

## Seasonal and spatial distribution of heterotrophic bacteria in relation to physico-chemical properties along Ennore coastal waters

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Water samples were examined for total viable count and pollution indicator bacteria (total coliform, fecal coliform, *Escherichia coli* and *Streptococcus faecalis*). Salinity, pH, dissolved oxygen, biological oxygen demand, nutrients and Chlorophyll-*a* were examined to assess the physico-chemical condition of water. Statistically, insignificant seasonal variation was found in bacterial populations. Average abundances (CFU/ml) of the different heterotrophic bacteria of surface waters were: total viable count ( $2.1 \times 10^6$ ), total coliforms ( $1.3 \times 10^5$ ), fecal coliforms ( $1.5 \times 10^4$ ), *Escherichia coli* ( $1.1 \times 10^4$ ) and *Streptococcus faecalis* ( $0.4 \times 10^4$ ). Spatially, the higher bacterial population was recorded in creek and shore regions with high concentration of nutrients. Significant positive correlation ( $p < 0.05$ ) was observed between bacterial population and the nutrient concentration which suggest that elevated nutrient load favors the bacterial growth. However, bacterial populations showed positive correlations with biological oxygen demand, and negative relationship ( $p < 0.05$ ) with salinity, indicating the dominating influence of anthropogenic activities in the coastal area.

[**Keywords:** Heterotrophic bacteria; BOD; DO; Correlation analysis; Ennore]

### Introduction

India is bestowed with long coastline of 8,129 km and of this 6,000 km is rich in estuaries, creeks, brackish water, lagoons and lakes<sup>1</sup>. The southeast coast of India is an important stretch of coastline, where many major rivers drain into the Bay of Bengal and they are also richer in marine fauna and flora<sup>1</sup>. In recent years, the human interference through urbanization and industrialization has severe impact on the microbial population of coastal water of the Bay of Bengal<sup>2</sup>. Various anthropogenic activities and some natural processes, bring about adverse environmental conditions that affect physiological responses such as viability<sup>3-5</sup>, metabolism, resting (dormant) stages and death<sup>6</sup> among microorganisms in situ. Coastal water generally contains both pathogenic and non-pathogenic microbes derived from river run off, sewage, industrial effluents, agricultural activities, wild

life and indigenous microorganisms<sup>2</sup>. Responses of heterotrophic bacteria to environmental changes are very rapid and quite consistent when compared with those of higher forms of marine biota<sup>7</sup>.

Pollution level is increasing day by day due to non availability of appropriate drainage system for industrial units and housing societies established along the banks. Hence, prevention of coastal pollution necessitates effective monitoring of physicochemical and microbiological parameters. Microbiological quality of marine water is the health indicator to identify polluted sites and also to estimate the extent of pollution<sup>8</sup>. Total coliform, fecal coliform, *Escherichia coli* and *Streptococcus faecalis* counts are the most widely used bacteriological procedures for assessment of the quality of water<sup>8</sup>. Thomann and Mueller<sup>9</sup> pointed out that Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and

nutrients, which help in assessing the quality of a water body, also assist in understanding the waste assimilative capacity of water. Along Ennore coastal waters, the distribution of physico-chemical properties<sup>1, 10</sup> and heterotrophic bacteria<sup>11-12</sup> reported in previous studies. Presence of fecal indicator organisms in water samples have been reported in several stations along the Indian coasts<sup>13-16</sup>. But scarce information is available about variations in contribution of pollution indicator bacteria along Ennore coast and their dispersion towards offshore with respect to physico-chemical properties.

The present study consists the microbial population (Total viable count, total coliform, fecal coliform, *Escherichia coli* and *Streptococcus faecalis*) from coastal water of Ennore during 2011 in four seasons (postmonsoon, summer, premonsoon and monsoon). This aids in understanding the seasonal variation, so as to provide an annual cycle of microbial load and to assess the distribution of pollution indicator bacteria in surface waters and sediments in the Ennore coast. Efforts have been made to found their relationship and dependency with physico-chemical parameters.

### Materials and Methods

The study was carried out along the coastline of Ennore (Fig. 1). Sampling was undertaken in the month of February (post monsoon), June (summer), September (premonsoon) and December (monsoon), 2011 covering four distinct seasons. Samples were collected during sunny weather. To understand the spatial variability of physicochemical and microbiological properties along Ennore coastal waters four stations were selected representing creek, shore (0.5 km from coast), inshore (2 km) and off shore (5 km) (Fig. 1). For microbial analysis, surface water samples were collected and aseptically, transferred into sterilized glass bottles. Sediment samples were collected using a Van Veen grab and sub samples were aseptically transferred into fresh polythene bags with a sterile spatula before disturbing the sediment for other analyses. Immediately after collection both water and sediment samples were stored in ice and transported to the field laboratory. Water quality parameters such as temperature, salinity, pH, DO, BOD nutrients (ammonia, nitrate, nitrite, phosphate, silicate, total nitrogen and total phosphorous) concentrations and Chlorophyll-*a* (Chl-*a*) concentration were also monitored for

their impact on microbial count for all four seasons.



Fig.1-Station locations in the Ennore coastal waters.

Surface water temperature was measured using mercury thermometer. Salinity was estimated with a hand held refractometer (Atago, Japan) and pH was measured using digital pH meter. Water transparency was measured by Secchi disc. For the analysis of nutrients, surface water samples were collected in clean polyethylene bottles and kept in an ice box and transported immediately to the laboratory. The water samples were analyzed for ammonia, dissolved inorganic phosphate, nitrate, nitrite, reactive silicate, total nitrogen (TN) and total phosphorous (TP) by adopting the standard methods described by Grasshoff *et al*<sup>17</sup>. Water samples for Chl-*a* determination were filtered through Whatmann GF/F (47mm) glass fiber filters and pigment extraction was performed using 90% acetone. Chl-*a* pigment concentrations were measured by using UV-visible spectrophotometer<sup>18</sup>. DO and BOD was estimated by the modified Winkler's method<sup>18</sup> and is expressed as mg/L. For Total Suspended Matter (TSM) estimation, about 500 ml of water samples were filtered through pre weighed 0.22  $\mu$ m

polycarbonate filters (Millipore), dried at 40° C and reweighed. The difference between two weights was taken as TSM content (mg/L).

