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RESEARCH ARTICLE

MICROBIAL STATUS OF THE COASTAL HABITATS IN THE ANDAMANS, INDIA

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ABSTRACT

Since ancient period, humans depend on coastal areas for their livelihood. Therefore, coastal monitoring process is necessary to detect contaminations. In the present investigation, distribution of bacteria including pathogens in water and sediments of different coastal habitats (mangrove, coral, seagrass and beach) of the Havelock Island, the Andamans, India, was studied. From nine stations, the study has found 47 total heterotrophic bacterial strains, belonging to 13 genera (*Escherichia*, *Pseudomonas, Vibrio*, *Aeromonas, Enterococcus, corynebacterium, Salmonella, Klebsiella, Streptococcus, Staphylococcus, Flavobacterium, Micrococcus* and *Shigella*). The genus *Escherichia,* was dominant with 21%, followed by *Pseudomonas* (13%) *and Vibrio* (13%). THB population density varied in water samples from 43 $X10^5$ CFU/ml to 182 $X10^5$ CFU/ml and in the sediment samples, it varied from 79 $X10^5$ CFU/mg to 259 $X10^4$CFU/mg. This study is significant as it would pave way for future workers to elucidate the importance of coastal sanitation, for keeping the environment clean.

INTRODUCTION

Microorganisms are cosmopolitan, diverse and distributed in open seawaters, sediments, estuaries, and hydrothermal vents. Marine ecosystems are governed by decomposition of organic matter to inorganic form and cycling of nutrients, mediated by microorganisms, which are responsible to sustain all the living things in the oceans (Cevera *et al.,* 2005). Transformation of organic detritus through the mediation of microorganisms is now recognized as an important process in the sea (Surajit Das *et al.,* 2007). Further, marine microbes are accountable for most of the benthic biomass and it is well known that they play a significant ecological and biogeochemical role in the marine environment by regulating the transformation of major bioactive elements (i.e. carbon, nitrogen, phosphorus, oxygen and sulfur) and by affecting the degradability of organic matter (Polymenakou *et al.,* 2009).

In general, sea bathing and consumption of seafoods from coastal waters contaminated by the discharge of urban wastes could cause human health problems (Shuval, 1999). Contaminants are received in the sea either by direct discharge of waste water flowing from communities or from the runoff of rivers and streams, carrying the wastes disposed off by the up-steam communities (Shuval, 2005). Also many pathogens are transferred to the sea from vegetable wastes and fecal matters (Sharma and Chaturvedi, 2007; Williams *et al.,* 2007). Pathogens originating from such contaminations frequently cause a lot of diseases and sometimes menace even the human life (Elmanama *et al.,* 2005). The pathogens can also cause major diseases in the marine organisms like corals (Banin *et al.,* 2000; Kakim *et al.,* 2012), fishes (Austin, 2005), etc.

Humans have resided in close association with the coastal regions of the world for the past many years. So, a continuous monitoring of the marine environment (water and sediments) is necessary to detect the presence of THB and pathogenic bacteria for the betterment of coastal people. This will also help create public awareness on the health management for the coastal dwellers. In this context, present investigation focuses attention on THB and pathogenic bacteria occurring in both water and sediments of the Havelock island, the Andamans, India.

MATERIALS AND METHODS

Study area

The Andaman group of Islands in the Bay of Bengal, lie between the latitude 11°97' N and longitude 93°00' E. In these island groups, Havelock island clinch with mangrove, coral, seagrass and beach habitats was taken as our study area. The sample sites around this island were selected based on the different habitats: Station 1 (Mangrove I, Lat. 12˚02' 27.9" N and Long. 92˚ 58' 41.5" E), Station 2 (Coral I, Lat. 12˚ 02' 28.5 N and Long. 92˚ 58' 47.1" E) Station 3 (Beach I, Lat. 12˚ 02' 32.5" N and Long. 92˚58' 56.9" E), Station 4 (Mangrove II, Lat. 12˚02' 00.0" N and Long 92˚59' 52.7" E), Station 5 (EL Dorado - Coral II, Lat 12˚ 01' 31.6" N andLong93˚ 00'13.8" E), Station 6 (Dive India - Beach II, Lat 12˚ 01' 38.5" N and Long 93˚ 00' 15.8" E), Station 7 (Kalapathar –

