ISSN 1110-0354
Bull. Nat. Inst. of Oceanogr. & Fish., A.R.E., 1998. Vol. (24): 127 - 138

ENUMERATION AND BIOMASS OF BACTERIAL INDICATOR SAPROPHYTIC COMMUNITY IN ALEXANDRIA COASTAL WATER

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Key words: Biomass, Bacteriology, Mediterranean, Egypt.

ABSTRACT

Samples from Alexandria coastal water were surveyed monthly during 1995 at nine different sites. Allochthonous discharges of rainfall and also domestic sewage have considerable effects on the distribution of bacterial communities. Microbial assessment was carried out in a total of 108 samples. Total and feacal coliforms, the saprophytic bacteria as well as total bacterial counts and biomass were determined. The use of discriminant function analysis depending on some biological and physiochemical variables led to differentiate the water environment into four characterized seasons. The highly effective parameters were temperature, dissolved oxygen, total bacterial numbers, saprophytic bacteria and N/P ratio. In general, the bacterial flora of the coastal water was markedly affected by water temperature. Bacterial number and saprophytic bacterial density showed the same trend allover the year (fluctuated between 0.7 and 5 $x10^6$ cells/ml and 1.7 and 6.6 $x10^3$ cells/ml respectively). Twenty-three percent of the examined samples were found to be free from feacal bacteria. The bacterial biomass fell in the range of 17 to 824.3 $\mu g c l^{-1}$.

INTRODUCTION

Bacteria are a large and active part of marine ecosystem and are the main agents of the food web. Microorganisms (including bacteria) like all biological systems, respond to the biological and abiotic environments, and under stress the structure of the microbial community changes.

Allochthonous materials entering seawater via domestic and industrial wastes can adversely affect the biota in several ways: (a) by initiating proliferation of microorganisms which in turn increase the biomass; (b) by selecting species able to survive at elevated concentration of exogenous substances; and (c) by adding pathogenic microorganisms associated with domestic sewage causing a public health problem. And since faecal coliforms show a high specific positive correlation with faecal contamination, so they are considered the best indicator for the microbial water quality. Also, the saprophyte counts, which represent a small group of actively metabolizing bacteria, react immediately with the changes in the nutrient supply and thus, they are an important indicator group.

The purpose of the present study is to estimate the number of saprophytic bacteria, total and faecal coliforms, total bacterial number and its biomass in the coastal water of Alexandria extending about 20 kilometer, as an attempt to evaluate the microbial water quality of this area.

MATERIAL AND METHODS

Water samples from Alexandria coast were collected during 1995 from nine sites, designated as sites 1 to 9 (Fig. 1). Each site was sampled monthly and a total of 108 samples were randomly selected to provide comprehensive coverage of the study area which extends for about 20 km, from Mamoura in the East of Alexandria and Anfoushi in the West.

Water samples were collected at 30 meter away from the shore, where the water depth ranged between 2 and 4 meter, in sterile screw capped bottles below the sea surface with ample air space to permit shaking the samples. Total and faecal coliforms bacteria were detected and enumerated by the five-tube fermentation technique (M.P.N.) according to American Public Health Association (1984). Saprophytic bacteria were counted as colony forming units (C.F.U.) on yeast extract-peptone agar medium Zobell 2216E.(1984) Incubation temperature was 20°C. The resulting colonies were counted after 14 days. Total bacterial number (TBN) and bacterial biomass (BBM) was estimated by the acridine orange method described by Hobbie ***et al.*** (1977).

Data for physico-chemical parameters were compiled from Zaghloul and Siam (1996) where; temperature, hydrogen ion concentration (pH), salinity, dissolved oxygen, oxidizable organic matter, and nutrient salts (ammonia, nitrate, nitrite and reactive phosphorus). Chlorophyll-a content were measured following the methods of Strickland and Parsons (1972).

