Original Research Article

Physicochemical and marine actinomycetes characterization of soil samples from the coastal areas of Arabian sea

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Abstract

The marine actinomycetes are a rich source for novel biomolecules. Especially the exotic tropical marine habitat of the Kerala coastal region favours the actinomycete diversity. The present study focuses on the isolation, purification and morphological characterization of marine actinomycetes. A total of 280 morphologically distinct actinomycetes were isolated from marine soil and sediments of 10 different isolation sites along the coastal region of Thiruvananthapuram district Kerala, India. The physicochemical analysis of the soil samples collected from different stations was also done. Even though the soil/sediment samples were collected from geographically nearby places, the physicochemical parameters showed a significant variation. This may be one of the factors which may trigger the actinomycete diversity in these regions

Keywords: *Marine actinomycetes , biomolecules, marine, physicochemical, soil.*

Introduction

The life on earth was originated from sea. On earth more than 70% of the surface is covered by oceans and seas. Among the marine ecosystem, the deep sea floor and coral reefs were reported with biological diversity which is higher than that of the tropical rainforests (Edward et al., 2006). The marine environment is having exteme environmental conditions which are the reason for the occurance of rare and diverse actinomycetes with unique characteristics compared to those of its terrestrial counterparts (Fenical et al., 1999; Gesheva et al., 2005). The unique bioactive compounds reported from marine actinomycetes may be due to the diversity of marine habitats. The actinomycete genera such as Streptomyces and Nocardiopsis are the most frequent members distributed in the marine habitats. They are omnipresent in the marine habitats such as in sponges, marine sediments, sea sands and sea water (Goodfellow et.al., 1983).

The marine actinomycetes are capable of producing a wide range of secondary metabolites with a variety of biological activities such as antibiotics, anti-parasitic agents antitumor agents, enzymes cosmetics, secondary metabolites, glycopeptides, beta-lactams, aminoglycosides, polyenes, polyketides, macrolides, actinomycins and tetracyclins , nutritional materials immunosuppressive agents, vitamins, pesticides and herbicides (Valli et al., 2012; Imada 2005; Atta et al., 2009). From literatures of last century itself it is evident that the marine habitat harbors several novel species of actinomycetes which are a potential source of novel structured secondary metabolites with novel biological properties (Takizawa et al., 1993). In the present study we are focussing on the isolation of novel secondary metabolites with antimicrobial properties. The literatures were also in accordance with the diversity of novel antibiotics from the marine environment (Sujatha et al., 2005; Biabani et al., 1997; Maskey et al., 2003; Charan et al., 2004; Li et al., 2005).

The actinomycetes are Gram-positive bacteria with a high G+C content (Barka et al., 2015). They are free living, saprophytic, filamentous bacteria and are a key source for the industrial production of antibiotics (Valli et al., 2012). Actinomycetes are described by the arrangement of spreading strings or poles, as often as possible offering ascend to an average mycelium which is unicellular particularly during the beginning phases of development. The hyphae are commonly non septate, under certain extraordinary conditions, septa might be seen in certain structures. According to Ward et al. (2006) Actinomycetes comprise about 10% of the bacteria colonizing in marine aggregates and can be isolated from marine sediments. Streptomyces are the largest genus of Actinobacteria proposed by Waksman and Henrici in 1943 (Abussaud et al., 2013) and more than 500 species of this genus have been reported by Euzeby (2008).

Initially the actinobacteria were considered as an intermediate group between bacteria and fungi. But latter it has attained a distinct position in the taxonomy (Pandey et al., 2004). After the discovery of the broad spectrum antibiotic Streptomycin, more attention was paid towards the actinomycetes for isolation of novel antibiotics. They are the most economically and biotechnologically valuable prokaryote and are responsible for the production of more than half of the secondary metabolites reported so far. Marine actinomycetes are a potential source of novel compounds as the environmental conditions of the sea are entirely different from the terrestrial conditions (Meiying and Zhicheng, 1998). Most of the antibiotics in clinical use are direct natural products or semi synthetic derivatives from actinomycetes. Isolation of Streptomyces sp. from seawater collected from Jeju Island South Korea and assessment of its antioxidant, cytotoxicity and antimicrobial activity against major fish pathogens was done by Subramanian et al. in 2017. This study reveals that the S. carpaticus- MK-01 from Jeju Island seawater contains antioxidant and antimicrobial secondary metabolites that could be potentially used in the aquatic feed formulation as a natural antibiotic which may reduce the risk of side effects and lower the cost of fish feeding in the aquatic food industries.

In another study, the cytotoxic activity of different actinomycetes species isolated from the Red Sea coast in Sharm el-Sheikh, Egypt was carried out by Abdelfattah et al. in 2016. In this study forty actinomycetes strains were isolated from the Red Sea coast in Egypt and these showed that five ethyl acetate extracts exhibited cytotoxicity towards breast cancer cell lines MDA-MB-231. The isolate EGY3 was identified as a new Streptomyces species, while the actinomycete EGY22 was found to be a member of the genus Nocardiopsis sp. The crude extract of the isolate EGY8 showed slightly high antimicrobial activity against different test microorganisms.

Pure cultures of actinomycetes were recovered from samples taken from the seashore of Dachen Island, Zhejiang Province, China and elucidated their antagonistic activity against various plant pathogens by S. chumphonensis by Xiurong Hu et al. in 2019 shows that This

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strain had strong antagonistic activity against various plant pathogens and its fermentation extracts showed good control efficacy against the main citrus postharvest diseases. Girao et al. in 2019 investigated cultivable actinobacteria associated with the macroalgae Laminariaochroleuca collected from the intertidal area of the rocky shore at Mindelo, located in northern Portugal and assessed their potential to produce compounds with antimicrobial or anticancer activities. Isolates in this study exhibited antimicrobial activity, mostly antifungal activity against

C. albicans and antibacterial activity against S. aureus. Isolation of actinomycetes from the marine environment of South East coast of the Bay of Bengal and screen them for the production of secondary metabolites was performed by Mohan et al. in 2014. From the result of primary and secondary screening, three isolates VSBT-201, VSBT-501 and VSBT-503 were found to be the best strains as they showed broad spectrum antifungal activity.

In India, the works dealing with the isolation of marine actinomycetes with antibacterial activities are few in number. For instance Alice Maria in 2018 isolated Streptomyces from marine soil sample collected from Stella Maris college campus and Puducherry beach with significant antibacterial activity. In another study Devi

et al. in 2006 reported marine actinomycetes with antimicrobial activity against human pathogens from coastal water of Dhanushkodi, Ramanathapuram District, India. The study also reveals that Streptomyces sp. showed the best level of antibacterial and antifungal effect against selected human pathogens of Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Klebsiella sp. and Aspergillus niger.

Similar studies were also reported from the West coast region of India. For instance Sharma et al. in 2014 studied the production of bioactive compounds from marine actinomycetes isolated from Arabian Sea (Tithal), and Salt pan of Charwada -Valsad district, India. The study concluded that marine environment of west coast of India is a rich source of deriving economically important actinomycetes that are able to produce different bioactive compounds. All these studies reported so far is an evidence for the secondary metabolite diversity in marine actinomycetes isolated from the coastal regions of Indian subcontinent. Eventhough, there were still gaps to be filled since less than 1 percentage of the marine actinomycetes were screened so far. Keeping these facts in mind we started our present study for the isolation and characterization of marine actinomycetes to combat the MDR in UTI pathogens.

Materials and methods

3.1.1 Sample Collection

Sea shore soil and marine sediment from a depth of approximately two meters and four meters were collected in sterile, disposable polythene container and brought to the laboratory in ice-box and stored at 4.0^0C in the refrigerator till the isolation of actinomycetes (Pisano et al., 1989).

