
6.1. Introduction

The marine environment, a complex system is mainly influenced by various physical and chemical processes. One of the basic goals for the study of the physico-chemical parameters is to understand the factors that play an important role in the distribution pattern of organisms in the marine environment (Muraleedharan et al., 2010). Physico-chemical properties in a coastal environment, particularly in the near shore waters and sediments, exhibit considerable variations depending upon the regional environmental set up such as rainfall, quantum of fresh water inflow, tidal incursion and also biological activities (Satpathy et al., 2010). Microbial density is largely influenced by nutrients and physiochemical parameters (Kathiresan and Bingham, 2001).

Considering the importance of physico-chemical parameters on the productivity potential of coastal waters and sediments, numerous studies have been made in coastal waters and sediments (Jayaraman, 1951, 1954; Sankaranarayanan and Reddy, 1968; Naqvi et al., 1978; Rajendran et al., 1980; Panigrahy et al., 1984; Sasmal et al., 1986; Choudhary and Panigrahy, 1991) and their impacts on the occurrence and abundance of actinomycetes density (Goodfellow and Haynes, 1984; Bercina et al., 1987; Takizawa et al., 1993; Colquhoun et al., 1998; Zheng et al., 2000).

Hence, the influence of the environmental factors such as temperature, salinity, pH, DO, EC, Total organic carbon, nutrients and soil texture on the actinobacterial density of the coastal habitats of the Neil island, the Andamans, was studied.
6.2. Materials and methods

6.2.1. Collection of samples

Samples for the estimation of various parameters were collected from the Neil Island, the Andamans, in the month of November, 2011. Surface water and sediment samples were collected from the six stations, covering the mangrove, coral reef and beach habitats of the Neil island.

6.2.2. Physico-chemical parameters

Air, water and sediment temperatures were measured with a mercury thermometer. With the use of a hand held refractometer (Erma company, Japan), salinity of the samples was measured. pH was measured by pH Tester Model DM – 13, Takemura Electric Works Ltd Tokyo, Japan.

Water samples collected for dissolved oxygen estimation were transferred carefully to BOD bottles. The dissolved oxygen was immediately fixed and the samples were brought to the field laboratory for further analysis. The modified Winkler’s method described by Strickland and Parsons (1972) was adopted for the estimation of dissolved oxygen.

6.2.3. Nitrogen, Phosphorus and Potassium (NPK)

In the powdered digested samples of the sediments, levels of Nitrogen, Phosphorus and Potassium were analyzed using Kjeldahl method (Subbiah and Asija, 1956) and colorimetric methods (Olsen et al., 1954) respectively, in the Sugarcane Breeding Research Institute, Cuddalore. The values obtained are expressed in mg g$^{-1}$. 
6.2.4. Sediment texture

Sample weighing 100 g was taken and sieved through a mesh (62 µ) for ten minutes in a sieve shaker. The sample remained in the sieve was weighed and treated as sand. The sediment samples which passed through the 62 µ sieve were the silt and clay. The silt and clay were then separated by means of pipette method, described by Lindholm (1987).

6.2.5. Enumeration of actinobacteria

For the actinobacterial assessment, the sediment samples were collected by a spatula sterilized with alcohol before sampling. The sample was then transferred to a sterilized sample collector. Sediments were aseptically air dried to increase the isolation of overall actinobacteria and the dried samples were pretreated and mixed with equal weight of CaCO$_3$ (1:1) and incubated at 55°C for 5 min. (Balagurunathan, 1992).

One g of pretreated sample was serially diluted and plated by spread plate method using Kuster’s agar as detailed in the Chapter 5. The plates were incubated at 30°C in inverted position for 7 to 15 days. Colonies from the isolation plate were sub-cultured in nutrient agar prepared in 50% sea water. The slants of the above media were stored and used for further studies. The leathery colonies of actinobacteria that appeared on the petriplates were counted from the 5$^{th}$ day onwards upto 28$^{th}$ day. The population density of actinobacteria has been expressed as Colony Forming Units (CFU) per gm of the sample. All the colonies that grew on the petriplates were separately streaked for sub-culturing so as to ensure axenicity and were maintained in the slants.
6.2.6. Statistical analysis

Graphical representation of the physico-chemical parameters of the water and sediments were prepared using ORIGIN 6 software. Statistical analysis was performed to find out the relationship between the physico-chemical parameters of the water and sediments with actinobacterial population density, using SPSS version 11.5. Principal Component Analysis (PCA) and Cluster Analysis were used to identify the trends between the highly correlating physico-chemical parameters and actinobacterial population density, using PRIMER 6.1 software (Table 6.1).