Enumeration of bacteria was carried out by using standard procedures, water sample and sediment samples were prepared in 50% of aged sea water separately. Samples are serially diluted, surface plated in duplicate in appropriate media before microbial analysis. Standard spread plate technique was adopted for enumeration of Total Viable Count (TVC), Total Coliforms (TC), Fecal Coliforms (FC), *Escherichia coli* (EC) and *Streptococcus faecalis* (SF). Nutrient agar prepared in 10% seawater was used for enumerating TVC. Nutrient Agar plates are incubated at 28±1°C and counted after 24 hrs and 48 hrs. TC, FC, EC and SF using a membrane filtration technique<sup>19</sup>. Briefly, water sample (100 ml) was filtered through a gridded sterile cellulose-nitrate membrane filter (0.45 µm pore size, 47 mm diameter, Sartorius type filters) under partial vacuum (Millipore). TC was enumerated using Mac Conkeys agar which was incubated for 24 h at 37°C. All colonies showing pink-red colour were counted as total coliforms. FC were enumerated using M-FC agar with rosolic acid as an indicator and incubated at 44.5°C for 24 h. Typical blue-green colonies were counted for fecal coliforms. HiCrome E.coli agar was the selective media for the enumeration of EC. Agar plates are incubated at 37°C for 48 hrs and colonies appeared blue in colour. SF were enumerated on M-Enterococcus agar with maroon colour colonies after 48 hrs. The colonies were counted and the population density expressed as colony forming unit (CFU) per ml (water sample) or g (sediment sample) of the sample.

Correlation matrix of microbial population and physico-chemical parameters were analyzed with the help of *STASTICA 7.0* software. One such method employed is the Spearman rank-order correlation, which gives an idea of the inter-relation between various water quality parameters and this method was considered to be not significant when the value of the probability of significance (*p*) was greater than 0.05.

## Results and Discussions

The seasonal and spatial variations of water quality parameters with their mean and standard deviation along Ennore coast are

summarized in Table 1. Temperature controls behavioral characteristics of organisms, solubility of gases and salts in water<sup>20</sup>. During the study period, surface water temperature ranged from 28.0°C to 32.0°C with the minimum (28.3±0.6°C) during the monsoon season (Table 1). The variation in surface water temperature depends mainly on the climatic conditions, sampling times and the number of sunshine hours<sup>21</sup>. Salinity acts as a limiting factor in the distribution of living organisms, its variation caused by dilution and evaporation which influence the characteristic change of fauna in the intertidal zone<sup>22</sup> (Table 1). Salinity ranged between 25.8 and 34.4 PSU, the lowest salinity was found during monsoon period (29.6±3.2 PSU) due to heavy rainfall and large quantity of freshwater inflow to the coast. Similar trend in the salinity values were also observed from various parts in southeast coast of India<sup>23-28</sup>. Spatially, salinity values were found to be lower towards creek and were gradually increase towards offshore (Table 1). Turbidity is a good indicator of the amount of material suspended in water because it measures the amount of light that is scattered within the medium<sup>12</sup>. High concentrations of TSM were observed during monsoon (58±5.7 mg/L) whereas low concentrations were recorded during premonsoon (18.8±3.1 mg/L). Highest concentration of TSM coincided with heavy silt-laden land runoff during monsoon seasons. Transparency of the Ennore coastal waters ranged from 0.2 to 7 m with higher transparency in summer (3.9±2.8 m). The high transparency of the water column during summer (Table 1) can be due to the consequences of intensity of sunlight. pH maintenance (buffering capacity) is one of the most important attributes of any aquatic system since all the biochemical activities depend on pH of the surrounding water<sup>29</sup>. The pH values of water samples hardly varied from sample to another but generally were falling in the alkaline side ranging between 7.9 and 8.2. The high values of pH during non monsoon seasons (postmonsoon, summer and premonsoon) are due to the influence of seawater penetration and the biological activities (Table 1). The lower pH during monsoon may be due to freshwater influx, dilution of sea water, low temperature and decomposition of organic matter as suggested by Ganesan *et al*<sup>30</sup>.

Table 1: Variations of environmental properties along Ennore coastal waters during distinct seasons (postmonsoon, summer, premonsoon and monsoon) and creek to offshore. TSM, TN,TP,Chl-*a*, DO and BOD indicates Total suspended matter, Total nitrogen, Total phosphorous, Chlorophyll-*a*, Dissolved oxygen and Biological oxygen demand respectively.