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Mangrove III, Lat 11˚ 57' 37.9" N and Long 93˚ 00 46.6" E), Station 8 (Silver sand – Coral + Seagrass I, Lat 12˚ 00' 36.1"N and Long 93˚ 00' 31.0" E) and Station 9 (Radha Nagar, Beach III, 11˚ 59' 04.2" N and 92˚ 57' 04.0" E) (Fig. 1).

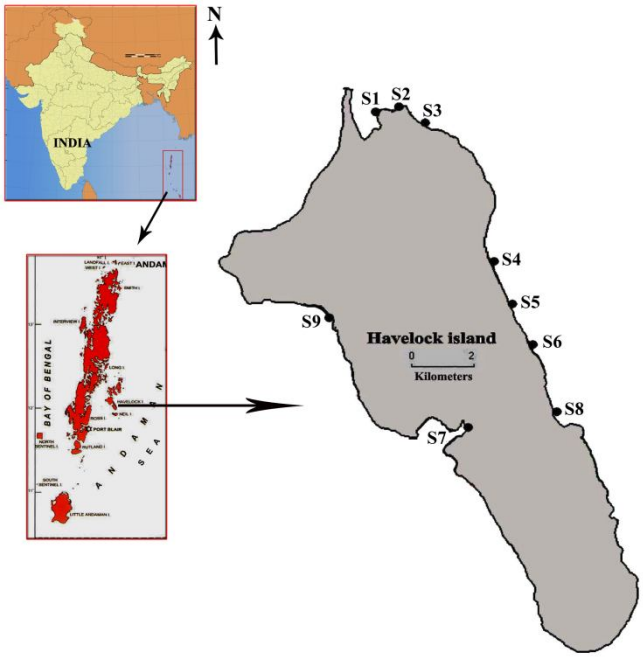

Fig.1 Map showing the study area and sampling stations in the Havelock Island

Sampling

Field collections were carried out in January 2011(non-monsoon) at the above nine stations. Surface water samples were collected in 100 ml sterile screw cap bottles for bacteriological assessment. Sediment samples were collected by employing an alcohol rinsed and air- dried small Peterson's grab. The central portion of the collected sediments was aseptically transferred into sterile polythene bags using sterile spatula. All samples were brought to the field laboratory (at Havelock Island) in portable icebox within 4 hours. Immediately after arrival, inoculations were made using suitable media with necessary dilutions and pure cultures were established.

Bacterial enumeration

Total heterotrophic bacterial (THB) population was enumerated by using spread plate method with Marine Agar medium. The plates after inoculation were incubated in an inverted position at a temperature of 28 ± 2°C for 24 to 48 hours. After incubation, colonies in the triplicate samples were counted and expressed as colony forming units in water (CFU/ml) and sediments (CFU/mg). Bacterial colonies were picked out from the petridishes and restreaked in appropriate nutrient agar plates and pure cultures were stored in agar slants for further identification. Specific media were used for *Escherichia coli* (MacConkey agar), *Vibrio cholera* and *V. parahaemolyticus* (TCBS agar), Species of *Salmonella, Shigella* and *Klebsilla* (XLD agar), *Pseudomonous aeruginosa* (Cetrimide agar), *Streptococcus faecalis* (M-enterococcus agar), *Aeromonas hydrophila* (AMB agar) and *Staphylococcus aureus* (Monnitol Salt agar). Different morphological and biochemical characteristics of the isolates were studied according to the Bergey's Manual of Determinative Bacteriology. All the chemicals and media were purchased from Hi-media, Mumbai.