6.3. Results

6.3.1. Air temperature (Fig. 6.1)

Air temperature varied from 25 to 29°C; lower value was recorded at station 5 (Coral reef) and higher value was recorded at stations 3 (Beach).

![Air temperature graph]

Fig. 6.1. Air temperature recorded at six stations of the Neil island.
6.3.2. Surface water temperature (Fig. 6.2)

Surface water temperature varied from 25 to 27°C, recording the lower value at stations 4 and 6 (Mangrove and Beach respectively) and higher value at stations 2 and 3 (Coral reef and Beach respectively).

![Surface water temperature graph](image)

**Fig. 6.2.** Surface water temperature recorded at six stations of the Neil island.

6.3.3. Sediment temperature (Fig. 6.3)

Sediment temperature varied from 26 to 28°C, recording the lower value at station 5 (Coral reef) and higher value at stations 2 and 3 (Coral reef and Beach respectively).
6.3.4. Water salinity (Fig. 6.4)

Water salinity fluctuated from 14 to 34 psu, registering the lower value (14 psu), at station 6 (Beach) and higher value at station 5 (Coral reef).
6.3.5. Sediment salinity (Fig. 6.5)

Sediment salinity varied between 28 to 35 psu, registering the lower value (28 psu) at station 6 (Beach) and higher value (35 psu) at station 5 (Coral reef).

![Sediment salinity graph]

Fig. 6.5. Sediment salinity recorded at six stations of the Neil island.

6.3.6. Hydrogen- ion concentration (pH) in water (Fig. 6.6)

Water pH fluctuated between 7.1 to 8.0 and registered the higher value (8.0 pH) at stations 3 and 5 (Beach and Coral reef respectively) and the lower value (7.1 pH), at station 6 (Beach).
Fig. 6.6. Hydrogen-ion concentration (pH) of water recorded at six stations of the Neil island.

6.3.7. Hydrogen-ion concentration (pH) in sediments (Fig. 6.7)

Sediment pH exhibited variations, registering the lower value (7.4 pH) at stations 2 and 6 (Coral reef and Beach respectively) and higher value (8.2 pH) at station 3 (Beach).
Fig. 6.7. Hydrogen-ion concentration (pH) of the sediments recorded at six stations of the Neil Island.

6.3.8. Dissolved oxygen concentration (DO) (Fig. 6.8)

DO content of water ranged between 2.4 to 4.5 ml l\(^{-1}\). At station 5 (Coral reef), higher DO concentration was recorded and the lower value was recorded at station 1 (Mangrove).
Fig. 6.8. Dissolved oxygen concentration recorded at six stations of the Neil island.

6.3.9. Electrical Conductivity (Fig. 6.9).

Electrical conductivity of the sediments fluctuated widely at all the stations investigated. It ranged from 1.3 to 4.9 dS m\(^{-1}\), registering the lower value (1.3 dS m\(^{-1}\)) at station 6 (Beach) and higher value (4.9 dS m\(^{-1}\)) at station 3 (Beach).
Fig. 6.9. Electrical conductivity of sediments recorded at six stations of the Neil island.

6.3.10. Sediment nitrogen (Fig. 6.10)

Sediment nitrogen content varied from 7.38 to 12.72 mg g\(^{-1}\) recording the lower value at station 6 (Beach) and higher value at station 1 (Mangrove).
Fig. 6.10. Sediment nitrogen content recorded at six stations of the Neil island.

6.3.11. Sediment phosphorus (Fig. 6.11)

Sediment phosphorus content varied from 3.7 to 5.6 mg g\(^{-1}\), recording the lower value at station 5 (Coral reef) and higher value at station 2 (Coral reef).
Fig. 6.11. Sediment phosphorus content recorded at six stations of the Neil island.

6.3.12. Sediment potassium (Fig. 6.12)

Sediment potassium content showed fluctuations, registering higher value (13.75 mg g\(^{-1}\)) at station 1 (Mangrove) and lower value (8.09 mg g\(^{-1}\)) at station 6 (Beach).
Fig. 6.12. Sediment potassium content recorded at six stations of the Neil island.

