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Seasonal Distribution of Bacterial Diversity in Marine Soil of Kodiyakarai, Vedaranyam, Nagappattinam (DT.)

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A B S T R A C T

Soil plays a vital role in sustaining life on planet. The marine soil bacteria as a source of bioactive compounds. The present study was physical and chemical characteristics of soil from three different marine environment of different places mangrove, saltpan, coastal of Kodiyakarai area in Vedaranyam, Nagapattinam district of Tamil Nadu. The soil sediments were collected in the season of Pre-monsoon, monsoon, post-monsoon and summer season. The marine soil sediment were selected for the following boundaries like soil texture, calcium carbonate, Electrical conductivity, Micronutrients like (Zinc, copper, iron, manganese), Macronutrients like (nitrogen, phosphorus, potassium) were studied. This study deals with the seasonal distribution of bacterial population in relation to the physicochemical parameters of marine soil. Totally 19 species of 12 genera were isolated and identified by using standard manuals. Data of the present study revealed that the total number of bacterial count was significantly correlated with physicochemical parameters of marine soil.

***Key words*:** Marine soil, Physicochemical analysis, Bacterial diversity

The marine is one of the world most important sources of bioactive compounds. Vedaranyam is situated on the coast of Bay of Bengal and one of the coastal blocks of Nagapattinam district in Tamil Nadu. The cape calimere and kodiyakarai is a low headland on the Coromandel Coast in the Nagapattinam district on the state of Tamil Nadu, India. Soil is a major and costly property of the nature. Soil is the essential component for sustaining plant growth in the terrestrial ecosystem. Soil plays an important role to human and living organisms. Microbes like fungus, bacteria, surrounded by the soil [1]. The mangroves are second most important ecosystem in productivity. Coastal environment consists of rich biodiversity [2]. The soil quality is depending on its physical properties (color, texture, moisture contents, pH, organic matter content etc.) and chemical properties (cation exchange capacity, organic matter contents, phosphate phosphorous, nitrate nitrogen, nitrite nitrogen etc.) [3]. Marine macro-organisms are a rich source of biologically active secondary metabolites. The bacteria present in seawater at concentrations of approximately one million cells per milliliter [4]. The mangrove forests surrounded the tropical and subtropical coasts of the world. They are developed in estuarine areas of the tropics. The World's mangroves span over 30 countries with a total area of 99,300 km^2. The largest mangrove area occurs in Indonesia (30%), Brazil (10%), Australia (8%), India and Nigeria (7%, each) [5]. The chemically unique bioactive molecules are too produced by marine bacteria. Microorganisms were reported to produce around 23,000 bioactive secondary metabolites with a wide range of industrially valuable activities, such as antibacterial, antifungal, anti-malarial, cytotoxic, anticancer etc. [6].

The soil containing huge microbial population counts of mass organic matter on earth. Bacterial population in soil play a vital role in nutrient cycles including *Nitrosomonas* and *Nitrobacter* (nitrification), *Thiobacillus* (sulfur and iron oxidation), *Rhizobium* and *Frankia* (N_2 fixation), *Bacillus* and *Clostridium* (carbon cycling) and *Caulobacter* and *Pseudomonas* (manganese oxidation) [7].

Halophiles are the source of enzymes stable in salts and organic solvents. Halophiles inhabit hypersaline environments where salinity often reaches saturation and accumulate large concentrations of salts and organic compatible solutes in their cells as an adaptation. Halophiles present or adapt the alkaline environment, because of Phylogenetic diversity, unique salt tolerance mechanisms and potential applications. Bacteria and probably the fungi are responsible for the degradation of organic matter and the conversion of organic nitrogen to ammonia which can be used by the algae and perhaps brine shrimp and brine flies so common to hypersaline environments [8]. Bacteria play a major role in biogeochemical cycles and also sustainable development of the biosphere. The seasonal variations influence the physiochemical parameters of soil and bacterial population. Soils and Sediments are one of the last

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great boundaries for biodiversity. Microbes in the seas play a vital role in maintaining the health of the planet and also making it habitable for people [9].

