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Isolation and Identification of Total Heterotrophic Bacteria and Human Pathogens in Water and Sediment from Cuddalore Fishing Harbour after the Tsunami

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ABSTRACT

Water and sediment samples were collected from Uppanar estuary (harbor) and analyzed the distribution of THB and pathogenic bacteria. Totally 10 strains were isolated from those samples and three strains were selected based on the growth which were identified up to species level, Viz, *Vibrio cholerae* and *Vibrio parahaemolyticus* and *Escherichia coli*. In the present study the population density of pathogenic bacteria were recorded as maximum values of *E. coli* (5.9×10^4 cuf mL^{-1}, 4.7×10^4 cfu g^{-1}), *V. cholerae* (6.3×10^4 cfu mL^{-1}, 5.7×10^4 cfu g^{-1}) and *V. parahaemolyticus* (6.2×10^4 cfu mL^{-1}, 7.3×10^4 cfu g^{-1}). After tsunami there is no detailed work in this aspect (Cuddalore fishing harbor). Having with this in mind the present study was carried out to detect the bacterial densities.

Key words: Cuddalore fishing harbor, water, sediment, THB, *Vibrio* sp., *E. coli*

INTRODUCTION

Marine ecosystem is being threatened by the discharge of unrated sewage 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. Some microbial pathogens in the coastal environment are indigenous to the oceans, including *Vibrios*. Whereas others like *E. coli*, *Salmonella* sp. and *Shigella* sp. are allochthonous which introduced through agriculture, urban surface runoff, waste water discharges and from domestic and wild animals. Most of the *Vibrios* and *Salmonella* sp. Are pathogenic to humans and some have fatal infections (Blacke *et al*., 1981; Grimes, 1975; Carlson *et al*., 1968; Gerba and Sehalberger, 1975).

Heterotrophic bacterial biomass and production in coastal waters have been reported almost from all parts of the world away from the immediate influence of rivers, the heterotrophic microorganism and autotrophic microorganism are the major agents shaping the organic composition of ocean. The heterotrophic bacterial distribution, diversity and activities are controlled by various hydro biological factors and nutrient levels present in the aquatic environment and have been well studied in marine environment (Azam *et al*., 1983; Ducklow and Hill, 1985). Distribution of bacteria depends on changes in water temperature, salinity and physicochemical parameters.

Microorganisms distributed in the marine and brackish environments play an important role in the decomposition of organic matter and mineralization (Hollibaugh *et al*., 1980). The existing

bacterial communities are likely to play very active role in the rapid *in situ* degradative process. Especially, the salinity, dissolved oxygen, pH, organic matter, nutrients and trace metals play a key role in the biological process. Temperature and pH are limiting factors for the survival of bacteria in the environment (Whipple and Rohovec, 1994). In view of their importance and involvement in the biological processes, attempts have been made in the present study to quantify THB. Their seasonal variations have also been studied in water, sediment in the study area.

MATERIALS AND METHODS

Study area: The Uppanar estuary is located at Cuddalore (Lat. 11 43' N; Long 79 49' E) on the South East coast of the peninsular India (Harbor). Field collections were carried out during July-December, 2008 at two seasons *viz,* pre monsoon and monsoon (Fig. 1).

Sample collection: Water quality parameters such as temperature, salinity and pH were monitored. Atmospheric and surface water temperatures were measured using standard mercury filled centigrade thermometer, salinity was estimated with the help of a hand Refractometer (Atago, Japan) and pH was measured using Elico pH meter (Model LC-120). The surface water sample was collected from sterile screw capped bottles for bacteriological assessment and the sediment sample was collected by employing an alcohol rinsed and air-dried small Peterson's grab, which were aseptically transferred into new polyethylene bags using a sterile spatula. All samples were brought to the field laboratory in portable icebox with in 2 h.