RESULTS

Generic composition of Total Heterotrophic Bacteria (THB)

A total of 13 genera [*Escherichia* (21%), *Pseudomonas* (13%), *Vibrio* (13%), *Aeromonas* (11%), *Enterococcus* (9%), *corynebacterium* (7%), *Salmonella* (6%), *Klebsiella* (6%), *Streptococcus* (4%) *Staphylococcus* (4 %), *Flavobacterium* (2%), *Micrococcus* (2%) and *Shigella* (2%)] were identified (Fig. 2) from 47 total heterotrophic bacterial strains, isolated from the water and sediment samples of nine stations of the Havelock island. Among them, 8 genera *viz. Escherichia, Pseudomonas, Salmonella, corynebacterium, Vibrio, Klebsiella, Enterococcus* and *Aeromonas,* from 22 strains, were isolated from the water samples, and 13 genera *viz. Escherichia, Pseudomonas, Flavobacterium, Salmonella, Vibrio, Shigella, Klebsiella, Micrococcus, Cornybacterium, Enterococcus, Aeromonas, Streptococcus* and *Staphylococcus,* from 25 strains, were isolated from the sediment samples. In the water and sediment samples, gram negative bacteria were more (64.36%) as compared to the gram positive bacteria (35.64%).

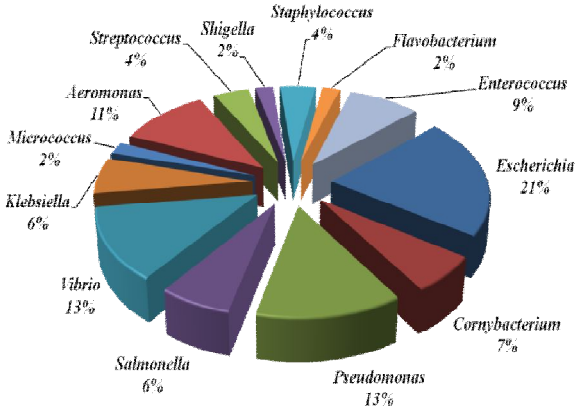

Fig. 2 Percentage composition of THB genera isolated from the water and sediment samples of the Havelock island

Population density of THB and pathogenic bacteria

Population density of THB in water samples varied from 43 $X10^5$ CFU/ml (Station 9) to 182 $X10^5$ CFU/ml (Station 7). In the case of the sediment samples, It varied from 79 $X10^5$ CFU/mg (station 9) to 259 $X10^4$CFU/mg (station 7) (Fig.3).

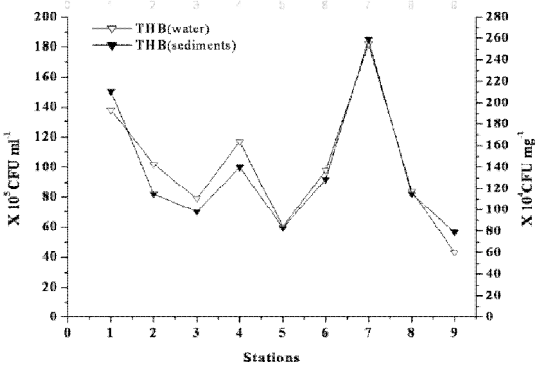

Fig.3 Population density of THB in the water and sediment samples of the Havelock island.

Population density of *E. coli* was lower in water (3 X10^5 CFU/ml) and sediments (8 X10^{4}CFU/mg) at station 3. While, station 7 recorded higher density both in water (83 X10^5 CFU/ml) and sediments (195 X10^{4}CFU/mg) (Fig.4).