3.1.2 Sampling site description

Marine sediment samples and shore soil were collected from 10 sampling sites along the coastal regions of Arabian Sea, Thiruvananthapuram District, Kerala, India. The details of the sampling sites were given in Figure 3.1. S1-Kovalam, S2-Poovar, S3-Vizhinjam, S4-Veli, S5-Shanghumugam, S6-Azhimala, S7-Perumathura, S8- Poonthura, S9-Valiyathura, S10-Menamkulam

3.1.3 Optimization of growth media for marine actinomycetes

The marine samples were decimally diluted up to 10-6 dilutions using sterile seawater, stirred well with a cyclo-mixer. After settling of debris one ml aliquot from 10-2 to 10-4 dilutions were aseptically inoculated on actinomycete isolation agar and starch casein agar plates by spread plate method. The inoculated plates were inverted and incubated in a bacteriological incubator at 28.0□C for a period of two to four weeks (Kuster and Williams, 1964).

3.1.4 Pre-treatment

For the enrichment and selective isolation of actinomycetes, the marine samples were pre-treated with calcium carbonate, humic acid, antibiotics and heat.

3.1.5 Calcium carbonate pre-treatment

Air-dried sediment samples were re-moistened, mixed with calcium carbonate (10:1) and incubated at 28.0ºC for a week in a humid chamber (Tsao et al., 1960).

3.1.6 Humic acid pre-treatment

The sediment samples were treated with Humic acid (Himedia, India) 1gram per 10gram sample for a week in a humid chamber at 28.0□C (Hayakawa and Nonomura, 1987).

3.1.7 Antibiotic pre-treatment

The samples were pre-treated with nystatin and actidione complex in a concentration of 1μg/ml per gram sample. The mixture was kept overnight in a humid chamber at 28.0□C (Porter et al., 1960).

3.1.8 Heat treatment

The sediment samples were pre-incubated at 55° C for one hour. The pre-treated sediment samples were decimally diluted and inoculated on Starch Casein Agar plates by spread plate method. The inoculated plates were inverted and incubated in a bacteriological incubator at 28.0□C for a period of 21 days (Kuster and Williams, 1964).

3.1.9 Soil physicochemical parameters analysis

The standard methods used for the analysis of physicochemical parameters are given below (Table 3.1).

Table 3.1 Methods used in Pedological Analysis

Sl. No.	Factor	Method
1	pH	Potentiometry in a 1:2.5 soil : water suspension using pH meter
2	EC	Using Conductivity meter in a 1:2.5 soil water suspension
3	OC	Walkely- Black acid digestion method (Gelman *et al*., 2012)
4	P	Bray and Kurtz method using spectrophotometer (Irving and McLaughlin 1990)
5	K	Flame photometrically using 1 N ammonium acetate as extractant
6	Ca	Flame photometrically using 1 N ammonium acetate as extractant
7	S	Using spectrophotometer with Sodium Acetate Buffer as extractant
8	Boron	Using spectrophotometer with Azomethine-H

3.1.10 Isolation and Enumeration of actinomycetes (from marine samples)

Marine samples (sediment and shore soil) one gram was aseptically serial diluted in sterile saline. One ml sample from 10-2 to 10-4 dilutions were pour plated in starch casein agar (Himedia, India) prepared with 0.5% Sodium chloride (in triplicates). The plates were incubated at 28 degree Celsius for 4 to 21 days in an inverted position at room temperature. After proper incubation, the plates were observed for actinomycete colony formation, and the colonies developed were counted using colony counter (LAPIZ INDIA). The actinomycete load in each samples were calculated by standard method.
The actinomycete load in each samples were calculated using the formula,

$$\text{The actinomycete load in each sample} = \frac{\text{Average number of colonies} \times \text{dilution factor}}{\text{Volume of sample}}$$

3.1.11 Selection of Actinomycetes Isolates
Morphologically distinct actinomycetes colonies were selected for further studies based on their macroscopic qualities.

Purification and preservation of Actinomycetes Isolates

The purified and selected actinomycete isolates were stocked in sterile starch casein agar vials overlaid with sterile liquid paraffin and kept in deep freezer at -20^{0}C (Maniatis et al., 1989). Further five days old broth cultures were lyophilized. The broth culture were centrifuged (Remi, India) at 7000 rpm for 5 minutes after discarding the supernatant the pelleted biomass was kept in deep freezer at -20^{0}C for 24 hours and lyophilized using a lyophilizer. The freeze dried cultures were used for further studies.

Selected actinomycetes isolates were microbiologically purified by repeated sub- culturing on starch casein agar and the purified isolates were stored at four degree celsius in starch casein agar slants for further studies.

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Result

The sampling sites and the samples collected were showed in Figure 3.2.

Sampling sites Samples

Figure 3.1 Sampling sites and samples

A-Kovalam, B-Poovar, C-Vizhinjam, D-Azhimala, E-Perumathura, F-Menamkulam, G-Shanghumugam, H-Valiyathura, I- Poonthura, J-Veli,

The samples were serially diluted Figure 3.3, from 10^{-1} to 10^{-4}. The dilutions 10^{-2} to 10^{-4} were spread plated.

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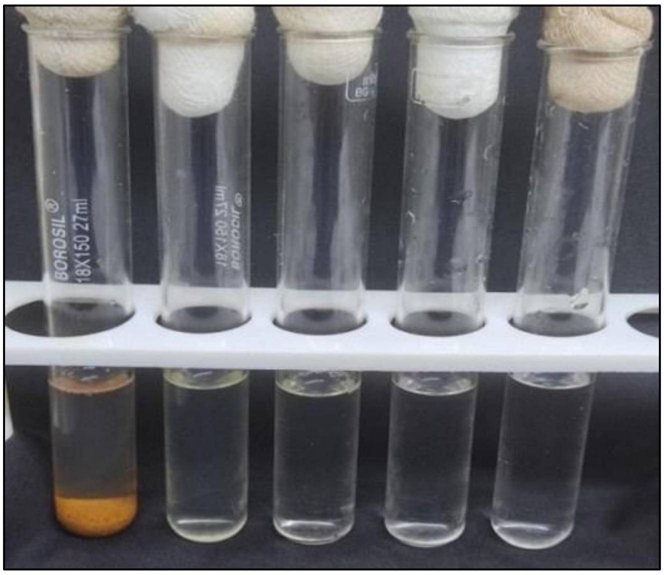