Thirteen biological and chemical parameters were selected for statistical analysis using the SPSS® for Windows™, Release 6.0 (Norusis, 1993). Discriminant function analysis (DFA) was used to classify Alexandria coastal water according to water quality in different seasons. According to the determined variables. The data of the present work were represented in Box and Whisker plots in order to display the median, rang and extremes of the distribution. i.e., the maximum and minimum values.

RESULTS AND DISCUSSIONS

In the current study, the purpose of using the discriminant function analysis (DFA) is to distinguish between water quality of Alexandria coastal area, when some chemical and biological parameters characterize it (Table 1).

The application of the direct method of the DFA for Alexandria coastal area data indicated that a significant variation exists between the four seasons.

The first two discriminant functions accounted for 90.8% of the variation between these seasons (Table 2). Figure 2 shows all groups (seasons) plotted on the these two functions. Function one separates group 1 (Spring) from the other three groups 2, 3 and 4 (Summer, Autumn and Winter), while function 2 separates group 2 (Summer) from group 4 (Winter). Table 3 summarizes the classification results obtained through the discriminant analysis. It is evident that all examined sites have been correctly classified into 4 groups. Spring (1), Summer (2), Autumn (3) and Winter (4), and each one has its own characteristics. This table shows that the classification of all sites into four groups (1 to 4) is perfect (i.e. 100% assignment efficiency).

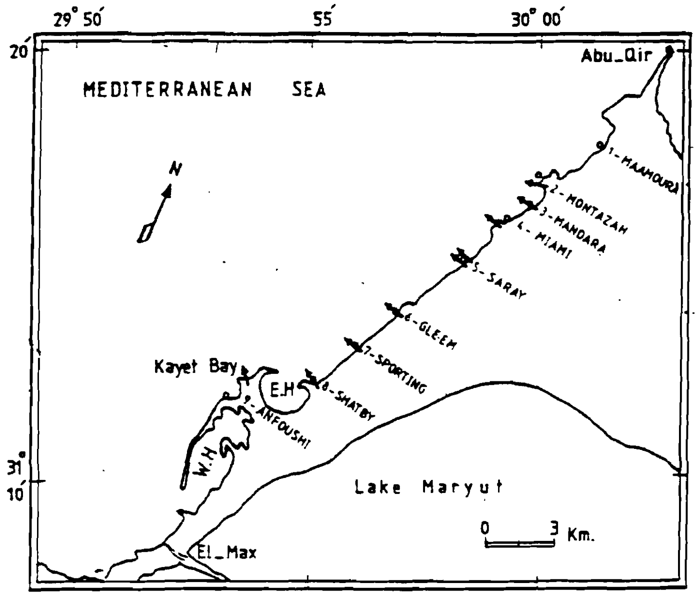

Fig. 1:Alexandria beach sampling sites (1-9) and waste water outfalls.