Halophilic bacteria are organisms that survive under high salt environments such as solar salterns, salt lakes and salt mines contain large population's organisms. The halophiles are diverse when compared to their terrestrial counterpart and obligate halophiles. Recently many reports conclude on the production of pigments from halophilic bacteria and its huge applications. Many marine organisms like micro and macroalgae to produce antioxidant substances [10].

Marine bacterial diversity has been assessed as a resource for drug discovery. We know very little about how marine organisms respond to the bacteria and also how bacteria respond to the physiochemical environment of a potential host, however bacterial responses to host chemical cues appear to explain some of the epibacterial distributions were observed in nature [11]. Bacteria that able to grow in the absence of salt and also presence of high salt concentrations are called halo tolerant. Halophilic and halotolerant bacteria can produce enzymes and essential for salty foods production [12]. Mangroves are the productive coastal ecosystems of the world that protect coastal people from natural calamities and support livelihood. Bacteria influence nutrient cycling and thus contribute to soil and vegetation patterns. Mangroves provide a unique ecological environment for diverse bacterial communities [13].

MATERIALS AND METHODS

The marine soil samples were collected from post-monsoon, summer, Pre-monsoon and Monsoon. The marine soil sediments were collected from kodiyakarai area in vedaranyam, Nagapattinam district of Tamil Nadu.

Physicochemical analysis of soil samples [14]

The physicochemical parameters of collected soil samples were analyzed by standard methods. The analysis of physicochemical parameters of the soil sample was done at soil testing laboratory, Department of Microbiology, Government of Tamil Nadu. The physico-chemical Parameters were recorded.

Isolation of bacteria from Marine soil samples [15]

The serial dilution technique was used for isolation of bacterial colonies from different site of marine environment soil (Saltpan, Mangrove, and Coastal). One gram of each site of soil were suspended in 10ml of sterile distilled water prepared as a stock. From this stock various dilutions were prepared 10^{-4}, 10^{-5} and 10^{-6} using sterile distilled water. One milliliters of the diluted sample was poured in to Petri plates containing Zobell marine agar medium. Triplicates were maintained for each dilution. The plates were incubated at 37 °C for 24 Hours. The colonies growing on Zobell marine agar medium containing plates with different colonies were observed.

Identification of bacteria from marine soil samples [16-17]

Statistical analysis

Correlation coefficient was done to test the relationship between bacterial population and the physicochemical properties. Correlation between the number of colonies and Physicochemical Parameters of the coastal soil revealed pH, AC, AL, AMN were negatively correlated and ECC, AN, AP, AK, AZ, AMN were positively correlated. However, the relationship in ECC, AMN, A was statistically significant at 0.05% and AK was statistically significant at 0.01% level. Correlation between the number of colonies and Physicochemical Parameters of the mangrove soil revealed pH, AC, AMN were negatively correlated and ECC, AN, AP, AK, AZ, AL were positively correlated. However, the relationship in AP, AK, and AMN were statistically significant at 0.05%. Correlation between the number of colonies and Physicochemical Parameters of the mangrove soil revealed AN, AC were negatively correlated and pH, ECC, AK, AZ, AL, AMN were positively correlated. However, the relationship in AP, AMN were statistically significant at 0.05% and AK were statistically significant at 0.01% level.

RESULTS AND DISCUSSION

The current study of physicochemical factor including Soil texture, Calcium Carbonate, Electrical conductivity, Power of hydrogen, Macronutrients like (Organic carbon, Nitrogen, Phosphorus, Potassium), Micronutrients like (Iron, Manganese, Zinc, Copper) soil samples were studied (Table 1).