Figure 3.3 Serial dilution of samples

Isolation of Marine Actinomycetes

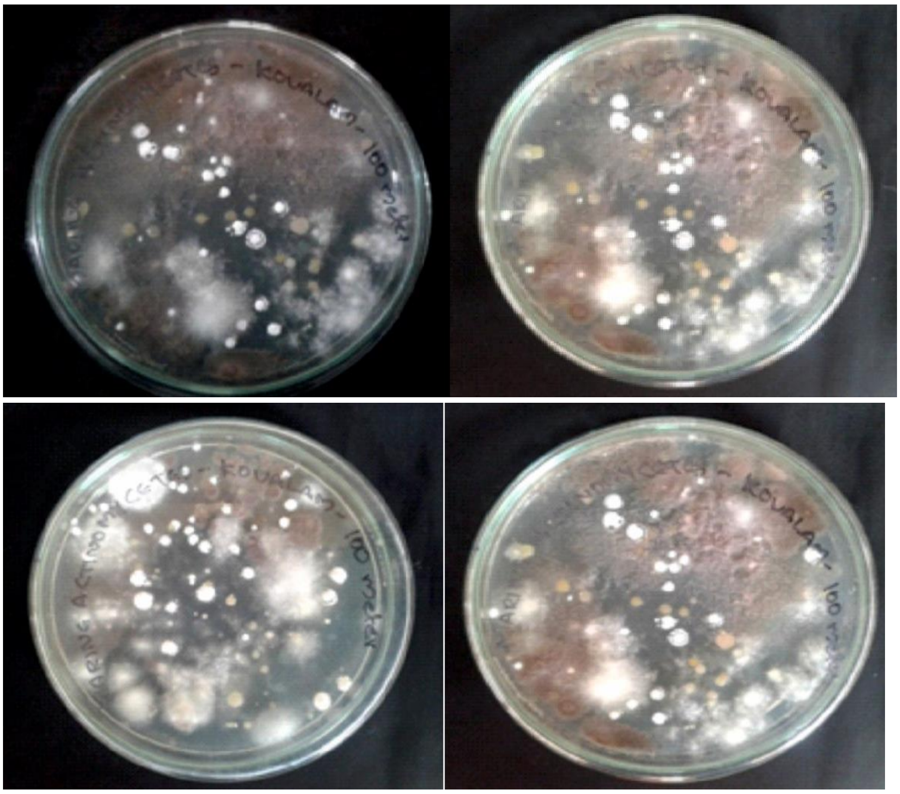

Figure 3.4 Isolation of marine actinomycetes

The samples were spread plated and incubated for 4-21 days at room temperature

Figure 3.5 Single actinomycetes colonies on starch casein agar plates

The morphologically distinct colonies were selected and subjected to quadrant streaking and purified Figure 3.6.

Figure 3.6 Isolated actnomycetes on Starch Casein Agar (SCA) and Actnomycetes Isolation Agar (AIA)

From sediment from all the samples 156 isolates were obtained (SD 1-SD 156), while from sea shore samples 124 isolates were obtained (V 1-V 124). All these results show that the marine habitat of Kerala coastal region is rich in actinomycete diversity.

The physicochemical parameters of the samples were analysed and the results were given in table 3.3

The results showed that in general the soil/sediment of the Kerala coastal region is slightly alkaline in nature. Organic carbon (C), Phosphorus (P) and Potassium (K) were found to be low, while Sulphur (S) ranged between 100-300 ppm and Calcium (Ca) ranged between 100-800 ppm. The available Boron (B) was found in the range between 0.5 to 2.6 ppm. The availability of these mineral nutrients as well as the salinity and pH must have a direct role in determining the actinomycete diversity of these regions. In this chapter we have isolated marine actinomycetes from the soil/sediments of Kerala coastal region. Also the physiochemical characterization of the soil samples was done. The variation of pH in differentsoil/sediment samples collected was given in the Figure 3.7

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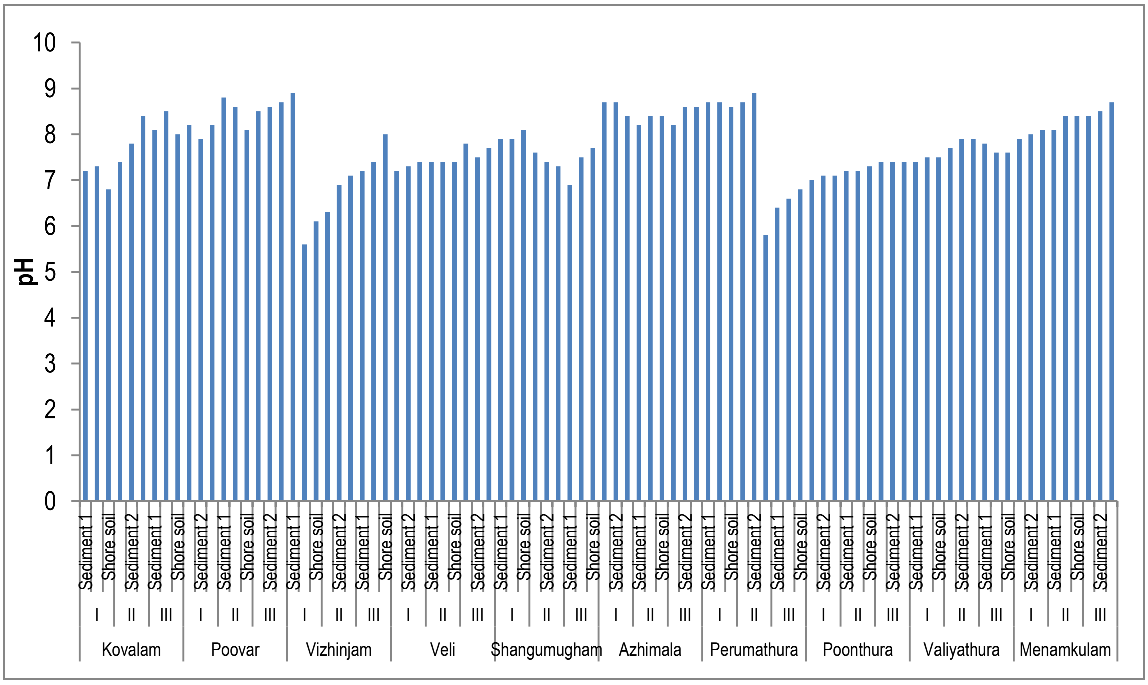

Figure 3.7 Variation of pH observed in collected samples

Due global warming and climate change, the pH of the seas is changing to acidic. In our study also many of the soil/sediment samples were showed neutral pH which is an indicator of the pH shift of the marine environment from alkaline to acidic. Similar studies were done along the coast of Tamilnadu by Manikandan and Vijayakumar (2016). They observed that the pH of many of the soil/sediment samples collected along the Palk Straitregion were acidic in nature, which substantiates the acidification of oceans.

The variation in salinity of various soil/ sediment samples was depicted in Figure 3.8.

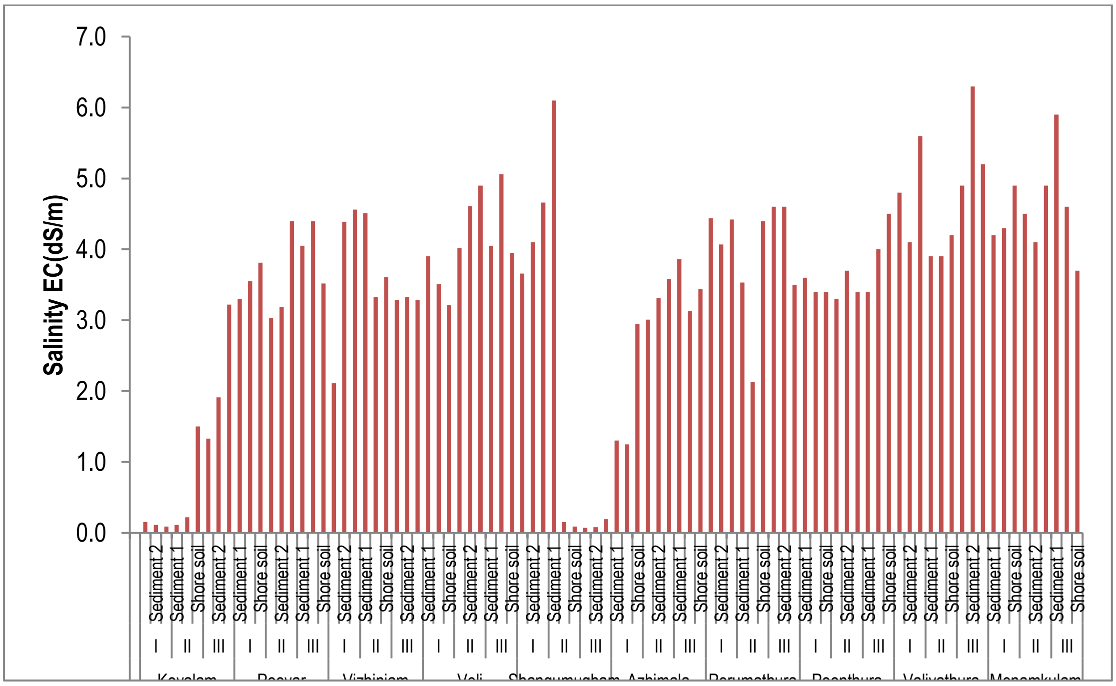

Figure 3.8 Variation of salinity - EC(dS/m)

The samples from Kovalam and Shangumugham showed less salinity compared to other samples. The variation in these sampling sites may affect the actinomycete diversity pattern also.

