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MICROBIAL CONTAMINATION OF SEAWATER AND SEDIMENTS FROM THOOTHUKUDI COASTAL REGION, TAMIL NADU, INDIA

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Abstract: Water is essential for all living organisms. Microbial contamination of coastal ecosystem causes waterborne diseases in human beings so regular monitoring of microbial contamination is essential. In the present study, total heterotrophic bacteria, *Escherichia coli* and faecal coliform were measured in the seawater and sediments collected from three stations, namely Threspuram, Inigo Nagar and Tharuvaikulam located in Thoothukudi coastal region. The average abundance of Total heterotrophic bacteria, *Escherichia coli* and faecal coliform in the seawater are 11967 CFU/ml, 10100 CFU/ml and 207 MPN/100ml and in the sediment are 55000 CFU/g, 8733 CFU/g and 70 MPN/g in Thoothukudi coastal region. Untreated sewage and improper disposal of domestic waste could be the leading cause of high microbial content in seawater and sediments.

Keywords: Marine pollution; Coastal ecosystem; Total heterotrophic bacteria; *Escherichia coli*; Faecal coliform; Waterborne disease

INTRODUCTION

Water is a primary source of all living organisms. Hence, the pollution of the sea not only affects the marine ecosystem but also other living things on earth. The Inter-Governmental Oceanographic Commission defines marine pollution as the introduction of substances or energy into the marine environment by human beings, which harms living organisms, cause illness to humans, hamper marine activities such as fishing, affect the quality of seawater that is used or the comfort of the marine environment. There are numerous marine pollutants. Microbial contamination is one of the major marine pollutions as it could result in fatal illness. Sediments act as a repository for microbes. Karbasdehi *et al.* (2017) reported that the microbial counts in sediment are ten to hundred times higher than that of the surrounding water. The microbes present in sediments are transferred back to seawater when the sediments are disturbed by natural forces such as flood or anthropogenic forces including recreational activities. Hence, numerous studies have been done to know the microbial abundance of seawater and sediments. Vaidaya *et al.* (2001), Nallathambi *et al.* (2002), Arasamuthu *et al.* (2017), Helen *et al.* (2014) and Subramani *et al.* (2006) have investigated the microbial abundance in the coastal regions of India. Sugumar *et al.* (2008) investigated the microbial abundance of seawater and beach sand at four fish landing in Tuticorin. They reported that the count of total coliform bacteria, faecal coliform bacteria and *Escherichia coli* varied from below the detectable limit to higher than the maximum detectable limit (>140 MPN/ml for water and >140 MPN/g for sand). They also reported no significant seasonal variation and concluded that the coastal water of Tuticorin post health risk. Selvam *et al.* (2014) investigated the sediments of Tuticorin corporation. They found that the coastal regions are highly contaminated due to fishing and human waste disposal through Buckle Channel.

Untreated domestic waste dumped into the ocean directly or carried by runoff water is the leading cause of microbial abundance of marine ecosystems. The disease-causing microbes in the marine ecosystem come back to humans through the consumption of

marine products such as fish, and recreational activity such as swimming, surfing and boating. Faecal coliforms, *Escherichia coli* and faecal streptococci are a few microbial pathogens that can spread through seawater. Hence, a study on the microbial abundance of seawater and sediments is essential to prevent diseases caused by a contaminated marine ecosystem.

MATERIALS AND METHODS

Site description

The study was carried out in the coastal region of Thoothukudi, a highly polluted city in the Coromandel Coast of Bay of Bengal. Air pollution is the major environmental issue in Thoothukudi. Ministry of Environment has listed Thoothukudi as a Non-attainment city due to high particulate matter (PM) concentration. Water pollution is an equally important concern in Thoothukudi region. Environmental Information System (ENVIS, 2020) estimated that 155 and 84 lakh litres of sewages are generated by Thoothukudi municipal and Thoothukudi town panchayats respectively. The district does not have a proper system for sewage disposal. Open drainage systems are very common in this locality. Improper handling of solid waste also results in marine pollution through land runoff. Thoothukudi municipality and town panchayats respectively generate 35.1 and 23.45 tonnes of solid waste. The solid wastes are dumped at Threspuram, which is one of the study locations. Figure 1 depicts the stations were the microbial abundance were investigated. The other two study locations are Inigo Nagar and Tharuvaikulam. The latitude and longitude location of the stations are given below. This study investigates the abundance of Total Heterotrophic Bacteria, *Escherichia coli* and Faecal coliform in the seawater and sediments from the three stations mentioned in Fig. 1, which include Station 1, Threspuram (8.8163 N; 78.1636 E), Station 2, Inigo Nagar (8.7907 N; 78.1613 E) and Station 3, Tharuvaikulam (8.8887 N; 78.1738 E).

