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SEASONAL VARIATION OF MICROBIAL POPULATION FROM SEDIMENTS OF VELLAR ESTUARY, SOUTH INDIA

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ABSTRACT - A seasonal study on the occurrence and distribution of total aerobic microbial population (TMP), total aerobic heterotrophic bacteria (THB), actinomycetes and fungi in the sediments of Vellar estuary, South India (11°29'N, 79°46'E) was carried out during Jan. to Dec. 1975. Sediment samples were collected at monthly intervals, from four stations and analysed microbiologically. TMP varied between 1.26 to 428.93 x 10^6/g, THB 1.2 to 428. 3 x 10^6/g, actinomycetes 1.3 to 78.87 x 10^4/g and fungi 2.5 to 18.9 x 10^4/g dry wt of the sediment. In general, bacterial population was found to be dominant followed by actinomycetes and fungi. The maximum peak for TMP, THB and fungi were recorded in November in all stations. Monsoon season supported TMP, THB and fungi to a high level and it was summer for actinomycetes. The statistical analysis (Kendall's tau) showed a significantly positive correlation among TMP, THB, actinomycetes and fungi in station 1 and 2. There existed a significantly positive correlation of TMP, THB, with organic carbon in station 2 and 3. Though fungi exhibited a significantly positive correlation with organic carbon, total nitrogen, total phosphorus, clay, sand and a significant negative correlation with C/N ratio, such relationship was not consistent in all stations. While a significantly negative correlation of TMP, THB with temperature was found, no such positive or negative significant correlation could be observed for actinomycetes.

RÉSUMÉ - Une étude saisonnière a été faite de janvier à décembre 1975 sur l'incidence et la distribution de la population microbienne aérobie totale (TMP), les bactéries aérobies hétérotrophes totales (THB), les actinomycètes et les champignons. Elle a été réalisée dans les sédiments de l'estuaire Vellar en Inde du Sud (11°29'N, 79°46'E). Les analyses microbiologiques sont faites sur des échantillons de sédiment prélevés mensuellement dans quatre stations. Les variations des différents groupes microbiens, exprimés par g de sédiment sec, oscillent entre 1,26 et 428 x 93 x 10^6 pour les TMP, entre 1,2 et 428,3 x 10^6 pour les THB, entre 1,3 et 78,87 x 10^4 pour les actinomycètes et entre 2,5 et 18,9 x 10^4 pour les champignons. En général, ce sont les populations bactériennes qui sont les plus importantes, suivies par les actinomycètes puis par les champignons. La densité maximale pour TMP, THB et les champignons, est atteinte en novembre dans toutes les stations. Ces trois groupes sont les plus abondants en période de mousson tandis que les actinomycètes sont plus nombreux en été. L'analyse statistique montre une corrélation significative et positive entre TMP, THB, les actinomycètes et les champignons dans les stations 1 et 2. TMP, THB sont corrélés significativement et de façon positive avec le carbone organique, dans les stations 2 et 3 tandis que les champignons montrent une corrélation significative et positive avec le carbone organique, l'azote total, le phosphore total, les argiles et le sable et une corrélation significative mais négative avec le rapport C/N. Cependant, il faut noter qu'une telle relation n'existe pas dans toutes les stations. TMP et THB, sont également corrélés significativement et de façon négative à la température. De telles corrélations significatives, qu'elles soient positives ou négatives, n'ont pas été observées pour les actinomycètes.

INTRODUCTION

The role of microbial communities in the process of decomposition, nutrient regeneration, cycling and production of particulate matter and relationship with other organisms in marine environment is well known (Rheinheimer, 1980). However, reports on the seasonal distribution of various microbial taxa (bacteria, actinomycetes and fungi) in the marine and estuarine environments are limited (Grein and Meyers, 1958; Boeye *et al*, 1975; Walker and Colwell, 1975; Erkenbrecher and Stevenson, 1977). During recent years, few studies have been conducted on the quantitative distribution of heterotrophic bacteria in Indian estuarine sediments (Dhevendaran, 1977) and information on bacteria, actinomycetes and fungi is totally lacking. This prompted us to attempt on the distribution of bacteria, actinomycetes, fungi and total microbial population (TMP i.e. bacteria, actinomycetes and fungi) in the sediments of a tropical estuary like Vellar. This paper reports the seasonal variation and the influence of physico-chemical parameters on the number and distribution of the microbial taxa in the sediments of Vellar estuary, South India.

MATERIALS AND METHODS

Sampling area

Vellar estuary (lat 11°29'N and long 79°47'E) is comparatively a shallow estuary within an average depth of about 2 m. This estuary is demarcated and defined, in terms of salinity characteristics, into four zones viz marine zone, gradient zone, tidal zone and freshwater zone (Ramamoorthi, 1954). Four stations (station 1 in marine zone, station 2 in gradient zone, station 3 in tidal zone and station 4 in freshwater zone) were selected for the study (Fig. 1). Monthly samples of sediment and overlying water were taken from Jan. to Dec. 1975.