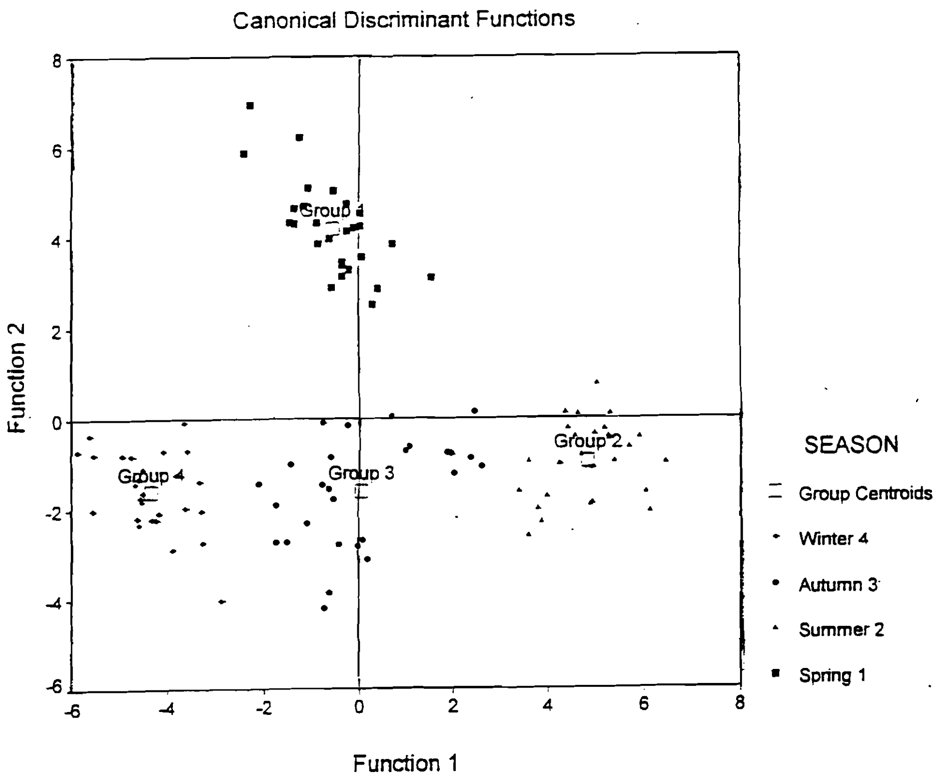

Fig. 2: Scatterplot of the studied sites on two discriminant functions derived from data matrix, Alexandria beach, Egypt.

Table 1: Seasonal variations in the mean value of the determined variables

Variable	Spring	Summer	Autumn	Winter
No. of samples	27	27	27	27
Total bacterial number/ml	710370	198407	5070370	1020370
Saprophytic bacteria/ml	634	972	1673	632
Coliforms Bacteria/ml	1232	217	112	197
Bacterial biomass μgCgm^3	99.9	505.8	17.0	824.3
Chlorophyll-a μg at./l	2.81	3.48	2.23	1.26
Temperature °Co	20.97	28.33	23.92	17.44
PH	8.09	8.08	8.15	8.18
Dissolved Oxygen mO_2/l	6.02	3.82	3.51	3.32
Dissolved organic matter mg O/l	4.12	1.07	0.36	0.65
Total inorganic nitrogen μ g at./l	4.04	3.96	6.48	7.18
Phosphate μg/l	0.18	5.63	0.55	0.77
Salinity ‰	37.04	38.46	34.56	34.41

Table 2: Evaluation of discriminant function for the studies water masses of Alexandria Coastal Area.

Function No.	Eigen Value	Variance %	Cumulative	Canonical correlation	After Function	Wilks Lambda	Chi-Square
1	11.0116	58.38	58.38	0.9575	0	0.0043	545.41
2	6.1137	32.42	90.80	0.9271	1	0.0514	296.83
3	1.7351	9.20	100.00	0.7965	2	0.3656	100.62

In order to determine which parameters are most important in distinguishing each group. Table 4 shows that the first function is correlated most highly with dissolved oxygen, bacterial biomass, organic matter and total coliform.

Seasonal distribution of total bacterial number and saprophytic bacterial density (CFU) exhibit the same trend with pronounced maxima during Spring (Fig.3). The larger the box, the greater the spread of the variable. This is supported by Gocke ***et al.***, 1990 in coastal water of Germany. Also this may be due to high Phytoplankton production in euphotic zone which, by its death creates a favorable condition for the growth of bacteria (Gast and Gocke 1988).

The presence of high counts of saprophytic bacteria (non-indicator organisms) minimized the number of coliform. This is probably due to the masked detection as indicated by many investigators (Geldreich ***et al.*** 1972, Hassan 1993 and Pagel ***et al.*** 1982) or because saprophytic bacteria have the ability to grow on wide spectrum of organic material and salinity (Rheinheimer 1987).

