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Occurrence and distribution of some enteric bacteria along the southern coast of Kerala

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Six major groups of enteric bacteria, viz, Faecal coliforms, *E. coli, Shigella* spp., *Salmonella* spp., *Vibrio parahaemolyticus* and *Vibrio cholerae* were screened for the present study. The overall percentage occurrence of enteric bacteria in water and sediment was maximum accounted for *Vibrio parahaemolyticus* (81.7%) and minimum for *Salmonella* spp. (9.6%) and moderate reported against *Vibrio cholerae* (60.6%). The distribution of enteric bacteria was more in the water sample than sediment except *Vibrio* spp. and the highest occurrence was found to be at Cochin transect, which is the most polluted transect due to enteric microbes. Relationship between the stations on the occurrence of enteric bacteria was linear and significant variations was observed (R^2=0.899) and the same pattern of linear regression model was also obtained in source wise occurrence (R^2=0.777). The present study elucidates that the health status of the Kerala coast may deteriorates and will be detrimental to the coastal community.

[**Keywords:** Enteric bacteria, allochthonous, bioindicators, indigenous, storm water]

Introduction

Marine ecosystem is being threatened by the discharge of untreated sewage wastes and industrial effluents which ultimately affects the sustainability of living resources and public health. These wastes carry enormous level of microbial pathogens to the marine environment and results in negative impact on the marine resources thus causing economic loss [1]. Some microbial pathogens in the coastal environment are indigenous to the oceans, including Vibrios. Wheras others like *E. coli*, *Salmonella* spp. and *Shigella* spp. are allochthonous which introduced through agricultural, urban surface runoff, waste water discharges and from domestic and wild animals. Most of the Vibrios and *Salmonella* spp. are pathogenic to humans and some have fatal infections[2-5]. Infections with Vibrios are known to be associated with either consumption of seafood or exposure to marine environment[6]. The presence of faecal coliforms forms representative for the assessment of coastal recreational water quality[7]. The incidence and distribution pattern of enteric bacteria along the south-west coast of India is well documented[8-12]. However, the prevalence of major enteric bacteria along the southern coast of Kerala has not been studied well. The present investigation highlights the occurrence and distribution pattern of enteric pathogens in marine water and sediment source and it also evaluating the influence of anthropogenic inputs and raw sewage on the incidence of these bacteria at inshore and offshore region along Kerala coast.

Materials and Methods

Three stations (Fig.1) were selected viz. Veli (lat. 8° 29′ 39 ″ N; long. 76° 50′ 56″ S) Neendakara (lat. 8°56′ 52.7″ N; long. 76° 31′ 46″ S) and Cochin (lat. 9° 57′ 6.9″ N; long. 76° 14′ 29″ S).Sampling was carried out at 0 km (nearshore) 1, 3, 5 and 10 km. The water and sediment samples were collected during the cruise programme of CRV *Sagar Purvi*, from 24thSep to 1stOct 2004. Water sample were made from depths varying 3m to 50m (<3m-surface; 3 or <10m-both surface and bottom; 10 or <10m - surface, mid and bottom collected from surface, mid and bottom) using Niskin water sampler and aseptically transferred into sterilized glass bottles. Duplicate sampling and analysis were performed for each depth and station. Sediment samples were collected using a Van Veen grab and sub samples were aseptically transferred into sterilized petri dishes with sterile spatula before disturbing the sediment for other analysis. The environmental parameters monitored in this study were water temperature, pH, salinity, dissolved oxygen, nitrite-N, nitrate-N and inorganic phosphate. Temperature, pH and salinity were measured at the sites by using a field thermometer and model Multi