Table 1 Physiochemical properties of coastal, mangrove and saltpan environment of Vedaranyam, Nagappattinam (Dt)

Physicochemical properties	Coastal				Mangrove				Saltpan			
	Pre-monsoon	Monsoon	Post-monsoon	Summer	Pre-monsoon	Monsoon	Post-monsoon	Summer	Pre-monsoon	Monsoon	Post-monsoon	Summer
pH	8.56	8.49	8.64	8.45	7.93	8.28	8.27	8.11	7.49	7.85	7.85	7.53
EC (dsm^{-1})	2.39	3.53	2.22	2.08	24.3	4.44	4.19	3.14	0.44	13.50	17.02	9.55
Available nitrogen (mg/g)	35	37.8	35	34	29.4	57.4	32.2	27.8	54.6	29.4	33.6	26.3
Available phosphorus (mg/g)	0.50	2.0	0.50	1	0.85	1.98	1.05	1.10	3.0	0.82	0.99	1.05
Available potassium (mg/g)	128	161	136	127	247	201	211	218	60	240	217	74
Available zinc (ppm)	0.85	0.91	0.95	0.89	0.99	1.37	1.19	1.05	0.93	0.89	0.94	0.79
Available copper (ppm)	1.05	0.82	1.10	1.01	1.06	1.03	1.19	1.11	0.99	0.81	0.98	0.85
Available iron (ppm)	4.43	4.39	4.53	4.31	4.54	4.37	4.47	4.29	4.58	4.28	0	0
Available manganese (ppm)	3.11	3.49	3.20	3.14	3.03	3.73	3.63	3.58	3.57	3.73	3.79	3.55

The physicochemical characterization of three marine sediment samples was investigated and collected from Kodiyakarai, Vedaranyam, Nagapattinam, Tamil Nadu, India. The physicochemical parameters of each site of soil were tabulated. This study of some Physicochemical analysis of Keezhathottam mangrove water parameters such as atmospheric temperature, surface water temperature, pH, alkalinity, salinity, TDS, calcium, magnesium, chloride,

ammonia, nitrate, nitrite inorganic and organic phosphate in all the four seasons examined. Most of the parameters tested were slightly higher in summer than the monsoon seasons. Alkalinity and salinity were observed more during the summer. In general, the characteristics of water tested in all the four seasons were not varied much [18]. The present studies saltpan, mangrove and coastal soil sediments of physicochemical parameters were analyzed. The 9 site of each soil parameters were calculated and also physical and chemical correlation coefficient were performed. The soil parameters like texture, calcium carbonate content, electrical conductivity (EC), pH, organic carbon (OC), available nitrogen, available phosphorus, available potassium iron, manganese, zinc, copper and cation exchange capacity were studied by the standard methods. Further, the correlation coefficient analysis between the physical and chemical boundaries of the marine soil samples and total action bacterial isolates were performed [1].

Table 2 Morphological characteristics of Isolated Bacteria

Code of the isolated strains	Growth	Shape	Surface	Margin	Color	Elevation	Consistency	Opacity
ASGK 1	Slow	Circular	Rough	Irregular	Light green	Convex	Viscous	Opaque
ASGK 2	Rapid	Punctiform	Smooth	Even	White	Convex	Viscous	Transparent
ASGK 3	Slow	Punctiform	Rough	Irregular	White	Umbonate	Adhesive	Opaque
ASGK 4	Slow	Circular	Smooth shiny	Entire	Creamy white	Pulvinate	Buttery	Opaque
ASGK 5	Slow	Circular	Smooth shiny	Entire	Pale Yellow	Pulvinate	Buttery	Opaque
ASGK 6	Rapid	Rhizoid	Smooth	Lobate	Milky White	Flat	Viscous	Opaque
ASGK 7	Rapid	Circular	Rough	Entire	Creamy White	Raised	Viscous	Opaque
ASGK 8	Slow	Irregular	Glistening	Entire	Pale yellow	Pulvinate	Buttery	Translucent
ASGK 9	Rapid	Punctiform	Glistening	Entire	Yellow	Convex	Viscous	Transparent
ASGK 10	Slow	Punctiform	Smooth	Wavy	White	Flat	Buttery	Opaque
ASGK 11	Slow	Irregular	Glistening	Irregular	Greyish White	Effuse	Buttery	Translucent
ASGK 12	Rapid	Punctiform	Rough	Erose	White	Convex	Viscous	Opaque
ASGK 13	Rapid	Sperical	Concentric	Lobate	White	Flat	Buttery	Opaque
ASGK 14	Slow	Circular	Smooth shiny	Entire	Pale yellow	Pulvinate	Buttery	Opaque
ASGK 15	Slow	Irregular	Smooth	Even	White	Convex	Adhesive	Translucent
ASGK 16	Slow	Circular	Smooth shiny	Entire	Pale yellow	Pulvinate	Buttery	Opaque
ASGK 17	Slow	Circular	Rough	Irregular	White	Convex	Viscous	Opaque
ASGK 18	Rapid	Punctiform	Smooth	Even	White	Convex	Viscous	Opaque
ASGK 19	Slow	Circular	Smooth shiny	Entire	White	Pulvinate	Buttery	Opaque

