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A SYNOPTIC SURVEY ON NUMBER OF BACTERIA AND BIOMASS IN RELATION TO PHYSICO-CHEMICAL PARAMETERS IN EASTERN HARBOUR OF ALEXANDRIA, EGYPT

BY

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Key words: Bacteriology, Hydrobiology, Alexandria, Mediterranean.

ABSTRACT

The bacterial count, and its biomass were estimated in the seawater of the Eastern Harbour of Alexandria in relation to some physicochemical parameters to show their effect on the bacterial abundance and its biomass in a water body subjected to fast changing.

The bacteriological count in the area showed pronounced differences, ranging between 0.47 to 5.4 million cells/ml for total bacterial number (TBN) and from 540 to 5400 CFU/ml for viable saprophytic bacteria. The bacterial biomass (BBM) ranged from 97.6 $\mu gCgm^3$ in winter to 190.4 $\mu gCgm^3$ in summer.

INTRODUCTION

The Eastern Harbour (E.H.) is a relatively shallow semi-enclosed basin sheltered from the sea by concrete break-water about 750 meter in length leaving two openings through which the exchange of water between the harbour and the inshore water take place. Its area is about $2.53x10^6$ m^2 with an average depth of about 6.0 meters.

The water of Eastern Harbour is subjected to pollution from eleven small sewage outfalls inside the harbour with an annual discharge of about $35.2x10^6m^3$ of untreated domestic sewage (Aboul Kassim, 1987). This quantity is about 2.3 times the water volume of this basin (Zaghloul, 1996).

High numbers of different microorganisms are introduced with domestic sewage into the harbour along with great amounts of organic and inorganic nutrients, which greatly enhanced the growth of microbial mass.

Microorganisms especially bacteria play an important role in changing the marine ecosystem. Bacteria are the first component of the marine habitat to be affected by altered environmental conditions. The autochthonous bacterial community of aquatic systems may therefore provides a mean of detecting environmental perturbations (Bond, 1967, Bell ***et al.***, 1982)

The bacterial populations play an important role in the turn over of organic materials and thereby, in the self-purification of seawater (Rheinheimer, 1977). Also the composition of bacterial biomass is important since this defines the quality of food available to bacteriovorus organisms (Gast & Gocke, 1988).

The present work deals with a synoptic survey on bacterial numbers- and biomass in seawater of the E.H. in relation to some physico-chemical parameters.

MATERIALS AND METHODS

Surface water samples were collected seasonally during the period between June 1994 and May 1995 from 6 sites of the Eastern Harbour representing the different habitats of the investigated area Fig. (1).

For viable saprophyte count [Colony forming unite CFU] Zobell's medium 2216 E (1946) with 1.5% agar was used by performing the "Pour plate method" Colonies were counted after 14 days incubation at 20°C.

Total bacterial number (TBN) and bacterial biomass (BBM): were determined immediately after sampling as follows: one hundred of water was transferred into brown glass bottle and preserved with 2 ml freshly filtered formaline (37%).

