28 - 8.71% and 3.06 - 5.06%, respectively. Bacteriological analysis showed that the highest counts of the studied bacteria were recorded in Bahr El-Baqar and Hadous Drains compared with Lake Manzala' stations.Conversly , the lowest counts of nitrifying bacteria were recorded in Bahr El-Baqar Drain and its discharge points.

INTRODUCTION

The extensive use of water by agriculture in Egypt produces a large volume of agricultural drainage water which is collected in several drains. In addition, these drains receive various domestic and industrial water effluents. The majority of this wastewater (85% by volume) is discharged into the Northern lakes of the Nile Delta (Manzala, Burullus, Edku and Mariut) (Mancy, 1986). Lake Manzala can be used as stabilization lagoon and nutrient assimilation system. Its capacity is relatively efficient because of its 6 week retention time, high temperature shallow depth and high productivity (Mancy and Ibrahim, 1988). In addition. it is the largest and most economically important of Egypt's coastal lakes where it provides an abundance of fish and an internationally important wintering area for waterbirds and staging area for migratory birds. Accordingly, the eastern part of the Lake (Ashtum El-Gamil) was declared as a Natural Protectorate in June 1988 (Meininger and Atta, 1990).

Nitrogen is a vital element in aquatic systems, accordingly, the nitrogen cycle occupies an important position among the biological processes accomplished in waters. On the other hand, distortions in the nitrogen cycle, resulting in the accumulation of certain toxic compounds, are one of the major causes of pollution (Rabeh, 1993, 1996).

Although considerable work was done on the role of bacteria in nitrogen cycle in Egyptian soils (Naguib *et al.*, 1983; Soraya *et al.*, 1987 and Mansour *et al.*, 1995) and aquatic systems (El-Samra, 1983; Elewa and Azazy, 1986; Abo-Sedera, 1990; Sabae, 1993 and 1996; Rabeh, 1993 & 1996), little is known about their role in Lake Manzala.

So,the object of the present investigation is to study the bacteria involved in the nitrogen cycle in lake Manzala. In this respect, N_2-fixing; nitrifying and denitrifying bacteria were selected for periodic monitoring.

MATERIAL AND METHODS

Study area

Lake Manzala occupies the north eastern corner of the Nile Delta between the Mediterranean Sea (North) and Suez Canal (East), while 5 Governorates share the western and southern Lake borders. The lake is presumed to have resulted from the accumulation of the Nile flood water, before the construction of the High Dam, in the low lying land which it occupies. Its area has been gradually decreasing since the earliest decades of the twentieth century. In 1900 its area was 1,709 km^2 reaching 1,200 km^2 in 1970s. As measured by Landsat imagery in 1981 the area of the lake was 904,785 km^2, while the area of open water was only 699,215 km^2 due to the presence of a large number (1022) of islets in the Lake. Widespread land reclamation and establishment of fish farms have resulted in major reduction in the area of the Lake and its marshlands. (Meininger and Atta, 1990). The present area of the lake is only 120 km^2.

The feed waters to the lake (4 billion m^3/year) is mainly from the Nile through the Damietta Branch, and from the wastewater (7.7 billion m^3/year). About 530 million m^3/year of Lake water drains to the Mediterranean Sea through 14 narrow openings; each is not more than 5 m wide, and 11 of them are man-made (Dewedar *et al*, 1995).

Bahr El-Baqar Drain (total length is about 97 km) receives untreated and/or only primary treated wastewater from East Cairo. From its upstream end, starting at the discharge point from El-Gabal El-Asfar treatment works into the Belbeis Drain, down to the confluence with the Qaliubia Drain at its discharge end into the southeastern region (El-Genki) of Lake Manzala. It contributes 25% of the inflow to Lake Manzala and about 60% of the nutrient (Toews & Ishac, 1984). Recently, the Drain discharges most of its water in El-Bashteir region. The Drain receives sewage, industrial as well as agricultural drainage water, with an annual discharge abut 1678 million m^3 (El-Bokhty, 1996).