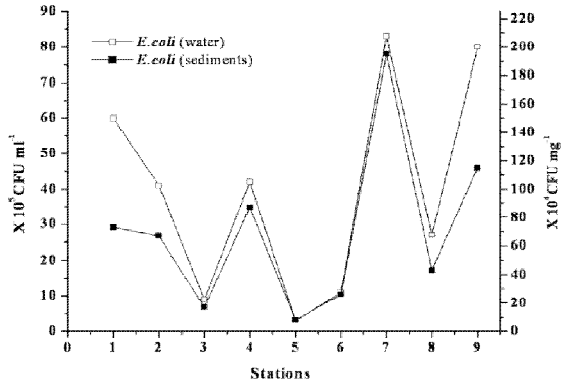

Fig.4 Population density of *E.coli* in the water and sediment samples of the Havelock island

Population density of both *V. cholerae* and *V. parahaemolyticus* was estimated in both water and sediment samples at all the nine stations. In the water, *V. cholerae* showed the maximum (21 X10^5 CFU/ml) at station 9 and the minimum (1 X10^5 CFU/ml), at station 3. *V. parahaemolyticus* registered lower population density (2 X10^5 CFU/ml) at station 3 and higher population density (28 X10^5 CFU/ml), at station 4. In the sediments, *V.parahaemolyticus* recorded the maximum density (41 X10^{4}CFU/mg) at station 7 and the minimum (3 X10^{4}CFU/mg), at station 3. *V. cholerae* showed the maximum (55 X10^{4}CFU/mg) at station 7 and the minimum (5 X10^{4}CFU/mg), at station 3 (Fig. 5).

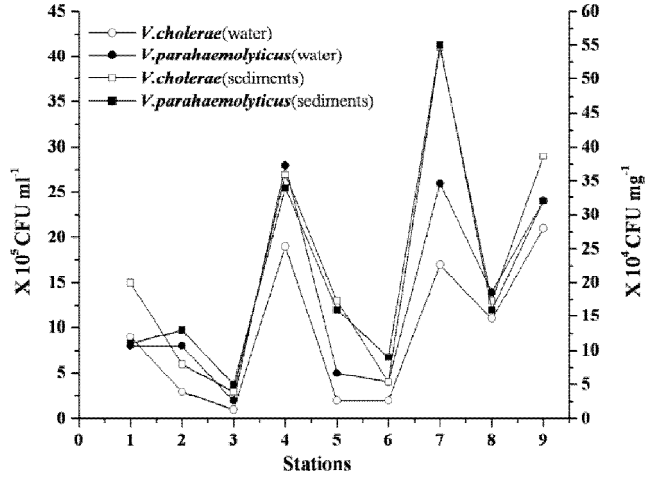

Fig.5 Population density of *Vibrio* spp. in the water and sediment samples of the Havelock island

In water, lower population density of *S. typhimurium* (2 X10^5 CFU/ml) was recorded at stations 3, 5 and 9 and higher density (31 X10^5 CFU/ml), at station 4. *S. paratyphi* recorded the maximum density (19 X10^5 CFU/ml) at station 4 and the minimum (1 X10^5 CFU/ml), at stations 3 and 5. *S. dysenteriae* recorded the maximum (24 X10^5 CFU/ml) at station 7 and the minimum (1 X10^5 CFU/ml), at stations 3 and 5. In *K. pneumonia,* the density was minimum (1 X10^5 CFU/ml) at stations 5 and 9, and maximum (23 X10^5 CFU/ml) at station 4. In the case of sediments, lower population density of *S. typhimurium* (6 X10^{4}CFU/mg) was recorded at station 5 and higher density (53 X10^{4}CFU/mg), at station 7. *S. paratyphi* recorded the maximum (39 X10^{4}CFU/mg) at station 7 and the minimum (2 X10^4 CFU/mg), at station 5. *S. dysenteriae* showed the maximum (26 X10^{4}CFU/mg) at station 7 and the minimum (2 X10^{4}CFU/mg), at station 3. *K. pneumonia* showed the minimum (3X10^{4}CFU/mg) at stations 3, 5 and 9 and the maximum (24 X10^{4}CFU/mg), at station 7 (Fig.6).