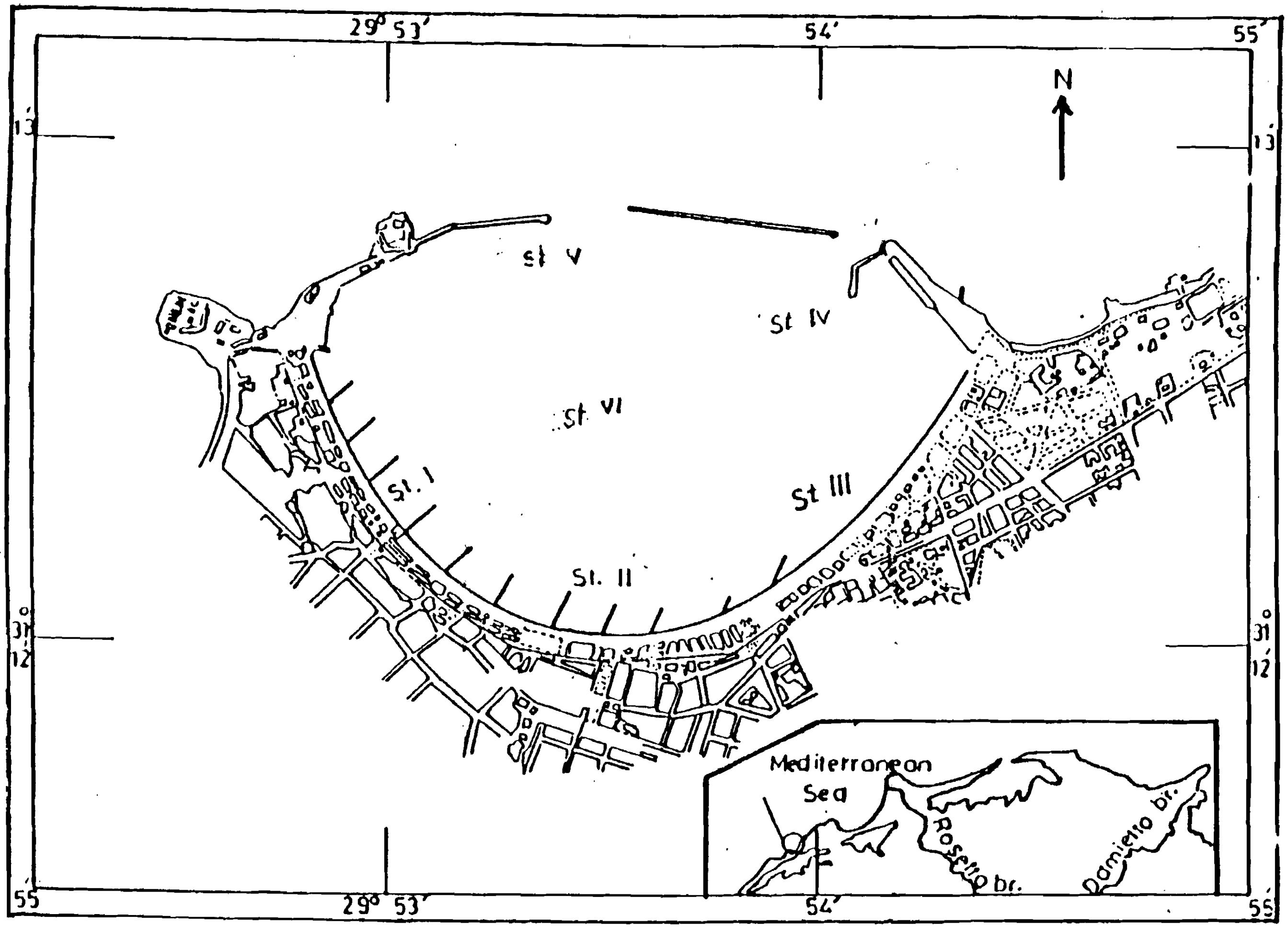

Fig. 1. A map showing the sampling stations of the Eastern Harbour, Alexandria, Egypt.

For BBM the methodology of Zimmermann (1977) was used using Millipore filters (0.2 μm pore size) previously stained with 0.004% solution of Sudan black, then the bacteria cells were stained with a 0.01% solution of acridine orange for 3 minutes; and counted with a standard Zeiss microscope equipped with an epifllouresence apparatus. From each slide 10 randomly chosen microscopic fields were assessed for count bacteria, lengths were measured with a graduated eyepiece. Cell volume and its biomass was calculated according to Zimmermann (1977) using a conversion factor of $1x10^{-7}$ μg C/m^3.

For physico-chemical parameters subsurface water samples were taken using one litre capacity Ruttener sampler.

In case of bacterial, water samples, it was collected in sterile screw capped bottles below the sea surface with ample air space to permit shaking the samples. Both temperature and hydrogen ion concentration (pH) were recorded for each sample at the time of sampling using an ordinary thermometer (1-50°C) and portable pH meter. Dissolved oxygen (DO) ml O_2/l was carried out according to Winkler method (Strickland and Parsons, 1972). The biological oxygen demand (BOD) ml O_2/l was carried out according to the standard methods for the examination of water and wastewater (American Public Health Association, 1982). Oxidizable organic matter (OOM) mg O_2/l was determined by the method of *Taras et al.* (1974). Dissolved nitrite and nitrate μg-at/l were measured according to Strickland and Parson (1972). Dissolved ammonia μg-at/l was determined using the endophenol blue method as described by Karolefft (1969). Dissolved phosphate μg-at/l was analysed according to the method described by Grasshoff (1976).

