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Bacterial Indicators of Both Sewage Pollution and Trophic Status in Abu Za'baal Lakes, Egypt

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Abstract: Abu Za'baal Lakes are three newly formed lakes lying in North of El-Qalubiya Governorate. Water samples were collected seasonally from surface and near-bottom waters of these lakes. Physico-chemical and bacteriological analysis of the studied lakes were investigated. The results revealed that the recorded pH values of the studied lakes' waters were on alkaline side. High total bacterial counts (saprophytic and parasitic) and high bacterial indicators of sewage pollution (total and faecal coliforms as well as faecal streptococci) were recorded in Summer. The total counts of gram-negative bacteria were also recorded, where *E. coli*, *Klebsiella pneumonia*, *Salmonella choleraesuis*, *Enterobacter aerogenes*, *Yersinia pseudotuberculosis* and *Citrobacter freundii* were found to be dominant organisms. As indication of trophic status of the studied lakes, seasonal and regional variations of bacterial reproduction rate (generation time), bacterioplankton biomass, daily production of bacterial biomass and hydrolytic bacterial activity were determined. Generally, first lake maintained the highest values of bacterial reproduction rate, bacterial biomass, daily production of bacterial biomass and hydrolytic bacterial activity compared with the other two lakes. Also, the near-bottom water recorded the highest bacteriological parameters compared with those in surface water layers of the investigated lakes. Seasonally, these parameters recorded the highest levels during Summer.

Key words: Abu Za'baal Lakes, bacterial sewage pollution, gram-negative bacteria, bacterial biomass, bacterial activity, Egypt

Introduction

Abu Za'baal Lakes (three) were formed during the last century. The 1st and the 2nd lakes were formed during the fifth and eighth decades, respectively. The 3rd lake was formed in the ninth decade and there is a small depression still in the filling phase now (Fig. 1). These lakes were formed probably due to fracture and extraction of basalt rocks and they became gradually filled up by ground water and seepage (Abd Ellah, 2003). The seepage source may be from Imailiya Canal, Bahr El-Baqar drain and the cultivated areas close to the lakes. Abu Za'baal Lakes are located in north of El-Qalubiya Governorate. They occupy the area between latitude 30° 16.62' and 30° 17.58' N and longitude 31° 20.90' and 31° 21.69' E. The 1st lake lies between latitudes 30° 16.84' and 30° 17.58' N and longitudes 31° 20.94' and 31° 21.53' E (375.846×10³ m²). The 2nd lake extends between latitudes 30° 16.78' and 30° 17.15' N and longitudes 31° 20.90' and 31° 21.22' E (151.848×10³ m²). The 3rd lake lies between latitudes 30° 16.62' and 30° 16.82' N and longitudes 31° 21.09' and 31° 21.29' E (80.386×10³ m²). The filling phase depression extends between latitudes 30° 6.71' and 30° 16.82' N

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and longitudes 31° 21.59' and 31° 21.69' E. The lakes waters cover an area of 608.050×10³ m². The water depth in the 1st lake was sharply increased northward (0.6 to 20.0 m with average of 10.219 m). The water depth of the 2nd lake varied from 2.9 to 7.6 m with average 6.12 m and the 3rd lake depth varied from 0.8 to 7.1 m with average of 5.791 m. The highest water storage was 3840.464×10³ m³ in the 1st lake while the lowest water storage was 465.516×10³ m³ in the 3rd lake. The water storage in the 2nd lake was 928.095×10³ m³. The total water storage was 5234.075×10³ m³. The 1st lake is the widest (61.81% of total water surface area), deepest and largest (73.38% of the total water storage) basin. The 3rd lake is narrower (13.22% of total water surface area), shallower and smaller (8.89 of the total water storage) (Abd Ellah, 2003).

Formation of Abu Za'baal Lakes has led to a number of changes in the existing microhabitats that a large number of different species of fauna and flora became re-established in such new ecological setting. The area thus opened several possibilities for various forms of development in tourism and recreational activities, fisheries and limited agricultural or soil reclamatory activities. Water can be rendered unsatisfactory from technical or aesthetic points of view by the microorganisms it contains. Thus, the bacteriological examination of water is necessary to disclose the presence of microorganisms that might constitute a health hazard.

Phytoplankton composition, chemical and morphometrical characteristics of Abu Za'baal Lakes' waters were studied by Abd Ellah (2003) and Hussian (2005). The heavy metals pollution and its effect on some fish species and pathological conditions of *Tilapia zillii* caught from these lakes were also investigated (Gaber and Fadel, 2005; Mohamed and Gad, 2005; El-Mansy, 2005).