Hadous Drain (65 km long) is the drain in the Eastern Delta which receives mainly agricultural drainage water. It contributes 49% of the inflow to the Lake and about 16% of the nutrients (Toews & Ishac, 1984). It receives about 2200 million m^3/year of which almost 50% was delivered to El-Salam Canal.

Sampling

Water and sediment samples were collected seasonally (Autumn, 1999 - Summer, 2000) using sterile glass bottles and the dredge, respectively.

Sampling stations

For the present investigation six stations were chosen to represent the southern part of Lake Manzala. In addition, Bahr El-Baqar and Hadous Drains were represented (one Stn. each). The eight sampling stations (Map 1) might be described as follows:

Station (1).	Recent mixing area in the Lake's recent outlet of Bahr El-Baqar Drain" "El-Bashteir".
Station (2).	Lies in the Navigation Canal at the middle between Stn 1 and Stn. 5.
Station (3)	Old mixing area in the Lake, in front of the old outlet of Bahr El-Baqar Drain.
Station (4)	Mixing area in the Lake, in front of Hadous Drain's outlet.
Station (5)	"Control El-Genki" lies in the middle of the southern part of the Lake far from El-Genki region.

Station (6) Lies in front of El-Matariya city at the beginning of the Navigation Canal "El-legan".

"Bahr El-Baqar Drain Station" lies at 100 m up-stream from the end point of discharge into the Lake.

Hadous Drain' station. located at 100 m upstream from the end point of discharge into the Lake.

Physico-chemical measurements

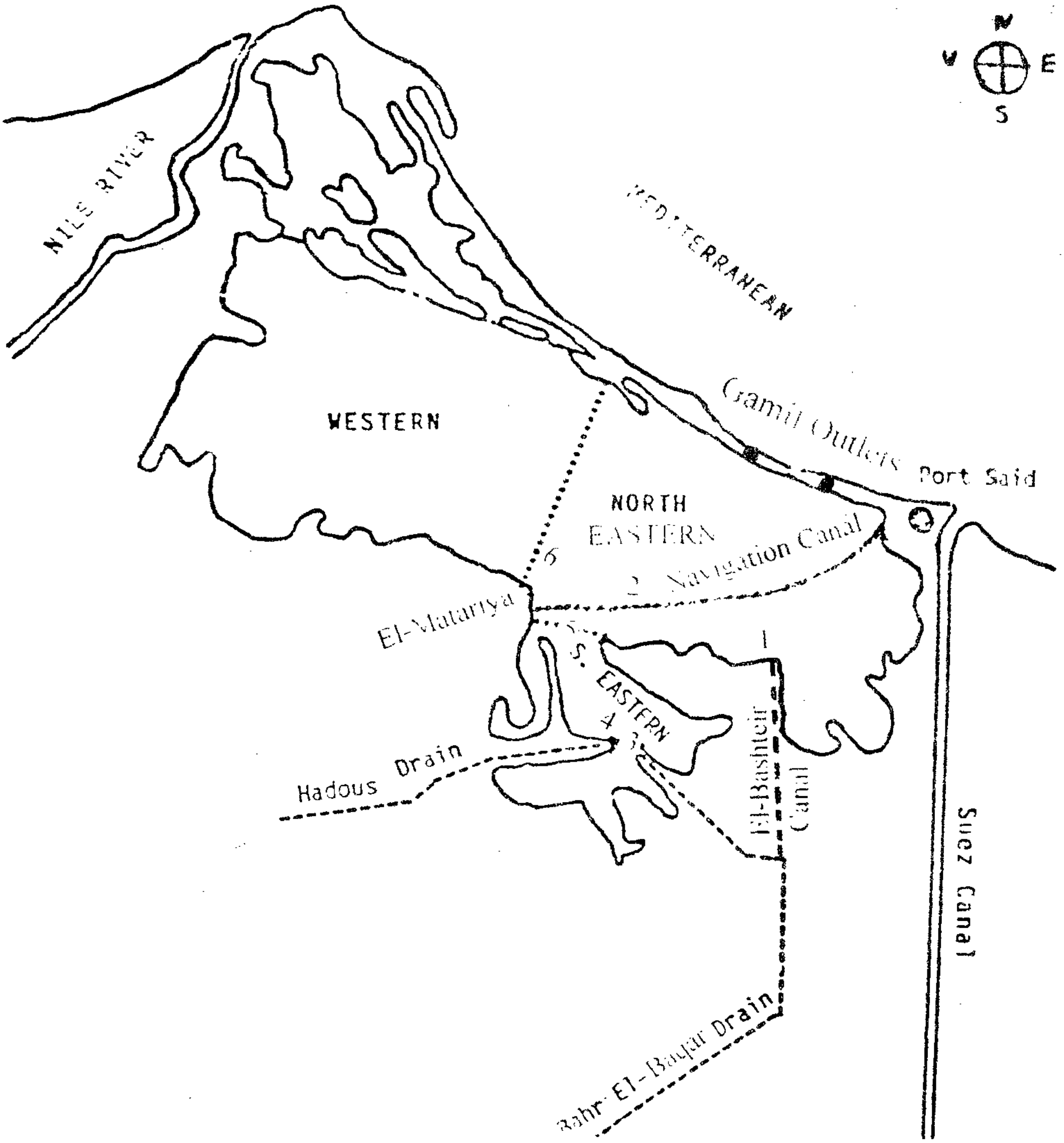
Air and water temperatures were measured *in situ* using a thermometer graduated to 0.1°C. Water transparency was measured by black/white standard 25 cm ϕ Secchi disc. Hydrogen ion concentration (pH) was measured using a portable pH-meter. Organic matter determination in bottom sediment was expressed as "loss on ignition" (Allen, 1974).