Chlorophyll-a $mg.m^{-3}$ content was measured following the method of Strickland and Parson (1972). Statistical analyses including Stepwise multiple regression equations at a confidence limit of 95% were carried out according to Freund, (1988).

RESULTS AND DISCUSSION

Temperature:

The mean seasonal water temperature ranged between 19.7°C and 26.2°C. The lowest temperature was recorded in winter, increasing gradually throughout Spring attaining its maximum in Summer and then gradually decreased during Autumn Fig. 2, Table 1.

Salinity:

Seasonal salinity values ranged between 37.57S‰ and 38.43S‰. Generally salinity attained lower values at the coastal marginal stations II) & (I due to the sewage discharge near these stations. Seasonal salinity changes are given in Table (1) and illustrated in Fig. 2.

Hydrogen ion concentration (pH):

The pH values in E.H. tend to be on the alkaline side, showing seasonal variations between 7.43 in summer and 8.25 in autumn and winter. The higher values recorded during this period may be due to the high standing crop of Phytoplankton.

Dissolved Oxygen (DO):

Dissolved Oxygen shows more or less narrow, range of seasonal variations, (2.26 – 2.97ml O_2/l) with the highest values during winter and spring. This way is due to low water temperature and low OOM concentration during these two seasons.

Biological Oxygen Demand (BOD):

The BOD ranged between 1.16 to 2.13ml O_2/l, the higher values was recorded during autumn especially at marginal stations I & II near sewage outfalls. The seasonal averages are given in Table (1) and illustrated in Fig 2.

Oxidizable Organic Matter (OOM):

The spatial OOM fluctuated between 8.3 to 2.2 mg $O_2.l^{-1}$. The higher value recorded during summer.