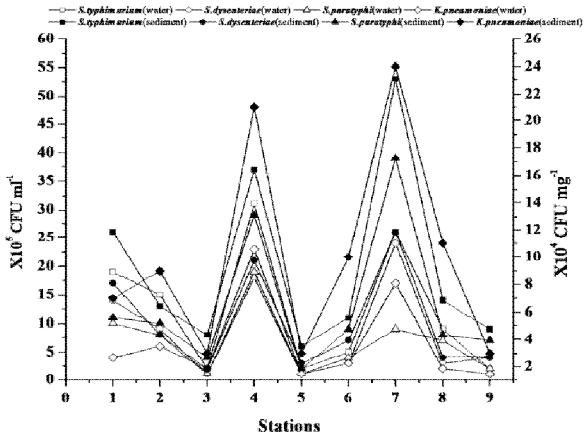

Fig.6 Population density of the species of *Salmonella, Shigella* and *Klebsiella* in water and sediment samples of the Havelock island.

Population density of *P. aeruginosa* in water was lower (1 X10^5 CFU/ml) at station 6 and higher (47 X10^5 CFU/ml) at station 7. While in the sediments, lower density (7 X10^{4}CFU/mg) was noticed at station 6 and higher density (63 X10^{4}CFU/mg), at station 7 (Fig.7).

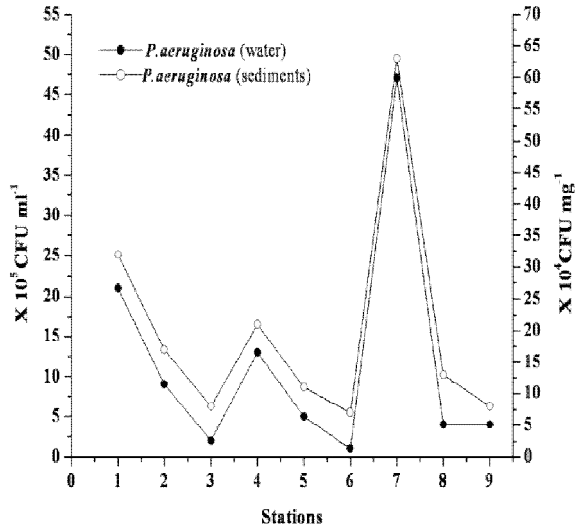

Fig.7. Population density of *P. aeruginosa* in the water and sediment samples of the Havelock island.

In the water samples, population density of *S. faecalis* was lower (5X10^5 CFU/ml) at station 5 and higher (49X10^5 CFU/ml), at station 1. In the sediments, lower population density (11 X10^{4}CFU/mg) was noticed at station 5 and higher density (106 X10^{4}CFU/mg), at station 4 (Fig.8).

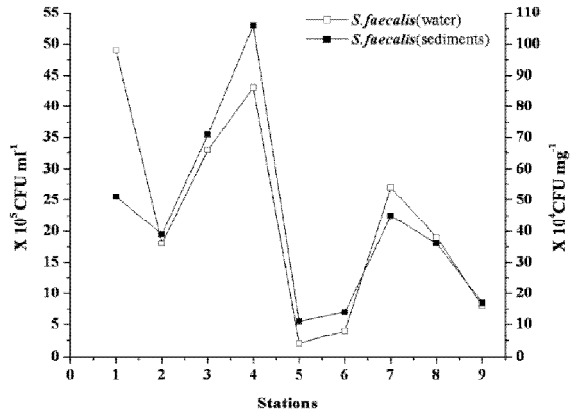

Fig.8 Population density of *S. faecalis* in the water and sediment samples of the Havelock island.