	Seasons			
	Postmonsoon	Summer	Premonsoon	Monsoon
Temperature (°C)	28.9 ± 0.3		29.5 ± 1.7	28.3 ± 0.6
Salinity (PSU)	32.3 ± 2.1	33.3 ± 0.8	34.2 ± 0.2	29.6 ± 3.2
Transparency (m)	2 ± 1.6	3.9 ± 2.8	3.7 ± 3.2	3 ± 0.5
TSM (mg/L)	52.7 ± 7.4	47.3 ± 9.3	18.8 ± 3.1	58 ± 5.7
pH	8.2 ± 0.1	8.2 ± 0.1	8.1 ± 0.4	7.9 ± 0.3
Ammonia (µM)	0.3 ± 0.4	0.4 ± 0.4	0.9 ± 1.2	0.4 ± 0.2
Nitrite (µM)	0.8 ± 0.8	0.8 ± 0.6	1.5 ± 0.9	0.6 ± 0.2
Nitrate (µM)	7.8 ± 3.3	7.7 ± 1.6	10.2 ± 1.5	9.1 ± 1.8
Phosphate (µM)	0.3 ± 0.1	1.1 ± 0.6	0.9 ± 0.9	1.6 ± 0.6
Silicate (µM)	14.5 ± 3.1	8.3 ± 3.7	19.1 ± 5	33.9 ± 1.9
TN (µM)	26.7 ± 4.8	24.6 ± 3.4	25.1 ± 3.7	26.2 ± 4.8
TP (µM)	2.7 ± 2	1.8 ± 1.2	2 ± 1.2	2.8 ± 0.6
Chl- <i>a</i> (mg/m <sup>3</sup> )	5.4 ± 1	2.9 ± 2.7	2.8 ± 1.3	0.4 ± 0.2
DO (mg/L)	4.8 ± 1.6	4.8 ± 1.4	5 ± 1.8	4.6 ± 0.7
BOD (mg/L)	2.2 ± 2.8	1.5 ± 0.8	1.9 ± 1.9	1.5 ± 1.2
	Stations			
	Creek	Shore	Inshore	Offshore
Temperature (°C)	29.8 ± 1.9	28.5 ± 0.5	28.7 ± 0.6	28.8 ± 0.4
Salinity (PSU)	29.1 ± 3.3	32.3 ± 2.4	32.8 ± 1.9	33.9 ± 0.4
Transparency (m)	0.2 ± 0.3	3.1 ± 1.4	4 ± 1.9	4.8 ± 1.9
TSM (mg/L)	48.3 ± 4.3	43.8 ± 19.1	41.7 ± 19.5	50.4 ± 18.8
pH	7.8 ± 0.3	8.1 ± 0.2	8.2 ± 0.1	8.3 ± 0.1
Ammonia (µM)	1.3 ± 0.9	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.2
Nitrite (µM)	1.8 ± 0.8	0.8 ± 0.4	0.6 ± 0.4	0.5 ± 0.3
Nitrate (µM)	11.8 ± 0.7	8.2 ± 1.6	8.8 ± 1.5	7 ± 2.3
Phosphate (µM)	2.2 ± 0.2	0.7 ± 0.3	0.8 ± 0.4	0.7 ± 0.6
Silicate (µM)	24 ± 9.5	20.3 ± 12.3	16.6 ± 11.6	17.2 ± 11.5
TN (µM)	30.7 ± 2.9	24.3 ± 3	25 ± 2.2	22.6 ± 1.9
TP (µM)	4.2 ± 1	1.7 ± 0.8	1.6 ± 0.7	1.7 ± 0.5
Chl- <i>a</i> (mg/m <sup>3</sup> )	2.2 ± 2.4	3.7 ± 2.2	2 ± 2.6	3.4 ± 2.6
DO (mg/L)	2.9 ± 0.6	5.4 ± 0.6	5.4 ± 0.3	5.7 ± 0.4
BOD (mg/L)	4.2 ± 1.6	1 ± 0.5	1 ± 0.3	0.8 ± 0.4

In the coastal environment, the supply of nutrients is mainly influenced by the amount of fresh water inflow, rate of the rainfall, invasion of tidal pattern and also biological activities<sup>31</sup>. Terrestrial runoff from the industrial, agricultural, residential and urban areas may also raises substantially the nutrient loads to the near shore

and inland waters in the ocean<sup>32</sup>. The variations of nutrients concentrations along Ennore coastal water ranged from 0.01 to 2.6 µM of ammonia, 0.2 to 2.8 µM of nitrite, 4.0 to 12.3 of nitrate, 0.2 to 2.5 of phosphate and 5.5 to 36.7 of silicate. Nutrient concentrations (except silicate) showed significant spatial variability than seasonal

variability (Table 2). Along Ennore coastal waters these nutrient concentrations (except silicate) are indicating that the source of the nutrients is from localized anthropogenic input rather than monsoonal runoff. The seasonal distribution showed well defined pattern for silicate (Table 2), higher concentrations of silicate were noticed during monsoon ( $33.9 \pm 1.9$ ). During monsoon high concentrations of silicate is attributed by input of more siliceous sediment gathered from its catchments<sup>33-36</sup>. The concentrations of TN and TP along Ennore coastal waters ranged from 19.9 to

33.7 and 0.9 to 5.6  $\mu\text{M}$ . No significant seasonal variability was noticed in the concentrations of TN and TP due to influx of surplus discharge of industrial and domestic sewage. The difference between dissolved inorganic nitrogen (ammonia + nitrite + nitrate) and TN is much higher during all seasons, indicating that a higher value of the organic form of nitrogen contributed through industrial and domestic sewage along Ennore coastal waters. High concentrations of nutrients detected in Ennore Creek region reflect the domination of industries around the region.

Table 2: Results of analysis of variance (ANOVA) for nutrients and bacterial population

Parameters	Seasons		Stations	
	F-value	P-value	F-value	P-value
Total nitrogen	0.22	0.878	7.55***	0.004
Total phosphorous	0.49	0.693	10.33***	0.001
Dissolved inorganic silicate	35.36***	0	0.3	0.824
Total viable count	0.06	0.979	91.70***	0
Total coliforms	0.21	0.888	1524.19***	0
Fecal coliforms	0.16	0.923	15.22***	0
<i>Escherichia coli</i>	0.64	0.602	5.71**	0.012
<i>Streptococcus faecalis</i>	0.31	0.815	2.63*	0.09

DO may be present in water due to direct diffusion from air and photosynthetic activity of autotrophs<sup>37</sup>. Seasonal average of DO values oscillated between  $4.6 \pm 0.7$  in monsoon and  $5 \pm 1.8$  in premonsoon (Table 1). The depletion of DO during monsoon could be anticipated to the higher rate of microbial decomposition of the excessive organic matter discharge directly into water body which in turn is controlled by temperature<sup>38-39</sup>. Among stations, low DO values were noticed at creek region may be due to the closeness of the stations to the effluent discharge points in the creek. Amount of BOD is used to find out the level of organic pollution in water. Obtained data recorded higher BOD values at creek region may be due to the high level of organic matter loaded from sewage and industrial discharge. There is no significant seasonal cycle was observed in BOD values. Present study recorded higher Chl-*a* concentrations during non monsoon period (Postmonsoon, summer and premonsoon respectively) than monsoon season (Table 1). Similarly, during monsoon season minimum concentration of Chl-*a* has also been reported by several authors<sup>40-42</sup>. During monsoon season, the

sharp decline in Chl-*a* concentration encountered due to the changes in hydrographic parameters due to large volume of freshwater influx, increased turbidity and decreased pH.