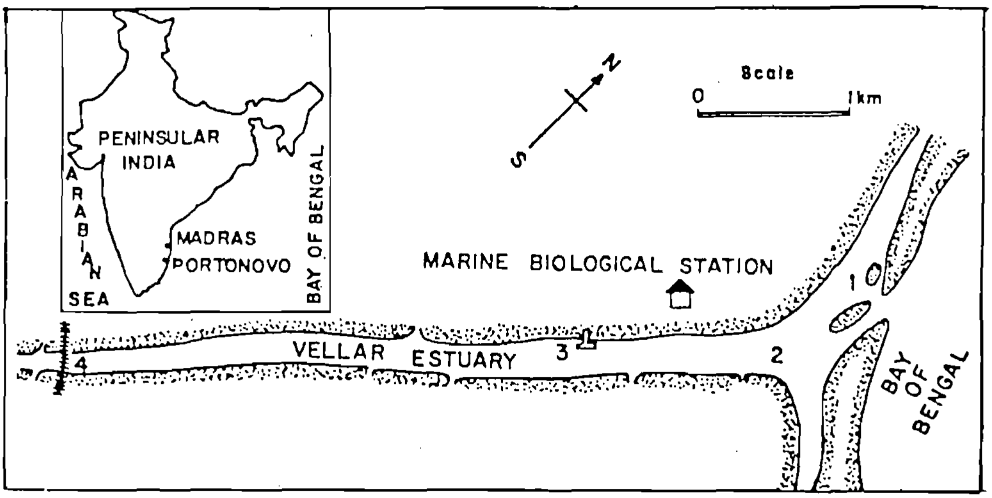

Figure 1 : Map of Vellar estuary showing sampling stations.

Microbiological analysis

Estimations of microbial fractions in the sediment samples were made according to pour plate method. The media used were listed in Table 1. The plates were incubated at 28 ± 2°C for varied lengths of time (3-7 days for bacteria; 7-10 days for fungi; 14-20 days for actinomycetes). The media were prepared in 25,50 and 100% filtered aged seawater (32.4 ‰).

Medium	Composition		Medium	Composition	
ZoBell	Peptone	: 5 g			
	Yeast extract	: 1 g			
	K_2HPO_4	: 0.5 g	Kusters	Glycerol	: 10 g
	$FeSO_4$	: Trace		Casein	: 0.3 g
	Agar (Difco)	: 15 g		KNO_3	: 2 g
	Seawater*	: 1 l		K_2HPO_4	: 2 g
	pH	: 7.0-7.1		$MgSO_4$	: 0.05 g
				$CaCO_3$	: 0.02 g
Grein & Meyers	Soluble starch	: 10 g		$FeSO_4$	: 0.01 g
	Casein (dissolved in NaOH)	: 1 g		Agar (Difco)	: 18 g
				Seawater	: 1 l
	Seawater	: 1 l		pH	: 7.0-7.1
	pH	: 7.0-7.1			
				Dextrose	: 10 g
Glucose Asparagine	Glucose	: 10 g	Martin	Peptone	: 5 g
	Asparagine	: 0.5 g		K_2HPO_4	: 1 g
	K_2HPO_4	: 0.5 g	Rose-Bengal	Rose bengal**	
	Agar (Difco)	: 15 g		Agar (Difco)	: 15 g
	Seawater	: 1 l		Seawater	: 1 l
	pH	: 7.0-7.1		pH	: 7.0-7.1

Table 1. Media used for the enumeration of microbial taxa.

* Filtered aged seawater (32.4 ‰), dilutions were with distilled water.
** 1g of streptomycin sulphate dissolved in 100 ml of sterile distilled water and from this 0.1 ml added to 1 l medium.

Measurement of environmental variables

The temperature, salinity and hydrogen-ion concentration of the overlying water were estimated using maximum minimum thermometer, standard argentimetric method (Harvey, 1955) with necessary corrections and a pH meter (ELICO MODEL L1-10) respectively. The sediment composition (sand, silt, clay) was analysed following pipette method (Krumbein and Pettijohn, 1938). Total organic carbon (Wakeel and Riley, 1956), total nitrogen (Barnes, 1959) and total phosphorus (Murphy and Riley, 1962) were also estimated.

Statistical analysis

Kendall's tau rank correlation method (Sokal and Rohlf, 1969) was employed to find out the significance of parameters and population using IBM 1130 computer.

RESULTS AND DISCUSSION

Seasonal distribution

Bacterial population was found to be dominant followed by actinomycetes and fungi. These groups could be recorded in all samples during the period of study but their numbers fluctuated widely depending on season and location of the sample. Sediment contained bacteria in the magnitude of 10^6-10^8/g, actinomycetes of 10^4-10^5/g and fungi of 10^3-10^4 g (Fig 2-5). Since bacterial population constituted more than 90 % of the total microbial population, fluctuations of TMP, in general, was similar to bacteria.

