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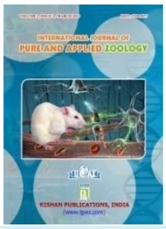

RESEARCH ARTICLE **OPEN ACCESS**

CHARACTERIZATION OF BACTERIAL DIVERSITY IN MARINE SEDIMENT AT DIFFERENT SEASONS IN KARANKADU, TAMILNADU, INDIA

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ABSTRACT

Microbes in diverse communities interact with other organisms and its environment, making their impact difficult to predict. Marine organisms have offered an endless list of novel bioactive metabolites. In search of new bioactive entities, investigation was expanded to marine habitats including marine sediments and organisms. In the present study, the soil sample was collected from Karankadu, located in east coast of Thanjavur District at four different seasons viz., post monsoon, summer, premonsoon and monsoon, and investigation were carried out. Four soil samples were subjected to physico-chemical analysis. Seasonal variations of different parameters investigated were as follows: pH (7.29-7.63), Electrical conductivity (0.26-0.52 Dsm^{-1}), Organic carbon (0.42-0.60%), Clay (10.36-18.54) and other soil parameters such as available Nitrogen and Phosphorous (84.2-95.60Kg/ac and 3.25-3.75 Kg/ac, available micronutrients (ppm) such as Zinc, Copper, Iron, Manganese (0.74-0.89,0.78-1.05,4.85-8.23,3.15-3.48) respectively and the presence of heavy metal were also analyzed for all different four seasons. pH, temperature, clay and organic matter indicated a correlation at $P<0.01$. Spatial and seasonal fluctuations of twenty five important groups of bacterial isolates were evaluated from the marine sediments during different seasons, along with soil physico-chemical parameters. Determination of bacterial diversity in the Karankura marine soil by culture method showed the predominance of bacterial genera such as *Melissococcus sp., Marinococcus albus, Salinococcus sp., Rohella sp., Oscillospira sp., Saccharococcus sp., Sulfidobacillus sp., Brucella sp., Plannococcus sp., Edwardsiella sp., Brochothrix sp., Veillonella sp.* and *Syntrophococcus sp.*

Key words: Physico-chemical parameters, sediment, seasonal fluctuations, organic carbon, soil nutrients.

INTRODUCTION

Biodiversity in extreme habitats attracts great attention among researchers because a study of these systems can increase our understanding of the relationship between organisms and their environment, and the unraveling of mechanisms of their adaptation to extreme conditions (Horikoshi and Grant,1998). However, the most fundamental meaning of biodiversity probably lies in the concept of species richness (Baltanas, 1992), that is, the number of species occurring at a site, in a region or ecosystem. Ecologists are used to measuring diversity through a number of indices all of which relate, more or less directly, the number of species to their abundance and/or numerical dominance (Magurran, 1988). Microbial number and species composition in the soil habitat differ from place to place depending upon the physical, chemical and biological factors of the particular habitat. Microbes in the seas play an important role in maintaining the health of the planet, making it habitable for people. Half the quantity of oxygen is being generated by the photosynthesis of marine microorganisms. By recycling nutrients, marine microbes maintain productivity and also alter atmospheric chemistry. Soils and Sediments are one of the last great frontiers for biodiversity research and are home to an extraordinary range of microbial and animal groups. Sediments form a natural buffer and filter system and often play an important role in the storage and release of nutrients in the aquatic ecosystems.

Biological activities in soil drive inevitable ecosystem processes, especially in the cycling of elements such as carbon, nitrogen and phosphorus in general services. Sediments can also be defined as the material deposited at the bottom of rivers, which are silt and deposits (Davies *et al*, 2009). Sediments are indicators of quality of overlying water and its study is a useful tool in the assessment of environmental pollution status. Microbial survival in polluted soils depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation (Wuertz and Mergeay, 1997). Sediments of temperate climate zones represent a temporally and spatially highly dynamic marine environment characterized by strong physical mixing and seasonal variation. Despite the large proportion and turnover of rare organisms, the overall community patterns were driven by deterministic relationships associated with seasonal fluctuations in key biogeochemical parameters related to primary productivity. Especially Bacteria play a crucial role in biogeochemical cycles and in sustainable development of the biosphere (Diaz, 2004). So this present study aimed to investigate how the seasonal variations influence the physiochemical parameters of soil and bacterial population.