Microbiological examination of coastal water have a special status in marine pollution studies, as it is a direct measurement of deleterious effect of coastal pollution on human health through food chain<sup>43</sup>. It is necessary to monitor marine microbial population along coastal waters, as these waters are used for many purposes. Spatial and seasonal variations of TVC and indicator bacteria (TC, FC, EC and SF) abundance of collected water samples during 2011 were summarized in Fig. 2. From the analysis of variance of these data it was clear that the numerical differences of TVC, TC, FC, EC and SF among seasons during the year 2011 were not statistically significant (Table 2). Total heterotrophic bacteria (THB) count can be reliable indicator of water quality since the number of bacteria present depends on the degree of contamination<sup>44</sup>. The distribution of TVC of the collected samples during the course of the study ranged from 0.04 to  $6.5 \times 10^6$  CFU/ml

recording their minimum number during postmonsoon (average  $2.1 \times 10^6$ ) and rest of the seasons (summer, premonsoon and monsoon) showed high numbers and did not show statistically significant differences among seasons ( $p < 0.9$ ). During study period, the heterotropic bacterial population showed relatively high numbers of TVC. Warmer temperature and influx of surplus discharge of industrial and domestic sewage are prime cause for these higher bacterial counts. Population was found to be quite comparable to other areas of Southeast coast of India. Mahalakshmi *et al*<sup>45</sup> recorded THB load in Uppanar estuarine sediments were ranged from  $11.3 \times 10^3$  -  $3.0 \times 10^6$  CFU/g and water were  $10.5 \times 10^3$  -  $3.2 \times 10^6$  CFU/ml and revealed that the Uppanar estuarine region is highly polluted with pathogenic microbes. Premalatha<sup>46</sup> recorded the distribution of THB in Uppanar estuary during the month of monsoon season, the higher population was observed like  $8.7 \times 10^6$  CFU/ml in water and  $9.4 \times 10^7$  CFU/g in sediment.

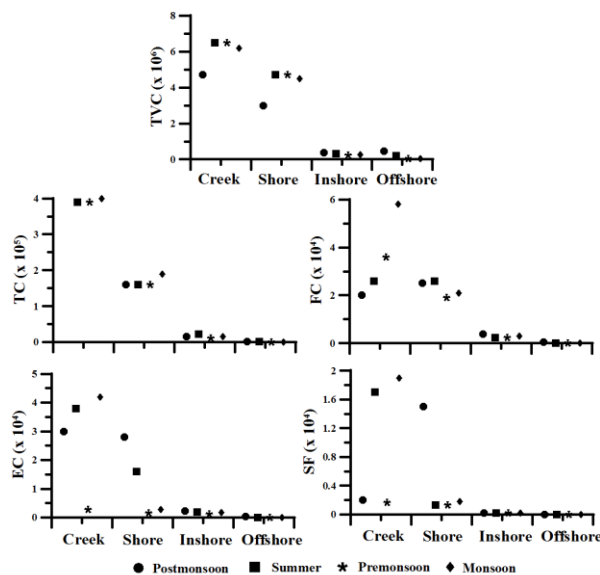


Fig.2-Spatial and seasonal distribution of bacterial populations from water samples along Ennore coastal waters. TVC, TC, FC, EC and SF indicates Total viable count, Total coliform, Fecal coliform, *Escherichia coli* and *Streptococcus faecalis*.

TC population varied between 0.001 and  $4.0 \times 10^5$  CFU/ml, showing high numbers throughout the year along Ennore coastal waters (Fig. 2), since present study area receives huge waste water from adjacent areas. Coliforms are the dominant bacteria in the waste water, and their presence in natural water is an indicator of water pollution<sup>47</sup>. Coliform bacteria themselves are not necessarily pathogenic, but they indicate the likelihood of more dangerous organisms that

cause salmonellosis, cholera or hepatitis<sup>8</sup>. FC bacteria are indicators of recent contamination by fecal matter from warm blooded creatures, including humans, other mammals and birds<sup>8</sup>. Along Ennore coast, FC population ranged from 0.002 to  $5.8 \times 10^4$  CFU/ml and their average counts were higher during monsoon season ( $2.0 \times 10^4 \pm 3.0 \times 10^4$  CFU/ml), which clearly suggests that, the role of precipitation on the sources and the level of microbial populations. High coliform numbers have been reported in waters used for dumping of sewage<sup>48</sup>. TC counts are always higher than FC counts, since total coliform can originate from nonfaecal sources such as plants and soils<sup>49</sup>. The number of EC and SF ranged from 0.002 to  $4.2 \times 10^4$  CFU/ml and 0.001 to  $1.9 \times 10^4$  CFU/ml respectively. EC is a well-known indicator of fecal pollution and it is capable of causing many diseases, also major cause of gastroenteritis.

On the whole, bacterial abundance often decreased with distance from the coast during four seasons (Fig. 2) and the spatial variability is statistically significant (Table 2). Creek and shore waters were recorded much higher population of TVC and indicator bacteria than in the offshore water, might be due to decomposition of organic matter and anthropogenic activities. Relatively low bacterial load in the offshore stations compared to near shore may be attributed to salinity variations and marginal stress from the saline water.