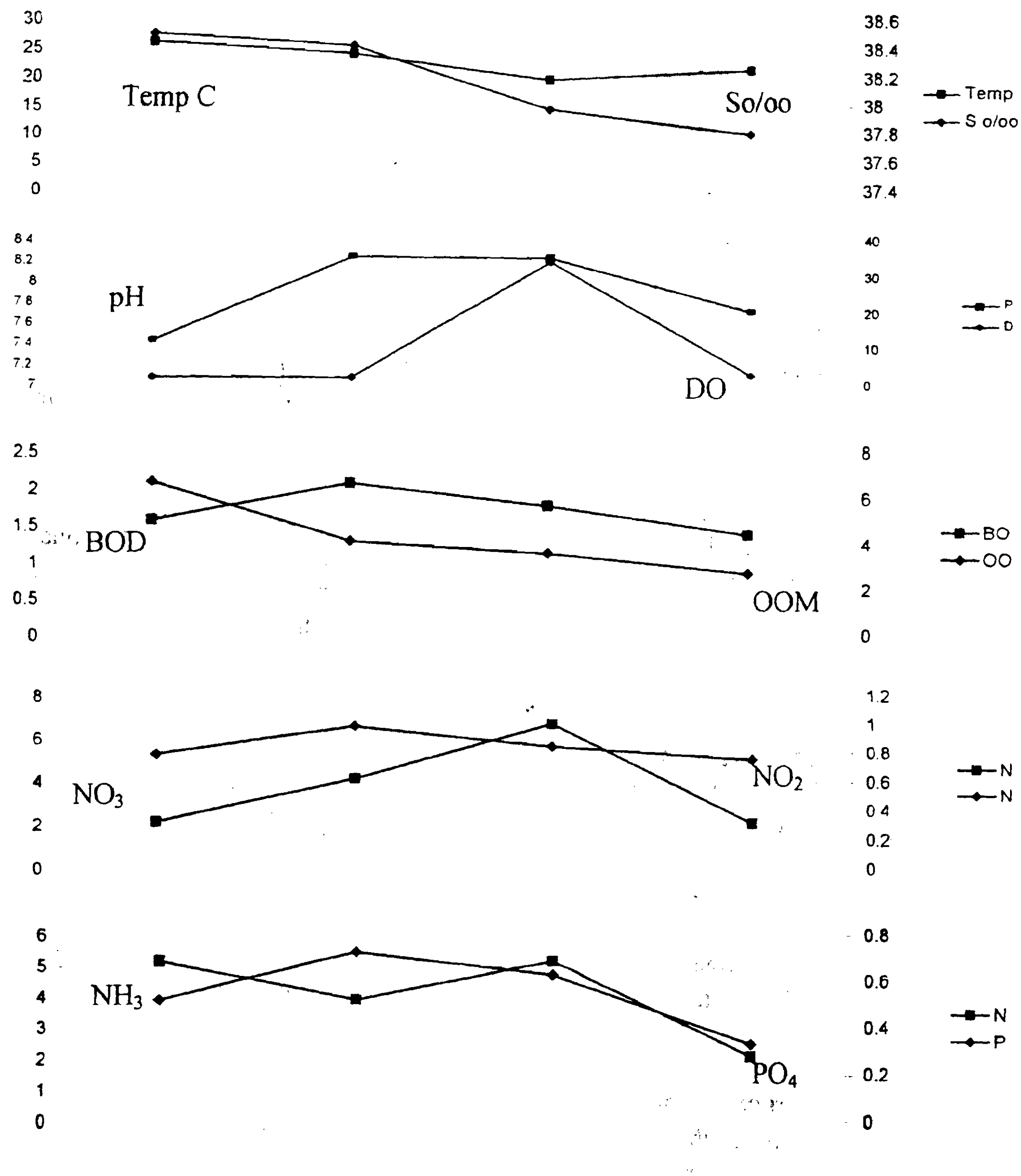

Fig. (1): Some seasonal surface water parameters variation during the period between June 1994 and May 1995 from the Eastern Harbour.

Table (1) : Some seasonal surface water parameters variation during the period between June 1994 and May 1995 from the Eastern Harbour.