MATERIALS AND METHODS

Collection of soil sample & Sampling Schedule

Soil samples were collected seasonally from the village, Karankadu, Sethbavachattram Taluk, Thanjavur District, Tamil Nadu, India for a period of one year from January 2009 to December 2009. The climate is monsoonic and the calendar year has been divided into four season viz., Post monsoon (January - March), summer (April - June), Pre monsoon (July - September) and Monsoon (October- December).

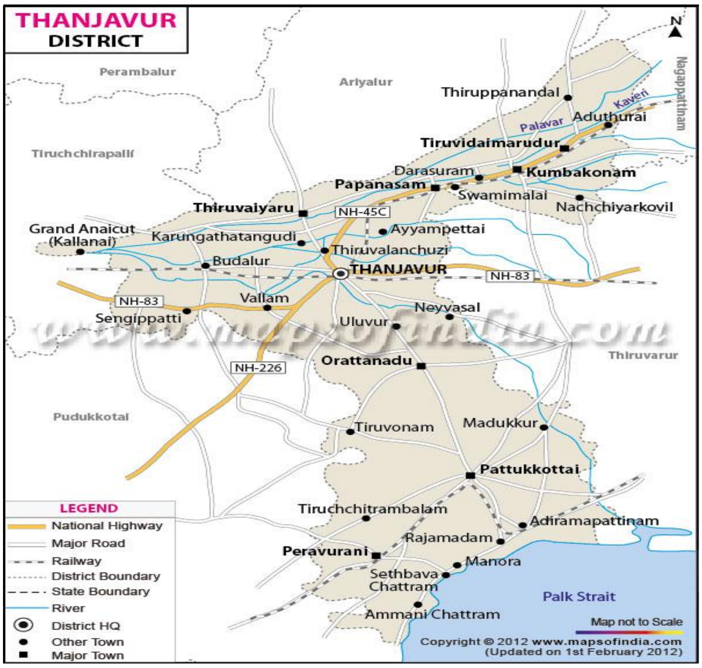
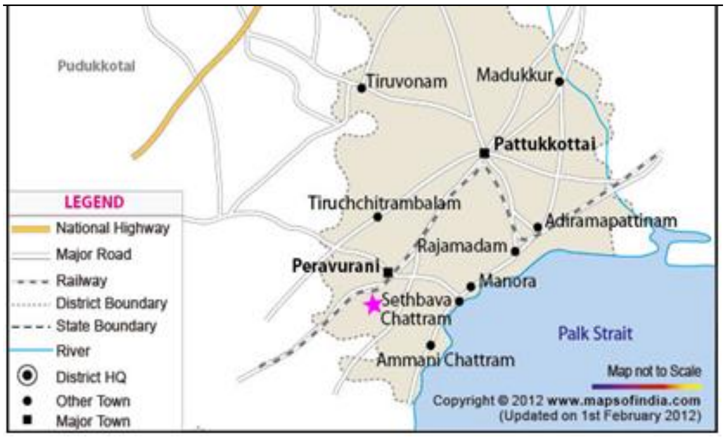


Figure 1. Location of Sample Collection.



* Sethbavachattram; Sample collection site.

Physico-chemical analysis of soil

The sediment samples were collected in zip-lock polythene bags from selected Study site at monthly interval for the period of one year from June-2010 to May-2011. The collected sediment samples were first air dried at room temperature, then crushed using a porcelain mortar and pestle and then sieved for further analysis. The pH of the suspension was read using pH meter (Systronics, India), to find out the soil pH. Electrical conductivity of soil was determined in the filtrate of the water extract using Conductivity Bridge and Cation exchange capacity (CEC) of the soil was determined by using 1 N ammonium acetate solution as described by Jackson (1973). Organic carbon content was determined by adopting chromic acid wet digestion method as described by Walkley and Black (1934), available nitrogen was estimated by alkaline permanganate method as described by Subbiah and Asija (1956) and available phosphorus by Brayl method as described by Bray and Kutz (1945). Available potassium was extracted from soil with neutral 1 N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer, calcium (Neutral 1 N NH4 OAC extractable 1:5) was extracted with neutral 1 N ammonium acetate and the available calcium in the extract was determined by Versenate method (Jackson, 1973). Other nutrient based parameters i.e. available phosphate and total nitrogen were estimated using standard methods of APHA (1987). Available micronutrients such as Zn, Cu and Mn were determined in the diethylene triamine pentaacetic extract of soil using Perkin-Elmer model 2280 Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978). Other nutrients such as magnesium, sodium and available iron were analyzed following the method of Muthuvel and Udayasoorian (1999). The reagents used for the analysis were AR grade and double distilled water was used for the preparation of solutions. The analyzed Physico-chemical parameters for four different seasons were represented in Table 1.