During the investigated period, in surface sediments, TVC varied from 0.03 to  $5.0 \times 10^7$  CFU/g, TC 0.01 to  $2.2 \times 10^6$  CFU/g, FC 0.01 to  $4.0 \times 10^5$  CFU/g, EC 0.001 to  $2.9 \times 10^5$  CFU/g and SF 0.01 to  $3.0 \times 10^4$  CFU/g (Fig. 3). Abundance of all groups of bacteria were observed more (2-23 times) in the sediment than in the water column (Fig. 3) which was also reported in other studies<sup>50-51</sup> and their averages did not vary much between seasons. Higher population in the sediment is generally attributed to the rich organic content<sup>52</sup>. Besides, it could also be due to the less residence time of the microorganism in the water column compared to sediment<sup>53</sup>. Dale<sup>54</sup> and Davies *et al*<sup>55</sup> pointed out that the interaction of microorganisms with sediments may enhance their survival by reducing exposure to stressors such as infrared radiation and predation or by increasing the availability of nutrients. The presence of these bacteria in bottom sediments reflects fecal pollution even when at the given moment there are no such bacteria in the water<sup>56</sup> they can also cause secondary bacteriological pollution of water<sup>57</sup>.

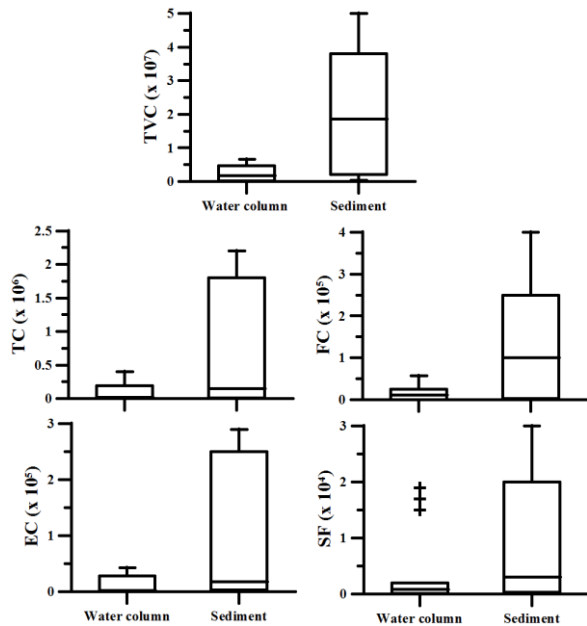


Fig.3-Variations of bacterial population between surface waters and surface sediments. TVC, TC, FC, EC and SF indicate Total viable count, Total coliform, Fecal coliform, *Escherichia coli* and *Streptococcus faecalis*.

Spearman correlations between the environmental variables and microbial populations can provide interesting information on the relationship of the factors studied and their sources. Several investigators have examined the distribution of indicator and human pathogenic bacteria as well as certain viruses in coastal waters with a view to quantify and understand their relationships and their relevant environmental variables<sup>2, 58-59</sup>. Microbial and physiochemical parameters along Ennore coastal waters showed significant correlations between them which indicated that those were strongly associated with each other statistically due to same origin. Results indicate a strong significant positive correlation between salinity and pH ( $p < 0.05$ ). Coastal water pH found to vary with salinity<sup>60</sup>. Insignificant correlation was noticed in case of TSM vs. nutrients (Table 3). This lack of significant correlations indicates the influx of anthropogenic inputs and waste discharges containing nutrients from adjacent effluents irrespective of monsoonal influence.

Number of viable bacterial count was significantly and positively correlated with only the TN and TP content rather than available

dissolved inorganic nitrate and dissolved inorganic phosphate indicating the importance of the TN in driving the bacterial population in the study area. The result of the present study corresponded with the findings of other study that reported that dissolved organic nitrogen and amino acids were more important than inorganic nitrogen for bacteria in the marine habitats<sup>61</sup>. Fig. 4 is depicting that elevated nutrient load (TP, TN) supports the bacteria growth throughout the year irrespective of the season. Unlike phosphorous and nitrogen, input of dissolved silicate is largely unaffected by human activity and mostly originated by way of weathering of silicate rocks<sup>62</sup>. Hence, silicate showed significant seasonal variability ( $p < 0.001$ ) than spatial variability (Fig. 4). Correlation matrix showed a positive relationship (statistically insignificant) of BOD, with most of the bacterial counts (TVC, TC, FC, EC and SF). This is clearly indicating that the microbial population in the coastal water contributes the BOD. Overall, correlation matrix showed a very good positive relationship ( $p < 0.05$ ) with ammonia with almost all the microbial populations during study period which indicates the influence of effluents in the coastal areas. The significant negative correlation ( $p < 0.05$ ) was found between variables (DO and pH) and microbial population indicating dominant influence human activities. A negative relationship of salinity exists with the bacterial populations due to sewage influence decreases vice versa salinity increases towards offshore.

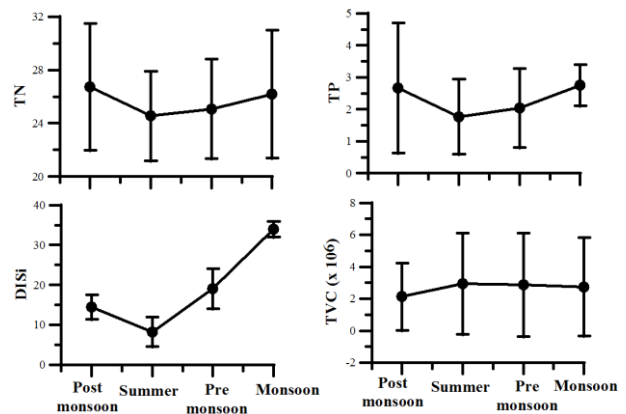


Fig.4-Seasonal variability in concentrations of TN, TP, DISi (dissolved inorganic silicate) and TVC.