	Station	Temp.	S%	ph	DO	BOD	OOM	NO3	NO2	NH3	PO4	Chl-a	TBN	CFU	BBM
	I	26.2	38.3	7.4	1.72	1.49	5.8	3.14	0.78	7.54	0.35	5.35	3.15E+06	2.50E+03	140.4
	ii	25.9	37.7	7.3	1.87	1.34	6.7	2.78	1.08	6.25	0.6	4.8	2.96E+06	2.20E+03	166.5
Summer	iii	26.4	37.8	7.6	2.1	1.7	8.3	1.23	0.75	6.5	0.55	3.57	4.20E+06	3.90E+03	190.4
	iv	26.4	38.2	7.3	2.36	1.62	7.2	2.24	0.92	2.36	0.7	3.94	5.10E+06	5.40E+03	186
	v	26.2	38	7.55	2.96	1.35	5.8	3.46	0.54	3.38	0.5	2.37	3.09E+08	2.80E+03	176.1
	vi	26.1	38.3	7.45	2.52	1.52	6.6	1.62	0.67	4.93	0.45	2.06	5.40E+06	3.30E+03	180
	Mean	26.2	38.05	7.43333	2.255	1.50333	6.73333	2.41167	0.79	5.16	0.525	3.6816667	5.49E+07	3.35E+03	173.23
	I	24.3	38.4	8.22	1.82	1.7	6.2	6.2	0.9	5.44	0.35	3.27	2.10E+06	2.10E+03	150.8
	ii	24	38.5	8.32	2.2	2.04	2.9	2.9	1.32	4.93	0.6	3.65	3.10E+06	1.10E+03	120
AUTUMN	iii	24.4	38.6	8.21	2.84	2.01	4.8	4.8	1.2	3	0.55	5.23	1.90E+06	2.20E+03	144
	iv	24.2	38.4	8.23	2.42	1.61	2.7	2.7	0.95	3.12	0.7	3.93	2.80E+06	1.09E+03	112.2
	v	24	38.5	8.22	2.75	2.1	4.5	4.5	0.83	3.87	0.5	3.76	1.40E+06	2.02E+03	126.4
	vi	24.2	38.2	8.28	2.05	1.8	3.9	3.9	0.77	3.97	0.45	3.87	8.40E+06	1.10E+03	119.2
	Mean	24.1833	38.4333	8.24667	2.34667	1.87667	4.16667	4.16667	0.995	4.055	0.525	3.9516667	3.28E+06	1.60E+03	128.76
	I	20	38.4	8.7	2.52	2.03	4.8	6.54	0.84	5.33	0.82	[illegible].35	8.80E+05	8.00E+02	110.[illegible]
	ii	19.3	38.5	8.13	3.1	2.13	3.2	8.21	1.1	5.57	0.92	3.33	6.40E+05	6.00E+02	101.2
WINTER	iii	20.9	37	8.12	2.42	1.63	2.5	6.24	0.98	4.96	0.43	3.15	4.70E+05	1.10E+02	118.6
	iv	19.5	36.8	8.14	3.82	2.03	4.7	6.5	0.83	4.33	0.7	[illegible].11	6.20E+05	8.10E+02	109.4
	v	19.2	37.5	8.17	2.6	1.22	2.6	5.96	0.62	4.79	0.39	1.93	9.20E+05	6.00E+02	98.8
	vi	19.1	37.2	8.15	1.92	1.68	4.1	6.8	0.72	5.22	0.53	2.38	8.40E+05	5.60E+02	97.6
	Mean	19.6667	37.5667	8.235	2.73	1.78667	3.65	6.70833	0.84833	5.16667	0.63167	2.5466667	7.28E+05	5.80E+02	106.03
	I	20.2	38	7.7	2.44	1.53	2.9	2.34	2.34	0.7	0.42	1.12	1.40E+06	5.40E+02	98.4
	ii	20.5	38	7.82	3.06	1.86	3.8	2.36	2.36	1.03	0.32	1.93	1.62E+06	6.40E+02	106
SPRING	iii	22.4	37.2	7.51	2.83	1.36	2.2	2.49	2.49	0.88	0.3	2.1	1.90E+06	7.10E+02	108
	iv	22.1	38.1	7.91	2.9	1.23	2.7	1.97	1.97	0.72	0.22	3.12	1.40E+06	7.40E+02	112.5
	v	21.3	38	7.86	3.24	1.16	2.3	1.62	1.62	0.53	0.42	2.32	1.62E+06	6.70E+02	107.4
	vi	22	37.5	7.5	3.34	1.43	2.6	1.43	1.43	0.68	0.33	2.79	1.90E+06	6.90E+02	106.2
	Mean	21.4167	37.8	7.71667	2.96833	1.42833	2.75	2.035	2.035	0.75667	0.335	2.23	1.64E+06	6.65E+02	106.41

(TBN = Total Bacterial Numbers, CFU = Colony Forming Unit counts and BBM = Bacterial BioMass)

Dissolved Inorganic Nitrogen:

The range of inorganic nitrogen concentration μg at/l is the summation of the concentration of ammonia, nitrite, and nitrate. The higher values were mostly recorded near sewage outfalls. The seasonal averages of the different forms of nitrogen are given in Table (1) and represented in Fig 2.

Reactive Phosphate:

Reactive phosphate pO_4 is considered as a potential pollutant brought into the coastal water mainly by sewage and wastewater. Its value were fluctuated between a maximum value of 1.1 μg/l at station 1 near the outfalls and a minimum value of 0.22 μg/l far from the outfalls (Station IV) Table (1).

Chlorophyll-a:

Chlorophyll-a content in the seawater E.H. that is reflecting Phytoplankton biomass during the period of study fluctuated between 1.12 to 5.35μg at.l^{-1}. These values were considerably less than those previously recorded in the same area during the last decade. The higher level of Chl-a was recorded specially at the marginal station I & II especially in summer.