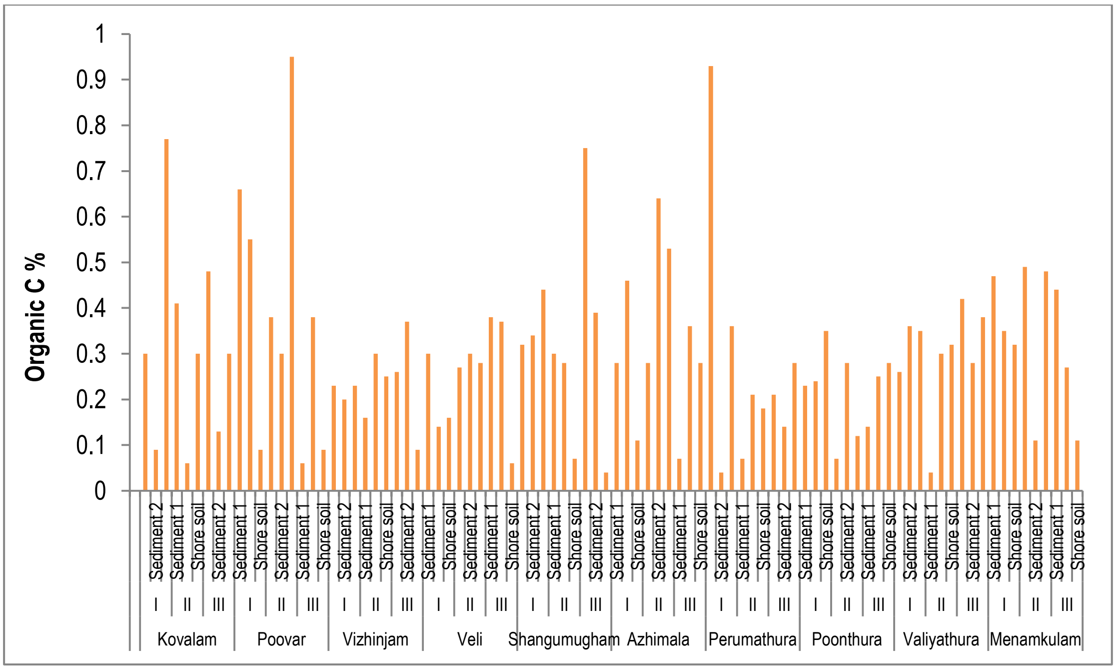

Figure 3.9 Organic Carbon %

Organic C content showed a high variation among samples Figure 3.9. The highest ones were reported from Poovar and Perumathura. The P content in different soil samples werealso analysed and results were pictured in Figure 3.10.

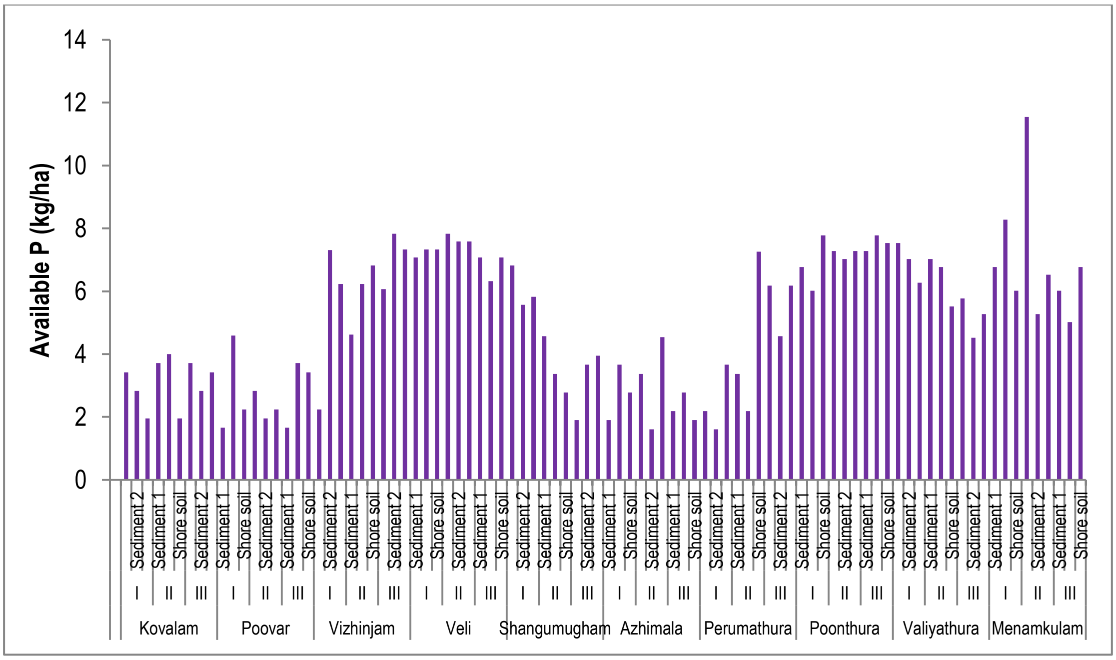

Figure 3.10 Available Phosphorus (kg/ha)

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Available K, B, S and Ca were given in Fig 3.11, Fig 3.12, Fig 3.13, Fig 3.14 respectively. All these parameters showed a high range of variation among samples.