340i/set (Germany) and dissolved oxygen was measured by Winkler method[13] and nutrients by the method of Grasshoff[14]. Six major groups of enteric bacteria were selected and the selective media used for the growth of bacteria were Membrane Filter Coliform (MFC) agar for faeal coliforms, M-7hr FC Agar for *E. coli*, Xylose Lysine Deoxycholate Agar for *Shigella* spp. and *Salmonella* spp. and Thiosulphate Citrate Bile Sucrose Agar for the isolation of *Vibrio parahaemolyticus* and *Vibrio cholerae*. The spread plate technique was adopted to enumerate the enteric bacteria except *E. coli* using 0.1 to 0.5 ml of the sample and the results were reported as Colony Forming Units (CFU/ml). Membrane filter technique was used for the isolation of *E. coli* using 10 ml sample. The sediment sample (1 g) was dissolved in 100 ml distilled water that was used for the isolation of enteric bacteria from the sediment samples and the 9 results were expressed in CFU/g. The counts were recorded after 48 to 72 hr. and each group was characterized using AOAC method[15]. Coefficient of determination (R^2) by regression analysis was computed for determining the relationship between the stations and sources on the occurrence of enteric bacteria.

Results

The water temperature ranged between 27-30 °C, for pH 5.8-8.12, salinity 20.4-34.0 psu, dissolved oxygen 3.7-4.95 mg/l, nitrite-N 0.52-1.70 µmol/l, nitrate-N 3.80-5.22 µmol/l and inorganic phosphate 0.90-2.40 µmol/l. The distribution patterns of different enteric groups in the water column at various stations are illustrated in Fig. 2 (A-F). The population of faecal coliforms (Fig. 2A) was found to vary between 5 and 850 CFU/ml. The minimum population was reported from the mid and bottom water at 5 and 10 km from Neendakara coast and mid water depth of 10 km at the Veli transect. The maximum population was observed in the nearshore surficial water at Cochin. *E. coli* (Fig. 2B) ranged between a minimum of 5 CFU/ml from 5 and 10 km (mid water) and 1 and 5 km (bottom water) at Neendakara and maximum population density of 1500 CFU/ml was reported from nearshore surface water at Cochin. The population of *Shigella* spp. (Fig. 2C) ranged from 10 to 3600 CFU/ml and the minimum was reported 1, 5 and 10 km (mid water) and 1, 3, 5 and 10 km (bottom water) at Veli transect. The maximum number of *Shigella* spp. was observed from the surface water of nearshore at Cochin. The population density of *Salmonella* spp. (Fig.2D) at various stations were ranged from 5 to 10 CFU/ml and the maximum population was observed in the mid water of 5 km and bottom water of 10 km at Veli transect. It also noticed from nearshore (surface) and nearshore, 1 and 3 km (bottom) at Cochin. The minimum population was reported from the mid water of 1 km and bottom water of 1 and 5 km at Veli. The microbial population of *Vibrio parahaemolyticus* (Fig.2E) at different transects ranged between 5 and 580 CFU/ml. The lowest and highest population was recorded from 5 and 1 km (bottom water) at Veli and Cochin transect respectively. The distribution of microbial population of *Vibrio cholerae* (Fig.2F) varied from 5 to 980 CFU/ml and the lowest was noticed from 3 km (mid water) and 1, 3 and 5 km (bottom water) at Veli. The highest population (980) was reported from nearshore (surface water) at Cochin.

The distribution patterns of different enteric bacteria in the sediment sample at various stations are depicted in Fig.3 (A-E). There was no incidence of faecal coliforms, *E. coli, Shigella* spp. and *Salmonella* spp. reported from the sediment sample at Veli and Neendakara transect (Fig.3A). The population density of faecal coliforms varied from 500 to 5500 CFU/g and the minimum (nearshore) and maximum (1 km) of faecal coliforms was observed from Cochin transect. The sediment counts of *E. coli* (Fig.3B) were found to 2000 CFU/g reported from the sediments of nearshore at Cochin. The lowest population of *Shigella* spp. was 1500 CFU/g at 1 km of Cochin and the highest value was 3000 CFU/g at nearshore of Cochin (Fig.3C). *Salmonella* spp. was totally absent in the sediment samples at all stations.The population density of V*ibrio parahaemolyticus* (Fig.3D) ranged between 500 and 5000 CFU/g in the sediment sample of various stations and the minimum was observed at 3 and 5 km of Veli and 1 and 3.0 km of Neendakara. The minimum value was also noticed at 5 and 10 km off Cochin transect. The highest population density was registered at 1.0 km off Cochin. The microbial counts of V*ibrio cholerae* (Fig.3E) fluctuated between 200 and 9500 CFU/g and the lowest value was noticed from 3 km at Veli and the highest was reported from nearshore at Cochin.

