

BACTERIOLOGICAL STUDIES OFF MANGALORE COAST

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ABSTRACT

Bacteriological studies of sediment and water in the near shore region off Mangalore showed that both limnotolerant and halotolerant bacteria were present. Significant inter-correlation (quantitative and qualitative) could be noted between various groups of bacteria in the sediment. It is therefore indicated that a change in the sediment population would reflect any changes in the environment.

Key words: Limnotolerant, halotolerant.

INTRODUCTION

The bacterial population forms an important constituent of any ecosystem and can be considered as an index for monitoring environmental stresses and changes. A preliminary survey was carried out with a view to understand the bacteriological characteristics of a coastal region off Mangalore as a part of environmental studies sponsored by Kudremukh Iron Ore Co. Ltd. in order to monitor the effects of effluents discharged into the nearby sea.

MATERIAL AND METHODS

Water and sediment samples were collected from six stations in the coastal region off Mangalore (Fig. 1) with the help of sterile bottles and Petersen grab respectively over a period of seven months (October 1979-May 1980) at fixed intervals. The samples were transported to the laboratory in ice boxes for further analyses. Colony forming units (CFU) in various samples were estimated by plating technique after serial dilution using three different media, viz. (1) ZoBell 2216E (2) Nutrient agar in aged sea water (30‰) and (3) Nutrient agar in distilled water. Even though media 1 and 2 were prepared with aged sea water, they differ in their composition. The CFU appearing in media 1 and 2 are referred as halotolerant and those in medium 3 as limnotolerant. The enumeration of CFU was done after incubation for 48 hrs at room temperature ($28 \pm 2^\circ\text{C}$). The population of sediment was expressed as number per gram of dry weight and that of water as number per ml.

For qualitative analysis, several hundred colonies from different plates were isolated at random, checked for their purity and identified to the generic level following the scheme of Shewan, Hobbs and Hodgkins (1969) and Bergey's Manual of Determinative Bacteriology (1974).

The population and percentage occurrence of various generic groups were subjected to correlation coefficient analyses and the significance levels were estimated using student's T test ($df = 22$).

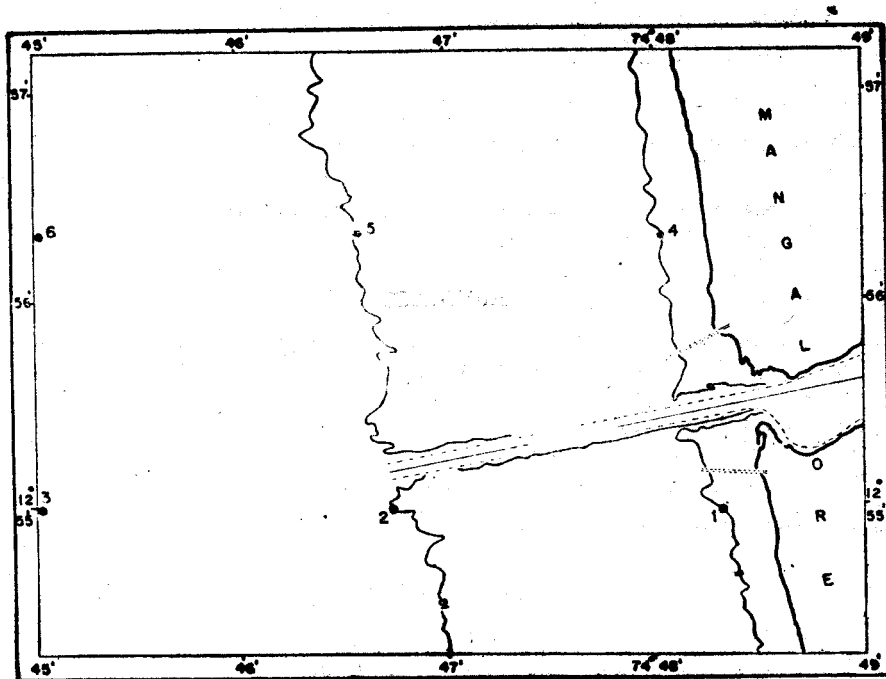


Fig. 1. Location of sampling stations.