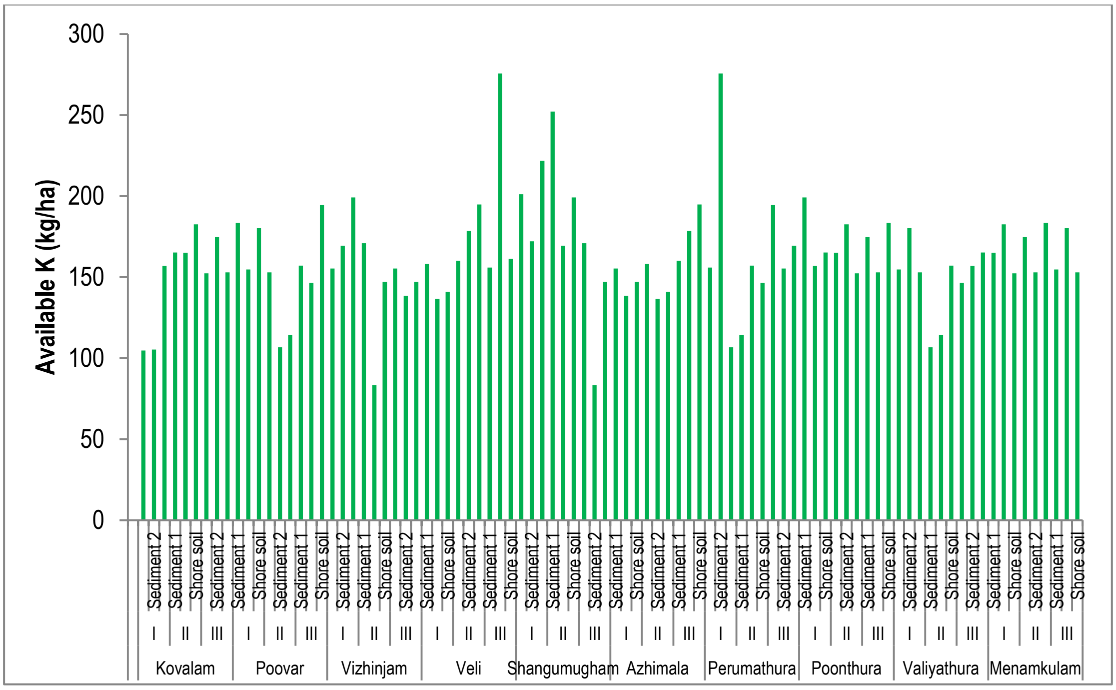

Figure 3.11 Available Potassium (kg/ha)

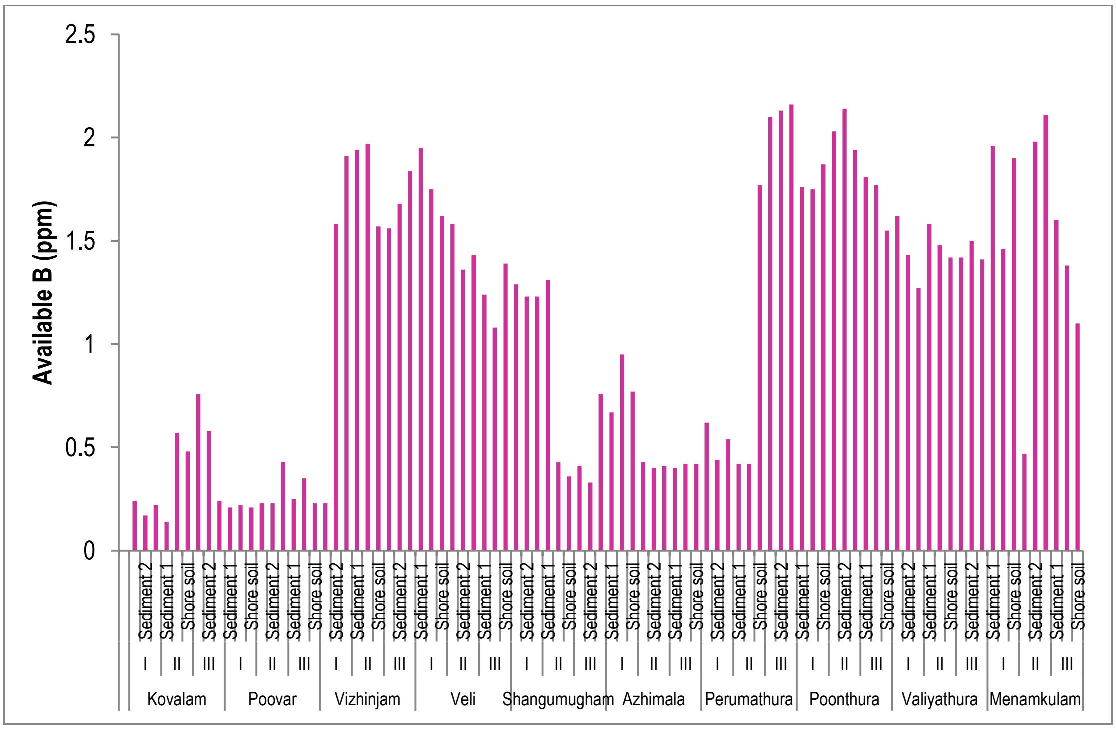

Figure 3.12 Available Boron (ppm)

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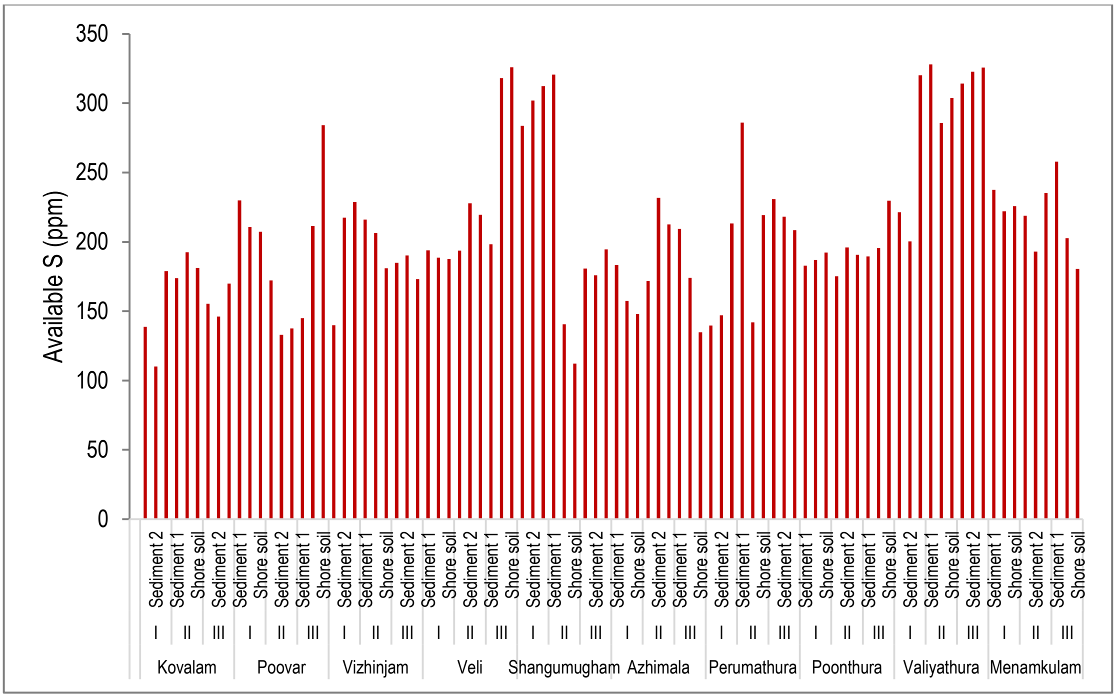

Figure 3.13 Available Sulphur (ppm)