The present investigation is concerned with studying of the total bacterial counts at 22 and 37°C and bacterial indicators of sewage pollution (total and faecal coliforms, in addition to faecal streptococci). These parameters are usually used for evaluating the sanitary quality of water. Rate of bacterial reproduction, cell volumes, biomass, daily production and activity of bacteria as indications of trophic status in the studied lakes were also investigated. As far as we know, this work represents the first bacteriological study on Abu Za'baal Lakes.

Materials and Methods

Sampling and Sampling Stations

Water samples were collected seasonally during the period of Autumn 2003-Summer 2004 from the three lakes of Abu Za'baal. The largest first lake was represented by 5 stations, while the other two lakes were represented by 2 stations (one for each) (Fig. 1).

Physico-chemical Parameters

The temperatures of surface and near-bottom waters (SW and N-BW) were measured using a digital thermometer and the pH values were recorded using a pH meter. Water transparency was measured by black/white standard 25 φ Sechi disc.

Bacteriological Analysis

Total bacterial Counts

The total counts of bacteria at 22 and 37°C on plate count agar, was carried out, using the standard spread method (Clark, 1971).

Bacterial Indicators

Most probable numbers (MPN) of bacterial indicators of sewage pollution (total and faecal coliforms and faecal streptococci) were determined using Multiple Fermentation Tube (MFT) method as described by APHA (1992).

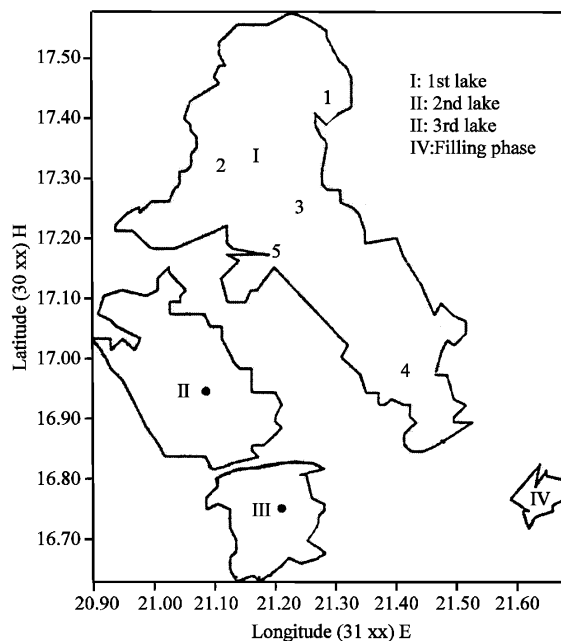


Fig. 1: Location of the sampling stations in Abu Za'baal Lakes

Total Gram-Negative Bacteria

The total gram-negative bacteria in the studied lakes during different seasons were determined, using MacConkey agar supplemented with 0.001 g L⁻¹ crystal violet (Oxoid Manual, 1981). Several isolates of these gram-negative bacteria were grouped, selected and characterized morphologically and biochemically and confirmed by API 20E strip system (Biomerieux).

Rate of Reproduction

Rate of reproduction (generation time) of bacterioplankton was calculated using the formula of Kuznecov and Romanenko (Niewolak and Sinica, 1981).

Volume of Bacterial Cells

To determine the bacterial cell volume in the tested water, the dimensions of the cells were determined by transmission electron microscopy (JEOL, JEM-1230) according to the method described by Zimmermann (1977). Cell volumes were calculated according to the formula of Loferer-Krößbacher *et al.* (1998) and then the average volume of one cell was calculated.

Bacterioplankton Biomass

Bacterioplankton biomass in mg of org. C m⁻³ and in a water column of m² in area, was calculated according to Romanenko's formula (Niewolak and Sinica, 1981).

Daily Production of Bacterial Biomass

Daily production of bacterial biomass was estimated by using average values of generation time and bacterial biomass according to the formula described by Niewolak and Sinica (1981).

Bacterial Activity

Bacterial activity in the studied lakes was estimated by the method described by Fontvieille *et al.* (1992). The assay depends on measuring of the bacterial hydrolytic activity using

fluorescein diacetate (FDA). One hundred milliliter of water collected from the studied lakes was filtered through 0.2 μm filter and the filter was washed in 3 mL phosphate buffer (pH 7). 0.1 mL of FDA solution (2 g L^{-1}) was added and the mixture was incubated at 15°C overnight and then frozen to stop the reaction. The active bacteria in the water samples hydrolyzed the colorless fluorescein diacetate to fluorescein (yellowish green in color). The concentration of this compound in one ml of water sample was estimated from the optical density measured at 490 nm.