Bacteriological analysis: One milliliter of water sample and 1 g of sediment sample was added in 99 mL of 50% of aged sea water separately and then serially diluted using the same diluents. A 0.1 mL of the serially diluted sample was inoculated in to the Zobell marine agar and some selective media (TCBS, EMB) to enumerate the THB and also to isolate the specific pathogens. After inoculation the plates were incubated in an inverted position at a temperature of 28±2°C for 24 to

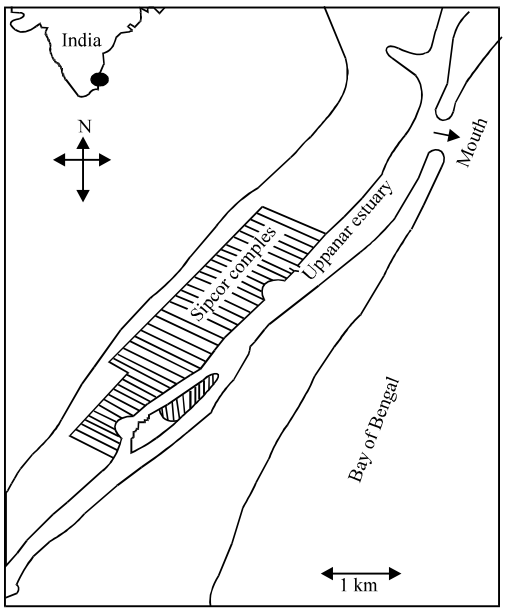

Fig. 1: Map showing the location of sampling site in Cuddalore Fishing Harbour

48 h. After incubation the colony was enumerated. Heterotrophic bacterial population was expressed as colony forming units in water (cuf mL^{-1}) and sediments (cfu g^{-1}).

RESULTS

Seasonal variation in water quality parameters: Temperature is considered one of the most important environmental factors affecting growth and survival of microorganism which ranged from (27-36.5°C) (Fig. 2). Another important factor is salinity which profoundly influences the abundance and distribution of the microorganism in the estuarine environment which ranged from (20-30.4 ppt) (Fig. 3). The hydrogen ion concentration was recorded minimum in the month of July and maximum in the month of August and November which ranged from (8-8.3) (Fig. 4). Thus the seasonal variations of water quality have been change depends upon the temperature, salinity and pH of overlying water and nature of effluent discharges from industries.

Total heterotrophic bacterial population: THB load in sediments were ranged from 11.3×10^{3} cfu g^{-1} as maximum and 3.0×10^{6} cfu g^{-1} as minimum and in water 10.5×10^{3} cfu mL^{-1} as maximum and 3.2×10^{6} cfu mL^{-1} as minimum in monsoon season. A total of 10 strains were isolated

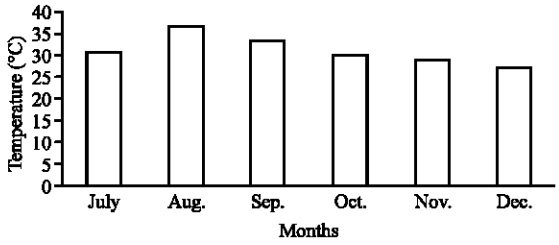

Fig. 2: Water temperature of the Uppanar estuary from the July to December 2008

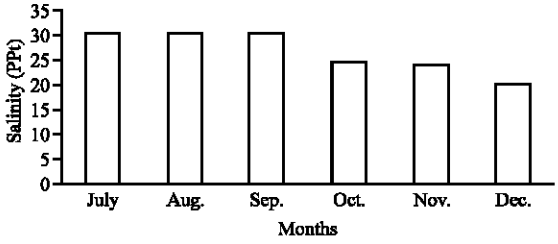

Fig. 3: Water Salinity of the Uppanar estuary from the July to December 2008

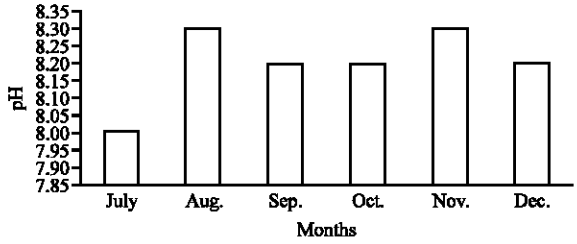

Fig. 4: pH of the Uppanar estuary from the July to December 2008

from water and sediment samples from Cuddalore fishing harbour (Fig. 5). Based on colony morphology 3 genera were identified (Table 1) viz., *V. cholerae, V. parahaemolyticus* and *E. coli* (Fig. 6, 7).