Population density of *A. hydrophila* in water was lower (2 $X10^5$ CFU/ml) at station 9 and higher (23 $X10^5$ CFU/ml) at station 7. In the sediments, lower density (5 $X10^4$CFU/mg) at station 9 and higher density (34 $X10^4$CFU/mg) at station 7 were recorded (Fig.9).

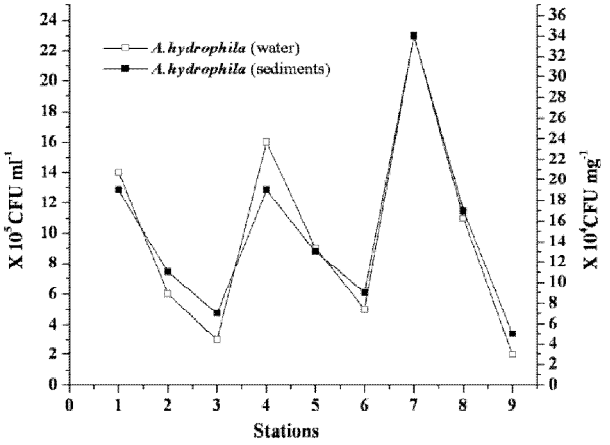

Fig.9 Population density of *A. hydrophila* in the water and sediment samples of the Havelock island.

In water, population density of *S. aureus* was found to be lower (4 $X10^5$ CFU/ml) at station 3 and higher ($33X10^5$ CFU/ml) at station 7. In the sediments, lower density of $9X10^4$ CFU/mg at station 3 and higher density of 49 $X10^4$ CFU/mg at station 7 were recorded (Fig.10).

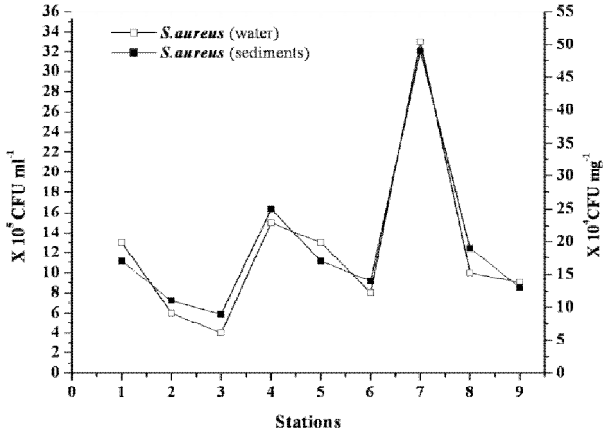

Fig.10 Population density of *S. aureus* in the water and sediment samples of the Havelock island

DISCUSSION

Many researchers have reported on the marine bacterial diversity from various parts of the world, such as Coast of Spitsbergen, Arctic Ocean (Ravenschlag *et al.,* 1999), Balltic Sea, Mediterranean Sea, Southern CaMornia Bight, Skagerrak, Weddell Sea and Andaman Sea (Hagstrom *et al.,* 2000), Southern Baltic Sea coast (Mudryk, 2005), Bay of Bengal (Surajit Das *et al.,* 2007), East and South China Sea (Hailian Du *et al.,* 2006), Northern Baffin Bay (Fouilland *et al.,* 2007), Wadden Sea of the German North Sea coast (Stevens *et al.,* 2007), Northeastern Pacific Ocean (Hongxiang *et al.,* 2008) and Sindh and Baluchistan coast of Pakistan (Uzair *et al.,* 2009), Eastern Antarctica (Yong Yu *et al.,* 2011), Kottaipattinam, Southeast coast of India (Ramkumar *et al.,* 2011) and Muthukuda Mangroves, Southeast Coast of India (Govindasamy *et al.,* 2011).