Results

Physico-Chemical Parameters

In this study, the temperature and pH of both SW and N-BW in addition to water transparency of the studied lakes were recorded. The highest temperatures of both SW and N-BW were recorded in Summer (31 and 29°C , respectively), while the lowest ones were recorded in Winter (18 and 16°C) (Fig. 2 a and b). The highest pH values were recorded in the SW (7.8 to 8.2 during Autumn and Spring, respectively), while the lowest were recorded in N-BW (7.6 to 8.1). Generally, the water of the 3rd lake was more alkaline compared to the other 2 lakes (Fig. 2c and d). Water transparency of the studied

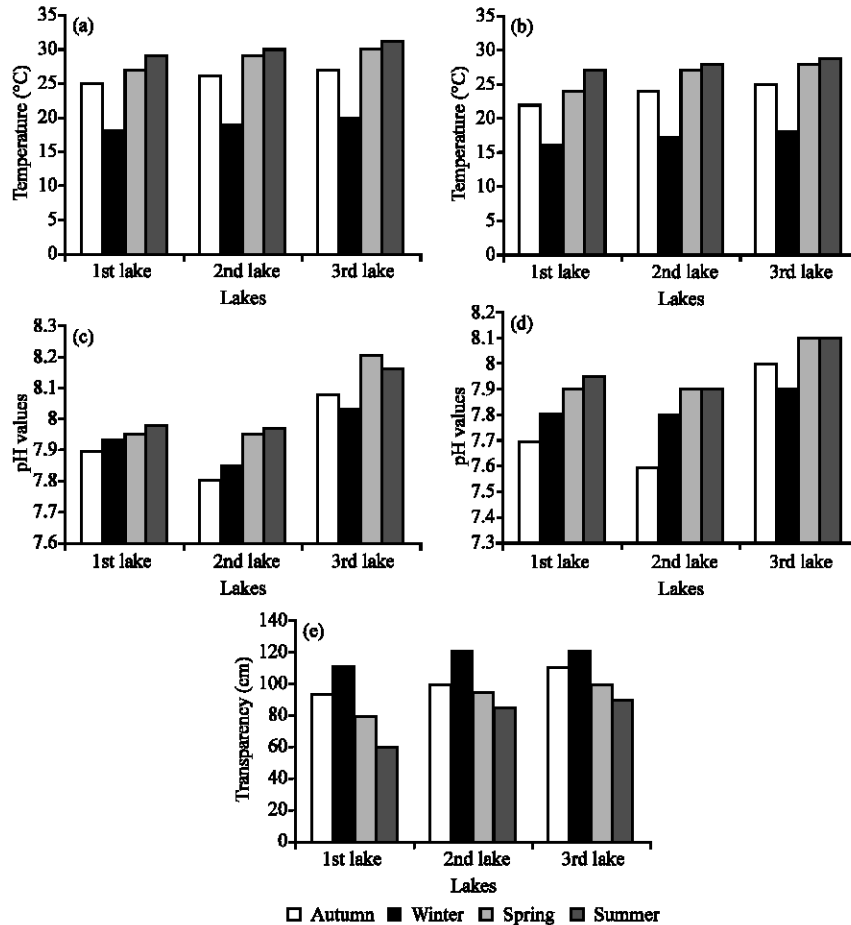


Fig. 2: Seasonal variations in surface and near-bottom waters of (a) and (b) temperature (c) and (d) pH, respectively and (e) transparency

lakes also varied seasonally from 60 and 85 cm during Summer to 110 and 120 cm during Winter in the 1st and 2nd lakes, respectively. In the 3rd lake, transparency ranged from 90 to 120 cm during Summer and Winter, respectively (Fig. 2e).

Total Bacterial Counts

The total bacterial counts at 22 and 37°C in the studied lakes were determined seasonally. Compared to the other two lakes, the 1st lake maintained the highest average of bacterial counts at 22 and 37°C, recording 21.8×10^6 and 17.8×10^6 cfu mL⁻¹ in SW and 80.9×10^6 and 74.62×10^6 cfu mL⁻¹ in N-BW, respectively during Summer (Fig. 3a-d). Low bacterial counts were recorded in the SW of the 3rd lake (0.6×10^6 and 1.4×10^6 cfu mL⁻¹ at 22 and 37°C, respectively) and the 2nd lake (0.8×10^6 and 1.5×10^6 cfu mL⁻¹) during Winter. On the other hand, the bacterial counts were higher in N-BW compared to those in the SW.