Isolation and identification of bacteria

The soil samples were passed through a sieve (1.7 mm mesh) to remove large pieces of debris and vegetation. The bacteria were originally isolated by plating dilutions of soils in saline solution (0.9% NaCl) on nutrient agar, was incubated at 37^{o}C for 48 h. The developed colonies were counted in the plates and the average number of colonies per three plates was determined. The number of total bacteria (CFU) per gram dry weight soil was determined. Individual colonies of bacteria which vary in shape and color were picked up and purified by streaking on nutrient agar. The bacterial isolates were identified on the basis of classification schemes published in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984) based on the characters such as morphology, physiology and nutrition, cultural characteristics and biochemical tests were presented in Table 2.

Table 1. Soil samples analytical report.

S.No.	Name of the Parameter	Sample Details			
		Post Monsoon	**Summer**	**Premonsoon**	**Monsoon**
1	pH	7.56	7.50	7.29	7.63
2	EC (dsm^{-1})	0.52	0.36	0.42	0.26
3	Organic Carbon (%)	0.62	0.42	0.52	0.60
Available Macronutrients					
4	Available Nitrogen (Kg/ac)	95.6	84.2	89.6	85.0
5	Available Phosphorus (Kg/ac)	3.50	3.45	3.25	3.75
6	Available Potassium (Kg/ac)	135	145	125	140
Available Micronutrients					
7	Available Zinc (ppm)	0.85	0.89	0.74	0.85
8	Available Copper (ppm)	0.98	0.96	0.78	1.05
9	Available Iron (ppm)	4.85	7.62	4.96	8.23
10	Available Manganese (ppm)	3.26	3.15	3.48	3.69
Soil Fractions					
11	Clay	15.33	18.54	10.36	13.41
18.54Cat ion Exchange Capacity (C.Mole Proton^{+}/Kg) Exchangeable Bases (C. Mole Proton^{+}/Kg)					
12	Calcium	10.8	11.2	12.3	11.2
13	Magnesium	9.5	8.9	9.4	8.7
14	Sodium	2.16	2.15	2.38	2.25
15	Potassium	0.18	0.19	0.16	0.15
16	Heavy metals (ppm)	Present	Present	Present	Present

Table 2. Biochemical characteristics of the bacterial isolates.

S.No	Gram Staining	Motility	Indole	MR	VP	TSI	Citrate	Catalase	Urease	Oxidase	Glucose	Lactose	Reduction of nitrate	Organism Name
1	+ve Cocci	Non-Motile	-	+	-	Alkaline production	+	+	-	+	-	+	-	*Melissococcus sp.*
2	+ve Cocci chain	Motile	-	-	-	Alkaline production	+	+	-	+	-	+	+	*Marinococcus albus*
3	+ve rod	Non-Motile	-	-	-	Alkaline production	+	+	-	+	+	+	-	*Sulfidobacillus sp.*
4	-ve rod	Motile	-	+	-	Alkaline production	+	+	-	-	+	-	+	*Buttiauexlla agrestis*
5	-ve rod	Motile	+	+	-	Alkaline production	+	+	-	+	+	-	-	*Oscillospira sp.*
6	+ve Cocci	Non-Motile	+	-	-	Alkaline production	+	+	-	+	-	+	-	*Deinococcus sp.*
7	-ve Cocci, rod	Non-Motile	+	+	-	Alkaline production	-	+	-	+	+	+	+	*Mesophilobacter sp.*
8	+ve Cocci	Motile	+	+	-	Alkaline production	+ve	+	-	-	+	+	-	Planococcus sp.
9	+ve rod	Non-Motile	+	+	-	H_2S	+	+	-	+	-	-	-	*Syntrophospora bryantii*
10	-ve rod	Non-Motile	-	+	-	H_2S is not produced	-	+	-	-	-	+	+	*Shigella sp.*
11	+ve rod	Non-Motile	-	+	+	Alkaline production	-	+	+	+	+	-	-	*Brochothrix sp.*
12	+ve Cocci	Non-Motile	+	+	-	Acid more gas production	-	+	+	-	+	+	-	*Megasphacera sp.*
13	+ve cocci	Non-Motile	-	+	-	Acid production	+	+	+	+	+	+	-	*Saccharococcus sp.*