Analysis of Microbial Abundance

Sample collection and storage: Seawater samples were collected using Mayer water sampler from 30 cm under the surface, to avoid the layer of water directly affected by the ultraviolet radiation of the sun. Water samples were collected in sterile 500 ml glass vessel for bacterial analyses. Surface sediments

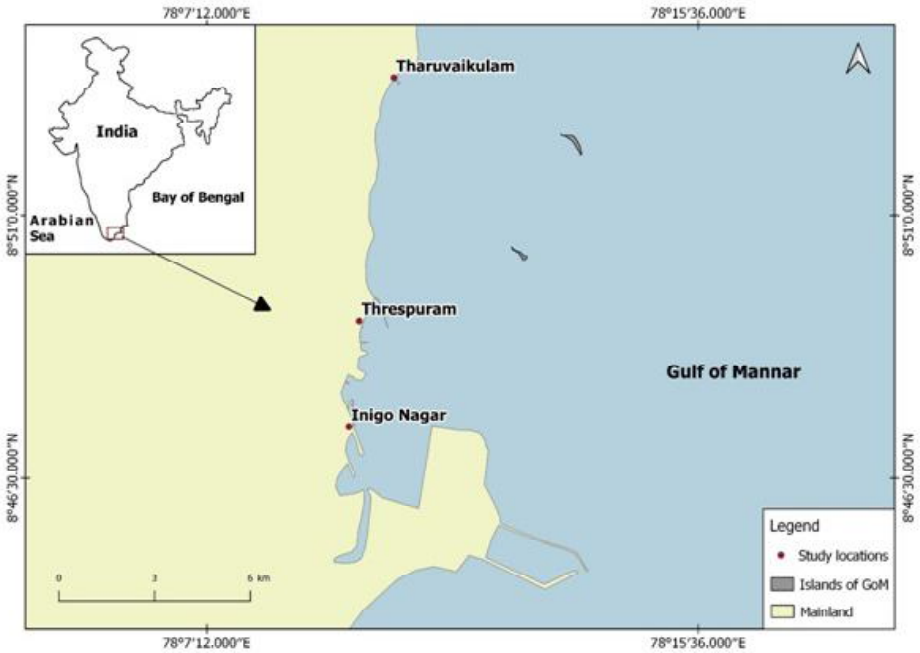

Fig. 1. Study locations

were collected from top 4 cm using a Van Veen Grab Sampler and stored in a sterile bag. Both samples were stored in a cool box (4°C) and immediately they were transported to the laboratory for analysis. The time between sampling and analysis was less than 4 h.

Preparation of Samples for Analysis: The water samples from the three stations are taken from the storage bottle and prepared separately for microbial analysis. From each of the station, 10 ml of water sample was taken and diluted serially (10^{-1} to10^{-5}) with sterile physiological saline (0.85% wt/vol NaCl) in deionized water. A 10 g sediment sample from each station was blended with 100 ml of sterile 0.1% sodium chloride for 1 min in a blender to produce a well-dispersed suspension, which was serially diluted (10^{-1} to10^{-5}). For both seawater and sediment sample, the analysis was done in triplicate and the average counts are presented in this paper.

Enumeration of Total Heterotrophic Bacteria (THB): Total heterotrophic bacteria were enumerated using the spread plate method. Petri plate containing Zobell marine agar medium (Himedia Laboratory, Mumbai) was taken and 0.1 ml of diluted sample was spread over it with the aid of an L shaped spreader. The plates were incubated at room temperature (28±2°C) for a duration of 24 to 48 hours at the inverted position. The number of colonies formed was counted and the results were expressed in colony-forming unit per millilitre (CFU/ml) for water samples and colony-forming unit per gram (CFU/g) for sediment samples.