Bacterial Indicators of Sewage Pollution

MPN of total and faecal coliforms (TC and FC) as well as faecal streptococci (FS) recorded the highest average values in the 1st lake, giving rise to 522, 350 and 271/100 mL in SW and 194×10^2 , 89×10^2 and $90.8 \times 10^2/100$ mL in N-BW, respectively, during Summer, while the lowest values (55.8, 26.6 and 21.4/100 mL in SW and 3.8×10^2 , 2.8×10^2 and $2.28 \times 10^2/100$ ml in N-BW) were recorded in Winter. Again here, the counts of bacterial indicators of sewage pollution were higher in the 1st lake compared to those recorded in the other 2 lakes (Table 1 and 2). Seasonally, the highest counts of bacterial indicators were recorded during Summer and the lowest values during Winter in all of the studied lakes.

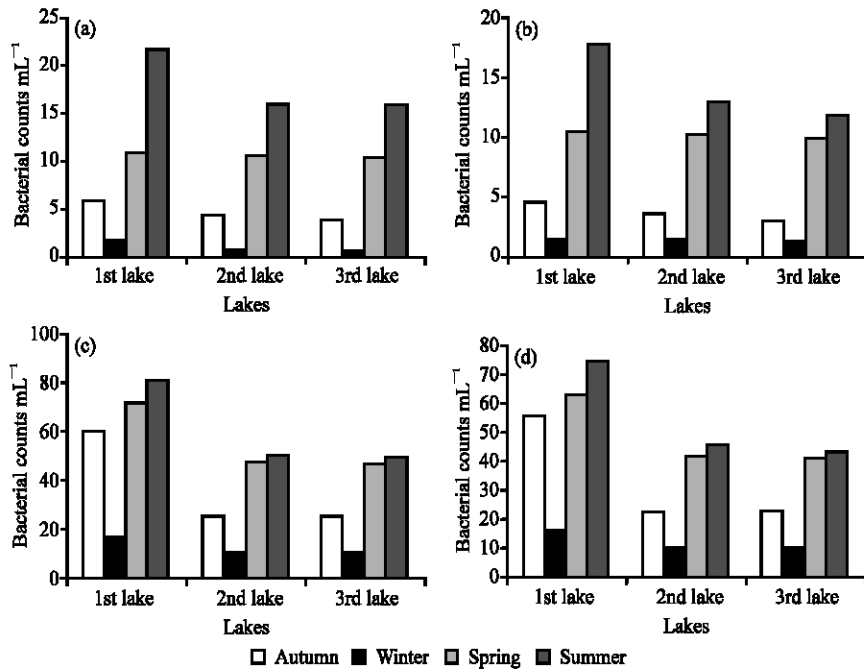


Fig. 3: Seasonal variations in total bacterial counts at 22 and 37°C in (a) and (b) surface, (c) and (d) near-bottom waters, respectively

Table 1: Most probable number of bacterial indicators of sewage pollution in surface water of Abu Za'baal Lakes

Lakes	Autumn			Winter			Spring			Summer		
	TC	FC	FS	TC	FC	FS	TC	FC	FS	TC	FC	FS
1st lake	132.2	71.8	41.6	55.8	26.6	21.4	288.8	166.6	186.4	522	350	271
2nd lake	46.0	24.0	21.0	43.0	34.0	16.0	75.0	43.0	43.0	160	90	90
3rd lake	46.0	24.0	21.0	7.0	3.0	3.0	53.0	36.0	43.0	75	64	75

Table 2: Most probable number of bacterial indicators of sewage pollution ($\times 10^2$) in near-bottom water of Abu Za'baal Lakes

Lakes	Autumn			Winter			Spring			Summer		
	TC	FC	FS	TC	FC	FS	TC	FC	FS	TC	FC	FS
1st lake	9.72	4.16	4.74	3.8	2.28	1.64	95.4	60.4	73.6	194	89	90.8
2nd lake	6.40	4.60	3.50	2.6	2.10	2.00	64.0	46.0	35.0	95	75	93
3rd lake	4.60	2.40	2.40	2.3	2.10	1.10	46.0	29.0	28.0	95	75	93

Table 3: Total counts of gram-negative bacteria in surface and near-bottom waters of Abu Za'baal Lakes

Lakes	Autumn		Winter		Spring		Summer	
	SW	N-BW	SW	N-BW	SW	N-BW	SW	N-BW
1st lake	460	6000	90	1900	1400	19500	1750	20600
2nd lake	350	4100	60	1100	950	11300	1050	16600
3rd lake	220	3300	50	900	560	9500	750	10300