The counts of bacteria ranged between log 6.4 to log 8.4/g. The numbers of bacteria were similar to those found in other sediment studies of St. Lawrence river outlet (Vanderpost and Dutka, 1971) and Rhode river estuary of Maryland (Rublee and Dornseif, 1978). It fluctuated widely between months and no similar pattern of distribution was noticed in all the stations. The maximum counts were obtained in station 4 and peak values in all stations were encountered in November when salinity was found to be very low except at station 1. A reduction in the microbial fractions from station 4 to station 1 was well pronounced in November. However such decrease was not noticed during other months. The maximum extinction of fresh water forms was observed in station 1 which is situated at the mouth of the estuary. Also salinity of the station 1 was above 24 ‰ during flood condition and this could be detrimental to freshwater bacteria. According to Larsen (1962), various true marine bacteria did not find optimal living conditions in brackish water areas and many fresh water bacteria were inhibited by salinities of over 5 ‰. Similar pattern of reduction in fresh water bacteria was reported in Schwentine river mouth region (Rheinheimer, 1984). The antimicrobial activity of seawater and the salt concentration of the overlying water and sediment might have inhibited the freshwater bacteria. The annual mean values varied from 30.7 to 61.47 x 10^6/g and the maximum bacterial counts were observed in monsoon season (Fig. 6). The high population recorded during monsoon season may be attributed to the increasing quantity of flood by monsoon rains. A similar increase in total bacterial content of water run off was also observed during flooding of northern German rivers (Muller-Haeckel and Rheinheimer, 1983) The other peaks observed in post monsoon period coincides with the primary production (Santhanam, 1976) and also might be due to nutrient content (Walker and Colwell, 1975; Rublee, 1982).

Annual curves for actinomycetes also exhibited a completely different pattern than to the curves of bacteria which demonstrated a peak in November in all stations. In general, a bimodel distribution could be found. In station 2 and station 3, primary peaks and secondary peaks were recorded in May and November respectively. But in station 1 and 4 primary peaks were recorded in november and secondary peaks in april and may respectively. A similar bimodel periodicity was noticed showing peak with greatest incidence in March and November in Eastern Bay sediments (Walker and Colwell, 1975). However, the shifting of peaks in station 1 and station 4 here may be due to non-static conditions prevailing there. The number varied form log 4 to log 6/g and maximum occurred in station 2 during May. Mean values fluctuated between 4.37 to 15.58 x 10^4/g and unlike bacteria maximum were recovered in summer followed by monsoon season. The population reported here is higher than the number of actinomycetes reported in Chesapeake Bay estuarine sediments (Walker and Colwell, 1975) and Weser estuarine sediments (Weyland, 1969). The higher numbers in summer may be due to stable conditions of available nutrients.

The population of fungi varied from log 3 to log 6/g and maximum was recorded in station 2. The annual mean value ranged between 15.36 to 33.19 x 10^3/g. Higher numbers were encoutered in the monsoon season. Monthly fluctuations vary widely and did not show any common pattern of distribution in all stations. Also, no uniformity could be observed in the distribution pattern of fungi with actinomycetes and bacteria in estuarine sediments.

Influence of environmental parameters

The distribution of microbial populations in sediment is known to be controlled by physico-chemical and biological characteristics of the sediment. To understand their influences on bacteria, actinomycetes, fungi and total microbial load of the sediments, a number of factors such as temperature, salinity, pH, organic carbon, total nitrogen, total