Bacteriological examinations

Surface plate (SP) and most probable number (MPN) methods were used for determining the aerobic heterotrophic bacteria (AHB) and nitrogen cycle bacteria, respectively. The MPN was calculated using Cochran's Tables (1950).

Experimental media

- Plate count agar medium (APHA, 1980) as used for AHB after 72 hr at 22°C.
- Modified Ashby's medium (Abdel-Malek and Ishac, 1968) was used for the aerobic free-living nitrogen-fixing Azotobacter at 30°C for 15 days. The presence of Azotobacter was detected by visible turbidity and presence of a pellicle formed over the medium surface.
- Anaerobic free living nitrogen-fixing *Clostridium* was grown on modified Winogradisky's medium (Naguib, 1961). The dilutions were pasteurised at 80°C for 15 min. before incubation to kill all vegetative cells. The presence of *Clostridium pasteurianum* was detected after 12 days by the accumulation of gases (stormy fermentation) and was confirmed microscopically for plectridial sporangia.
- Stephenson's ammonium sulphate medium with $CaCO_3$ (Allen, 1953) was used for nitrifying bacteria. The culture was tested for nitrite and nitrate after 21 days' incubation by diphenylamine indicator (blue colour with either radicals) indicating the presence of nitrifying bacteria.



Map (1) Sampling Stations in Lake Manzala

Table ([illegible]): Ranges and annual averages of some physico-chemical parameters of the Lake Manzala during the period of study.