Total Counts of Gram-Negative Bacteria

The total gram-negative (TG-N) bacteria in the collected water samples of the studied lakes was counted along the period of study (Table 3). Generally, as recorded in the total bacterial counts and bacterial indicators, the highest level of TG-N bacteria were recorded in the 1st lake during Summer in N-BW, while the lowest were determined in the 3rd lake during Winter in SW. The morphological, biochemical data in addition to API 20E revealed that *E. coli*, *Klebsiella pneumonia*, *Salmonella choleraesuis*, *Enterobacter aerogenes* *Yersinia pseudotuberculosis* and *Citrobacter freundii* were dominant gram-negative organisms.

Rate of Bacterial Reproduction

To determine the rate of reproduction as a parameter of bacterial biomass production, the generation time of bacterioplankton in the studied lakes was performed. Bacterial generation time in the SW of 1st lake ranged from 3 h at station 1 in Summer to 25 h at station 4 in Winter, while seasonal average ranged from 3.77 h during Summer to 17.95 h during Winter. On the other hand, the values in N-BW ranged from 4.9 h at station 1 to 33.33 h at station 4 with seasonal average ranged from 6.79 to 27.28 h during Summer and Winter, respectively. Regionally, there was a gradual increase in generation time from the first lake to the third one (Fig. 4a and b).

Bacterial Cell Volume

The transmission electron micrograph indicated that almost all bacterial cells in the water of Abu Za'baal Lakes are bacilli (Fig. 5). The volume of bacterial cells ranged from 0.156 to 0.427 μm^3 and their average cell volume was 0.258 μm^3 .

Bacterioplankton Biomass

The bacterioplankton biomass as indication of trophic level in the studied lakes was determined. The seasonal averages of bacterioplankton biomass in the SW of the 1st lake ranged from 34.05 mg org. C m^{-3} in Winter to 421.83 mg org. C m^{-3} in Summer, while their averages in N-BW

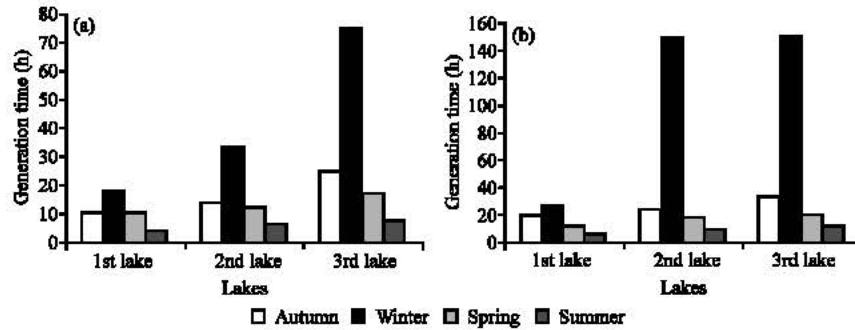


Fig. 4: Seasonal variations in rate of bacterial reproduction in (a) surface and (b) near-bottom waters

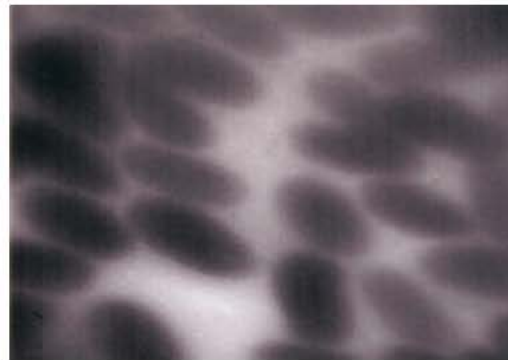


Fig. 5: Transmission electron micrograph showing the rod-shaped bacteria in Abu Za'baal Lakes

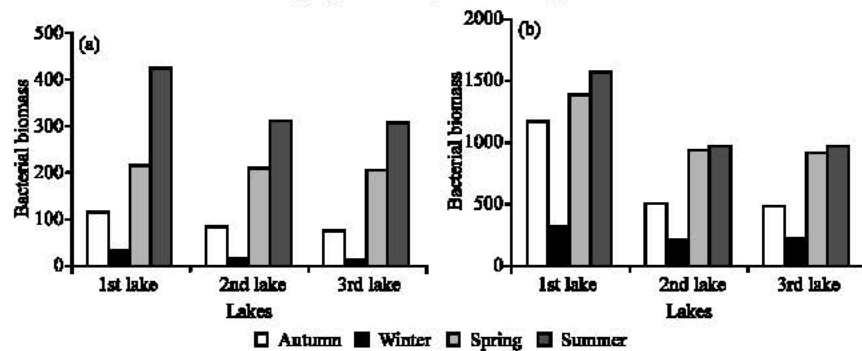


Fig. 6: Seasonal variations of bacterial biomass (mg org. C m^{-3}) in (a) surface and (b) near-bottom waters