Present investigation highlights the occurrence of THB and pathogenic bacteria in the water and sediment samples, collected from nine stations along the Havelock island, the Andamans, covering mangrove, coral, seagrass and beach habitats. THB and pathogenic bactera were more in the sediments than the water samples due to the rich organic content of the former and lesser residential time of the microorganisms in the water column than the sediments (Anon, 1997), in addition to the sediments retaining substantial amounts of naturally occurring organic matter such as sugars, amino acids, phenolic substances, lipids, polypepdides, polysaccharides and other constituents of living organisms (Premuzic *et al.,* 1982); especially sediment CO_2 and pH (Yanagowa *et al.,* 2012) were favourable for microbial growth. Further, coastal and shelf sediments play a significant role in the demineralization of organic matter (Swarnakumar *et al.,* 2008) which would enhance the microbial load in the sediments.

Swarnakumar *et al.* (2008) reported that *Vibrio* was dominant followed by *Pseudomonas* and *Escherichia* in the Little Andaman island. Sahu *et al.* (2006) also isolated *Vibrio* spp. from the sediments of the coral reef environment of the Little Andaman island. In the present study, a total of 13 bacterial genera were identified: *Escherichia Pseudomonas*, *Vibrio, Aeromonas*, *Enterococcus, Cornybacterium, Salmonella, Klebsiella, Streptococcus*, *Staphylococcus*, *Flavobacterium*, *Micrococcus* and *Shigella. Escherichia* (21%) followed by *Pseudomonas* (13%) *and Vibrio* (13%) contributed more than the other genera in the Havelock island. But, Mohapatra *et al.* (2003) isolated 102 bacterial strains from three sedentary organisms of Havelock and among them *Bacillus*, *Flavobacterium* and *Micrococcus* were found to be dominant. Thus, this coastal area thronged by tourists with higher anthropogenic activity might contribute to the THB and pathogenic bacteria in addition to the other processes, indicating a higher risk of pathogens being present, as suggested by Fujioka (2001) in his study from Hawaii.

Mangrove ecosystems are the major ecosystems along the tropical coastlines. They play a vital role in regulation and optimization of marine environments (Zhang *et al.,* 2009). In this investigation, most of the THB and pathogenic bacterial communities were more at stations 1, 4 and 7, the mangrove sites (as compared to coral, seagrass and beach sites), which might act as a major nutrients transformation system

responsible for microbial activity (Alongi *et al.,* 1993; Holguin *et al.,* 2001). Further, bacterial colonies, after the mangrove litter fall, can appear shortly, grow quickly and reach very high densities (Kathiresan, 2007). As homeland of microbes, mangrove area also offers the major substrate for the proliferation of bacteria by providing with favorable conditions, due to the presence of rich sources of nutrients (Sahoo and Dhal, 2009; Lakshmipriya and Sivakumar, 2012).

Escherichia has a great genetic diversity and is disseminated all over the world (Korfmann *et al.,* 1983): Atlantic Ocean (76.8 %) (Regine *et al.,* 1998), South China Sea (55.1%) and Spain Sea (32-34%) (Barcina *et al.,* 1990). In this investigation, only 21% of *Escherichia* was found, suggesting that the coastal region of Havelock island is less polluted with *Escherichia* as compared to other parts of the world. Continuous water exchange with the oceanic waters might reduce the pathogenic bacterial density, as opined by Nallathambi *et al.* (2002) form their study from the Port Blair Bay, the Andamans.

Presence of *S. faecalis* suggests that the occurrence of such microbes in seawater could exist anytime as sewage from human or animal origin is discharged into the coast (Metcalf, 1982) and the higher THB population density (Hatha *et al.,* 2008) and fecal coliform density (Shehane *et al.,* 2005) could be due to the land run off from various sources after the rainfall. Similarly, Cheung *et al.* (1990) from the Hong Kong beach area observed the presence of *Streptococci* and used them as indicators of fecal pollution.

In conclusion, this study, to the best of our knowledge, is the first of its kind in the Havelock coast of India and this study is significant as it would pave way for future workers to elucidate the importance of coastal sanitation, for keeping the environment clean.

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