Table 3 Biochemical characteristics of isolated bacterial strains from marine associated soil sample

Code of the Isolated strains	Gram staining	Indole	MR reaction	Voges Proskaure reaction	Citrate	Catalase	Carbohydrate fermentation			Oxidase	TSI	Nitrate reduction	Name of the organisms
							Glucose	Mannitol	Sucrose				
ASGK 1	-ve	+	+	-	+	+	Invalid	-	Invalid	+	A/A H_2S	+	*P. facilis*
ASGK 2	- ve	-	-	+	-	+	-	+	-	+	-	+	*S. aureus*
ASGK 3	- ve	+	+	+	+	+	-	-	Invalid	+	A/A H_2S	+	*P. aerogenosa*
ASGK 4	+ ve	-	-	+	-	+	Invalid	+	+	-	A/A H_2S	+	*Bacillus sp.*
ASGK 5	+ ve	+	-	-	+	+	+	+	+	+	A/A H_2S	+	*B. subtilis*
ASGK 6	- ve	+	-	-	+	-	+	+	+	+	A/A H_2S	+	*S. fonticola*
ASGK 7	- ve	-	+	+	+	-	-	Invalid	+	+	H_2S	-	*F. odoratum*
ASGK 8	- ve	-	+	-	+	+	+	+	+	-	A/A H_2S	+	*Proteus sp.*
ASGK 9	+ ve	-	-	+	-	-	-	-	+	+	A/A H_2S	-	*B. cereus*
ASGK 10	- ve	+	-	+	+	+	+	Invalid	Invalid	+	A/A	+	*E. coli*
ASGK 11	- ve	-	+	-	+	+	+	+	-	+	-	-	*P. vulgaris*
ASGK 12	+ ve	+	+	+	+	-	+	+	+	+	A/A H_2S	-	*C. butyricum*
ASGK 13	- ve	+	-	-	+	+	+	Invalid	-	+	A/A H_2S	-	*Aeromonas sp.*
ASGK 14	+ ve	+	-	+	+	+	+	Invalid	Invalid	-	A/A H_2S	+	*S. pneumonia*
ASGK 15	+ ve	-	+	-	+	-	Invalid	+	+	-	A/A	-	*K. pneumonia*
ASGK 16	- ve	-	-	-	-	+	+	+	-	-	A/A H_2S	+	*E. tarda*
ASGK 17	- ve	+	+	+	+	+	-	-	Invalid	+	A/A H_2S	+	*Pseudomonas sp.*
ASGK 18	- ve	-	-	+	-	+	-	+	-	+	-	+	*Staphylococcus sp.*
ASGK 19	+ ve	-	-	+	-	+	Invalid	+	+	-	A/A H_2S	+	*Bacillus sp.*

+ = Positive; - = Negative

Correlation coefficient was done to test the relationship between bacterial population and the soil physicochemical properties. The different types of bacterial species was isolated during Pre-monsoon, Monsoon, Post-monsoon and summer seasons. The seasonal distribution of bacterial diversity was isolated and identified from marine soil result were recorded (Table 2-3, Fig 1). Physiochemical analyses were performed to study the soil characteristics related to fertility and chemical nature. Soil physicochemical parameters studied, it clearly indicates that, the soil collected at monsoon and post-monsoon

seasons showed higher values compared to pre-monsoon and summer seasons. This study could be concluded that there is no uniformity in the diversity of marine bacterial populations and their distribution pattern in different geographical regions studied by [9].