ranged from $327.78 \text{ mg org. C m}^{-3}$ to $1565.41 \text{ mg org. C m}^{-3}$ during Winter and Summer, respectively. The values of biomass in the SW of the 2nd lake varied from $15.48 \text{ mg org. C m}^{-3}$ during Winter to $311.53 \text{ mg org. C m}^{-3}$ during Summer and recorded $212.85 \text{ mg org. C m}^{-3}$ during Winter and $973.3 \text{ mg org. C m}^{-3}$ during Summer in N-BW. Compared to the other lakes, the 3rd one, maintained the lowest bacterial biomass recording $11.61 \text{ mg org. C m}^{-3}$ during Winter and $307.66 \text{ mg org. C m}^{-3}$ during Summer in SW and $212.95 \text{ mg org. C m}^{-3}$ during Winter and $967.5 \text{ mg org. C m}^{-3}$ during Summer in N-BW. As regards site-wise variation, there was a gradual decrease in bacterial biomass from the first to the third lake. In general, a higher bacterial biomass was found in N-BW while a lower one was in SW (Fig. 6a and b).

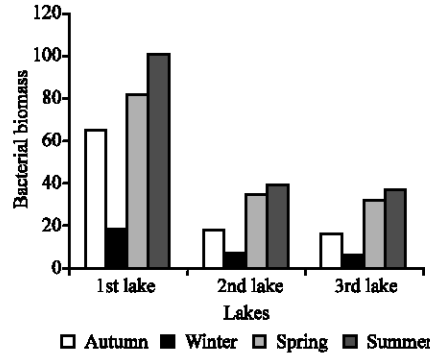


Fig. 7: Seasonal variations in bacterial biomass $\times 10^2$ (mg C m⁻²) of water column

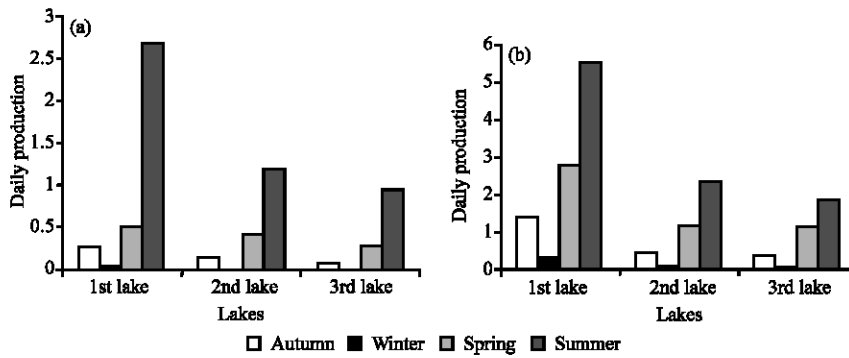


Fig. 8: Seasonal variations of daily bacterial production in (a) surface and (b) near-bottom waters

The lowest bacterial biomass in water column m⁻² in area (649.92 mg org. C m⁻²) was found in the 3rd lake during Winter, while the highest (10153.8 mg org. C m⁻²) was recorded in the deepest 1st lake during Summer (Fig. 7).

Daily Production of Bacterial Biomass

Daily production of the bacterial biomass in the SW of the 1st lake ranged from 0.0074 g org. C m⁻³ at station 4 during Winter to 3.870 g org. C m⁻³ at station 1 during Summer. On the other hand, their values ranged in N-BW between 0.0222 g org. C m⁻³ at station 4 during Winter and 9.47 at station 1 during Summer. The lowest values (0.0037 g org. C m⁻³ in SW and 0.034 g org. C m⁻³ in N-BW) were recorded in the 3rd lake. The highest averages of daily production (2.685 g org. C m⁻³ for SW and 5.533 g org. C m⁻³ for N-BW) of the 1st lake were recorded during Summer. Again here, the daily production of bacterial biomass was gradually decrease from the 1st lake to the 3rd one (Fig. 8a and b).