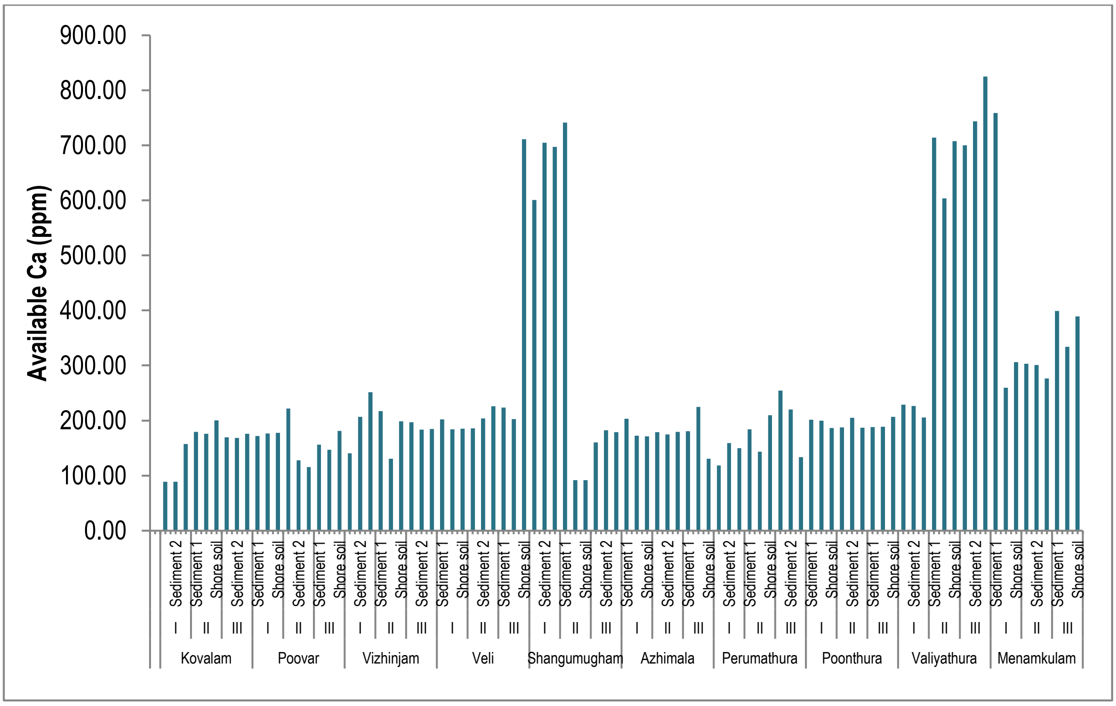

Figure 3.14 Available Calcium (ppm)

The highest Potassium was reported from samples from Veli and Perumathura. Other parameters like Boron, Sulphur and Calcium showed a wide variation among samples.

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Table 3.3 Physicochemical parameters of the soil and sediment samples

Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
Kovalam	I	Sediment 1	7.2	Mildly Alkaline	0.2	Non Saline	0.3	Low	3.42	Low	104.78	Low	0.24	138.62	88.55
		Sediment 2	7.3	Neutral	0.11	Non Saline	0.09	Low	2.83	Low	105.228	Low	0.17	110.12	88.70
		Shore soil	6.8	Neutral	0.09	Non Saline	0.77	Medium	1.95	Low	156.86	Medium	0.22	178.85	157.30
	II	Sediment 1	7.4	Mildly Alkaline	0.11	Non Saline	0.41	Low	3.71	Low	148	Medium	0.14	173.82	179.25
		Sediment 2	7.8	Strongly Alkaline	0.22	Non Saline	0.06	Low	4	Low	64.924	Medium	0.57	192.59	176.05
		Shore soil	8.4	Strongly Alkaline	1.5	Non Saline	0.3	Low	1.950	Low	182.508	Medium	0.48	181.19	200.15
	III	Sediment 1	8.1	Strongly Alkaline	1.33	Non Saline	0.48	Low	3.71	Low	152.268	Medium	0.76	155.38	169.75
		Sediment 2	8.5	Moderately Alkaline	1.91	Very Slightly Saline	0.13	Low	2.83	Low	174.556	Medium	0.58	145.99	168.20
		Shore soil	8	Moderately Alkaline	3.22	Very Slightly Saline	0.3	Low	3.42	Low	152.94	Medium	0.24	169.8	177.90

Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
	I	Sediment 1	8.2	Moderately Alkaline	3.3	Very Slightly Saline	0.66	Medium	1.66	Low	183.404	Medium	0.21	229.8	
		Sediment 2	7.9	Moderately Alkaline	3.55	Very Slightly Saline	0.55	Medium	4.59	Low	154.62	Medium	0.22	210.7	176.30
		Shore soil	8.2	Moderately Alkaline	3.81	Very Slightly Saline	0.09	Low	2.24	Low	180.268	Medium	0.21	207.34	177.90
Poovar	II	Sediment 1	8.8	Strongly Alkaline	3.03	Very Slightly Saline	0.38	Low	2.83	Low	152.828	Medium	0.23	172.14	221.75
		Sediment 2	8.6	Strongly Alkaline	3.19	Very Slightly Saline	0.3	Low	1.95	Low	106.684	Low	0.23	132.92	127.70
Poovar		Shore soil	8.1	Strongly Alkaline	4.4	Slightly Saline	0.95	Medium	2.24	Low	114.3	Medium	0.43	137.61	115.55
	III	Sediment 1	8.5	Strongly Alkaline	4.05	Slightly Saline	0.06	Low	1.66	Low	157.084	Medium	0.25	144.99	156.15
		Sediment 2	8.6	Strongly Alkaline	4.4	Slightly Saline	0.38	Low	3.71	Low	146.444	Medium	0.35	211.37	146.60

Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
		Shore soil	8.7	Strongly Alkaline	3.52	Very Slightly Saline	0.09	Low	3.42	Low	194.38	Medium	0.23	284.11	180.95
Vizhinjam	I	Sediment 1	8.9	Strongly Alkaline	2.11	Very Slightly Saline	0.23	Low	2.24	Low	155.292	Medium	0.23	139.96	140.40
		Sediment 2	5.6	MediumAcid	4.39	Slightly Saline	0.2	Low	7.31	Low	169.292	Medium	1.58	217.4	206.9
		Shore soil	6.1	SlightlyAcid	4.56	Slightly Saline	0.23	Low	6.23	Low	199.196	Medium	1.91	228.8	251.6
	II	Sediment 1	6.3	Neutral	4.51	Slightly Saline	0.16	Low	4.62	Low	170.972	Medium	1.94	216.06	217.4
		Sediment 2	6.9	Neutral	3.33	Very Slightly Saline	0.3	Low	6.23	Low	83.276	Low	1.97	206.34	130.3
		Shore soil	7.1	Neutral	3.61	Very Slightly Saline	0.25	Low	6.82	Low	147.004	Medium	1.57	180.86	198.4
	III	Sediment 1	7.2	Neutral	3.29	Very Slightly Saline	0.26	Low	6.07	Low	155.292	Medium	1.56	184.88	196.8
		Sediment 2	7.4	Neutral	3.33	Very Slightly Saline	0.37	Low	7.83	Low	138.492	Medium	1.68	190.25	183.4

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Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
		Shore soil	8	Neutral	3.29	Very Slightly Saline	0.09	Low	7.33	Low	146.892	Medium	1.84	173.15	184.9
	I	Sediment 1	7.2	Neutral	3.9	Very Slightly Saline	0.3	Low	7.07	Low	157.98	Medium	1.95	193.93	201.9
		Sediment 2	7.3	Neutral	3.51	Very Slightly Saline	0.14	Low	7.33	Low	136.476	Medium	1.75	188.57	183.9
		Shore soil	7.4	Mildly Alkaline	3.21	Very Slightly Saline	0.16	Low	7.33	Low	140.844	Medium	1.62	187.56	185.1
Veli	II	Sediment 1	7.4	Mildly Alkaline	4.0	Very Slightly Saline	0.27	Low	7.83	Low	159.996	Medium	1.58	193.6	186.1
		Sediment 2	7.4	Mildly Alkaline	4.61	Slightly Saline	0.3	Low	7.58	Low	178.364	Medium	1.36	227.79	203.9
Veli		Shore soil	7.4	Mildly Alkaline	4.9	Slightly Saline	0.28	Low	7.58	Low	194.716	Medium	1.43	219.41	225.7
	III	Sediment 1	7.8	Mildly Alkaline	4.05	Slightly Saline	0.38	Low	7.07	Low	155.852	Medium	1.24	198.29	223.6
		Sediment 2	7.5	Mildly Alkaline	5.06	Slightly Saline	0.37	Low	6.32	Low	275.692	High	1.08	318.16	202.4
		Shore soil	7.7	Mildly Alkaline	3.95	Very Slightly Saline	0.06	Low	7.07	Low	161.228	Medium	1.39	326.02	711.1