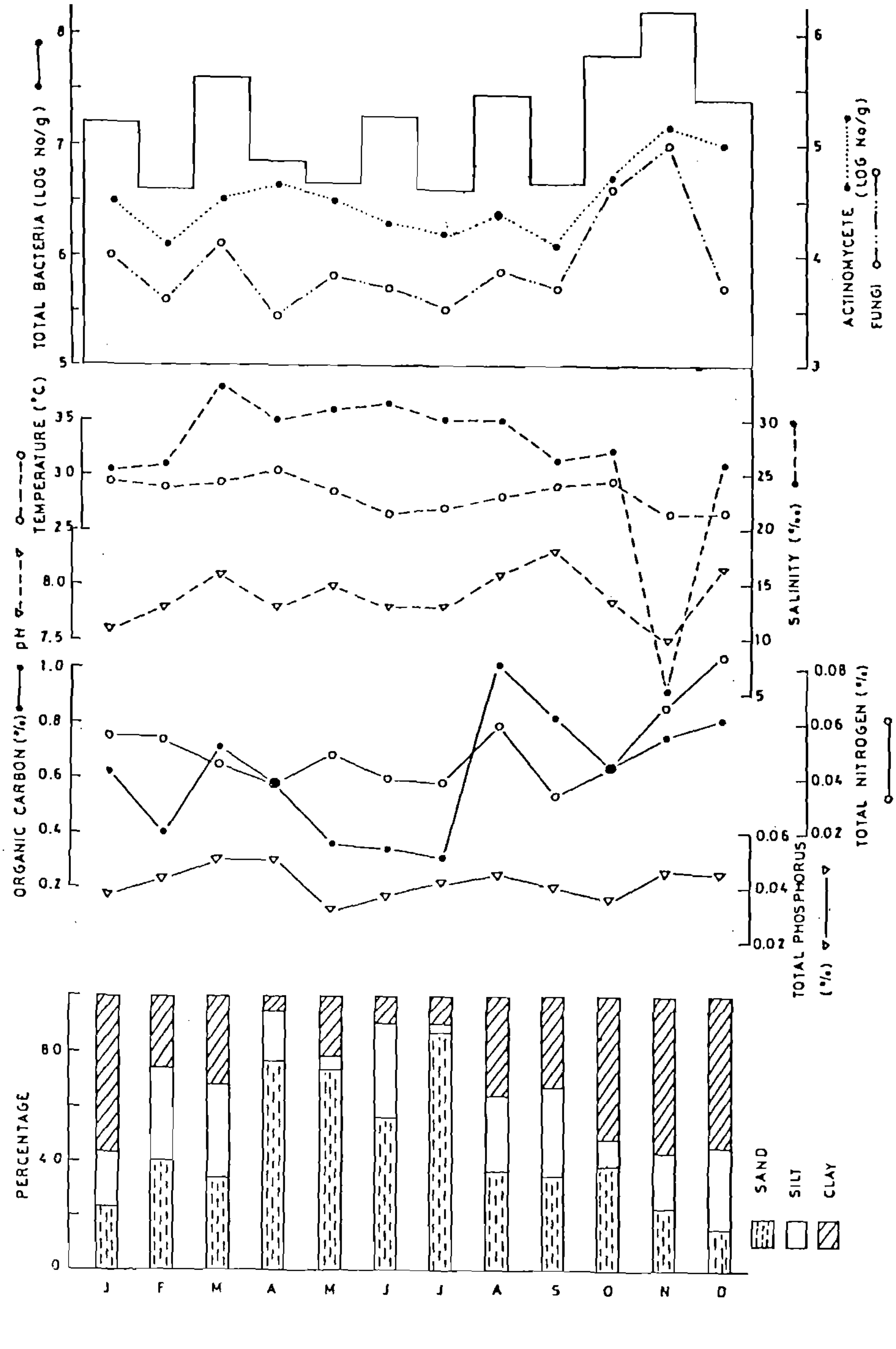

Figure 2 : Microbial populations and physico-chemical parameters at station 1.