RESULTS AND DISCUSSION

The distribution pattern of the aerobic bacteria in water and sediment at all the six stations is given in Fig. 2.

Temperature of water in the area ranged from 27.8 to 33.4 °C and salinity from 27.4 to 36.1‰ during the period of study.

In general, sediment samples showed higher population levels compared to overlying water. The limnotolerant bacterial population in sediment varied from 0.21×10^5 to 773.85×10^5 . For enumeration of the halotolerant population, in addition to ZoBell marine agar, nutrient agar prepared in sea water was also used so as to ascertain the changes in enumeration efficiency in various concentrations of the components in the medium. As can be seen from Fig. 2, differences could be noted between two media in the growth of bacterial population. The halotolerant population in nutrient agar showed a greater fluctuation than that obtained from ZoBell marine agar. Maximum number of halotolerant population was isolated in February at stns. 1 and 4. The halotolerant populations were higher in nutrient agar (828.25×10^5) than in ZoBell marine agar (373.04×10^5).

As seen from Fig. 2, bacterial population in water was lower than that in sediment by a magnitude of 10^3 , an observation which confirms the earlier findings (Meyer-Riel, Dawson, Liebezeit and Tiedge, 1978). Generally, the limnotolerant group was dominant. The halotolerant forms were lower in number compared to the limnotolerant population in water.

Table 1. Correlation between population in three media.

	Na (sediment)	Na (water)	NaS (sediment)	NaS (water)	Zb (sediment)	Zb (water)
Na (sediment)	1.000					
Na (water)	-0.005	1.000				
NaS (sediment)	0.504**	-0.010	1.000			
NaS (water)	-0.230	0.630***	-0.010	1.000		
Zb (sediment)	0.600**	0.070	0.530**	0.200	1.000	
Zb (water)	0.180	0.320	0.220	0.350	0.380	1.000

Na — Nutrient agar in distilled water, NaS— Nutrient agar in sea water, Zb — ZoBell 2216E, ** — Significant at 1.0% level, *** — Significant at 0.1% level.

There was a marked decline in bacterial population of sediment in May as compared to that of overlying water, though in general the sediment population was high. This exception may not only be due to the higher organic matter (ZoBell, 1972) but also due to the greater surface area available (Dale, 1974) since the sediment was clayey in nature at that period. Nair, Loka Bharathi and Achuthankutty (1978) reported the presence of limnotolerant bacteria in fairly high numbers at few near-shore stations near Mangalore. In the present study, the limnotolerant population was seen to be dominant which could be ascribed to the greater influence of fresh-water runoff in the area of study.

The sediment populations enumerated with three media showed significant correlation (Table I), whereas in water, such relation was noted only between populations from nutrient agar in distilled water and nutrient agar in sea water. This suggests that the population in the sediment was more adapted and tolerant to different media than that of water.

The percentage occurrence of various groups of bacteria in the sediment is presented in Table II. The predominant groups present in all the stations were *Acinetobacter*, *Pseudomonas*, *Bacillus* and *Alcaligenes*. The percentage occurrence of the different groups was found to vary with the media.

Pseudomonas was the most dominant genus noted in the sediments and was represented both in limnotolerant and halotolerant groups as seen from the table. Their occurrence varied from 0.4–89.5% in limnotolerant category. In general, higher percentage occurrence of *Pseudomonas* was noticed in October at all the stations.

Bacillus was less in percentage than *Pseudomonas* and mostly limnotolerant. Members of *Alcaligenes* were mainly halotolerant and could be recovered during all months at all the stations except at stn. 6 in May.