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Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
Shangumugham	I	Sediment 1	7.9	Moderately Alkaline	3.66	Very Slightly Saline	0.32	Low	6.82	Low	201.212	Medium	1.29	283.78	600.5
		Sediment 2	7.9	Moderately Alkaline	4.1	Slightly Saline	0.34	Low	5.57	Low	172.092	Medium	1.23	301.88	704.7
		Shore soil	8.1	Mildly Alkaline	4.66	Slightly Saline	0.44	Low	5.82	Low	221.708	Medium	1.23	312.27	696.9
	II	Sediment 1	7.6	Mildly Alkaline	6.1	Moderately Saline	0.3	Low	4.57	Low	252.172	Medium	1.31	320.64	741.1
		Sediment 2	7.4	Mildly Alkaline	0.2	Non Saline	0.28	Low	3.37	Low	169.292	Low	0.43	140.6	91.45
		Shore soil	7.3	Neutral	0.09	Non Saline	0.07	Low	2.78	Low	199.196	Low	0.36	112.1	91.60
	III	Sediment 1	6.9	Neutral	0.07	Non Saline	0.75	Medium	1.9	Low	170.972	Low	0.41	180.83	160.20
		Sediment 2	7.5	Mildly Alkaline	0.08	Non Saline	0.39	Low	3.66	Low	83.276	Low	0.33	175.8	182.15
		Shore soil	7.7	Strongly Alkaline	0.19	Non Saline	0.04	Low	3.95	Low	147.004	Low	0.76	194.57	178.95
Azhimala	I	Sediment 1	8.7	Strongly Alkaline	1.3	Non Saline	0.28	Low	1.9	Low	155.292	Low	0.67	183.17	203.05

Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
		Sediment 2	8.7	Strongly Alkaline	1.25	Non Saline	0.46	Low	3.66	Low	138.492	Low	0.95	157.36	172.65
		Shore soil	8.4	Moderately Alkaline	2.95	Very Slightly Saline	0.11	Low	2.78	Low	146.892	Low	0.77	147.97	171.10
	II	Sediment 1	8.2	Moderat ely Alkaline	3.01	Very Slightly Saline	0.28	Low	3.37	Low	157.98	Low	0.43	171.78	178.60
		Sediment 2	8.4	Moderately Alkaline	3.31	Very Slightly Saline	0.64	Medium	1.61	Low	136.476	Low	0.4	231.78	174.90
		Shore soil	8.4	Moderately Alkaline	3.58	Very Slightly Saline	0.53	Medium	4.54	Low	140.844	Low	0.41	212.68	179.20
	III	Sediment 1	8.2	Moderately Alkaline	3.86	Very Slightly Saline	0.07	Low	2.19	Low	159.996	Low	0.4	209.32	180.80
		Sediment 2	8.6	Strongly Alkaline	3.13	Very Slightly Saline	0.36	Low	2.78	Low	178.364	Low	0.42	174.12	224.65
		Shore soil	8.6	Strongly Alkaline	3.44	Very Slightly Saline	0.28	Low	1.9	Low	194.716	Low	0.42	134.9	130.6
Perumathura	I	Sediment 1	8.7	Strongly Alkaline	4.44	Slightly Saline	0.93	Medium	2.19	Low	155.852	Low	0.62	139.59	118.45

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Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
		Sediment 2	8.7	Strongly Alkaline	4.07	Slightly Saline	0.04	Low	1.61	Low	275.692	Low	0.44	146.97	159.05
		Shore soil	8.6	Strongly Alkaline	4.42	Slightly Saline	0.36	Low	3.66	Low	106.684	Low	0.54	213.35	149.5
	II	Sediment 1	8.7	Strongly Alkaline	3.53	Very Slightly Saline	0.07	Low	3.37	Low	114.3	Low	0.42	286.09	183.85
		Sediment 2	8.9	Strongly Alkaline	2.13	Very Slightly Saline	0.21	Low	2.19	Low	157.084	Low	0.42	141.94	143.30
		Shore soil	5.8	Medium Acid	4.4	Slightly Saline	0.18	Low	7.26	Low	146.444	Low	1.77	219.38	209.8
	III	Sediment 1	6.4	Slightly Acid	4.6	Slightly Saline	0.21	Low	6.18	Low	194.38	Low	2.1	230.78	254.5
Perumathura		Sediment 2	6.6	Neutral	4.6	Slightly Saline	0.14	Low	4.57	Low	155.292	Low	2.13	218.04	220.3
		Shore soil	6.8	Neutral	3.5	Very Slightly Saline	0.28	Low	6.18	Low	169.292	Low	2.16	208.32	133.2
Poonthura	I	Sediment 1	7	Neutral	3.6	Very Slightly Saline	0.23	Low	6.77	Low	199.196	Low	1.76	182.84	201.3

Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
		Sediment 2	7.1	Neutral	3.4	Very Slightly Saline	0.24	Low	6.02	Low	156.86	Low	1.75	186.86	199.7
		Shore soil	7.1	Neutral	3.4	Very Slightly Saline	0.35	Low	7.78	Low	165.148	Low	1.87	192.23	186.3
	II	Sediment 1	7.2	Neutral	3.3	Very Slightly Saline	0.07	Low	7.28	Low	164.924	Low	2.03	175.13	187.8
		Sediment 2	7.2	Neutral	3.7	Very Slightly Saline	0.28	Low	7.02	Low	182.508	Low	2.14	195.91	204.8
		Shore soil	7.3	Neutral	3.4	Very Slightly Saline	0.12	Low	7.28	Low	152.268	Low	1.94	190.55	186.8
	III	Sediment 1	7.4	Mildly Alkaline	3.4	Very Slightly Saline	0.14	Low	7.28	Low	174.556	Low	1.81	189.54	188.0
		Sediment 2	7.4	Mildly Alkaline	4	Very Slightly Saline	0.25	Low	7.78	Low	152.94	Low	1.77	195.58	189.0
		Shore soil	7.4	Mildly Alkaline	4.5	Slightly Saline	0.28	Low	7.53	Low	183.404	Low	1.55	229.77	206.8
	I	Sediment 1	7.4	Mildly Alkaline	4.8	Slightly Saline	0.26	Low	7.53	Low	154.62	Low	1.62	221.39	228.6

Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
Valiyathura		Sediment 2	7.5	Mildly Alkaline	4.1	Slightly Saline	0.36	Low	7.02	Low	180.268	Low	1.43	200.27	226.5
		Shore soil	7.5	Mildly Alkaline	5.6	Slightly Saline	0.35	Low	6.27	Low	152.828	Low	1.27	320.14	205.3
	II	Sediment 1	7.7	Mildly Alkaline	3.9	Very Slightly Saline	0.04	Low	7.02	Low	106.684	Low	1.58	328	714.0
Valiyathura		Sediment 2	7.9	Moderately Alkaline	3.9	Very Slightly Saline	0.3	Low	6.77	Low	114.3	Low	1.48	285.76	603.4
		Shore soil	7.9	Moderately Alkaline	4.2	Slightly Saline	0.32	Low	5.52	Low	157.084	Low	1.42	303.86	707.6
	III	Sediment 1	7.8	Mildly Alkaline	4.9	Slightly Saline	0.42	Low	5.77	Low	146.444	Low	1.42	314.25	699.8
		Sediment 2	7.6	Mildly Alkaline	6.3	Moderaely Saline	0.28	Low	4.52	Low	156.86	Low	1.5	322.62	744.0
		Shore soil	7.6	Mildly Alkaline	5.2	Slightly Saline	0.38	Low	5.27	Low	165.148	Low	1.41	325.65	825.0
Menamkulam	I	Sediment 1	7.9	Moderately Alkaline	4.2	Slightly Saline	0.47	Low	6.77	Low	164.924	Low	1.96	237.48	758.8
		Sediment 2	8	Moderately Alkaline	4.3	Slightly Saline	0.35	Low	8.28	Low	182.508	Low	1.46	222.06	259.4