Table 3: Rank correlation matrix (Spearman's) of bacterial populations with physical and chemical parameters along Ennore coastal waters (\* indicates  $p < 0.05$ ). Tem, Sal, Tran, TSM,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^-$ ,  $\text{SiO}_4^-$ , TN, TP, Chl-*a*, DO, BOD, TVC, TC, FC, EC and SF indicates Temperature, Salinity, Transparency, Total suspended matter, Ammonia, Nitrite, Nitrate, Phosphate, Silicate, Total nitrogen, Total phosphorous, Chlorophyll-*a*, Dissolved oxygen, Biological oxygen demand, Total viable count, Total coliform, Fecal coiform, *Escherichia coli* and *Streptococcus faecalis* respectively.

	Tem	Sal	Tran	pH	$\text{NH}_4^+$	$\text{NO}_2^-$	$\text{NO}_3^-$	$\text{PO}_4^-$	$\text{SiO}_4^-$	TN	TP	DO	BOD
Sal			0.7 *										
TSM	-0.4	-0.4		-0.5		-0.6 *					0.4		
pH		1 *	0.8 *										
$\text{NH}_4^+$		-0.5	-0.5	-0.6 *									
$\text{NO}_2^-$			-0.5		0.8 *								
$\text{NO}_3^-$				-0.4	0.5 *	0.7 *							
$\text{PO}_4^-$		-0.6 *		-0.7 *	0.8 *	0.5	0.4						
$\text{SiO}_4^-$	-0.5	-0.4		-0.5	0.4		0.5	0.4					
TN	0.4	-0.5 *	-0.7 *	-0.6 *	0.5	0.6 *	0.4	0.5					
TP		-0.7 *	-0.6 *	-0.8 *	0.7 *	0.4	0.6 *	0.7 *	0.6 *	0.5 *			
Chl- <i>a</i>		0.4		0.4		-0.4	-0.7 *	-0.6 *			-0.4		
DO		0.8 *	0.8 *	0.9 *	-0.7 *	-0.5	-0.4	-0.8 *	-0.4	-0.7 *	-0.8 *		
BOD			-0.4	-0.4	0.5	0.4	0.7 *	0.6 *		0.5	0.6 *	-0.5	
TVC		-0.6 *	-0.7 *	-0.6 *	0.8 *	0.6 *	0.4	0.4		0.6 *	0.6 *	-0.6 *	0.4
TC		-0.7 *	-0.7 *	-0.7 *	0.7 *	0.5 *	0.4	0.6 *		0.7 *	0.6 *	-0.6 *	0.4
FC		-0.7 *	-0.8 *	-0.7 *	0.7 *	0.6 *	0.4	0.5		0.7 *	0.5	-0.6 *	0.4
EC		-0.8 *	-0.8 *	-0.7 *	0.5 *	0.4		0.4		0.7 *	0.5	-0.6 *	0.5
SF		-0.7 *	-0.7 *	-0.6 *	0.7 *	0.5 *	0.5 *	0.5 *	0.4	0.6 *	0.6 *	-0.6 *	0.5 *



## Conclusions

The levels of various physical, chemical and biological parameters along Ennore coastal waters have been assessed and studied during four seasons in 2011. The study aims to understand the distribution of physicochemical characteristics and bacterial populations in the study area and also understand the relations between environmental properties and microbial features. Nutrient concentrations and microbial populations showed great spatial variability due to point and non point sources of sewage discharge from adjacent regions. The overall data indicates that creek and shore regions are adversely impacted by anthropogenic material than offshore waters. Insignificant seasonal variability was noticed in nutrient concentrations due to influx of surplus discharge of industrial and domestic sewage. Higher microbial populations were observed throughout the year irrespective of seasons due to the intensive anthropogenic activities. The correlation proved that the nutrients are strongly supported to microbial population due to addition of rich organic content in the coastal waters from point and non point sources. Present study generated baseline data on microbiological quality of Ennore coastal waters may serve as a biomonitoring and comparisons analysis for other coastal studies. Further studies are necessary to examine how different combinations of physicochemical properties are influencing coastal bacterial population and its impact food chain process.

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## References

- Mohan Raj, V., Padmavathy, S., and Sivakumar, S., Water quality parameters and its influences in the Ennore estuary and near Coastal environment with respect to industrial and domestic sewage, *Inter. Res. J. of Environ. Sci.*, 2(2013) 20-25.
- Patra, A.K., Acharya, B.C., and Mohapatra, A., Occurrence and distribution of bacterial indicators and pathogens in coastal waters of Orissa, *Ind. J. Mar. Sci.*, 38(2009) 474-480.
- Colwell, R.R., Brayton, P.R., Grimes, D.J., Roszak, D.B., Huq, S.A., and Palmer, L.M., Viable, but non-culturable *Vibrio cholera* and related pathogens in the environment: implications for release of genetically engineered microorganisms, *Nat. Biotechnol.*, 3(1985) 817-820.
- Oliver, J.D., Hite, F., McDougald, D., Andon, N.L., and Simpson, L.M., Entry into, and resuscitation from, the viable but nonculturable state by *Vibrio vulnificus* in an estuarine environment, *Appl Environ Microbiol.*, 61(1995) 2624-2630.
- Naganuma, T., Differential enumeration of intact and damaged marine planktonic bacteria based on cell membrane integrity, *J Aquat Ecosystem Health.*, 5(1996) 217-22.
- Amy, P.S., and Morita, R.Y., Starvation-survival patterns of sixteen freshly isolated open-ocean bacteria, *Appl Environ Microbiol.*, 45(1983) 1109-1115.
- Pickett A M, Growth in a changing environment, in: *Microbial population dynamics*, edited by M. J. Bazin, (CRC Press, Boca Raton, Florida) 1991, pp. 91-124.
- Banoo, S., Kar, R.N., and Panda, C.R., Seasonal variation and distribution of sewage pollution indicator and human pathogenic bacteria along Odisha coast, *Indian J Geomarine Sci.*, 43(2014) 859-869.
- Thomann R V, & Mueller J A, *Principles of surface water quality modelling and control*, (Harper & Row, Inc., New York) 1987.
- Jayaprakash, M., Srinivasalu, S., Jonathan, M.P., and Ram Mohan, V.A., Baseline study of physico-chemical parameters and trace metals in waters of Ennore Creek, Chennai, India, *Mar. Pollut. Bull.*, 50(2005) 583-589.
- Mahalaxmi, B., Revathy, K., Raghunathan, C., Anjalai, K., and Subashini, A., Distribution of microbial population associated with crabs from Ennore seacoast Bay of Bengal north east coast of India, *Int. J. Curr. Microbiol. App. Sci.*, 2(2013) 290-305.
- Shanmugam, P., Neelamani, S., Ahn, Y.H., Philip, L., and Hong, G.H., Assessment of the levels of coastal marine pollution of Chennai city, Southern India, *Water Resources Manage.*, 21(2007) 1187-1206.
- Raveendran, O., Gore, P.S., and Unnithan, R.V., Observations on faecal contamination of Cherai beach in Kerala (India), Short communication, *Ind. J. Mar. Sci.*, 7(1978) 128-129.
- Gore, P.S., Raveendran, O., and Unnithan, R.V., Pollution in Cochin backwaters with reference to indicator bacteria, *Ind. J. Mar. Sci.*, 8(1979) 43-46.
- Vaidya, S.Y., Vala, A.K., and Dube, H.C., Bacterial indicators of faecal pollution at Bhavnagar coast, *Ind. J. Microbiol.*, 41(2001) 37-39.
- Nallathambi, T., Eashwar, M., Kuberaraj, K., and Govindarajan, G., Abundance of indicator and general heterotrophic bacteria in Port Blair bay, Andamans, *Ind. J. Mar. Sci.*, 31(2002) 65-68.
- Grasshoff K, Kremling K & Ehrhardt M G, *Methods of Seawater Analysis*, (VCH Publishers) 1999, pp. 632.
- Strickland J D H, & Parsons T R, *A practical handbook of seawater analysis*, (Journal of the Fisheries Research Board of Canada Bulletin 167) 1972, pp. 310.
- APHA, *Standard methods for the examination of water and waste water. 21<sup>st</sup> edition*. (DC: American Public Health Association, Washington) 2005.