Parameter	Temperature (°C)				Depth (m)		Transparency (cm)		pH		Organic matter (%)		Organic carbon (%)	
	Air		Water											
Stations	range	aver.	range	aver.	range	aver.	range	aver	range	aver.	range	aver	range	aver.
1	14.2 - 30.0	23.75	15.2 - 26.6	22.27	0.75 - 1.6	1.16	15.0 - 25.0	19.5	7.16 - 7.6	7.3	[illegible] - 13.5	8.71	4.01 - 7.84	5.06
2	15.1 29.2	23.95	15.5 - 28.7	22.15	1.2 - 1.5	1.31	25.0 - 35.0	29.25	7.21 - 7.78	7.4	4.2 - 8.78	6.03	2.44 - 5.1	3.5
3	16.2 - 36.0	25.35	15.8 - 29.2	22.52	0.75 - 1.5	1.08	20 - 30.0	23.12	7.2 - 7.6	7.32	5.5 - [illegible]	7.19	3.19 - 5.75	4.17
4	16.9 - 38.0	26.32	16.4 - 30.0	23.35	0.5 - 1.2	0.85	25.0 - 30.0	27.0	7.26 - 7.91	7.41	3.6 - 8.19	5.28	2.09 - 4.76	3.06
5	17.5 - 35.0	25.92	16.9 - 29.0	23.7	0.5 - 1.5	1.05	30.0 - 35.0	32.5	7.3 - 7.92	7.65	4.3 - 12.44	6.7	2.5 - 7.23	3.89
6	17.3 - 37.5	27.7	17.1 - 28.0	24.07	0.9 - 1.0	0.95	25.0 - 40.0	30.75	7.31 - 7.90	7.56	4.8 - 8.85	6.78	2.79 - 5.14	3.94
Bahr El-Baqar	17.1 - 32.0	25.77	18.1 - 27.0	23.87	1.0 - 2.5	1.56	7.0 - 15.0	11.75	6.85 - 7.52	7.15	8.4 - 14.6	10.16	4.88 - 8.48	5.9
Hadous	17.0 38.0	26.32	17.2 - 29.1	23.7	1.2 - 3	1.8	15.0 - 20.0	17.5	6.95 - 7.61	7.3	5.5 - 9.5	7.25	3.19 - 5.5	4.21

to the oxygenation of the former and the anaerobic microsites and muddy deposits-rich organic matter of the latter. On the other hand, the number of clostridial spores was lower compared to the *Azotobacter* counts. This finding might be explained by the fact that *Clostridia* are strictly anaerobic, being unable to survive in the oxygenated waters of Lake Manzala. Moreover, the clostridial cells derived from irrigated soils are mainly in the vegetative form, due to the high moisture content, and are thus heat-labile compared to the spores (Rabeh, 1993).

Nitrifying bacteria in water ranged from $0.9 - 17.0 \times 10^2$/ ml, while they ranged from $2.7 - 19.0 \times 10^3$/g in bottom sediment (Table 5). The highest density during spring and summer may be due to higher water temperatures during these seasons. This may indicate that nitrifying bacteria, like almost all chemoautotrophs, prefer warm condition. This finding is in accordance with Engel (1960) and Rabeh (1993, 1996) who attributed this finding to the mesophilic nature of these bacteria. The seasonal variations of nitrifying bacteria and zooplankton (El-Sherif *et al.*, 1993) showed inverse relation as a result of grazing effect of the latter on the former Zooplanktons-feeding on nitrifying bacteria was observed by Gophen *et al.*(1974), Rabeh (1996) and Lavrentyev, *et al.*(1997). On the other hand, the bottom sediment maintained the highest counts of nitrifying bacteria compared with the overlying water. This may be due to passage from the water masses along with detritus particles to which they are attached (*Nitrosomonas*), or by way of sedimentation of the free-floating cells (*Nitrobacter*). These observations were in line with the known biological behaviour of nitrifying bacteria in aquatic systems which was previously reported by Niewolak and Korycka (1979) and Rabeh (1996). The low numbers of nitrifying bacteria were recorded in Bahr El-Baqar Drain's water and its discharge points (Stns. 1 & 3). This could be attributed to one of the following:

(1) Sewage is known to contain several substances such as fatty acids, complex organic nitrogen... etc. which might hinder the well development of nitrifying bacteria (Naguib, 1963).

(2) The anoxy and low oxygen content of the drain and its discharge points (Dwedar, 1995) which might control the growth of these aerobic bacteria.

Denitrifying bacteria recorded higher numbers during the hot seasons (Spring and Summer) compared with the two other seasons. Bacterial denitrification has been recorded at temperatures from close to $0^{o}C$ to over $30^{o}C$. With a rise in temperature $10^{o}C$, the denitrification rate increases by 1.5 - times. (Jansson *et al.*, 1994). Denitrification activities generally highest at pH 6 - 8 (Jansson *et al*, 1994). A similar situation was recorded in Lake Manzala where the averages of pH ranged from 7.3 - 7.56. On the other hand, large areas of the Lake are densely covered with macrophytes especially in the southeastern basin (Shaheen and Youssef, 1978 and El-Sherif *et al*, 1993). Weisner *et al.* (1994) reported that macrophytes support denitrifying bacteria through the following :-

(1) Supplying organic carbon which is released from plant litter or from living plants.