The bacterial species were noticed from marine soil of coastal, mangrove, saltpan region. Totally 19 species of 12 genera were isolated and identified by standard method (Fig 2). Among this *Aeromonas* 01 species, *Bacillus* 04 species, *Clostridium* 01 species, *Escherichia* 01 species, *Edwardsiella* 01 species, *Flavobacterium* 01 species, *Klebsiella* 01 species, *Proteus* 02 species, *Pseudomonas* 03 species, *Serratia* 01 species, *Staphylococcus* 02 species, *Streptococcus* 01 species were recorded (Fig 3).

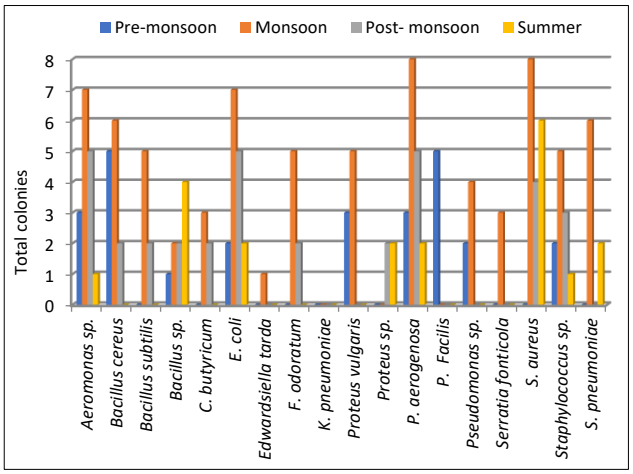

Fig 1 Bacterial diversity of Coastal region of Vedaranyam, Nagappattinam (Dt.)

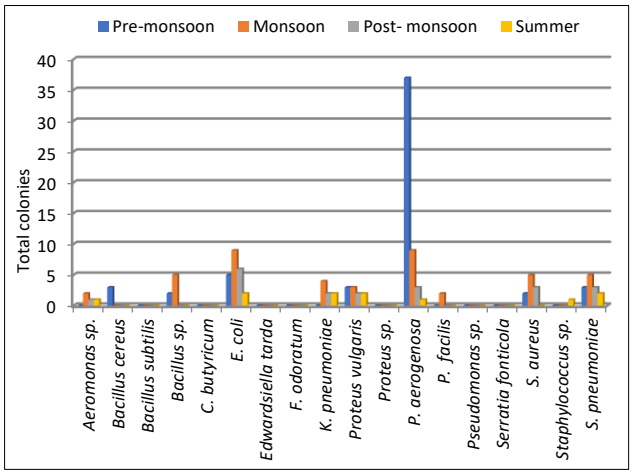

Fig 2 Bacterial diversity of Mangrove region of Vedaranyam, Nagappattinam (Dt.)

Table 4 Relationship between bacterial colonies and physicochemical properties of Coastal region of Vedaranyam, Nagappattinam (Dt.)

	TNC	pH	ECC	AN	AP	AK	AZ	AC	AI	AMN
TNC	1									
pH	-0.186	1								
ECC	0.983*	-0.258	1							
AN	0.988*	-0.100	0.986*	1						
AP	0.874	-0.621	0.872	0.807	1					
AK	0.992**	-0.141	0.952*	0.969*	0.862	1				
AZ	0.255	0.422	0.073	0.186	0.113	0.374	1			
AC	-0.885	0.620	-0.913	-0.841	-0.983*	-0.851	0.039	1		
AI	-0.003	0.982*	-0.085	0.078	-0.464	0.046	0.508	0.468	1	
AMN	0.984*	-0.234	0.943	0.947	0.907	0.994**	0.368	-0.886	-0.048	1

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

Table 5 Relationship between bacterial colonies and physicochemical properties of Mangrove region of Vedaranyam, Nagappattinam (Dt.)