The overall incidence of different enteric bacteria in water and sediment is presented in Table 1. Out of the 104 samples in each group of bacteria, the minimum occurrence was of *Salmonella* spp. at 9.6%.

Vibrio parahaemolyticus was found to be the highest at 81.7%. A moderate occurrence was reported for *Vibrio cholerae* at 60.6%. The source wise occurrence and distribution of different enteric bacteria at various transects are given in Table 2. Out of the 74 water samples and 30 sediment samples analyzed, the lowest occurrence was reported in sediment samples. The highest number of occurrence 79.7% for *Vibrio parahaemolyticus* in water and 86.7% in sediment samples. The occurrence *Salmonella* spp. in water was 13.5% and was not reported from the sediment samples. The number of positive occurrence of all enteric bacteria exhibited higher values in the water samples than the sediment samples.

Station wise occurrence of different enteric bacteria in water and sediment samples are given in Table 3. The maximum occurrence was reported at Cochin of all the groups except S*almonella* spp. and the minimum at Veli. All the 34 samples showed positive occurrence for V*ibrio parahaemolyticus* which was the most prominent bacteria (100%) at Cochin. This was found to be dominant one at Veli and Neendakara. Out of the 36 samples, 26 samples showed a positive occurrence (72.2%) at Veli and 25 showed positive occurrence 73.5% of the 34 samples analysed at Neendakara. Out of the 36 samples analysed at Veli transect, faecal coliforms were the least group in their occurrence at 2.8%. At Neendakara, there was no incidence of *Shigella* spp. and *Salmonella* spp. out of the 36 samples analysed. *Salmonella* spp. was the lowest group of bacteria which showed an occurrence of 4 out of the 34 samples analysed (11.8%) at Cochin transect. The result on regression analysis (Fig.4A) reveals that a significant variation was observed between the stations and R^2 values are 0.899 (r=0.95), 0.855(r=0.92) which is very high *i.e.* more than 85% of both the variation in observation and showed linear nature. A significant relation was also found to exist between the sources (Fig.4B) and the correlation coefficient for this was 0.88 significant at 77.7% level.