Enumeration of *Escherichia coli:* *Escherichia coli* bacteria were enumerated by spread plate method. 0.1 ml of diluted sample was spread on Eosin methylene blue (EMB) agar medium with the help of L shaped spreader. The inoculated plates were incubated at room temperature (28±2°C) for a period of 24 to 48 hours. After the incubation, the colonies were counted as CFU/ml for the water samples and CFU/g for the sediment samples.

Faecal coliform analysis: Faecal coliform was enumerated by multiple tube fermentation technique. The multiple tube technique for the enumeration of faecal coliforms gives a result expressed as the most probable number (MPN). MPN test is performed in 3 steps. 1. Presumptive test, 2. Confirmatory test, 3. Completed test (Shariq *et al.*, 2016).

Presumptive coliform test: Multiple tube fermentation was performed in the laboratory as described by Shariq *et al.*, (2016). The medium used for this isolation was Lauryl tryptose broth. Five broth tube series - the first series containing five double-strength broth tubes and the remaining two series comprising 10 single strength broth tubes were inoculated with 10 mL, 1 mL, and 0.1 mL of water (ratio 5:5:5), respectively. Tubes were incubated at 37°C and observed at 24 and 48 h. Presumptive tests are positive for coliform if acid and gas are produced in Durham tubes.

Confirmed test: The confirmed test was done by transferring a loopful of culture from a positive tube from the presumptive test into a tube of brilliant green lactose bile broth (Oxoid Ltd., Basingstoke, UK) with Durham tubes. The tubes were incubated at 44.5°C for 24 to 48 h for faecal coliform and observed for gas production.

Completed test: A loopful of broth from a positive tube was streaked on eosin methylene blue (EMB) agar plate for pure colonies and incubated at 37°C for 24 to 48 h. Colonies with a green metallic sheen were observed. The most probable number (MPN) per 100 mL of water was tabulated.

Statistical Analysis: Two-way ANOVA was performed to find out if the variations of microbial abundance among the stations are significance. The statistical analysis tool calculates the probability value. If the probability value is less than 0.05 then the difference is considered to be significant.

RESULTS AND DISCUSSION

Microbial abundance is the introduction of microbes such as bacteria and virus into the water bodies. Even though continues monitoring of microbial abundance in seawater and sediments is difficult and expensive (Enns *et al.*, 2012; Kinzelman *et al.*, 2006), it is essential to prevent the spread of waterborne diseases. The results of microbial abundance at three stations from the coastal region of Thoothukudi are presented and discussed below.

Seawater

The microbial counts in the monitored stations are presented in Fig. 2. The total heterotrophic bacteria,

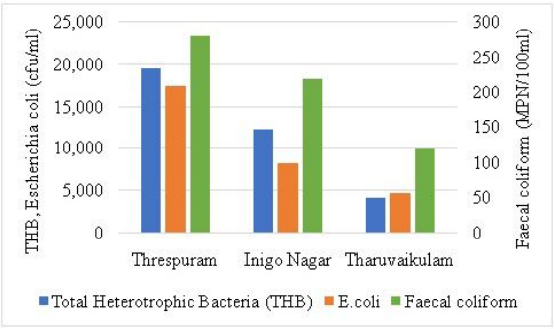

Fig. 2. Microbial contamination of water samples from three different sampling sites

Escherichia coli and faecal coliform were highest in the seawater of highly polluted Threspuram followed by Inigo Nagar and lowest at the least polluted Tharuvaikulam. With reference to Tharuvaikulam, the total heterotrophic bacteria, *Escherichia coli* and faecal coliform were 4.8, 3.8 and 2.3 times higher in Threspuram. The higher microbial abundance at Threspuram is attributed to the discharge of untreated sewage near the study location through Buckle Canal and runoff from the solid domestic waste dump site at Threspuram.