	TNC	pH	ECC	AN	AP	AK	AZ	AC	AI	AMN
TNC	1									
pH	-0.319	1								
ECC	0.713	-0.854	1							
AN	0.435	0.598	-0.310	1						
AP	0.219	0.665	-0.495	0.967*	1					
AK	0.377	-0.965*	0.917	-0.644	-0.759	1				
AZ	0.138	0.870	-0.591	0.912	0.911	-0.864	1			
AC	-0.681	0.292	-0.366	-0.565	-0.519	-0.142	-0.181	1		
AI	0.645	-0.400	0.768	-0.213	-0.453	0.612	-0.273	0.081	1	
AMN	-0.520	0.937	-0.969*	0.525	0.671	-0.987*	0.772	0.229	-0.693	1

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

Totally twenty-seven bacterial species were identified such as *Micrococcus* spp., *Bacillus* spp., *Acetobacter* spp., *Pseudomonas* spp., *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Sulfo bacillus* spp., *Escherichia coli*.,

Aeromonas spp., *Brevibacterium* spp., *Listeria* spp., *Azotobacter* spp., *Cellulomonas* spp., *Corynebacterium* spp., *Terrabacter* spp., *Aerococcus* spp., *Klebsiella* spp., *Marinococcus* spp., *Sacchrococcus* spp., *Enterobacter* spp., *Thiobacillus* spp., *Planococcus* spp., *Shigella* spp., *Dermobacter* spp., *Salinococcus* spp and *Deinococcus* spp [5]. The bacterial diversity was high on coastal soil in the duration of monsoon compare to pre-monsoon, post-monsoon and summer. In the mangrove soil have bacterial diversity high in the duration of pre-monsoon compare to monsoon, post-monsoon. The bacterial diversity high in the saltpan soil during the period of monsoon. The seasonal distribution of each marine soil bacterial diversity result was recorded (Table 4-6).

Table 6 Relationship between bacterial colonies and physicochemical properties of Saltpan region of Vedaranyam, Nagappattinam (Dt.)

	TNC	pH	ECC	AN	AP	AK	AZ	AC	AI	AMN
TNC	1									
pH	0.675	1								
ECC	0.352	0.870	1							
AN	-0.240	-0.478	-0.780	1						
AP	-0.367	-0.691	-0.912	0.964*	1					
AK	0.747	0.995**	0.836	-0.470	-0.679	1				
AZ	0.232	0.393	0.010	0.607	0.375	0.380	1			
AC	-0.557	-0.210	-0.305	0.734	0.637	-0.272	0.677	1		
AI	0.568	-0.094	-0.546	0.583	0.541	-0.010	0.390	-0.056	1	
AMN	0.536	0.967*	0.815	-0.305	-0.546	0.944	0.572	0.046	-0.124	1