20. Welch P S, *Limnological methods*, (Mc Graw Hill, New York) 1952.
21. Ezzat, S.M., Hesham, M., Mahdy, M.A., Abo-Stat, E., Abd El-Shakour, H., and El-Bahnasawy, M.A., Water quality assessment of river Nile at Rosetta branch: Impact of drains discharge, *Middle-East J. Sci. Res.*, 12(2012) 413-423.
22. Gibson, R.N., Recent studies on the biology of intertidal fishes, *Oceanogr. Mar. Biol. Ann. Rev.*, 20(1982) 363-414.
23. Seenivasan, R., *Spectral reflectance properties of the Vellar estuarine environment, southeast coast of India*, M.Phil. thesis, Annamalai University, India, 1998.
24. Palanichamy, S., and Rajendran, A., Heavy metal concentration in seawater and sediments of Gulf of Mannar and Palk Bay, southeast coast of India, *Ind. J. Mar. Sci.*, 29(2000) 116-119.
25. Sulochana, B., and Muniyandi, K., Hydrographic parameters off Gulf of Mannar and Palk Bay during an year of abnormal rainfall, *J. Mar. Biol. Ass. India.*, 47(2005) 198-200.
26. Prabu, V.A., Rajkumar, M., and Perumal, P., Seasonal variations in physico-chemical characteristics of Pichavaram mangroves, southeast coast of India, *J. Environ. Biol.*, 29(2008) 945-950.
27. Soundarapandian, P., Premkumar, T., and Dinakaran, G.K., Studies on the Physico-chemical Characteristic and Nutrients in the Uppanar Estuary of Cuddalore, South East Coast of India, *Curr. Res. J. Biol.Sci.*, 1(2009) 102-105.
28. Damotharan, P., Perumal, N.V., and Perumal, P., Seasonal variation of physico-chemical characteristics of Point Calimere coastal waters (South east coast of India), *Middle-East J. Sci. Res.*, 6(2010) 333-339.
29. Parihar, S.S., Ajit, K., Ajay, K., Gupta, R.N., Manoj, P., Archana, S., and Pandey, A.C., Physico-chemical and Microbiological analysis of underground water in and around Gwalior city, MP, India, *Res.J.Recent Sci.*, ISSN 2277(2012): 2502.
30. Ganesan, M., *Ecobiology of seaweed of the Gulf of Mannar with special reference to hydrography and heavy metals*, Ph.D. Thesis, Annamalai University, India, 1992.
31. Kathiravan, K., Usha, N., and Vishnunath, R., Water Quality of Rameswaram Island, Southeast Coast of India—A Statistical Assessment, *Int. Res. J. Environment Sci.*, 3(2014) 12-23.
32. Latha, T.P., Rao, K.H., Amminedu, E., Nagamani, P.V., Choudhury, S.B., Lakshmi, E., Sridhar, P.N., Dutt, C.B.S., and Dhadwal, V.K., Seasonal variability of phytoplankton blooms in the coastal waters along the East coast of India, *ISPRS - International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences*, 40(2014) 1065-1071.
33. Pai, R., and Reddy, M.P.M., Distribution of Nutrients of Malpe, South Kanara Coast, *Ind. J. Mar. Sci.*, 10(1981) 322-326.
34. Sharma, C.B., and Ghose, N.C., Pollution of the River Ganga by Municipal Waste: A Case Study from Patna, *J. Geol. Soc. India.*, 30(1987) 369-385.
35. Gouda, R., and Panigrahy, R.C., Seasonal Distribution and Behaviour of Silicate in the Rushikulya Estuary, East Coast of India, *Ind. J. Mar. Sci.*, 21(1992) 111-115.
36. Bhattacharya, A.K., Choudhury, A., and Mitra, A., Seasonal Distribution of Nutrients and Its Biological Importance in Upper Stretch of Gangetic West Bengal, *Indian J. Environ. Ecoplann.*, 6(2002) 421-424.
37. Agarwal, A.K., and Rajwar, G.S., Physico-chemical and microbiological study of Tehri dam reservoir, Garhwal Himalaya, India, *J. Am. Sci.*, 6(2010) 65-71.
38. Singh, S.P., Pathak, D., and Singh, R., Hydrobiology of two water bodies i.e. Jagatdev and Narayan ponds of Satna (M.P), *Ecol. Env. Cons.*, 8(2002) 289-292.
39. Kumar A, Mandal K K & and Kumar A, Aspects of water pollution scenario in lentic and lotic freshwater ecosystem namely pond 7 river in Jharkhand India, in: *Water pollution* edited by Prof. Arvind Kumar, (A.P.H. Publishing Corp) 2004.
40. Sarupria, J.S., and Bhargava, R.M.S., Seasonal distribution of chlorophyll 'a' in the exclusive economic zone (EEZ), *Ind. J. Mar. Sci.*, 27(1998) 292-297.
41. Raghuprasad, R., Plankton calendars of the inshore waters at Mandapam, with a note on the productivity of the area, *Indian J. Fish.*, 5(1958) 170-188.
42. Sridhar, R., Thangaradjou, T., and Kannan, L., Spatial and temporal variations in phytoplankton in coral reef and seagrass ecosystems of the Palk Bay, southeast coast of India, *J. Environ. Biol.*, 31(2010) 765-771.
43. Robin, R.S., Vardhan, K.V., Muduli, P.R., Srinivasan, M., and Balasubramanian, T., Preponderance of enteric pathogens along the coastal waters of Southern Kerala, Southwest coast of India. *Mar Sci.*, 2(2012), 6-11.
44. Bilgrami, K.S., and Kumar, S., Bacterial contamination in water of the River Ganga and its risk to human health. *Int J Environ Health Res.*, 8(1998) 5-13.
45. Mahalakshmi, M., Srinivasan, M., Murugan, M., Balakrishnan, S., and Devanathan, K., Isolation and identification of total heterotrophic bacteria and human pathogens in water and sediment from Cuddalore fishing harbour after the tsunami, *Asian J. Biol. Sci.*, 4(2011) 148-156.
46. Premalatha, K., *Studies on total heterotrophic bacteria and on human pathogens (V. cholerae and V. parahaemolyticus) from Uppanar estuary. Porto-Novo south India*, M. Phil. thesis, Annamalai University, India, 2001.
47. Prasad, V.R., Srinivas, T.N.R., and Sarma, V V S S., Influence of river discharge on abundance and dissemination of heterotrophic, indicator and pathogenic bacteria along the east coast of India, *Mar. Pollut. Bull.* 95(2011) 115-125.
48. Adingra, A.A., and Arfi, R., Organic and bacterial pollution in the Ebrie lagoon, Cote d'Ivoire, *Mar. Pollut. Bull.*, 36(1995) 689-695.
49. Goyal, S.M., Gerba, C.P., and Melnick, J.L., Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast, *Appl. Environ. Microbiol.*, 34(1977) 139-149.
50. Nagvenkar, G.S., and Ramaiah, N., Abundance of sewage-pollution indicator and human pathogenic bacteria in a tropical estuarine complex, *Environ. Monit. Assess.*, 155(2009) 245-256.
51. Haller, L., Pote, J., Loizeau, J.L., and Wildi, W., Distribution and survival of faecal indicator bacteria in the sediment of the Bay of Vidy, Lake Geneva, Switzerland, *Ecol. Indic.*, 9(2009) 540-547.
52. Mohandass, C., Nair, S., Achuthankutty, C.T., and LokaBharathi, P.A., Pollution monitoring of coastal and estuarine areas: I. Bacterial indicators along the south Gujarat Coast, *Indian J. Mar. Sci.*, 29(2000) 43-47.
53. Anon., Current measurements for proposed all weather ports at Redi, Vijaydurg and Ratnagiri, Maharashtra. Technical Report, National Institute of Oceanography, Goa, 1997. Dale, N.G., Bacteria in inter-tidal sediment:

- Factors related to their distribution, *Limnol Oceanogr.*, 19(1974) 509-518.
54. Dale, N.G., Bacteria in inter-tidal sediment: Factors related to their distribution, *Limnol Oceanogr.*, 19(1974) 509-518.
55. Davies, C.M., Long, J.A.H., Donald, M., and Ashbolt, N.J., Survival of faecal microorganisms in marine and fresh water sediments, *Appl. Environ. Microbiol.*, 61(1995) 1888-1896.
56. Babinchak, J.A., Graikoski, J.T., Dudley, S., and Nitkowski, M.F., Effect of dredge spoil deposition on fecal coliform counts in sediments at a disposal site, *Appl. Environ. Microbiol.*, 34(1977) 38-41.
57. Niewolak, S., Total viable count and concentration of enteric bacteria in bottom sediments from the Czarna Hancza River, Northeast Poland, *Pol j Environ Stud.*, 7(1998) 295-306.
58. Ruiz, G.M., Rawlings, T.K., Dobbs, F.C., Drake, L.A., Mullady, T., Huq, A., and Colwell, R.R., Global spread of microorganisms by ships, *Nature.*, 408(2000), 49-50.
59. Pathak, S.P., and Gopal, K., Rapid detection of *Escherichia coli* as an indicator of faecal pollution in water, *Ind. J. Microbiol.*, 41(2001)139-151.
60. Saraswat, R., Kouthanker, M., Kurtarkar, S., Nigam, R., and Linshy, V.N., Effect of salinity induced pH changes on benthic foraminifera: a laboratory culture experiment, *Biogeosci. Discuss.*, 8(2011), 8423-8450.
61. Wheeler, P.A., and Kirchman, D.L., Utilization of inorganic and organic nitrogen by bacteria in marine systems, *Limnol. Oceanogr.*, 31(1986) 998-1009.
62. Conley, D.J., Biogeochemical nutrient cycles and nutrient management strategies, *Hydrobiologia.*, 410(2000) 87-96.