Bacterial Activity

Bacterial activity as a criterion for trophic conditions of the studied lakes was assessed. The bacterial activity in the SW of the 1st lake varied between 0.13 U h⁻¹ and mL at station 4 during Winter and 0.71 U h⁻¹ and mL at station 1 during Summer, while the activity ranged in N-BW from 0.21 U h⁻¹ and mL at station 5 to 0.94 U h⁻¹ and mL at station 2 during Winter and Summer,

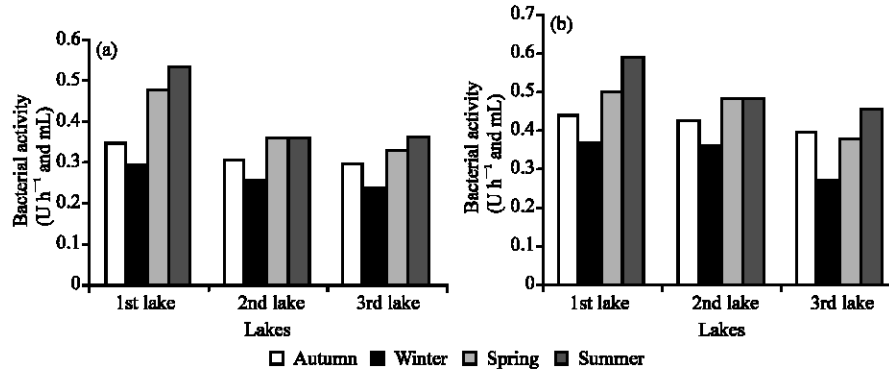


Fig. 9: Seasonal variations of bacterial activity in (a) surface and (b) near bottom waters

respectively. Compared to the other lakes, the 1st one, maintained the highest averages of bacterial activity (0.53 and 0.59 $U h^{-1}$ and mL in SW and N-BW, respectively) and the lowest was recorded in the 3rd lake. In general, higher values of bacterial activity were recorded in N-BW compared to those in SW (Fig. 9a and b).

Discussion

Lakes are extremely complex ecosystem. The unique inter-relationships of their physical, chemical and biological properties give each lake its own characters. Abu Za'baal Lakes are newly formed lakes in north of El-Qalubiya Governorate, lying in the Nile Delta. The life process of all aquatic microorganisms is affected by water temperature which enhance their active multiplications. Thus, the highest bacterial counts in both SW and N-BW of the studied lakes were recorded during Summer while the lowest were in Winter. The recorded pH values of the studied lakes were on alkaline side. Such pH levels are optimum for most microorganisms and fish species (Bagde and Varma, 1991; Haraguchi *et al.*, 2003). On the other hand, the highest pH values were recorded in the SW, while the lowest were recorded in N-BW. This might be due to the photosynthetic activity at the surface layer and bacterial degradation of organic matter at the bottom one (Rabeh, 2003a). In aquatic habitats, bacteria tend to adhere to the suspended matter. Therefore, a gradual increase in water transparency from the 1st lake to the third one was accompanied with a gradual decrease in the total bacterial counts in the same pattern.

The highest total bacterial counts at 22 and 37°C in SW and N-BW were found in the 1st lake compared to the other two lakes. This might be due to the relative ecological stability of the 1st one. On the other hand, the bacterial counts were higher in N-BW compared to those in the SW. This might be attributed to either of a lesser bactericidal effect of the solar UV rays than in SW, the N-BW being richer in organic and inorganic nutrients acting as a sink and being released from the sediment and/or sedimentation of bacteria with fine particles from the top layers.

First lake of Abu Za'baal maintained the highest bacterial indicators of faecal pollution, TC, FC and FS in both SW and N-BW compared to the other two lakes. The dominant gram-negative isolates recorded were *E. coli*, *Klebsiella pneumonia*, *Salmonella choleraesuis*, *Enterobacter aerogenes* *Yersinia pseudotuberculosis* and *Citrobacter freundii*. This might reflect the high load of bacterial pollution due to the various activities of fishermen at the 1st lake. On the other hand, these lakes are subjected to direct discharge of raw sewage from the near-by unsewered areas. The results of the present study

revealed that the counts of the bacterial indicators were high in N-BW compared to those of the SW. In addition to the earlier mentioned reasons, this may be due to the higher concentration of ammoniacal nitrogen in N-BW ($35\text{-}764 \mu\text{g L}^{-1}$) compared to those in SW ($26\text{-}466 \mu\text{g L}^{-1}$) (Hussain, 2005).