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

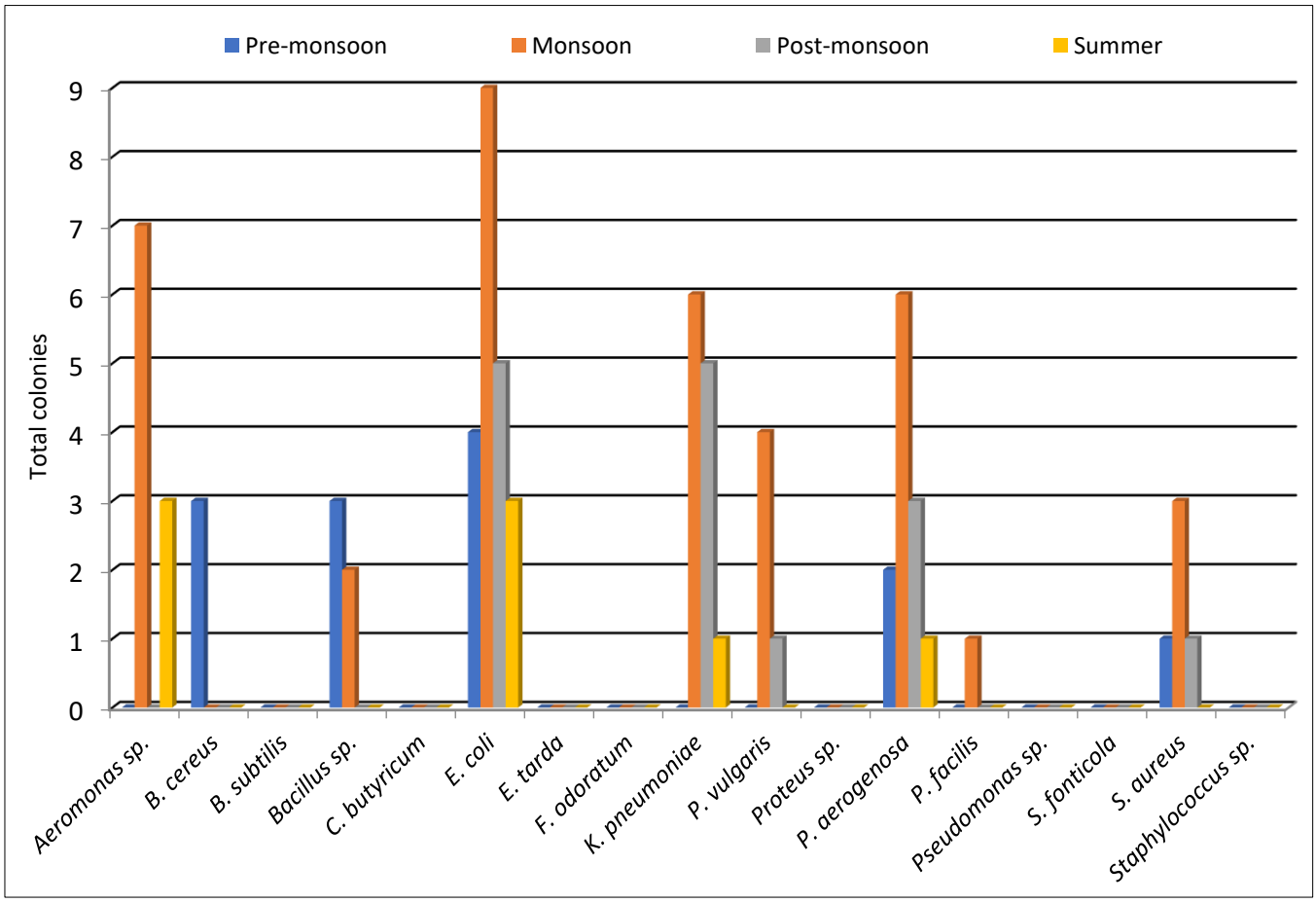

Fig 3 Bacterial diversity of Saltpan region of Vedaranyam, Nagappattinam (Dt.)

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 post-monsoon seasons showed higher values compared to pre-monsoon and summer seasons with very few exceptions were studied by Kalaivani *et al*. [9]. The physicochemical analysis of nine sites of three different types of areas of sample like saltpan, mangrove and coastal soil sediments were analyzed. Micronutrients and Macronutrients of soil were studied. The study of papers concluded the soil quality carried out by different parameters. The result reveals percentage of physicochemical parameters. Physicochemical parameters important for soil management.

CONCLUSION

It is getting recognized that research in marine environment including different places of mangrove, saltpan, coastal of Kodiyakarai area in Vedaranyam, Nagapattinam district of Tamil Nadu were analyzed physicochemical characteristics and organisms is ultimately aimed to focus on new bioactive compounds to severe diseases. Totally 19 species of 12 genera such as *Bacillus*, *Clostridium*, *Escherichia*, *Edwardsiella*, *Flavobacterium*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Serratia*, *Staphylococcus* and *Streptococcus* species were isolated and identified by using standard manuals. 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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