Continues…

S.No	Gram Staining	Motility	Indole	MR	VP	TSI	Citrate	Catalase	Urease	Oxidase	Glucose	Lactose	Reduction of nitrate	Organism Name
14	-ve cocci	Non-Motile	-	-	+	Acid, gas production	-	-	+	-	+	-	-	*Veillonella sp.*
15	+ve rod	Motile	-	-	+	Acid, gas production	-	+	+	+	+	-	-	*Curtobacterium sp.*
16	-ve rod	Motile	+	-	-	Acid, gas production	-	+	+	-	+	+	+	*Edwardsiella sp.*
17	-ve Cocci,	Non-Motile	-	+	+	No acid	-	+	+	+	+	-	-	*Salinicoccus sp.*
18	-ve rod	No-Motile	-	+	+	Acid production	+	+	-	+	+	-	+	*Rahella sp*
19	+ve cocci	Non-Motile	-	-	+	Acid, gas production	-	-	+	+	+	+	-	*Sarcina sp*
20	-ve cocci	Motile	-	+	-	Acid, gas	-	-	+	-	+	-	-	*Syntrophococcus sp.*
21	+ve rod	Non-Motile	+	-	-	Acid butt, alkaline production	+	+	-	+	+	+	-	*Dermobacter sp.*
22	+ve cocci	Non-Motile	+	-	-	Alkaline production	+	+	+	-	+	+	-	*Micrococcus sp.*
23	+vecocci	Non-motile	-	+	-	Alkaline production	+	+	+	+	+	-	-	*Brevibacterium sp.*
24	-ve cocci	Non-motile	-	+	-	Alkaline production	+	+	+	+	+	-	-	*Brucella sp.*
25	+ve cocci	Non-motile	+	+	-	Alkaline production	+	+	+	+	+	-	-	*Aerococcus sp.*

Presentation of Data

Number of species is referred as species diversity. Populations density expressed in terms of colony forming unit (CFU) per gram of soil with dilution factors. In order to assess the dominance of individual species in species site percentage contribution was worked out as follows

$$\% \text{ contribution} = \frac{\text{No. of bacterial colonies in a sample}}{\text{Total number of colonies of all the species in a sample}} \times 100$$

Statistical analysis

The Spearman rank test was used for the correlation analysis to compare the distribution of bacterial population with respect to its physico-chemical parameters and study area (Biodiversity PRO software). The findings were considered as significant if p value was < 0.005 by using ANOVA correlation method.

3. RESULTS AND DISCUSSION

1. Correlation co-efficient between the physico-chemical parameters of soil and bacterial isolates

The fluctuation in marine soil temperature usually depends on the season, geographic location, sampling time and temperature of effluent entering the stream (Ahipathy, 2006). Statistical analysis of these results suggests a much greater ‘hidden diversity’. Statistical analysis revealed that the correlation is significant at both the 0.01 level (2-tailed) and 0.05 level (2 tailed) (Table 3 & 4). Present investigation reveals that there was no great difference found in pH values in seasonal analysis which indicates the alkaline nature of marine water, may be due to high temperature which causes reduction in solubility of CO_2 (Mahananda *et al.*, 2010). While soil acidification is beneficial in the case of alkaline soils, it degrades land when it lowers crop productivity and increases soil vulnerability to contamination and erosion.Soils are often initially acid because their parent materials were acid and low in the basic cations (calcium,magnesium, potassium and sodium). Electrical conductivity of soils gives data of the soluble salts present in soil. Within the seasons studied, higher EC values were reported during pre- and post-monsoon seasons due to higher discharge of industrial effluent to the river. The monsoon season recorded lowest EC values which may due to dilution of soluble salts by rainwater. The Organic Carbon content in marine sediments is a key component in a number of chemical, physical and biological processes and contributes significantly to acidity through the formation of organic acids. The highest value of organic matter was recorded in the post monsoon season as natural processes and human activities have resulted in elevated content of organic carbon in soil, sediment and streams. This is supported by Kamaruzzaman *et al*. (2009) and Adeyemo *et al*. (2008). They reported that input from inappropriate animal waste disposals, forest clear cuttings, agricultural practices and changes in land usage rise the organic carbon content.