Place	Site	Type	pH (1:2.5)	Rating	EC (dS/m) (1:2.5)	Rating	OC %	Rating	Available P (kg/ha)	Rating	Available K (kg/ha)	Rating	Available B (ppm)	Available S (ppm)	Available Ca (ppm)
		Shore soil	8.1	Moderately Alkaline	4.9	Slightly Saline	0.32	Low	6.02	Low	152.268	Low	1.9	225.75	306.0
	II	Sediment 1	8.1	Mildly Alkaline	4.5	Slightly Saline	0.49	Low	11.54	Medium	174.556	Medium	0.47	218.7	303.0
		Sediment 2	8.4	Moderately Alkaline	4.1	Slightly Saline	0.11	Low	5.27	Low	152.94	Low	1.98	192.9	301.0
		Shore soil	8.4	Moderately Alkaline	4.9	Slightly Saline	0.48	Low	6.52	Low	183.404	Low	2.11	235.14	276.7
	III	Sediment 1	8.4	Moderately Alkaline	5.9	Slightly Saline	0.44	Low	6.02	Low	154.62	Low	1.6	257.93	398.0
		Sediment 2	8.5	Strongly Alkaline	4.6	Slightly Saline	0.27	Low	5.02	Low	180.268	Low	1.38	202.62	333.8
		Shore soil	8.7	Strongly Alkaline	3.7	Verly Slightly Saline	0.11	Low	6.77	Low	152.828	Low	1.1	180.49	389.0

Discussion

The identification of novel biomolecules from natural resources is a continuous process. The biomolecules with a wide range of biological activities are essential for the well-being of human race. The need for antimicrobial agents is essential for combating the drug resistance in the clinical scenario. So far many bacterial secondary metabolites with antimicrobial activity were reported from a variety of natural sources. Among them, the marine members of the order Actinomycetales holds the first position. They were reported with diverse phenotypic and genomic characteristics with utility in medical as well as in other fields. In medical field actinomycetes play a key role as a potential source of secondary metabolites that function as antibiotics, antifungals, antihelmintics, and antitumor agents (Barka *et al*., 2015). Many reports claimed that the Actinomycetes from marine origin were ideal sources of novel secondary molecules with diverse chemical diversity (Prabavathy *et al*., 2006; Almalki, 2020).

For isolation of Actinomycetes from marine environment in the present study we used starch casein agar supplemented with antibiotics. Many reports suggested that the actinomycetes could be isolated using starch casein agar medium supplemented with some antibiotics such as actidione and nalidixic acids (Sujatha *et al*., 2005; Al-Dhabi *et al*., 2019). Eventhough several studies were conducted in marine actinomycetes so far, many rare groups of actinomycetes are still under investigated. So it is essential to explore the unique marine habitats to discover the currently understudied ones for the identification of more potential biomolecules. Moreover, the study associated with plasmid mediated genes present in actinomycetes new gene loci not associated with bacterial genomes will be identified as new sources of antibiotics and other drugs. These innovations will be significant in making advancement in the combat against MDR bacteria by aiding as a resource for the improvement of novel antibiotic treatments. We presume that observing these marine actinomycetes will probable become more usual in the search for novel antibiotics. In the present study a total of 280 actinomycete isolates with distinct morphological differences were obtained from the marine samples. The result clearly indicates the unique actinomycete diversity present in the tropical seas.

The present physicochemical study was conducted in the shore and marine sediments of coastal region of Thiruvananthapuram district Kerala, India. Rainfall brings vital changesin the hydrological characteristics of the marine environment. Soil/sediment nutrient content varies according to the time of sampling. Especially the seasonal variations are very high. In our study we have done sampling at post monsoon season. From literatures it is evident that the actinomycete diversity is rich in post monsoon season. During post monsoon season, the sea water will be rich in micro and macro nutrients which trigger the growth and metabolism of marine actinomycetes. Rathore *et al*., (2019) reported that they observed most morphotypes of actinomycetes during post monsoon season than monsoon and pre monsoon season. Also they have reported that the isolation media, isolation method and season of isolation were also affect the actinomycete load in the marine habitat. The variations in the soil physicochemical parameters may be one of the reasons for the actinomycete diversity observed in this study.

There were only a few studies reported so far regarding the physicochemical analysis of marine sediments and soil across the coastal regions of Thiruvananthapuram District, Kerala, India. The coastal regions of Kerala are unique in its Geographical parameters since the presence of cliffed shoreline, barrier beaches and beaches backed by strand plain. The lower reaches of Neyyar river forms a backwater near Poovar, Backwater system also exist at where the river Karamana and Vamanapuram meets the Arabian Sea. There were also a few cliff section with Cenozoic sediments at Varkala and Karichal. Considering the complexity in physiography the coastal regions of Thiruvananthapuram are rich with microbial diversity. The beaches between Poovar and Kovalam are backed by lateral cliffs and full of medium sand. The sand loss from Kovalam beach is relatively low due to the protection of the beach from Vizhinjam harbour. All these physiographic matters may be contributing factors which reflects in the Actinomycete diversity along the Southern coastal regions of Kerala.

Nutrients are considered as one of the most important parameters in the marine environment influencing the growth, reproduction and metabolic activities of biotic components (Saravanakumar *et al*., 2008). The present study also analyzed the marine soil samples for physicochemical parameters. In the present study the salinity was found to be low at Kovalam and Shangumukham. The highest organic calcium content was reported at Poovar followed by Perumathura and Kovalam. Compared to other marine sediments the one from Poovar, Perumathura, Azhimala and Kovalam showed low phosphorus content. The calcium and boron were found to be low at Poovar and Kovalam Beach. While isolating actinomycetes the highest CFU were found to be reported from Kovalam, Poovar and Vizhinjam. The unique parameters such as low salinity, high organic calcium content, low phosphorus content, low calcium and boron content may be the contributory factors for the actinomycete richness reported from this area. Shanthi *et al*. (2017) also reported from the coastal regions of North Kerala, the sediments were rich in organic calcium content. Interestingly, the sediments sampled from Menamkulam region showed the highest phosphorus content. The sulphur content showed more or less similar across the sampling sites. The trace elements are from different sources-natural as well as anthropogenic. Since the coastal regions of Kerala are rich in population density the anthropogenic factors may also play equal role in the contribution of trace elements. Or in other words we can say that the overpopulation may cause environmental pollution that may be the reason affecting the available micro and macroelements present across the marine sediments among the sampling sites studied. The rich organic C content may be due to the rivers draining into the Sea.

Conclusion

The marine actinomycetes are a potential source of several bioactive molecules. The results in this chapter showed that the marine soil/sediments of Kerala regions harbour diverse group of actinomycetes which needs to be further characterized to elucidate its full biological potential. The characteristic features unique to the southern coastal regions of Kerala may be one of the contributing factors for the actinomycete richness we observed in this study.

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Conflicts of interest

The authors declare no conflicts of interest.

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