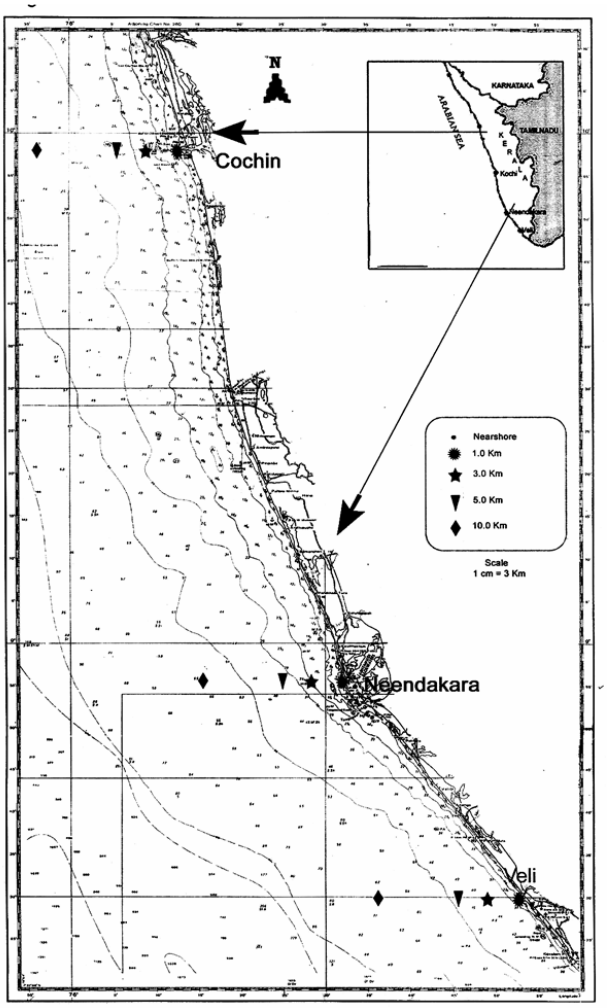
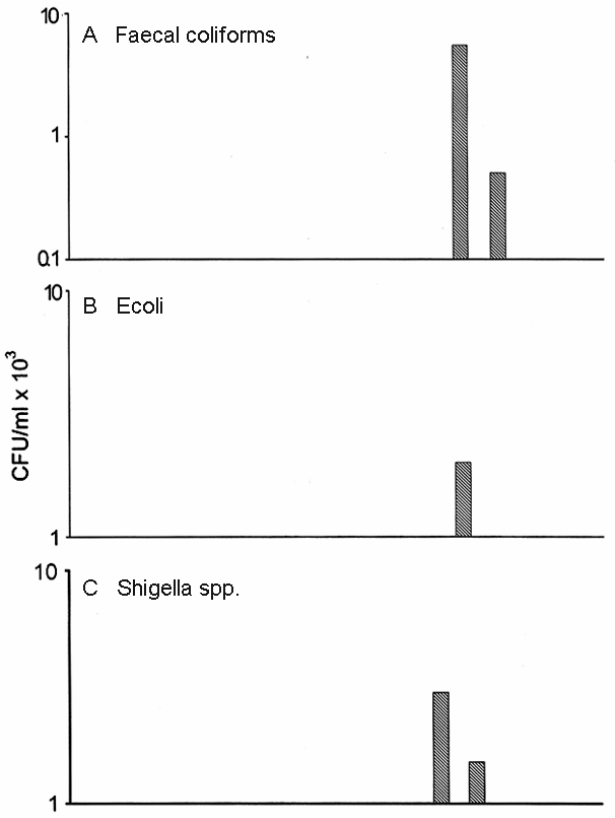