Twenty-five samples out of one hundred and eight were found to be free from faecal coliform but they were found to contain verified total coliforms. The sanitary quality of coastal water is best indicated by faecal coliforms because it exhibits highly specific positive correlation with the contamination by faecal material. In Fig (3) the boxes showing the distribution of total coliforms are large in Spring and Summer indicating the increase of the indicator bacteria in hot seasons. This is due to the large number of bathers in the studied sites, and also to the big quantity of sewage out flow to the sea (Zaghloul and Siam 1996). This finding was supported by Hassan (1993) in the Arabian Gulf, by (Hendery and Toth 1981) in Mississippi Lake and by Papapettro Paulou ***et al.*** (1994) in southern Greece coastal areas. Aubert ***et al.*** (1975) determined the main bacterial groups responsible for producing antagonistic substances to faecal coliforms in the Mediterranean Sea. They found that those bacteria belong to several genera, in particular Pseudomonas, Archromobacter, Flavobacterium, Chtomobacterium and Vibrio, which make up the bulk of the marine bacterial population, and since these organisms are strictly aerobes so they are limited to the well aerated euphotic level (studied area).

Table 3: Classification results of the studied water masses of Alexandria Coastal Area.

Actual Group	No. of samples	Predicted Group Membership			
		1	2	3	4
Group 1: Spring	27	27 100.0%	0.0 0.0%	0.0 0.0%	0.0 0.0%
Group 2: Summer	27	0 0%	27 100.0%	0.0 0.0%	0.0 0.0%
Group 3: Autumn	27	0 0%	0 0.0%	27 100.0%	0.0 0.0%
Group 4: Winter	27	0.0 0%	0.0 0.0%	0.0 0.0%	27 100.0%

Table (4): Pooled within–groups' correlation between discriminant functions and discriminating variables

Variables	Function 1	Function 2	Function 3
Temperature	0.65*	-0.11	0.30
Chlorophyll-a µg at./l	0.12*	-0.01	0.07
Dissolved oxygen mO_2/l	0.06	0.60*	0.04
Organic matter mg O/l	0.06	0.33*	-0.24
Bacterial Biomass µg Cgm^3	0.03	-0.16*	0.02
Total coliforms /m	0.02	-0.05*	0.02
Total bacterial number/m	0.09	-0.31	0.69*
Saprophytic bacteria/m	0.12	-0.22	0.67*
N/P	-0.37	0.19	0.50*
Salinity ‰	0.09	0.07	-0.10*

The water temperature recorded during sampling exhibited considerable seasonal variations and reached its maximum value during summer season and its minimum value in winter. The range between the maximum and minimum value was large in both spring and autumn (Fig. 3). This result may be because water temperature correlates with air temperature, which undergoes extreme changes in both seasons in this geographical area. While temperatures in summer and winter were more stables, also the shallowness and continuous mixing of beach water caused this temperature variation.

A remarkable seasonal variation in salinity was detected. A high mean value (38.46) was estimated during summer and a low mean value (34.41) in winter (Fig. 4). This wide variation reflects the influence of dilution of domestic, industrial and fresh water discharge as well as rainfall.

Relatively wide pH fluctuations were found in the nine sampling sites along the Alexandria coast during the present study. The minimum mean value was in summer (8.08) while the maximum mean (8.18) was in winter (Table1). This agrees with Dietrich and Kalle (1957). They reported that pH of the surface zone of the sea was 8.2.

The concentration of the dissolved oxygen in any water body reflects its sanitary status. The increase of pollutants lead to diminishing the amount of dissolved oxygen, which markedly affects the heterotrophic bacterial growth in the aquatic ecosystem (El Bestawy 1989). In the current study the average mean of the concentration of dissolved oxygen exhibits its maximum in spring (highly oxygenated period) but the differences between the other three seasons are limited (Table 1).

The oxidizable organic matter (OOM) determination gives an ideal characterization of the beach water quality (Carlberg 1972). The concentration of (OOM) is produced as a result of decomposition of domestic sewage, decaying of organic detritus and planktonic organisms by bacterial action (Hoppe 1978). In the present study the average seasonal changes exhibit the lowest value during autumn, while the highest average was recorded during spring.