6.3.13. Total Organic Carbon (TOC) (Fig. 6.13)

Sediment TOC content exhibited wide variations at all the stations investigated, registering the lower value (1.5 mg g$^{-1}$) at station 6 (Beach) and higher value (4.6 mg g$^{-1}$) at station 4 (Mangrove).
6.3.14. Sediment texture (Fig. 6.14)

Sand content varied from 80% to 95%, recording the lower value at stations 1 and 4 (Mangrove), and higher value at station 2 (Coral).

Silt content exhibited variations, registering lower value (3.05%) at station 3 (Beach), and higher value (3.65 %) at station 1 (Mangrove).

Clay content recorded higher value (16.6%) at station 4 (Mangrove) and lower value (1.8%) at station 2 (Coral reef ).
Ecology: Physico-chemical parameters and their influence on the actinobacterial density

Fig. 6.14. Sediment texture recorded at six stations of the Neil island.

6.3.15. Population density of actinobacteria (Fig. 6.15)

Actinobacterial population density varied from 8 to $19 \times 10^2$ CFU gm$^{-1}$. The minimum ($8 \times 10^2$ CFU gm$^{-1}$) was recorded at 6 (Beach) and the maximum ($19 \times 10^2$ CFU gm$^{-1}$) was recorded at station 4 (Mangrove).
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Fig. 6.15. Sediment actinobacterial population density on Kuster’s agar recorded at six stations of the Neil island.

6.4. Discussion

Ecological parameters are mainly constituted by the bioclimatic and edaphic criteria and this general assumption is valid for terrestrial as well as the coastal ecosystems. This would mean that whenever we want to establish the ecological relationships, we have to quantify the values of environmental parameters that are responsible for the presence of the biological systems like microbes including actinobacteria. Hence, the present study was made to analyse the physico-chemical properties of the coastal environment in relation to the actinobacterial population density.

Physical and chemical parameters investigated in the present study include air temperature, surface water temperature, sediment temperature, sediment salinity, water
salinity, water pH, sediment pH, dissolved oxygen, electrical conductivity, macronutrients *viz.* nitrogen, phosphorous and potassium and soil texture of six stations.

Temperature has a prominent role to play among all the environmental factors. Because, it influences all aspects of life as a result of fundamental thermodynamics (Bagi, 2013). The relationship between temperature and individual performance is reasonably well understood. In general, sea water temperature is influenced by sunshine, evaporation, cooling fresh water influx and admixture of ebb and flow from the adjoining neritic waters. In the present study, beach temperature was always higher than the coral reef and mangrove environments. However, all of them showed close similarity, due to the influence of the air temperature on the other two. Similarly, Sethubathi (2009) has reported that the air temperature ranged from 27°C to 32.5°C, surface water temperature ranged from 27°C to 30°C and sediment temperature ranged from 28°C to 33.5°C in the coastal water of the Andamans.

Salinity can play a significant role in the growth and size of aquatic and marine organisms. Fluctuation in salinity would definitely affect the biological system of the marine environment. In the present investigation, higher salinity was recorded in the coral reef habitat. Corals flourish in the salinity range of approximately 35 psu. However, many coral species can be found in localities that show significant variation in mean or extreme conditions. Interestingly, Karuppiah (2011) reported that the salinity ranged from 31 to 36 psu in the coral reef environment of the Gulf of Mannar.

Hydrogen-ion concentration (pH) plays an important role in the biological processes of almost all the aquatic organisms (Singare *et al.*, 2011) and pH is the factor which is controlling all the other parameters like dissolved oxygen (Manivasagan, 2009).
The stations showed alkaline nature (pH 7.2 to 8.2). Generally, fluctuation in pH occurs due to the removal of CO$_2$ by photosynthesis through bicarbonate degradation, dilution of seawater by freshwater influx, reduction of salinity. Swarnakumar et al. (2007) have found that alkaline and monomesohaline situations prevailed in the coastal waters of the Great Nicobar island of the Andamans.

Dissolved oxygen is vital for aquatic life and is needed to keep the organisms alive. It is the most significant parameter affecting the productivity of aquatic systems. The two main sources of dissolved oxygen in seawater are diffusion of oxygen from atmosphere and photosynthetic activity of aquatic flora (Archana and Babu, 2013). Gnanam (2010) reported that the observed dissolved oxygen level was between 3.7 ml 1$^{-1}$ to 5.33 ml 1$^{-1}$ in the Bay of Bengal. In the present study, lower value of dissolved oxygen (2.4 ml 1$^{-1}$) was observed at station 1 (Mangrove) which could be due to the microbial utilization of O$_2$ for the decomposition of biota and uptake of oxygen by the marine organisms (Karande, 1991). Higher value of dissolved oxygen (4.5 ml 1$^{-1}$) was observed at station 5 (Coral reef) and this could be correlated to the fresh water input and mixing, at this station.