Fig. 1—Map showing the sampling locations



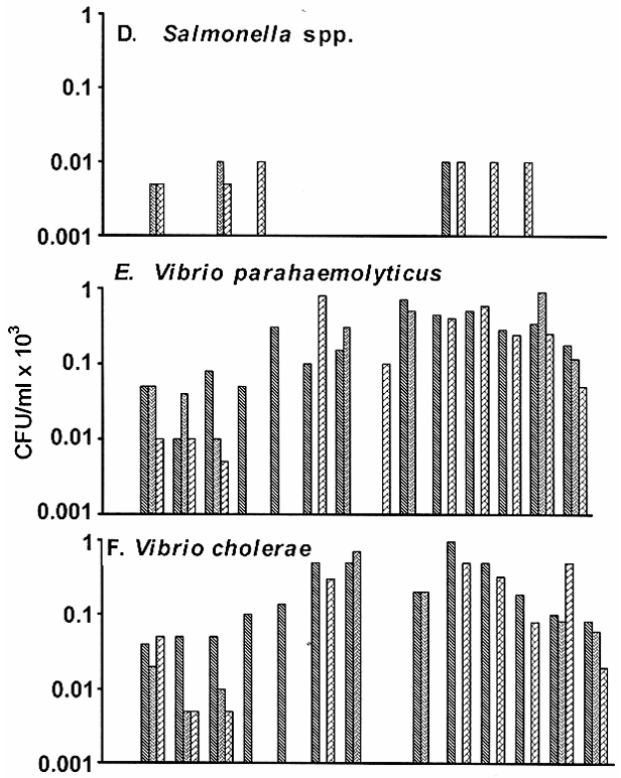
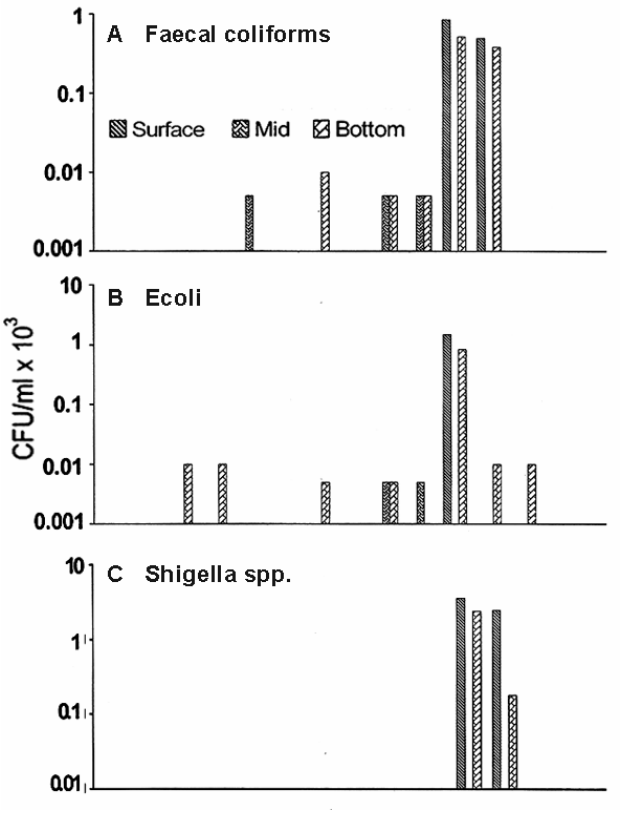


Fig 2 (A-F). — Population distribution of enteric bacteria in water sample at different stations.



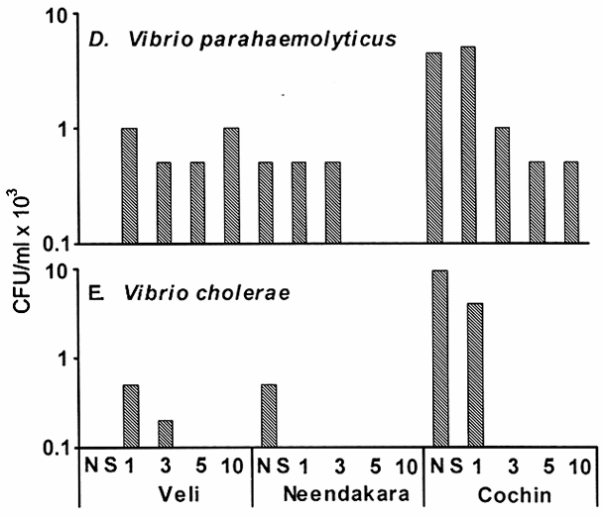


Fig 3 (A-E). — Population distribution of enteric bacteria in sediment sample at different stations

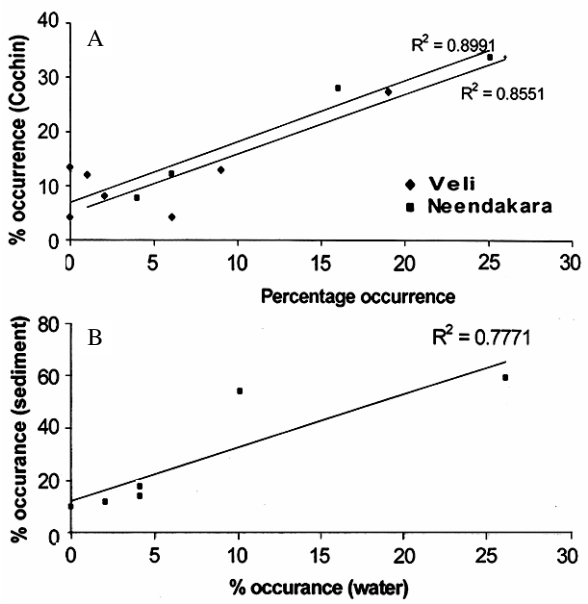


Fig 4 A.— Relationship showing the percentage occurrence of enteric bacteria at different stations: B.— Relationship showing the percentage occurrence of enteric bacteria at different sources.