Populations of Enterobacteria in sediment were found to be mostly halotolerant. They were absent in October at all the stations. However, strains of Entero-

		FEBRUARY						MAY					
		1	2	3	4	5	6	1	2	3	4	5	6
<i>Pseudomonas</i>	Na	64.4	35.1	27.7	24.8	16.8	37.8	18.2	20.0	69.2	55.6	33.3	40.0
	NaS	(—)	(—)	(—)	(3.3)	(25.4)	(23.9)	(—)	(24.1)	(39.8)	(22.2)	(32.8)	(16.1)
	Zb	(3.0)	(41.4)	(28.6)	(35.1)	(8.8)	(13.1)	(—)	(30.5)	(27.1)	(—)	(—)	(17.7)
<i>Bacillus</i>	Na	58.4	40.0	56.8	86.1	69.8	0.6	(—)	60.06	33.3	52.9	30.4	100.0
	NaS	(76.1)	(53.5)	(—)	(16.2)	(27.3)	(59.8)	(40.0)	(45.0)	(84.3)	(—)	(66.7)	(73.1)
<i>Alcaligenes</i>	Na	4.0	7.6	3.1	7.4	16.0	10.9	36.4	26.7	7.7	16.7	8.3	30.0
	NaS	(5.4)	(27.3)	(7.7)	(3.3)	(14.5)	(4.3)	(4.5)	(1.1)	(3.0)	(—)	(—)	(4.3)
	Zb	8.5	(—)	3.1	39.1	(—)	6.8	62.5	(—)	11.1	41.7	5.0	28.6
	Na	(27.0)	(30.0)	(71.4)	(64.9)	(35.3)	(60.9)	(62.5)	(—)	(10.4)	(75.0)	(30.3)	(4.8)
	NaS	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	8.3	(—)	(—)	(—)
<i>Micrococci</i>	Na	(—)	(—)	(—)	(—)	2.3	(—)	(—)	(—)	(0.5)	(—)	(—)	(—)
	NaS	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)
	Zb	41.7	58.0	16.0	13.9	30.0	99.4	100.0	36.4	50.0	25.0	23.3	4.8
<i>Enterobacteria</i>	Na	(23.9)	(46.5)	(100.0)	(83.8)	(72.7)	(40.0)	(60.0)	(32.5)	(1.6)	(50.0)	(33.3)	(17.3)
	NaS	(—)	(—)	(—)	(—)	(—)	(—)	36.4	13.3	(—)	(—)	(—)	(—)
<i>Moraxella</i>	Na	(—)	2.0	27.3	(—)	(—)	(—)	(27.3)	(4.3)	(8.9)	(22.2)	(21.3)	(2.2)
	NaS	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(22.5)	(13.6)	(50.0)	(—)	(7.7)
	Zb	(18.6)	(27.3)	(14.9)	(93.5)	(—)	(—)	(4.5)	(5.2)	(—)	(—)	(—)	(8.6)
<i>Flavobacter</i>	Na	91.6	37.5	40.6	47.5	60.2	17.7	83.3	63.4	(58.3)	(—)	50.0	57.2
	NaS	(—)	(28.6)	(—)	(—)	(52.9)	(26.0)	(18.8)	(50.8)	(—)	(—)	(46.5)	(72.6)
<i>Acinetobacter</i>	Na	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)
	NaS	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)
<i>Corynebacterium</i>	Na	(—)	12.5	28.1	(—)	15.3	(—)	(—)	(—)	(—)	(—)	(—)	(—)
	NaS	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)
<i>Staphylococcus</i>	Na	31.7	57.3	69.2	67.8	64.9	51.3	9.1	26.7	19.2	22.2	58.3	20.0
	NaS	(76.0)	(69.9)	(77.3)	(—)	(60.1)	(68.6)	(54.5)	(63.4)	(46.4)	(55.6)	(45.9)	(68.8)
<i>Unidentified groups</i>	Na	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	10.0
	NaS	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)
	Na	(—)	(—)	(—)	(—)	(—)	(—)	(—)	6.7	3.9	(—)	(—)	(—)
	NaS	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(2.1)	(1.0)	(—)	(—)	(—)
	Na	(—)	(—)	(—)	(—)	(—)	(—)	(—)	6.7	(—)	(—)	(—)	(—)
	NaS	(—)	(—)	(—)	(—)	(—)	(—)	(9.1)	(—)	(1.0)	(—)	(—)	(—)

* Na — Nutrient agar in distilled water, NaS — Nutrient agar in sea water, Zb — ZoBell 2216E. † Sediment, ‡ Water.