The paths through which microbial pollutants enter marine ecosystem includes untreated and partially treated sewage discharge, overflow of sewer, failure of sewage system, discharge from polluted river and estuaries, agricultural runoff, storm drains and wild birds droppings (Clark *et al.*, 1989; Vikas and Dwarakish, 2015). The source of microbial abundance could vary from one location to another (Colford Jr *et al.*, 2007). Untreated sewage increases the count of coliform bacteria, which is used as an indicator to know pollution by sewage. This is evident from the higher count of coliform bacteria at Threspuram station.

Two-way ANOVA results for the seawater contamination data are presented in Table 1. For comparison between stations, P-value is less than the critical value of 0.05 and F is higher than F_{crit}. This points out that the microbial abundance varies significantly between the stations. Physical and chemical parameters such as salinity, temperature, dissolved oxygen, pH and dissolved nutrient concentration have a major influence on the distribution of bacteria in the aquatic environment (Palaniappan, 1982). Biological contaminations have significant temporal and spatial variation. Hence, marine ecosystems require frequent monitoring of biological contaminants.

The intestine of warm-blooded animals including human have *Escherichia coli*. Hence, *Escherichia coli* is the best indicator of faecal contamination. The higher count of *Escherichia coli* at Threspuram points out that high faecal contamination of water at that location. From the measured data, it is difficult to point out if the faecal contamination is from humans, domestic animals or wildlife excreta or birds' droppings (Dickerson *et al.*, 2007; Malakoff, 2002). In general, the presence of *Escherichia coli* points out that other pathogens could be present in the water (Geldreich and Clarke, 1966). Hence in the past few decades, the quality of water is determined by faecal coliform *E. coli* levels in the water (Malakoff 2002; Pandey and Soupir 2012). But, in recent years, the use of indicator organisms to estimate the quality of water is challenged (Holt and Miller, 2010). Inability to distinguish between natural and anthropogenic causes in a few cases, scale-dependence, negative impact on endangered species which have different habitat requirement and oversimplification of complex ecosystems are the main criticisms against bioindicator.

Kaur *et al.* (2000) reported that the biological assessment is a useful alternative tool for assessing the ecological quality of an aquatic ecosystem. The biological contaminants from a marine environment reach human either through swimming in a contaminated environment or by consuming contaminated fish and other seafood. *Vibrio, Salmonella, Shigella,* and *Listeria* are a few

Table 1. Two-way ANOVA results for the microbial contamination of seawater

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between microbes	19082.89	2	9541.444	35.08601	0.002908	6.944272
Between stations	32861.56	2	16430.78	60.41961	0.001027	6.944272
Error	1087.778	4	271.9444			
Total	**53032.22**	**8**				

pathogens that cause seafood contamination. Infection by microbes during swimming in contaminated water is caused due to accidental swallowing or breathing of pathogens. Few studies have reported that coastal water adjacent to river and estuaries are highly contaminated due to high discharge from the rivers (Billen and Garnier, 1997; Ludwig *et al.*, 2009; Tilburg *et al.*, 2015). People who are bathing in such water bodies are exposed to the risk of infection by the microbes.

Sediments

Sediment ecosystems are very complex and are known to host diverse benthic organisms. Fig. 3 presents the microbial abundance of sediments in all three monitored stations. Microbial counts were higher in Threspuram and lowest in Tharuvaikulam. The abundance of total heterotrophic bacteria, E. coli and Faecal coliform in Threspuram were 92000 cfu/g, 15800 cfu/g and 170 MPN/g, the respective count in Tharuvaikulam were 15000 cfu/g, 1900 cfu/g and 70 MPN/g. In most of the sediments, microorganisms are the bountiful biomass (Fischer and Pusch, 2001; Gibbons *et al.*, 2014). Coastal sediment act as a reservoir for pathogenic microorganisms. This is evident from the higher count of heterotrophic bacteria in the marine sediments from all three stations compared to that of seawater. At Threspuram, Inigo Nagar and Tharuvaikulam, total heterotrophic bacteria levels in the sediments are 4.7, 4.8 and 3.7 times higher compared to that of the seawater. Other microbial contents are also higher in sediments than in seawater. This is due to the higher availability of nutrients in the marine sediment than in seawater. Dale (1974) pointed out that sediment enhances the microbial population due to the reduction of sunlight stress and predation. Tate (1978) and Sayler *et al.* (1975) pointed out that fine soil particle and organic content enhance the *E.coli* survival rate in sediment. The microbial community is affected by various parameters. The microbial content of sediments is affected considerably by the surrounding seawater.