Distribution of nutrients in the marine environment is mainly based on the tidal conditions and river flow. Nutrients such as N, P and K are responsible for promoting the microbial growth and diversity in the marine environments (Shiah and Ducklow, 1994; Rivkin and Anderson, 1997; Aarthi, 2013). In the present study, this was evidenced from the positive correlation obtained between the sediment nutrients (Nitrogen and Potassium) with the actinobacterial density (Nitrogen r = 0.822 < 0.05 and Potassium: r =
0.829 < 0.05). This clearly indicates that the nutrients are important and are capable of influencing the actinobacterial growth.

Distribution of total organic carbon is closely related with the sediment type. For example, if the clay content is lower in the sediments, the total organic carbon will also be low. In the present study, higher amount of clay was observed in the mangrove sediments and as a result, its total organic carbon content was also higher. Similarly, studies show that the increase of clay content would lead to the increase in total organic carbon level (Reddy and Hariharan, 1986; Saravanakumar et al., 2008). In the present study, higher amount of total organic carbon (4.6 mg g\(^{-1}\)) was recorded at station 4 (Mangrove) and lower (1.5 mg g\(^{-1}\)) was noticed at station 6 in the Beach environment, the former supporting higher actinobacterial population density, as compared to other stations. This is evidenced by the significant positive correlation obtained between the total organic carbon and the actinobacterial density (r = 0.927 < 0.01) in the sediments.

Soil texture plays an important role in the distribution and abundance of microorganisms, as revealed by the positive correlation obtained between the actinobacterial population density and clay (r = 0.952, < 0.01) and negative correlation, in sand (r = 0.953, < 0.01). Dhevendaran et al. (1987) have also reported that the clay sediments containing sufficient nutrients promote good propagation of the microbes than the sandy sediments. Studies of Lakshmanaperumalsamy (1978) further confirm this.

In the present study, hierarchical cluster analysis was performed to investigate the similarities or dissimilarities between the stations and physico-chemical parameters. Dendrogram of the analyzed stations and physico-chemical parameters provides with a visual summary of the clustering process (Figs. 6.16 and 6.17). Four clusters were
observed in the dendrogram (Fig. 6.16). Cluster 1 corresponds to stations 1 and 4. Cluster 2 corresponds to station 6, Cluster 3 corresponds to station 3 and Cluster 4 corresponds to stations 2 and 5.

Cluster 1 (stations 1 and 4) represents a dense mangrove habitat with favorable physico-chemical conditions, supporting higher actinobacterial density. In the Cluster 4 (stations 2 and 5), station 2 clade showed a very close distance similarity with the station 5 (4.951) and these two coral habitats were having moderate actinobacterial density in relation to the physico-chemical features. In the Cluster 3, station 3 (beach habitat) comprising sandy area is under the influence of anthropogenic activity. Similarly, station 6 in the Cluster 2 is also a sandy area with coral rubbles. Evidently these stations of the clusters 2 and 3 had lower actinobacterial density.

![Dendrogram of the cluster resemblance between physico-chemical parameters and six stations of the Neil island.](image)
Though the dendrogram can reveal the dataset structure, it does not allow the interpretation of the observed patterns in terms of the original parameters. Therefore, the target physico-chemical parameters dataset was subjected to Principal Component Analysis (PCA) in order to determine which parameter influences the variation in the actinobacterial density, in the six stations of the Neil island. PCA was used to identify the trends between the highly correlating physico-chemical parameters and actinobacterial density and it revealed the distinct relationship between the stations and the physico-chemical parameters with the actinobacterial population density.