In the marine environment the heterotrophic bacteria need a mixed diet of nitrogen, phosphorus and organic carbon in order to produce a new biomass, the balance between the concentration of these nutrients causes bacterial growth

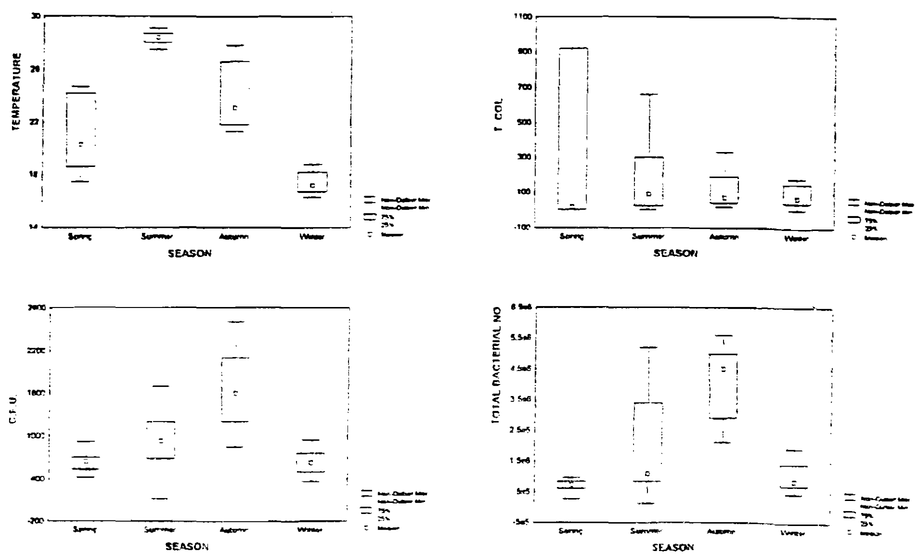

Fig. 3: Box- and - Whisker plot of temperature, saprophytic bacteria (C.F.U.), total coliforms bacteria (T. Col.) and total bacterial number of the studied water sites, Alexandria.

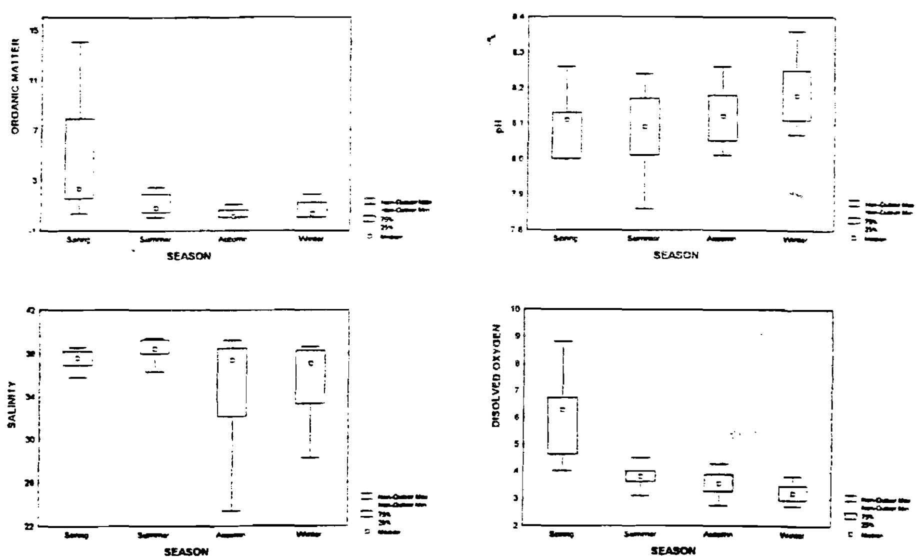

Fig. 4: Box- and - Whisker plot of organic matter, salinity, pH and dissolved oxygen of the studied water sites, Alexandria.

(Thingstad 1987). Spencer (1978) postulated that in a multilake study weak but significant correlation existed between bacterial number and orthophosphate uptake. Phosphate loading indicates that continuous supply existed through sewage pollution so that it exceeded bacterial and algae requirements. Phosphate fluctuation is governed by the interaction of two factors, the input of wastewater and the uptake by the heavy blooms (Hussein 1994).

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