Discussion

The coastal waters and the adjacent water bodies are increasingly used for waste disposal which has led to dreadful changes in the natural characteristics of the coastal ecosystem. Untreated sewage discharges, industrial effluents carry high load of pathogenic bacteria especially enteric groups and make potential threat to human health[16-17]. The lowest or absent enteric bacterial counts measured at Station I was found to be by the action of acidic effluents

Table 1—The overall occurrence of different enteric bacteria in water and sediment.

Sl. No.	Types of Bacteria	Number of Samples		Occurrence (%)
		No. of samples analysed (water + sediment)	Positive occurrence	
1.	*Faecal coliforms (FC)*	104	19	18.27
2.	*Escherichia coli*	104	14	13.46
3.	*Shigella* spp.	104	22	21.15
4.	*Salmonella* spp.	104	10	9.62
5.	*Vibrio parahaemolyticus*	104	85	81.73
6.	*Vibrio cholerae*	104	63	60.58

Table 2—The source wise distribution of different enteric bacteria at all stations

Sl. No.	Types of bacteria	Sample analysed	Water		Sample analysed	Sediment	
			Positive occurrence	Occurrence (%)		Positive occurrence	Occurrence (%)
1.	*Faecal coliforms (FC)*	74	15	20.27	30	4	13.33
2.	*Escherichia coli*	74	12	16.22	30	2	6.67
3.	*Shigella* spp.	74	18	24.32	30	4	13.33
4.	*Salmonella* spp.	74	10	13.51	30	Nil	--
5.	*Vibrio parahaemolyticus*	74	59	79.72	30	26	86.67
6.	*Vibrio cholerae*	74	54	72.98	30	10	33.33

Table 3—Station wise occurrence of different enteric bacteria in water and sediment samples

Sl. No.	Types of bacteria	Sample analysed	Stations								
			VELI			NEENDAKARA			COCHIN		
			Positive occurrence	%	Sample analysed	Positive occurrence	%	Sample analysed	Positive occurrence	%
1	*Faecal coliforms (FC)*	36	1	2.8	34	6	17.64	34	12	35.29
2	*Escherichia coli*	36	2	5.6	34	4	11.76	34	8	23.53
3	*Shigella* spp.	36	9	25.0	34	Nil	--	34	13	38.24
4	*Salmonella* spp.	36	6	16.7	34	Nil	--	34	4	11.76
5	*Vibrio parahaemolyticus*	36	26	72.2	34	25	73.53	34	34	100.00
6	Vibrio cholerae	36	19	52.78	34	16	47.06	34	28	82.35

discharged from nearby Travancore titanium products which imparts low *p*H (5.8) to the water column that highly reflects in the nearshore transect.

The overall occurrence of enteric bacteria in water and sediment shows that the most dominant group envisaged be the *Vibrio parahaemolyticus* (81.7%) followed by *Vibrio cholerae* (60.6%) and the least one for *Salmonella* spp. (9.6%). *Vibrio* spp. are widely distributed in marine environment and studied extensively by various authors.[18, 19] Watkins & Kabelli [20] documented that the probability of the existence and the distribution of Vibrios in coastal waters is mainly from polluted water and waste water effluents and their density was found more at near the water surface of polluted area and their habitat decreased from the source of pollutants of the site of waste water discharge. The results of this finding are similar to the published papers. The microbial count of *Salmonella* spp. in water was very low and was not detected in the sediment sample and accounts for 9.6% occurrence. *Salmonella* spp regarded as an index of pollution of coastal waters and studies shows that their incidence even in low numbers in marine water is of human or animal origin[21]. Baubart *et al*.[22] pointed out that the *Salmonella* contamination in the marine environments is mainly by rivers or storm generated discharges. The data from the present study highly corroborates with the earlier views and it focus the main source of *Salmonella* contamination in the coastal waters is of human or animal origin.The different microbial population structures may also be attributed to the different rates of growth and survival of these pathogens in the marine habitat.