In PCA, higher correlation component score was obtained between the actinobacterial population density and the physico-chemical parameters at stations 1 and 4 (76.2 and 79.5, respectively). In the PC1 axis, these two stations had positive significant correlation with clay, TOC, sediment pH, potassium (K), actinobacterial population density, nitrogen content, phosphorus content, air temperature and EC. It could be due to the fact that these stations are occupied by mangroves (stations 1 and 4). Whereas, stations 5, 2, 3 and 6 had negative correlation component scores (-33.1, -34, -34.6, and -53.9, respectively). It could be ascribed to the fact that these four stations are coral reef and sandy beach environments. In addition, in these stations, very poor vegetation exists, as compared to stations 1 and 4 which are mangrove habitats.
Fig. 6.17. PCA plot of the physicochemical parameters and actinobacterial population density recorded at six stations of the Neil island.
Table 6.1. Simple correlation co-efficient (r) between actinobacterial density in KUA with physico-chemical parameters

|                | Air temp | Sur water temp | Sedi temp | Water Sali | Sedi sali | Water pH | Sedi pH | DO | EC | N | P | K | TOC | Sand | Silt | Clay | Acti. density |
|----------------|----------|----------------|-----------|------------|-----------|----------|---------|----|----|---|---|---|-----|-------|------|------|------|---------------|
| Air temp       | 1        |                |           |            |           |          |         |    |    |   |   |   |     |       |      |      |     |               |
| Sur water temp | 0.304    | 1              |           |            |           |          |         |    |    |   |   |   |     |       |      |      |     |               |
| Sedi temp      | 0.446    | 0.383          | 1         |            |           |          |         |    |    |   |   |   |     |       |      |      |     |               |
| Water Sali     | 0.101    | 0.633          | -0.196    | 1          |           |          |         |    |    |   |   |   |     |       |      |      |     |               |
| Sedi sali      | -0.416   | 0.447          | -0.524    | 0.829*     | 1         |          |         |    |    |   |   |   |     |       |      |      |     |               |
| Water pH       | 0.378    | 0.340          | -0.433    | 0.614      | 0.445     | 1        |         |    |    |   |   |   |     |       |      |      |     |               |
| Sedi pH        | 0.571    | 0.199          | -0.296    | 0.507      | 0.205     | 0.946**  | 1       |    |    |   |   |   |     |       |      |      |     |               |
| DO             | -0.247   | 0.115          | -0.070    | -0.095     | 0.065     | 0.319    | 0.207   | 1  |    |   |   |   |     |       |      |      |     |               |
| EC             | 0.652    | 0.324          | 0.274     | 0.339      | -0.097    | 0.656    | 0.786   | 0.393 | 1  |   |   |   |     |       |      |      |     |               |
| N              | 0.135    | 0.125          | -0.448    | 0.785      | 0.622     | 0.481    | 0.482   | -0.525 | 0.100 | 1 |   |   |     |       |      |      |     |               |
| P              | 0.450    | 0.573          | 0.549     | 0.368      | 0.063     | -0.196   | -0.155  | -0.705 | -0.057 | 0.333 | 1 |   |   |     |       |      |      |     |               |
| K              | 0.218    | 0.174          | -0.290    | 0.813*     | 0.560     | 0.470    | 0.510   | -0.494 | 0.246 | 0.977** | 0.382 | 1 |   |   |     |       |      |      |     |               |
| TOC            | 0.328    | -0.429         | -0.244    | 0.244      | -0.047    | 0.247    | 0.464   | -0.503 | 0.282 | 0.693 | 0.062 | 0.737 | 1 |     |     |     |     |     |     |     |     |     |
| Sand           | -0.373   | 0.451          | 0.221     | -0.126     | 0.138     | -0.109   | -0.324  | 0.713  | -0.077 | -0.668 | -0.211 | -0.673 | -0.950** | 1 |     |     |     |     |     |     |     |     |     |
| Silt           | -0.157   | -0.510         | -0.754    | 0.076      | 0.231     | 0.108    | 0.106   | -0.580 | -0.464 | 0.618  | -0.066 | 0.471  | 0.559 | -0.685 | 1 |     |     |     |     |     |     |     |     |     |
| Clay           | 0.385    | -0.446         | -0.204    | 0.126      | -0.147    | 0.108    | 0.327   | -0.710 | 0.091  | 0.663  | 0.217  | 0.673  | 0.952** | -1.000** | 0.670 | 1 |     |     |     |     |     |     |     |     |     |
| Acti. density  | 0.226    | -0.310         | -0.261    | 0.351      | 0.122     | 0.112    | 0.271   | -0.748 | 0.011  | 0.822* | 0.312  | 0.829* | 0.927** | -0.953** | 0.692 | 0.952** | 1 |     |     |     |     |     |     |     |     |     |