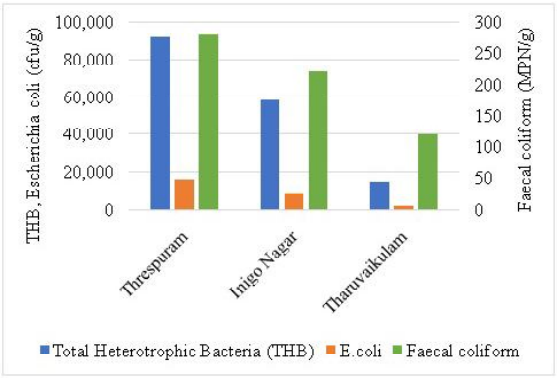

Fig. 3. Microbial contamination of sediment samples from three different sampling sites

Similar to the case of seawater, microbial abundance is high in the sediments collected from Threspuram than the other two stations. Thus, the microbial counts of sediments are influenced by the microbial count of seawater and local pollution levels. Copper and Mortia (1972) found that the temperature and salinity may affect the growth, metabolism, and survival of marine and freshwater bacteria. Table 2 presents the two-way ANOVA result for the microbial counts of marine sediments. For the comparison between stations, P-value is less than the critical value ($P< 0.05$). Hence, the microbial count variation between stations is significant.

The microbes can be from varied sources. Dissolved organic matter released into the water from the plankton may be a main source of nutrients that increased bacterial population. Swarnakumar et al. (2008) reported that *E.coli* density in the sediment of Andaman Island is mainly due to the faecal contamination from the human settlement along the coastal stretch and municipal sewage mixing. Joseph et al. (1982) stated that enteric bacteria present in the water column absorbed the soil particles that pose little danger to public health. Sometimes the re-suspension of sediment in response to storms, boat traffic, and changes in salinity and organic matter

Table 2: Two-way ANOVA results for the data presented in Table 4.5

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between microbes	8490.889	2	4245.444	12.2132	0.019801	6.944272
Between stations	16856.22	2	8428.111	24.2458	0.005807	6.944272
Error	1390.444	4	347.6111			
Total	**26737.56**	**8**				

can result in the release of absorbed bacteria into the overlying water and posing a hazard to human health. Microbial infection from a marine ecosystem can lead to respiratory tract infection, skin diseases, gastrointestinal problems and ear infection. It may also cause severe gastrointestinal and liver disorders. In a few cases, it could even be fatal. *Vibrio vulnificus* can cause acute gastroenteritis, vomit, abdominal pain and blistering dermatitis. Vibrio parahaemolyticus is known to cause bloody diarrhoea, nausea, vomiting and abdominal cramps. A bacteria group known as *Staphylococcus* may result in skin infection, bone infection, food poisoning, pneumonia and toxic shock syndrome. *Streptococcus pyogenes* may cause throat infection and fever. Dysentery, typhoid, cholera and a few other waterborne diseases are under control in developed countries, but they are still a major problem in underdeveloped and developing countries (Craun, 1991).

CONCLUSIONS

Total Heterotrophic Bacteria, *E. coli* and Faecal coliform were monitored in the sediments and seawater samples collected from three stations at Thoothukudi coastal region. Standard procedures were followed for the collection, preparation and analysis of samples. Microbial abundances were higher in sediments than in water. This is because of the availability of higher nutrition and lower sunlight stress. Among the three stations, Threspuram recorded higher microbial count both in sediments and in seawater samples. At Threspuram, the total heterotrophic bacteria, E. coli and Faecal coliform in seawater were 19600 cfm/ml, 17500 cfm/ml and 280 MPN/100ml and the respective counts in sediments were 92000 cfm/g, 15800 cfm/g and 170 MPN/g. The high microbial counts in Threspuram were due to the discharge of domestic water at Threspuram. The results were analysed using a statistical tool and significant variations were observed between stations.

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