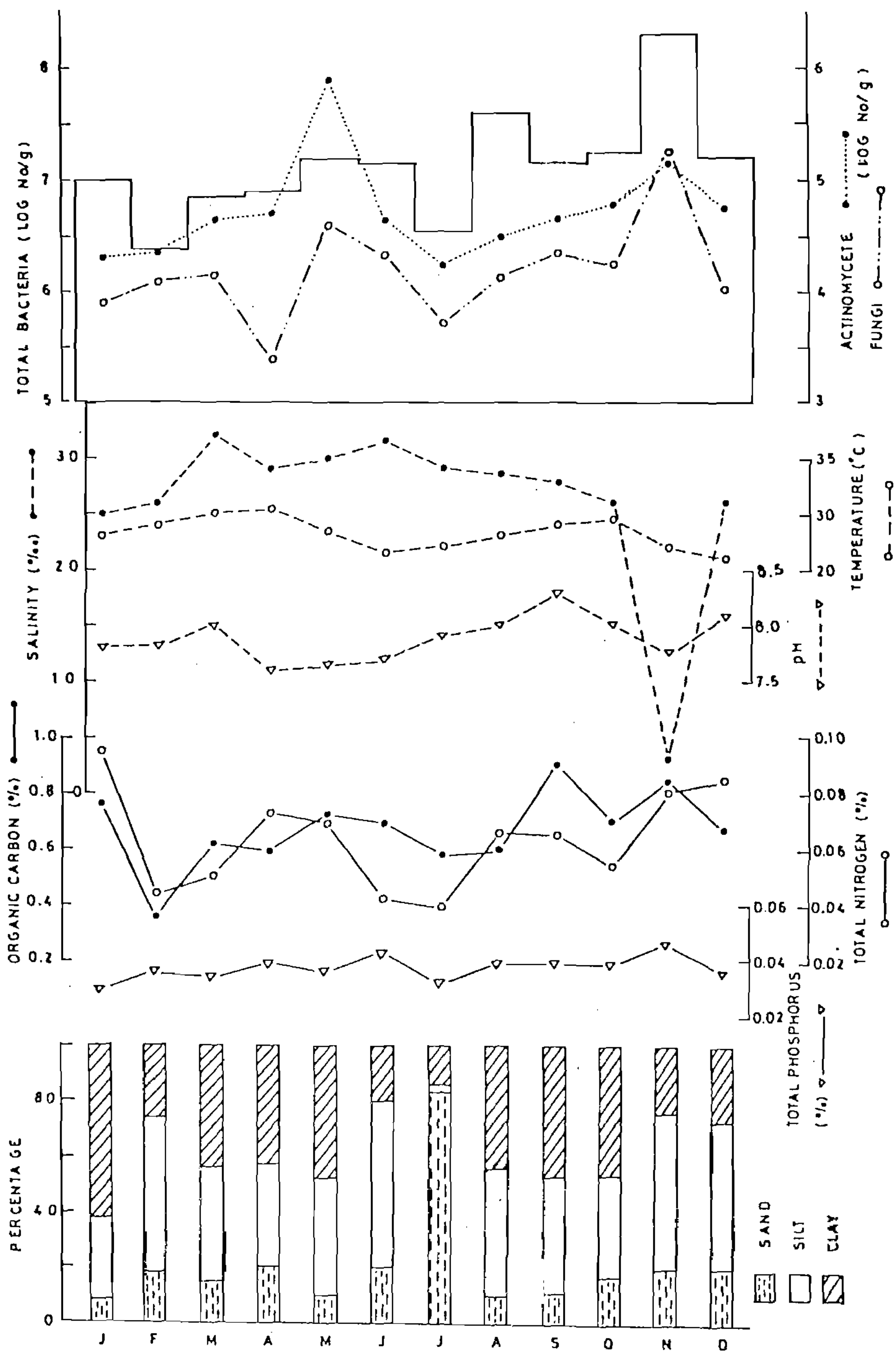

Figure 3 : Microbial populations and physico-chemical parameters at station 2.

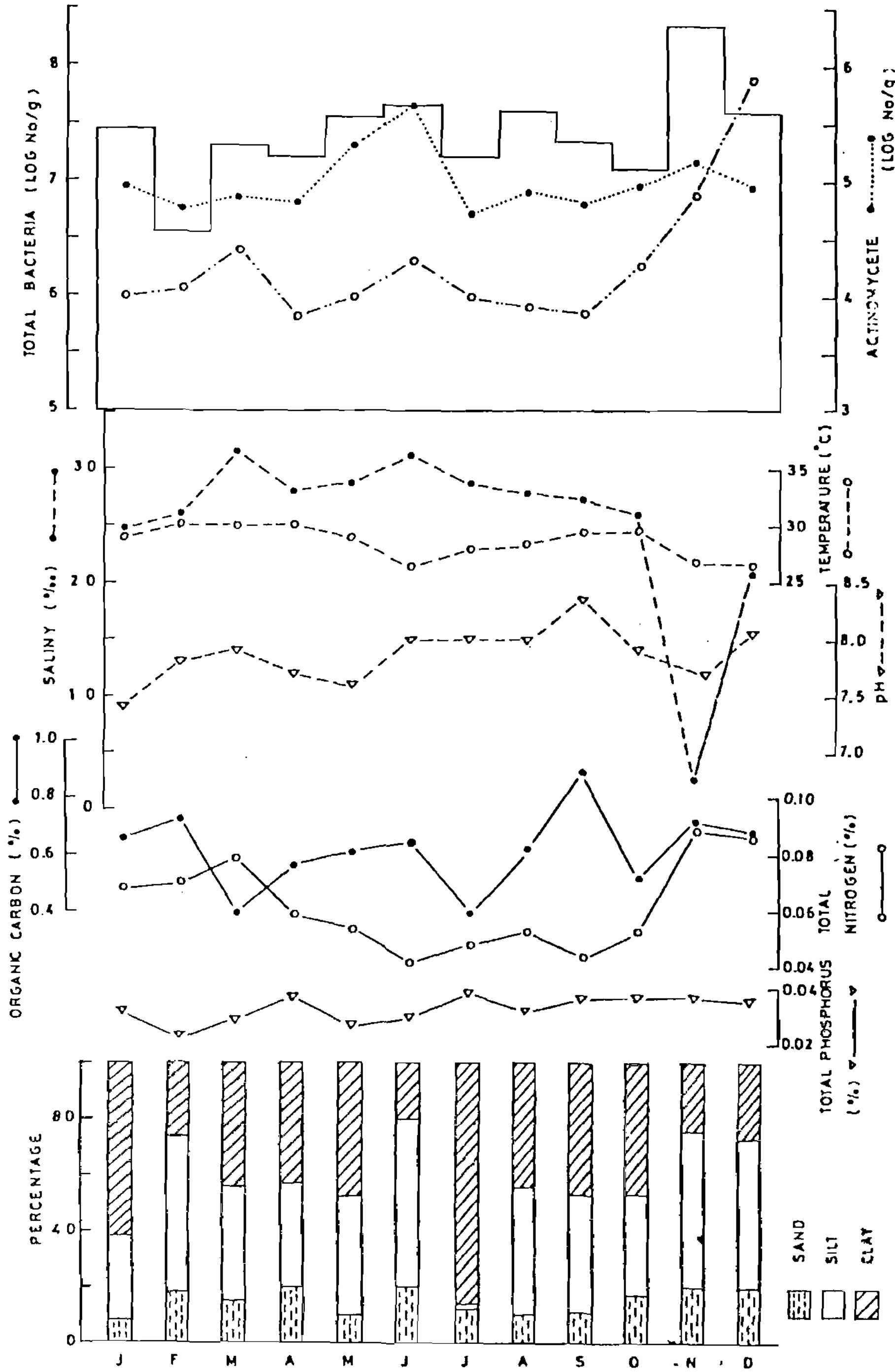

Figure 4 : Microbial populations and physico-chemical parameters at station 3.