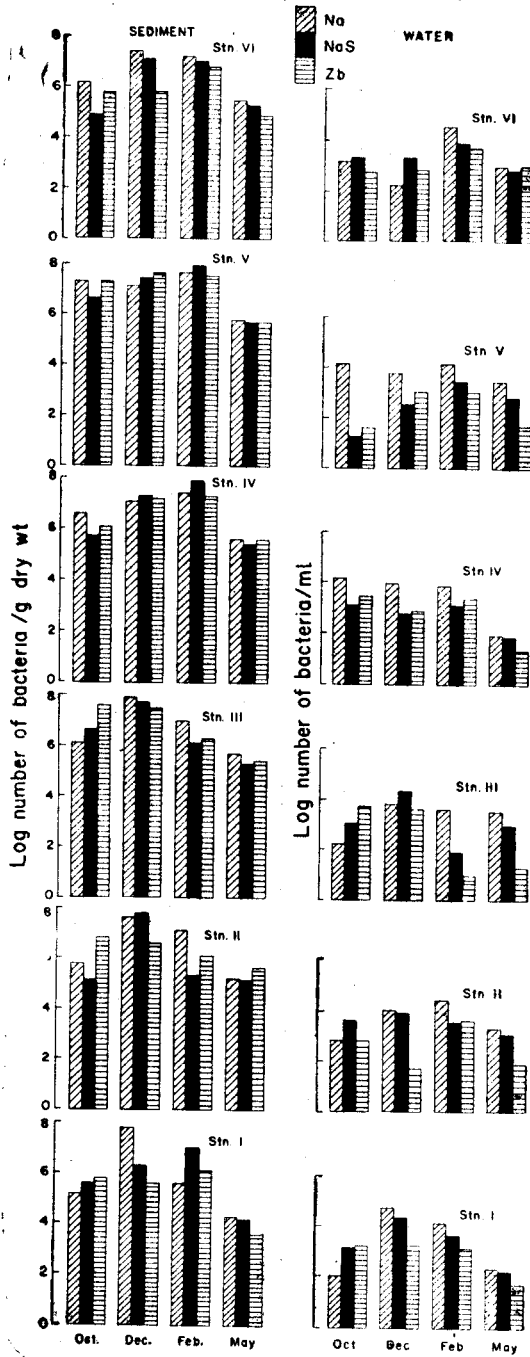


Fig. 2. Total bacterial population. (Na — Nutrient agar in distilled water, NaS — Nutrient agar in sea water, Zb — ZoBell 2216E.)

Table III. Group diversity of the bacteria

Monthwise	Sediment	Water	Stationwise	Sediment	Water
October	0.44	0.36	1	0.42	0.52
December	0.42	0.29	2	0.75	0.54
February	1.02	0.43	3	0.65	0.74
May	0.44	0.36	4	0.35	0.56
Mediumwise					
ZoBell 2216E	0.26	0.29	5	0.54	0.55
Nutrient agar in distilled water	0.66	0.49	6	0.57	0.45
Nutrient agar in sea water	0.42	0.43			

bacteria isolated from water were found to be both limnótolerant and halotolerant. Higher percentage of occurrence of these bacteria were noted in December. Enterobacteria encountered in water had a different distribution pattern than that in the sediment. The presence of these micro-organisms in high percentage in December and February in sediments could be due to land run off or due to low percentage of *Pseudomonas* (Mitchell and Morris, 1969).