(2) Offering attachment surfaces for epiphytes, also producing organic matter for denitrifying bacteria.

(3) Lowering redox potential which might result from the breakdown of organic matter or possible shading of water and sediment surface by the macrophytes.

On the other hand, their numbers were higher in bottom sediment than those in the overlying water (Table 6). This may be due to:

- High content of nitrate.
- High content of organic matter (3.6 - 13.5%) and organic carbon (2.09 - 7.84%).
- Type of the Lake sediment where the subsurface consists of silt (40%) clays (22%) with occasional layers of fine sand (38%) (El-Bokhty, 1995).
- Presence of the reduced micro-environment on organic sediment particles within the upper oxidized zone of the sediments (Rabeh, 1996). The occurrence of such micro-sites permits nitrification and denitrification processes to take place within the same environment although the two processes are generally considered to be mutually exclusive due to their different oxygen requirements (Robertson and Kuenen, 1984) and Rabeh (1996). In addition, the roots of macrophytes release oxygen into the rhizosphere enhancing coupled nitrification-denitrification processes in aquatic system (Weisner *et al.*, 1994). The tight coupling between nitrification and denitrification has been previously recorded (Jenkins and Kemp 1984; Seitzinger, 1988; Ahlgren *et al*, 1994a). On the other hand, Jansson *et al*, (1994) reported that the non-mineralised organic nitrogen in the shallow Lake Vallentuna (Sweden) reaches the sediment and is further metabolised in mineralisation-nitrification-denitrification. In addition, Jansson *et*

al., (1994) reported that the retention is probably greater in shallow lakes provided that water retention time was long enough to permit sedimentation, mineralisation, nitrification and denitrification. Thus nitrifying and denitrifying bacteria might play an important role in the removal of nitrogen from organic and high nitrate wastewaters to reduce the contamination and eutrophication of Lake Manzala. In this connection, Setizinger, *et al* (1984) found that about of 35% of the mineralised organic nitrogen in the sediment was removed from the ecosystem as N_2 through denitrification. On the other hand, several investigators, Brezonik and Lee (1968); Kremer and Nixon (1978); Seitzinger (1988); Pettersson and Bostrom (1990); Jensen *et al* (1990) Jansson *et al* (1994) Ahlegren *et al.* (1994a) and Ahlegren *et al* (1994b) reported that 11%, 25%, 1 - 36%, 50% or more, 33% ± 5%, 30 - 60%, 1/3.5 - 25%, , respectively, of the nitrogen acquired in freshwater lakes and rivers from various sources were lost through denitrification.

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- For denitrifying bacteria denitrifiers' medium (Emam, 1973) was used. The culture was tested for gas production (the gas replaces the medium in the Durham tube) after 14 days' incubation.