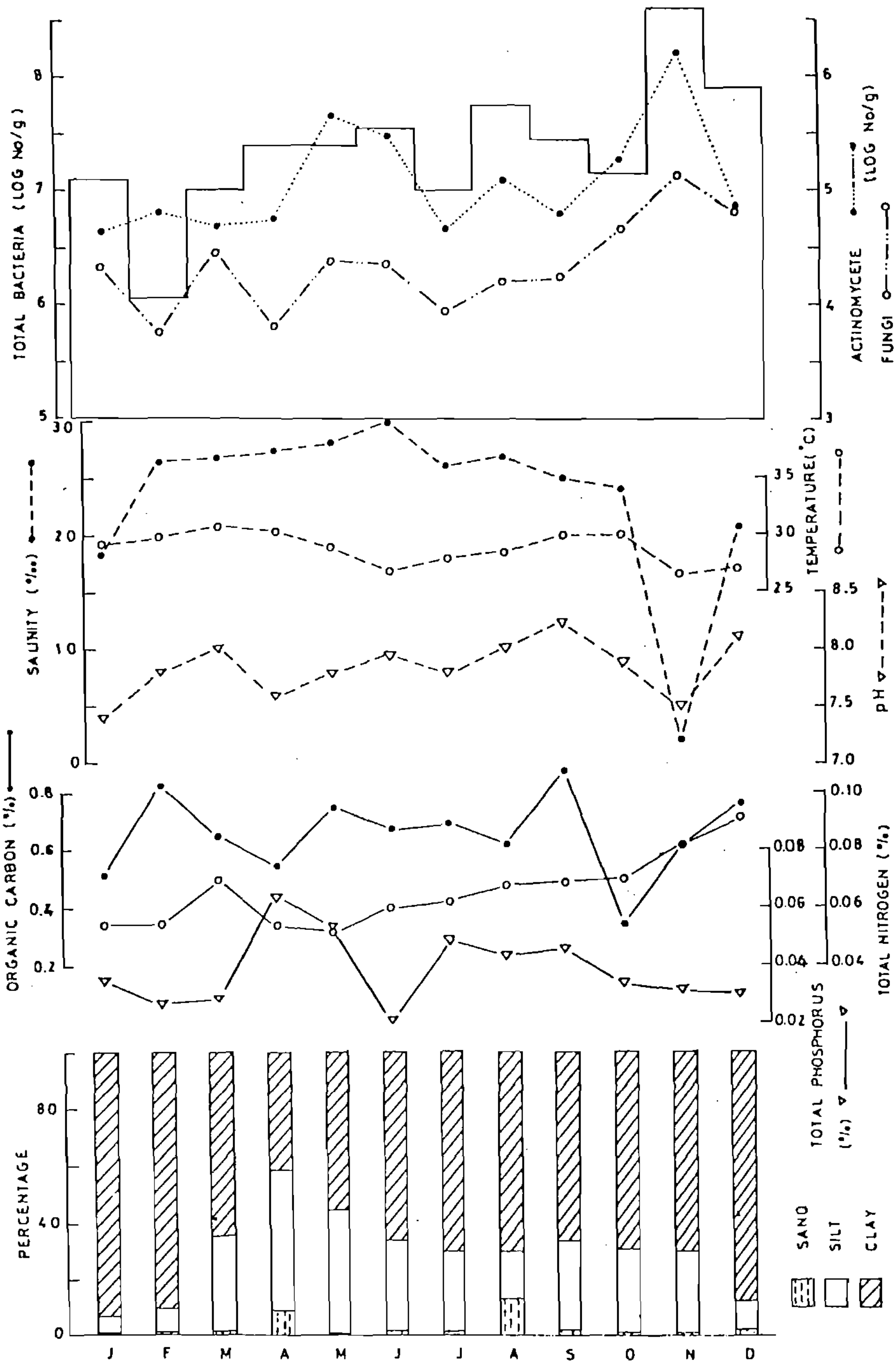

Figure 5 : Microbial populations and physico-chemical parameters at station 4.