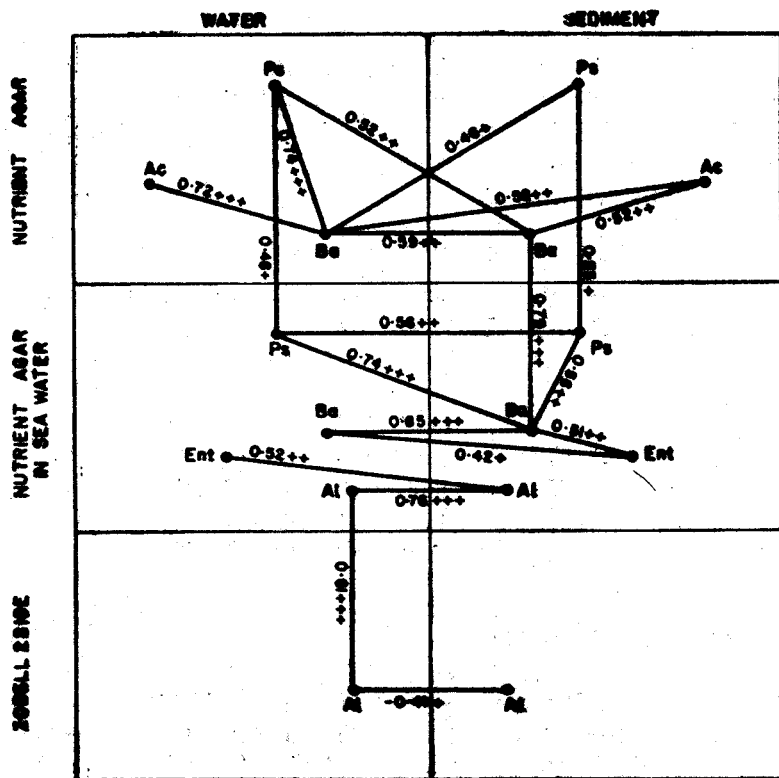


Fig. 3. Diagram of relationships between various groups of bacteria in sediment and water. Only significant correlations are presented. (+, ++, +++ significant at 5%, 1% and 0.1% levels respectively.)
 Al: Alkaligenes, Ac: Acinetobacter, Ps: Pseudomonas, Ent: Enterobacteria, Ba: Bacteria.

The Micrococci were of halotolerant and of limnotolerant types. *Coryneformae*, Staphylococci and other unidentified groups (- 10%) were also recorded sporadically.

The general pattern of distribution of different genera in water was similar to that in sediment (Table II). The diversity index was more in February in both water and sediment (Table III). Station 3 showed the maximum group diversity both in sediment and water. Nutrient agar prepared with distilled water was found to yield maximum variety of bacteria. The percentage occurrence of various genera when analysed statistically showed a good correlation. *Bacillus* showed significantly positive correlation with *Pseudomonas*, *Acinetobacter*, and Enterobacteria from water and sediment (Fig. 3). Limnotolerant and halotolerant *Bacillus* from sediment exhibited a significant correlation with those from overlying water ($r = 0.59$ at 1% and $r = 0.85$ at 0.1% level). *Alcaligenes*, from the sediment, showed significant correlation between themselves, even between those from the other two media. Various groups isolated from water and sediment samples thus seem to tolerate a wide range of salinity as evidenced from all the three media. Sediment population always showed consistent significant inter-correlation when compared to water suggesting that microbial studies of sediment may give a better evaluation of the change in the environment.

In conclusion the present study shows that the area of study, like any coastal area, is under terrestrial influence as indigenous halotolerant population was often dominated by limnotolerant forms. Though the distribution pattern of the populations was not identical in any two stations, qualitatively the correlation observed between various genera in sediment and water samples point out the stability of sediment populations which can be used with advantage for monitoring purposes.

ACKNOWLEDGEMENTS

The authors express their thanks to Dr. D. Chandramohan for his helpful suggestions and constructive criticism. They are also grateful to Dr. T. S. S. Rao and the Director, N.I.O. for the suggestions and facilities.

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