The fertility and biodiversity in an aquatic system are greatly influenced by nitrogen concentration of the sediment. The concentration of total nitrogen was the highest during premonsoon season 95.6 kg/ac which is due to the oxidation of organic matter that has settled on the top layer of sediment (Saravanakumar *et al*., 2008). The available phosphate content at study area varied between 3.25 to 3.75 kg/ac and showed higher values in monsoon followed by post monsoon and pre-monsoon. It might be due to the addition of fertilizers from agricultural runoff, sewage contaminated storm water out falls and other anthropogenic activities such as use of detergents, bathing; cattle wading etc. Saravankumar *et al.* (2008) also observed similar trend in case of inorganic phosphate. Among the months studied, higher calcium and sodium content were observed in pre and post-monsoon seasons. variations in magnesium contents showed that increased Mg contents ranging from 8.7 to 9.5 (mg/kg soil) were recorded .This excess Mg may be derived from the decomposition of litter accumulated for longer period in sediment favoured increased microbial activity. The lower concentration of magnesium and sodium during the monsoon season may be attributed to dilution by rainwater.

Increase in potassium concentration was recorded during the summer seasons which may be attributed to the decay of sacred grove litter. Zn showed higher concentrations in the post-monsoon season followed by pre-monsoon and monsoon season, the higher concentration of Zinc in sediment may be due to the presence of unused remains of Zinc sulphate in fertilizers (Reza & Singh, 2010).

2. Diversity in Marine Soil at Four different seasons

Soil samples from 4 different seasons representing the East Coast of Thanjavur District were examined for bacterial diversity. The study revealed the presence of 25 species of bacteria, among them 11 species were found in all the seasons (Table 5). Several studies suggested that soil microbial diversity had seasonal fluctuations (Lipson and Schmidt, 2004 and Smit *et al.*, 2001). In regards to its microbial diversity, this ecosystem is largely dominated by *Melissococcus sp., Marinococcus albus, Sulfidobacillus sp., Oscillospira sp, Planococcus sp, Saccharococcus sp, Edwardsiella sp., Salinococcus sp., Rahella sp., Syntrophococcus sp.*, which is the characteristic of saline soil. Presence or absence of particular bacterial genera may depend on soil parameters, as observed by Alexander (1971).

The present study revealed that the bacterial community varied at different seasons as well as area, and were both positively and negatively correlated with environmental factors such as pH, EC, and available micro & macro nutrients accounted for a significant amount of variability in bacterial community composition. This indicates that the organic matter content. pH and available soil nutrients could influence the structure of the bacterial community in marine sediments. The Gram negative population and higher O_2 level in sea water is conformed with previous reports by Gonzalez-Acosta *et al.* (2006). But the present study revealed the presence of higher Gram positive bacteria pool that they would be the sole decomposers. The site of the present study soil supports for the presence of higher population of gram positive than gram negative, nitrifier, denitrifiers, phosphate solubilizer, sulphur oxidizers, for major biogeo-chemical cycles and also bioluminescent bacteria responsible for quorum sing.

Table 3. Correlation between the physico-chemical and Biological parameters with distribution of Marine Bacteria.

SOIL	PH	EC	OC	AN	AP	AK	AZ	AC	AI	AM	CLAY	Ca	Mg	Na	K
PH	1														
EC	.058	1													
OC	.196	.960(*)	1												
AN	-.112	-.710	-.862	1											
AP	.506	-.320	-.041	-.362	1										
AK	.912	.305	.497	-.507	.629	1									
AZ	.802	-.214	.040	-.282	.921	.840	1								
AC	.99(**)	.098	.255	-.201	.558	.946	.835	1							
AI	.578	.625	.812	-.876	.528	.858	.611	.648	1						
AM	.118	.840	.684	-.224	-.657	.142	-.413	.103	.259	1					
CLAY	.514	-.442	-.173	-.226	.990*	.582	.920	.555	.420	-.719	1				
Ca	-.887	.403	.240	-.168	-.669	-.719	-.872	-.871	-.286	.318	-.727	1			
Mg	-.533	-.705	-.870	.893	-.435	-.821	-.525	-.602	-.994 **	-.351	-.320	.204	1		
Na	.238	.235	.488	-.845	.788	.580	.641	.329	.801	-.296	.695	-.184	-.759	1	
K	.012	-.804	-.620	.153	.755	.000	.538	.032	-.138	-.990*	.811	-.425	.236	.383	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

OC-Organic Carbon (%), EC-Electric conductivity, OC-Oxygen conductivity, AN-Available Nitrogen(Kg/ac), AP-Available Phosphorus(Kg/ac), APOT-Available Potassium(Kg/ac), AZ- Available Zinc(ppm), AC-Available Copper (ppm), AI-Available Iron(ppm) AM- Manganese(ppm).AK-Available Potassium((Kg/ac), AM-Available Magnesium(ppm), Ca-Calcium (ppm), Mg-Magnesium(ppm), Na- Sodium (ppm), K- Potassium (ppm).