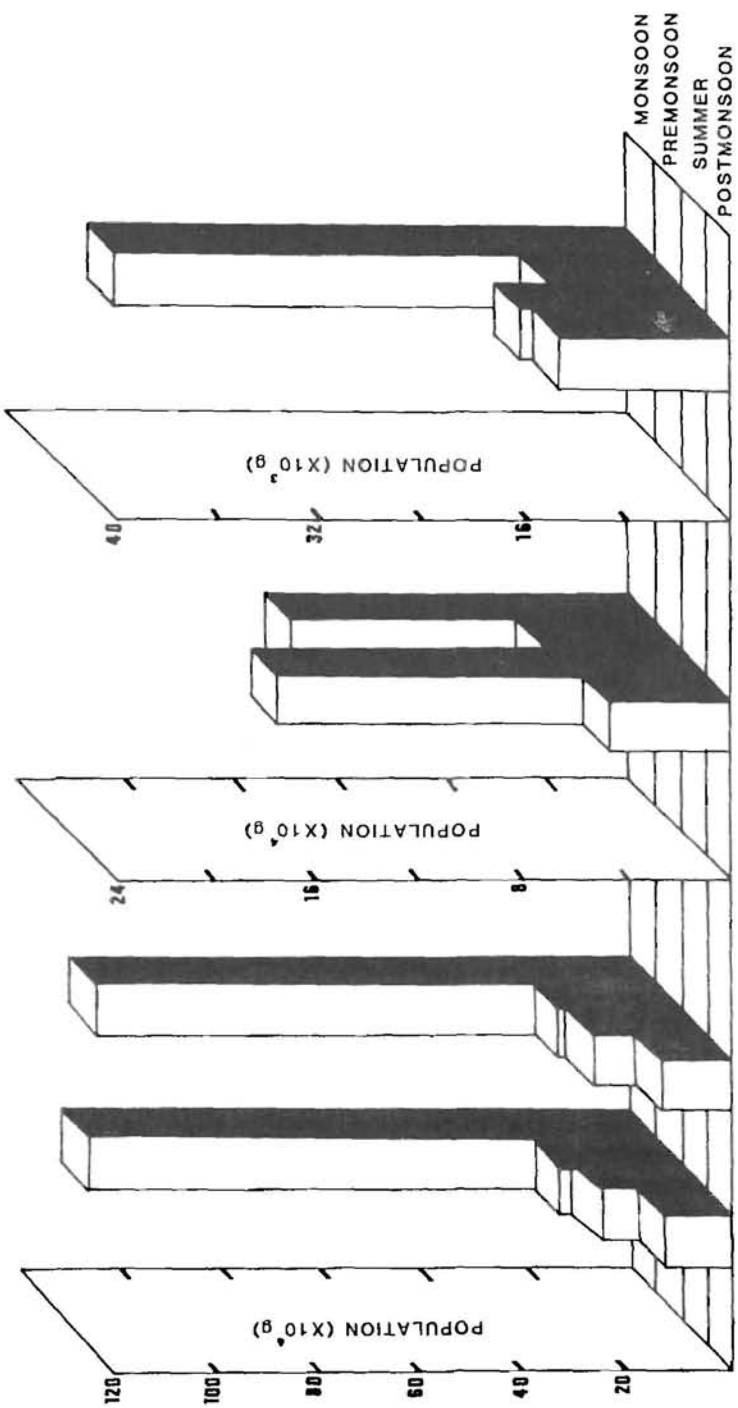

Figure 6 : Seasonal variation of microbial population.

phosphorus and sediment composition were estimated and related to the microbial fractions by a series of correlation coefficient matrices (Kendall's Tau). Results show that significant correlations between different factors and microbial taxa were not evident in all the stations (Table 2). Further it could be seen that in certain stations, physical factors like temperature and texture of the sediment were found to play a major role in determining the density of a given microbial population. Though fungi exhibited significant positive correlation with organic carbon, total nitrogen, total phosphorus, clay and sand and a significant negative correlation with C/N ratio, such relationships were not observed in a single station. Similarly, TMP and bacteria also showed significantly positive correlation with organic carbon in station 2 and station 3 and significantly negative correlation with temperature in station 3 and 4. It is thus indicated that the influence of various factors on the microbial population cannot be generalised.

	Temperature	Salinity	pH	Organic carbon	Total nitrogen	Total phosphorus	C/N ratio	N/P ratio	Clay	Silt	Sand
Station 1											
Actinomycetes	0.0788	0.1374	-0.1374	0.2901	0.3512	0.1395	0.0763	0.3078	0.3692	-0.2154	-0.3256
Bacteria	0.0156	0.0606	-0.1212	0.4242	0.3939	0.1846	0.2121	0.2290	0.4122	0.0458	-0.4308
Fungi	0.0782	-0.1818	-0.0606	0.3636	0.2727	0.0616	0.1515	0.2901	0.5954*	-0.0763	-0.4308
Total	0.0156	0.0303	-0.0909	0.3939	0.3636	0.1846	0.1818	0.1985	0.3817	0.0763	0.4001
Station 2											
Actinomycetes	0.0763	-0.0458	0.1515	0.3939	0.3333	0.3908	-0.0606	0.3030	0.1679	0.1818	0.0153
Bacteria	-0.2596	-0.1985	-0.0606	0.4849*	0.3636	0.4221	0.1515	0.3333	0.1679	0.2727	0.1985
Fungi	-0.1374	-0.0153	0.0606	0.4849*	0.0606	0.4534*	0.2727	-0.0303	0.1679	0.3939	0.1985
Total	0.2596	-0.1985	0.0606	0.4849*	0.3636	0.4221	0.1515	0.3333	0.1679	0.2727	-0.1985
Station 3											
Actinomycetes	-0.2016	-0.2290	0.2970	0.0764	0.2424	-0.0469	0.0606	0.1818	-0.3512	0.3512	0.1374
Bacteria	-0.6048**	-0.0763	0.1094	0.3206	0.0303	0.0782	0.0909	-0.0303	0.1069	0.1679	0.0458
Fungi	-0.2947	-0.0763	0.0156	0.0458	0.4546*	0.1720	-0.3333	0.3940	0.4428*	0.1679	0.5954**
Total	-0.4497*	0.1679	0.0782	0.4733*	0.1818	0.1407	0.1818	0.1818	-0.1069	0.0763	0.1069
Station 4											
Actinomycetes	0.2970	-0.0153	-0.0153	0.0909	0.1818	0.0303	0.2121	0.0909	0.1385	0.1212	0.0909
Bacteria	-0.4534*	0.1069	0.1374	0.0606	0.2727	0.0606	-0.3636	0.1212	-0.0462	0.0303	0.4242
Fungi	-0.2658	-0.3512	0.0763	0.1515	0.4849*	-0.2121	-0.4546*	0.3939	0.0154	0.0	0.0303
Total	-0.4534*	0.1069	0.1374	-0.0606	0.2727	0.0606	0.3636	0.1212	-0.0462	0.0303	0.4242
Estuarine biotope											
Actinomycetes	0.333	0.0	-0.333	-0.333	0.0	-0.333	0.0	-0.333	-0.667*	0.667*	0.0
Bacteria	0.0	-0.333	0.333	0.333	0.333	0.333	-0.333	0.0	0.0	0.0	1.000*
Fungi	-0.333	0.0	-0.333	-0.333	0.0	-0.333	-0.667*	0.667*	0.0	0.667*	0.333
Total	0.0	-0.333	0.333	0.333	0.333	0.333	-0.333	0.0	0.0	0.0	1.000*