The occurrence of enteric bacteria in water and sediment sample showed the maximum percentage was in water sample of all groups, except *Vibrio parahaemolyticus*. Coastal sediments act as reservoir for pathogenic microorganisms. Dale[23] and Davies *et*

al.[24] pointed out that the interaction of microorganisms with sediments may enhance their survival by reducing exposure to stressors such as sunlight and predation or by increasing the availability of nutrients. Van Donsel & Geldreich[25] and Goyal *et al*[26]. noticed that sediments appear to provide the most conducive ecological niche for the survival of the pathogens. Ramamoorthy & Natarajan[27] observed the high level of organic matter present in the sediment may also be a reason for promoting the survival of enteric pathogens especially Vibrios. This finding holds well in the present case also (Organic carbon & matter also estimated in our study but not included in this paper.). The percentage occurrence of faecal coliforms, *E. coli, Shigella* and *Salmonella* spp. was found to be more in the water than sediment. The presence of faecal coliforms in the coastal waters indicates that the faecal contamination is from the human and animal source. The enteric bacteria present in the water column adsorbed to the soil particles that pose little danger to public health and sometimes the resuspension of sediments in response to currents, storms, boat traffic, dredging and changes in salinity and organic matter can result in release of adsorbed bacteria into the overlying water and posing a hazard to human health[28-30]. All the stations in the present study are characterized by the above factors and that was more in Cochin.

The station wise occurrence of enteric bacteria indicates that Cochin transect was found to be highest population and percentage occurrence of all groups especially at nearshore waters. The river discharges which have been ascribed as stimulants for higher occurrence of microbial population in the coastal waters[31]. The coastal waters of Cochin transect receives fresh water from various rivers and large quantities of waste water from cities and variety of industrial effluents with minimal treatment which carries enormous level of pathogens. These pollutants attribute the microbial load of the coastal waters and sediment. The occurrence of *Salmonella* spp. and *Shigella* spp. in Cochin transect was probably be due to the discharge of polluted waters from an adjacent land or coastal area. Pradeep & Lakshmanaperumal samy[32] noticed the main source of faecal coliforms and other enteric pathogens in Cochin transect is through river water, which carries land runoff from urban and rural areas, sewage discharges and storm water. The present observation sure almost similar to is highly supported the above findings. No significant variation was observed on the microbial population and the percentage occurrence of faecal coliforms and *E. coli* at all stations, and its presence in the coastal waters is used as bio-indicator for the assessment of water quality and the reported value limited to the standard value. Based on the linear regression model study, Cochin transect (more than 85% of variation in the observation), which is characterized by high range occurrence of enteric microbes due to high load of anthropogenic inputs. The source wise occurrence (77% of variations in the observation) showed a significant variation between the water and sediment sample which attributed by overlying water easily harbour enteric microbes than sediment.

The results of the present study reveal that the coastal waters of Kerala being a dumping ground of the untreated sewage wastes and industrial effluents, which enhances the microbial load especially at nearshore water. The occurrence of Vibrios, *Salmonella* spp. and *Shigella* spp. in marine ecosystem is alarming, even though their count in low, to the people residing along the coast and their counts exceeds from the legal limit. The coastal contamination due to enteric bacteria leads to quality deterioration of marine resources that pose a human health hazard and subsequent economic loss. It can be concluded that Cochin is the most polluted site characterized by high rate of anthropogenic activities. The fishes inhabitant in these areas contaminates enteric bacteria and act as vectors for various diseases by cross contamination. It is suggested that the proper treatment of sewage and establishment of sanitary requirements in coastal regions would reduce the pollution load to protect the coastal livelihood.

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