RESULTS AND DISCUSSION

Ranges and averages of some physico-chemical parameters of the studied area are presented in Table (1). Air temperature ranged from 14.2°C during winter to 38°C during summer with an annual amplitude of 23.8°C. Because of the shallowness of the Lake, the water temperatures follow, more or less, the air temperatures ranging between 15.2°C during winter and 30°C during summer. The numbers of the aerobic heterotrophic bacteria (AHB) in water were low in Autumn, reaching their minimum in Winter and began to increase in Spring, reaching their maximum during Summer (Table 2). This pattern was in harmony with water temperature in the same seasons. In addition, the low number of AHB in Winter and Autumn may be due to the grazing effect of zooplankton (El-Sherif *et al*, 1993) on bacteria (Pace *et al* 1990 and Rabeh, 1996). A similar sequence of seasonal variation of development of AHB was also recorded for bottom sediment of Lake Manzala (Table 2). The amplitude of 14.8°C in water temperature of Lake Manzala might be translated into high aerobic bacterial counts and thus high rate of the biodegradation process during Summer and Spring, while the low Autumn and Winter temperature resulted in lower numbers of aerobic heterotrophic bacteria and slower oxidation of organic matter. With respect to the depth, Lake Manzala is very shallow with depth ranging from 0.5 to 2.3 m. This shallow depth assists in keeping the water column oxygenated through the complete vertical mixing which usually prevents the thermal and chemical stratification of the water column (Shaheen and Youssef, 1978). In addition, the shallow depth allows for release of nutrients directly into the water column from bottom sediment and prevents the sinking of nutrients to the bottom layer (Mancy and Ibrahim 1988). The transparency of the Lake water remained low throughout the present investigation ranging from 15 - 40 cm. Luettich *et al*. (1990) reported that the water quality in the shallow lake (Balaton, Hungary) is affected by resuspension and settling of bottom sediments at least in two ways. First, sediments suspended in the water column decrease light penetration, yielding low Secchi disc values. Second, the sediments are capable of acting as internal source of nutrients. On the other hand, Ibrahim *et al* (1988) attributed the low water transparency of Lake Manzala to one or more of the following factors:

1- The high amounts of fine soil particles (Clay), thus causing inorganic turbidity,
2- the quality of Drain's water entering the Lake,
3- High growth of phytoplankton (organic turbidity).

The lowest values of transparency were recorded in Bahr El-Baqar Drain and its discharge areas and increased gradually away from these Stations. On the contrary, the bacterial counts decreased in the same direction. The pH values in Lake Manzala range from 7.16 during Summer to 7 - 98 during Winter. On the other hand, the pH ranged from 6.87 - 7.52 and 6.94 - 7.61 for Bahr El-Baqar and Hadous Drains, respectively. Seasonal increases in pH of Lake Manzala during winter and Autumn may be due to the high photosynthetic activities while their spring and summer decreases may follow the degradation of organic matter in the sediment (Wahby *et al*,,1972).

Table (3) shows that the maximum and minimal counts of aerobic nitrogen-fixing *Azotobacter* were recorded during Summer and Winter, respectively. It is worthy mentioned here that the seasonal changes in AHB were generally concomitant with similar changes in *Azotobacter*. On the other hand, the higher counts of *Azotobacter* were recorded in Hadous Drain and its discharge point. These observations supported the idea that soil bacteria are the dominant one in agricultural drainage waters (Sabae 2000 and Rabeh, 2001). The high counts of *Azotobacter* in Lake Manzala may be due to the high organic and suspended matter content and the pH ranges of its water (7.16 - 7.97) which was suitable for the *Azotobacter* growth (Rabeh, 1993). Mahmoud *et al*. (1973) reported that the pH range for the growth and N_2-fixation of *Azotobacter* was between 6.5 - 9.5.

The high numbers of the anaerobic nitrogen-fixing *Clostridia* were recorded during Summer and Spring compared to Autumn and Winter (Table 4). On the other hand, the numbers of clostridial spores were high in both Drains and their mixing points. This may be due to high suspended matter. This finding confirms that of Elewa and Azazy (1986) and Rabeh (1993, 1996) as regards the association between *Clostridia* and suspended matter in Lake Nasser and Wadi El-Raiyan Lakes, respectively. The high numbers of *Clostridia* in the bottom sediment compared to those in the overlaying water may be attributed

Table (2). Aerobic heterotrophic bacteria × 10^9/ml of water or /g of sediment of Lake Manzala.

Seasons Stations	Autumn		Winter		Spring		Summer	
	W	S	W	S	W	S	W	S
1	3.3	10.0	1.5	5.3	5.5	28.9	8.7	30.2
2	0.022	0.48	0.005	0.12	0.03	0.57	0.034	0.67
3	0.031	9.5	1.4	4.7	4.9	23.2	7.8	28.1
4	0.02	0.32	0.031	0.15	0.027	0.54	0.031	0.62
5	0.03	0.2	0.029	0.13	0.055	0.37	0.067	0.39
6	0.028	0.1	0.01	0.07	0.037	0.2	0.039	0.32
Bahr El-Baqar	3.9	18.7	1.8	11.7	6.5	29.8	9.1	33.1
Hadous	0.026	0.35	0.0045	0.17	0.037	0.7	0.043	0.85

W = water S = sediment

Table (3). Most probable number of *Azotobacter* × 10^2/ml of water or × 10^3/g sediment of Lake Manzala.