Table 2 : Rank correlation coefficient matrix (Kendall's Tau) of microorganisms with environmental parameters. (Significant value * p.0.05 ; ** p.0.01).

When the data were computed on the basis of biotope instead of station and based on season than of individual months, a more reasonable picture emerged. The relationship of microbial taxa to sediment particle may be of extreme importance. Interestingly, the bacterial population was significantly positively correlated with sand and silt. Normally sandy type of substrate have been reported to harbour less number of microbial population (Ayyakkannu and Chandramohan, 1971 ; Vanderpost and Dutka, 1971). However the positive correlation might have been influenced by high organic matter (Rangaswami *et al.* 1967 ; Vanderpost and Dutka, 1971). The actinomycetes was significantly negatively correlated with clay. This is also another interesting observation since earlier reports showed that clay sediments always contain more microbial population including actinomycetes (Paulraj, 1973). The fungal population was significantly positively correlated with N/P ratio and silt. However a significant negative correlation was observed between fungal population and C/N ratio. The oceanic variables such as temperature and salinity vary greatly in nearshore environments where fresh water mixes with seawater. Such changes in temperature and salinity may affect growth, metabolism and survival of marine and freshwater bacteria. A good relationship seems to exist between salinity, temperature and marine bacteria (Stanley and Morita, 1968 ; Cooper and Morita, 1972). However, such relationship was not noticed in the sediments of Vellar estuary. No correlation between pH and microbial taxa was reported previously (Rangaswami *et al*, 1967) and similar observation was made here also.

The result on correlation between numerically dominant microbial groups reveal an existence of significantly positive correlation among bacteria, actinomycetes and fungi and TMP in station 1 and station 2 (Table 3). Nevertheless such relationship was not

	Actinomycetes	Bacteria	Fungi	Total
		Station 1		
Actinomycetes	1.0	0.6260**	0.4428*	0.5954**
Bacteria		1.0	0.6970**	0.9697**
Fungi			1.0	0.6666**
Total				1.0
		Station 2		
Actinomycetes	1.0	0.5455*	0.4849*	0.5455*
Bacteria		1.0	0.5152*	1.0000**
Fungi			1.0	0.5151*
Total				1.0
		Station 3		
Actinomycetes	1.0	0.3636	0.3636	0.2121
Bacteria		1.0	0.2121	0.7879**
Fungi			1.0	0.3030
Total				1.0
		Station 4		
Actinomycetes	1.0	0.4849*	0.3939	0.4849*
Bacteria		1.0	0.3636	1.0000**
Fungi			1.0	0.3636
Total				1.0
		Estuarine biotope		
Actinomycetes	1.0	0.333	0.333	0.333
Fungi		1.0	0.333	0.333
Bacteria			1.0	1.000*
Total				1.0

Table 3 : Rank correlation coefficient matrix (Kendall's Tau) of microbial populations, taking major groups in different stations and estuarine biotope as one. (Significant value * p.0.05 ; **-p.0.01).

observed in other two stations. A close correlation between bacteria and TMP in all stations is not surprising because as pointed out earlier, bacteria form the major component of the TMP. When the data were analysed biotope and season-wise, no significant relationship could be observed except for TMP and bacteria.

In conclusion, the present investigation reveals that (1) exogenous bacteria are predominantly supplied through floods (2) seasonal distribution may be influenced by the interaction of multiple environment factors and (3) each site will be different, due to physical and chemical factors regulating activity and distribution not only of microorganisms but also of predatory benthic organisms which in turn influence prey population.

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