Table 4. Correlation between the Seasonal variations and distribution of Bacterial population.

SEASON		**Post monsoon**		**Summer**		**Pre monsoon**		**Monsoon**		**Total Number of colonies**
		TNC	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**	
Postmonsoon	TNC	1								
	MD	.986(**)	1							
Summer	TNC	.337	.398(*)	1						
	MD	.363	.426(*)	.983(**)	1					
Pre monsoon	TNC	.048	.075	.140	.146	1				
	MD	.034	.059	.100	.116	.995(**)	1			
Monsoon	TNC	.058	.094	.175	.203	.445(*)	.459(*)	1		
	MD	.054	.088	.171	.198	.452(*)	.467(*)	.999(**)	1	
Total number of colonies		.486(*)	.534(**)	.310	.312	.593(**)	.571(**)	.586(**)	.582(**)	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 5. Status of Marine Bacterial isolates from Karankura, Thanjavur District.

S.No	Bacterial isolates	Premonsoon	Monsoon	Post monsoon	Summer
1	*Melissococcus sp*	+	+	+	+
2	*Marinococcus albus*	+	+	+	+
3	*Sulfidobacillus sp*	+	+	+	+
4	*Buttiauexlla agrestis*	-	-	-	+
5	*Oscillospira sp*	+	+	+	+
6	*Deinococcus sp*	-	-	-	-
7	*Mesophilobacter sp*	-	-	-	-
8	*Planococcus sp*	+	+	+	+
9	*Syntrophospora bryantii*	-	+	-	-
10	*Shigella sp*	+	-	-	-
11	*Brochothrix sp*	-	+	+	-
12	*Megasphacera sp*	-	-	+	-
13	*Saccharococcus sp*	+	+	+	+
14	*Veillonella sp*	-	-	+	-
15	*Curtobacterium sp*	+	-	-	-
16	*Edwardsiella sp*	+	+	+	+
17	*Salinicoccus sp*	+	+	+	+
18	*Rahella sp*	+	+	+	+
19	*Sarcina sp*	-	-	-	+
20	*Syntrophococcus sp*	+	+	+	+
21	*Dermobacter sp.*	-	-	+	+
22	*Micrococcus sp.*	-	-	-	+
23	*Brevibacterium sp.*	+	-	-	-
24	*Brucella sp.*	-	+	-	-
25	*Aerococcus sp.*	+	-	-	-

+ Denotes Present *- Denotes Absent*

4. CONCLUSIONS

It is getting established that research in marine habitats including marine sediments and organisms is ultimately aimed to focus on new bioactive compounds to improve the quality of our life and save ourselves from severe diseases. The soil of Karankadu, near sethubavachatram located in east coast of Thanjavur District have been investigated to study the monthly changes in soil moisture, soil pH, EC, organic carbon, available nitrogen and macronutrients like sodium, potassium, calcium and magnesium during the period of one year (2010-11) at four different seasons viz, post monsoon, summer and premonsoon and monsoon. Physiochemical analyses were performed to study the soil characteristics related to fertility and chemical nature. By monitoring the changes with respect to all soil physicochemical parameters studied, it clearly indicates that, the soil collected at monsoon and postmonsoon seasons showed higher values compared to premonsoon and summer seasons with very few exceptions. It was noticed that the regular addition of fertilizers from agricultural runoff, sewage contaminated water out falls, rain water and other anthropogenic activities contribute major changes in soil physiochemical properties that in turn significantly manifest the microbial populations. Different groups of bacterial populations observed in this study are uncommon and they were fewer in summer. Because there is a limitation in moisture during summer. So drought might be constituting a stress in microbial communities. Determination of bacterial diversity by culture method showed the predominance of bacterial genera such as *Melissococcus sp, Marinococcus albus, Salinococcus* *sp, Rohella sp, Oscillospira sp, Saccharococcus sp, Sulfidobacillus sp, Brucella sp, Plannococcus sp, Edwardsiella sp, Brochothrix sp, Veillonella sp,Syntrophococcus sp.* Venkatesharaju *et al.,* 2010 stated that the healthy aquatic ecosystem is depended on the biological diversity and Physico-chemical characteristics of water as well as its soil and sediments.

The present study could be concluded that there is no uniformity in the diversity of marine bacterial populations and their distribution pattern in different geographical regions. Several factors of salinity, origin, nature of substrata, pH and oceanic region affect the occurrence and diversity of marine bacteria. So it is obvious that a study based on biodiversity is a major challenging task as we try to predict the secret of nature.

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