Bacteria:

Total bacterial numbers (TBN) and the saprophytic bacteria plate counts (CFU) were ranged between 0.47 and 5.4 million **cells/mill** and between 540 and 5400 CFU/ML, respectively. The highest values for the two parameters were recorded **in** summer at stations 4 and 6, respectively, (Fig. 3 and 4) while the lowest values were recorded at station 5 in winter and spring respectively. The highest values of both TBN and CFU in summer may be due to higher temperature 26.4 'C or may be due to water current entering the Harbour from El-Silsila at station 4 and loaded by sewage with allochthonus bacteria. These results are in a good agreement with those reported by Rublee ***et al*** (1984).

Aboul Kassim (1987) indicated that the flourishing rate in the Eastern Harbour water attend 5.5 months during 1975 but recently it was estimated to be 10 days only (Zaghloul, 1996). So we expect fast changes in the bacterial biomass and total counts as the water body changed.

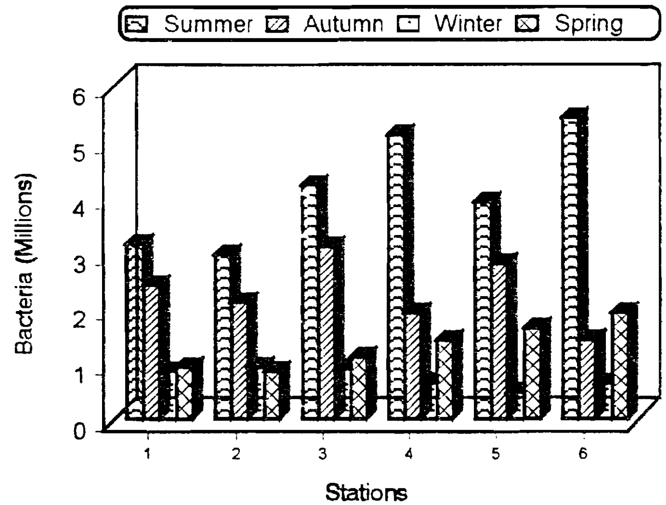

Fig. (3): Distribution of TBN at the different stations at the different seasons.

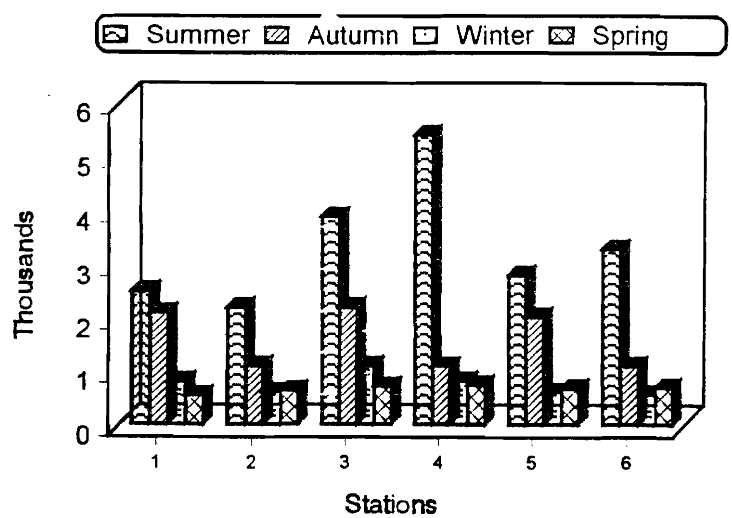

Fig. (4): Distribution of CFU at the different stations at the different seasons.

By applying the technique of stepwise multiple regression analysis between both TBN and CFU as dependent factors and the physico-chemical parameters as independent factors, it was found that TBN in the studying area was highly correlated with temperature, dissolved oxygen and oxidizable organic matter ($r=0.873, r=24$ at $p<0.05$). The same correlation was found by Rheinheimer (1977). Also, CFU was highly correlated with temperature, oxidizable organic matter and ammonia. ($r = 0.851$, $n=24$ at $p<0.05$) This correlation was also reported by Palumbo ***et al.*** (1 984) and Kunnis (1 99 1). The regression models for both parameters are described as follows:

TBN= -98.8x 15 +4 x 10^5 Temperature + 5.4 x 10^5 DO+ 2.8X 10^5 OOM.
CFU= -3950.4 + 175.2 Temperature + 500.25 OOM -154.1 NH_3

Highest value of bacterial biomass (190.4 µg Cμm^3) appeared during summer whereas lowest value (97.6 µg Cμm^3) occurred in winter (Fig. 5). The obtainable higher values are coincided with increase in both saprophytic bacteria (CFU) and total bacterial number reflecting the influence of environmental factors on the abundance of bacteria in water (Lee and Lee 199 1). Applying the stepwise multiple regression analysis between bacterial biomass as a dependent variable and physico-chemical parameters as independent ones separates the Most important environmental factors alerting the distribution of a dependent one, thus it was found that BBM was highly correlated with temperature, biological oxygen demand and oxidizable organic matter. Wright and coffin (1984), and Foog (1977) also reported these correlations.

The regression equation correlating them was as follows:

BBM= -19.485-10.78 BOD +10.37 OOM+ 5.3 Temperature

(r- 0.92, n=24, $p<0.05$).

Figures 6, 7 and 8 show the plotting of the observed and the calculated models of TBN and CFU and BBM respectively. The closeness of both lines reflects the goodness of the prediction models.

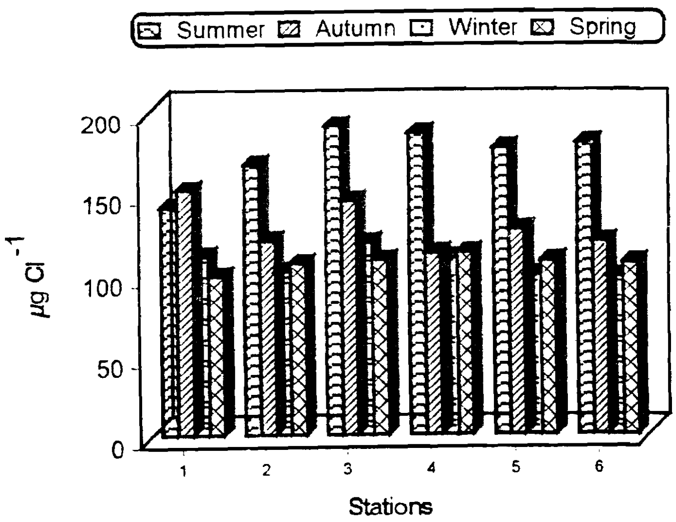

Fig. (5) : Distribution of BBM at the different stations at the different seasons.

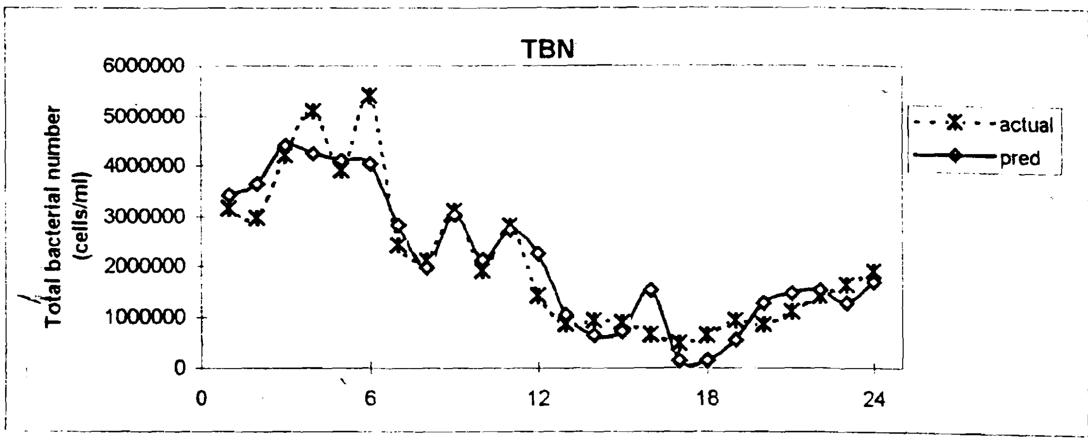

Fig. (6) : Actual and predicted values of total bacterial number with some physicochemical parameters (Temperature, DO and OOM) by the multiple stepwise regression model