Seasons Stations	Autumn		Winter		Spring		Summer	
	W	S	W	S	W	S	W	S
1	4.6	3.5	2.0	2.2	6.4	7.6	5.9	8.1
2	2.9	4.3	2.4	3.0	7.6	11.0	9.5	16.0
3	4.4	3.2	1.8	2.4	6.4	7.6	6.2	11.0
4	5.2	6.9	3.4	4.5	9.2	16.0	11.0	19.0
5	2.4	3.5	2.8	2.9	4.6	6.9	5.8	6.2
6	1.9	3.6	2.4	2.8	2.7	5.9	3.6	5.4
Bahr El-Baqar	4.6	5.9	3.4	5.0	7.6	11.0	14.0	14.0
Hadous	15.0	17.0	4.6	11.0	28.0	32.0	32.0	31.0

W = water S = sediment

Table (4). Most probable number of *Clostridium* × 10^2/ml of water or × 10^3/g sediment of Lake Manzala.

Seasons	Autumn		Winter		Spring		Summer	
Stations	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**
1	0.81	2.3	0.018	0.09	1.5	7.5	3.5	9.2
2	0.078	1.5	0.0	0.012	0.84	3.8	2.1	4.5
3	0.72	2.9	0.013	0.06	1.4	7.5	2.8	8.1
4	0.068	1.5	0.0	0.027	1.3	5.3	1.5	7.5
5	0.027	2.3	0.0	0.0	1.1	4.4	2.4	5.8
6	0.028	1.5	0.0	0.028	0.95	4.0	1.7	6.9
Bahr El-Baqar	1.8	2.9	0.37	0.13	2.8	8.1	4.1	9.5
Hadous	0.92	1.5	0.018	0.04	2.2	6.4	3.6	8.4

W = water S = sediment

Table (5). Most probable number of nitrifying bacteria × 10^2/ml of water or × 10^3/g sediment of Lake Manzala.

Seasons	Autumn		Winter		Spring		Summer	
Stations	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**
1	3.5	3.4	0.9	2.7	6.7	4.6	7.5	6.2
2	6.2	5.4	4.0	3.1	9.0	12.0	9.0	14.0
3	3.7	3.6	1.3	2.9	6.9	6.5	8.5	7.2
4	9.2	9.5	4.4	6.2	12.0	17.0	17.0	19.0
5	4.0	3.8	3.2	3.2	9.0	8.1	15.0	8.1
6	4.0	3.9	3.0	3.8	9.0	7.9	13.0	9.2
Bahr El-Baqar	3.1	3.0	1.5	2.1	6.2	4.4	7.7	6.2
Hadous	13.0	12.0	6.8	7.0	18.0	20.0	24.0	27.0

W = water S = sediment

Table (6). Most probable number of denitrifying bacteria × 10^2/ml of water or 10^3/g sediment of Lake Manzala.

Seasons / Stations	Autumn		Winter		Spring		Summer	
	W	S	W	S	W	S	W	S
1	0.95	6.4	0.018	0.09	3.2	4.5	4.0	9.2
2	0.22	2.2	0.0	0.027	1.6	2.3	2.5	4.6
3	0.92	6.2	0.018	0.09	2.9	4.6	3.8	7.2
4	0.095	3.5	0.0	0.28	1.4	2.3	2.4	4.3
5	0.081	2.8	0.0	0.04	1.2	4.3	2.8	4.4
6	0.075	2.8	0.0	0.07	1.5	4.3	2.9	6.4
Bahr El-Baqar	1.9	6.9	1.4	2.2	3.8	6.9	4.5	9.5
Hadous	0.95	5.8	0.7	1.8	2.7	4.3	3.7	6.2

W = water S = sediment