The mean counts of microbial indicators are greatly varied from method to method and from one standard to another. Thus, the ratios of 22/37°C, FC/TC and FC/FS represent a hard base of judgment, interpretation a decision making. The ratio of bacteria recovered at 22 and 37°C helps to explain any sudden fluctuation in the bacterial count. In non-polluted waters, the ratio was usually 10 or more to 1, while in polluted waters it was less than 10 (APHA, 1980). In the present study, 22/37 ratios ranged from 1.05 to 1.39 in SW and from 1.05 to 1.13 in N-BW of the 1st lake. On the other hand, the ratios ranged from 0.53 to 1.23 in SW and 1.06 to 1.14 in N-BW of the 2nd lake and between 0.42 to 1.33 in SW and 1.08 to 1.16 in N-BW in the 3rd lake. Accordingly, these lakes are heavily polluted with sewage. On the other hand, the FC/TC ratio confirms the faecal origin of the coliform bacteria. The ratios ranged from 0.47 to 0.72 and from 0.42 to 0.63 in SW and N-BW of the 1st lake, respectively. In the 2nd lake, these ratios ranged from 0.52 to 0.79 and from 0.71 to 0.8 in SW and N-BW, respectively. The ratios ranged from 0.42 to 0.85 in SW and from 0.52 to 0.91 in N-BW of the 3rd lake. These results indicate that the high levels of total coliform at the three lakes were faecal coliforms. The ratio FC/FS points to the source of faeces whether it is human (>4) or animal (<0.7) (Geldreich, 1974). Accordingly, the FC/FS ratios in SW and N-BW of the 1st lake (0.98 to 1.72 and 0.82 to 1.39), the 2nd lake (1 to 2.12 in SW and 0.17 to 1.31 in N-BW) and the 3rd lake (0.8 to 1.14 and 0.8 to 1.9 in SW and N-BW, respectively) may indicate the mixed origin of faecal pollution.

High rates of bacterial reproduction (short generation time) were recorded in the 1st lake compared to the other lakes. This might be due to high amount of organic matter in the form of phytoplankton, developing and dying in the 1st lake where the highest total phytoplankton crop ($1390 \times 10^5 \text{ U L}^{-1}$) was recorded (Hussain, 2005). In this connection, Niewolak and Sinica (1981) found that generation time ranged from 2.7 to 58.7 h, 2.6 to 59.5 h, 1.5 to 157.1 h and 2.8 to 163.4 h in dystrophic, weakly eutrophic and in 2 strongly eutrophic lakes, respectively. The intense development of bacterioplankton (short generation time) during Summer and Spring might be connected with high temperature which helps the active proliferation of bacteria and the intensive development of phytoplankton during these seasons.

The cell volume of aquatic bacteria varied between less than 0.005 to more than $5 \mu\text{m}^3$. The bacteria in a water sample, therefore, might differ in their volume in the ratio of 1:10000 (Zimmermann, 1977). In aquatic food chain, bacteria constitute a large biomass which is a continuous source of food for specialized predators (bacteriovors) (Wikner *et al.*, 1990). Moreover, they are considered a major secondary producers as they use dissolved organic matter (DOM) derived from primary producers, producing abundant biomass (Chin-Leo and Benner, 1990). Therefore, the quantification of bacterial biomass in Abu Za'baal Lakes becomes particularly important in case of growth and production rate determinations. The seasonal and regional variations of bacterioplankton biomass in the studied lakes were connected with fluctuations of the bacterial abundance and rate of multiplication. This finding was in accordance with those of Niewolak and Sinica (1981) and Rabeh (2003b) for some fertilized polish lakes and Lake Qarun (Egypt), respectively.

Daily production of bacterial biomass in the studied lakes was the highest in Summer as the high temperature enhance active proliferation of bacteria compared to other seasons. On the other hand, the gradual decrease in the production of bacterial biomass from the 1st to the 3rd lake was accompanied with the development of phytoplankton (Hussain, 2005).

Total microbial activity is a good measure of organic matter turnover in natural habitats, since generally more than 90% of the energy flow pass through microbial decomposers (Heal and Maclean,

1975). Thus, the heterotrophic activity might be a good indicator for eutrophic condition. The determination of FDA hydrolysis has the advantage of being simple, rapid and sensitive technique and it should be useful, especially for comparative studies of microbial activity in the natural habitats (Schnurer and Rosswall, 1982). In Abu Zabaal Lakes, the highest bacterial activities were recorded during Summer and Spring, where the high temperature may enhance the bacterial activities. Regionally, 1st lake maintained the highest bacterial activities compared to the other studied lakes. This might be attributed to the high organic and inorganic nutrients present in the 1st lake (Hussian, 2005).

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