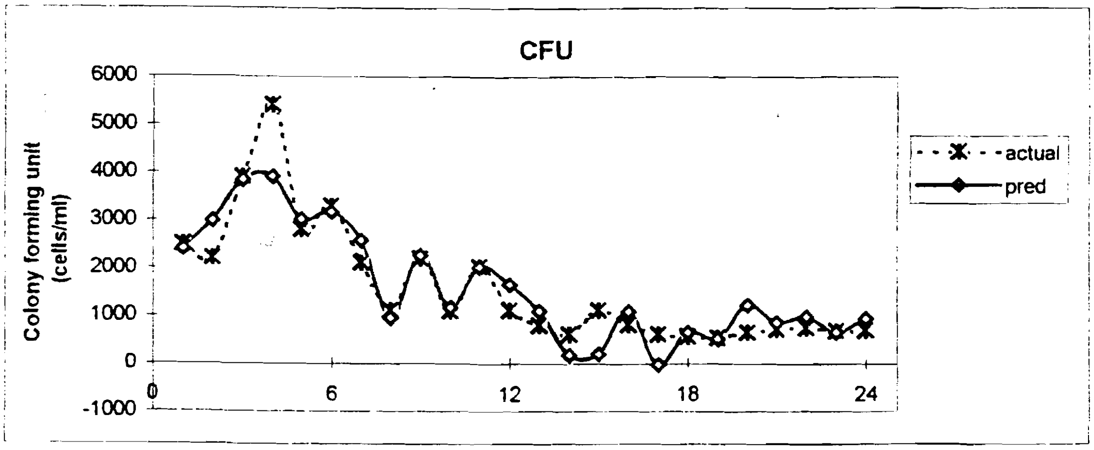

Fig. (7) : Actual and predicted values of colony forming unit with Some physicochemical parameters (Temperature, OOM and NH3) by the multiple stepwise regression model

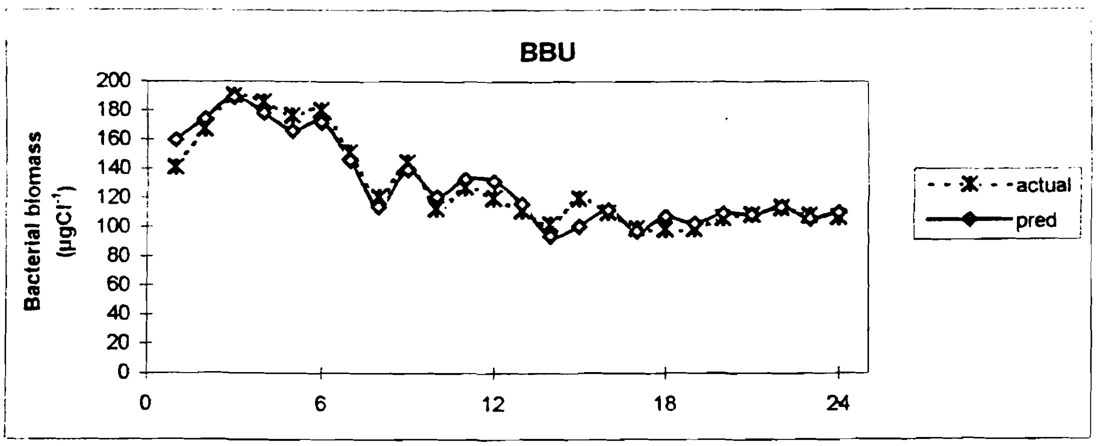

Fig. (8) : Actual and predicted values of bacterial biomass with some physicochemical parameters (Temperature, BOD and OOM) by the multiple stepwise regression model.

CONCLUSION

1. Temperature is the most important environmental parameter affecting total bacterial number, Saprophytic bacteria and bacterial biomass.

2. The effect of dissolved ammonia appears in calculating the saprophytic bacteria than the bacterial biomass.

3. Not all the environmental parameters have a noticeable effect on the count or the mass of bacteria.

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