Population density of *V cholerae* in the water sample was higher ($5.1x10^4$ cfu mL^{-1}) at monsoon and lower (2.4×10^4 cfu mL^{-1}) in premonsoon whereas in case of sediment ($5.9x10^3$ cfu g^{-1}) in monsoon and minimum (2.1×10^3 cfu g^{-1}) in pre monsoon were recorded *V. parahaemolyticus* the

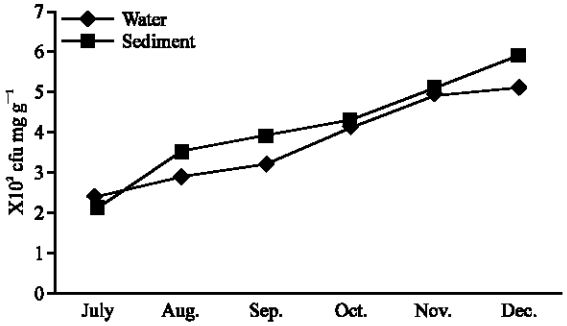

Fig. 5: Population density of *V. cholerae* in water and sediment samples collected from Cuddalore fishing harbour

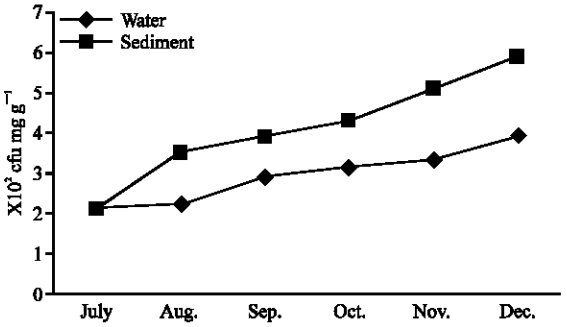

Fig. 6: Population density of *V. parahaemolyticus* in water and sediment samples collected from Cuddalore fishing harbour

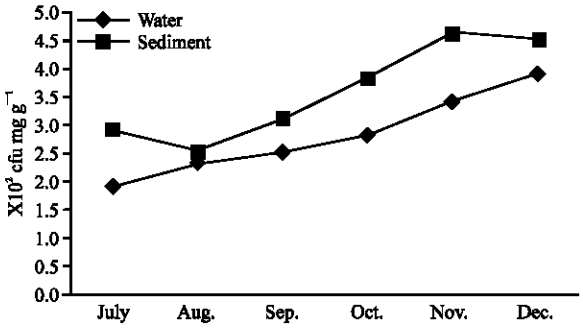

Fig. 7: Population density of *E. coli* in water and sediment samples collected from Cuddalore fishing harbour

population density was higher in water sample (3.3×10^{8} cfu mL^{-1}) at monsoon and lower (2.1×10^{8} cfu mL^{-1}) in pre monsoon but in case of sediment higher density (4.1×10^{8} cfu g^{-1}) in monsoon and the lower density (2.7×10^{8} Cfu g^{-1}) in pre monsoon were recorded. Population density of *E coli* was higher in water (3.4×10^{5} cfu mL^{-1}) in monsoon and lower in (1.9×10^{5} cfu mL^{-1}) in premonsoon and in sediment sample higher density (4.9×10^{8} cfu g^{-1}) in monsoon season and lower (1.7×10^{8} cfu g^{-1}) in pre monsoon (Fig. 8-12). Identification of pathogenic bacteria followed by Bergey's manual (Table 1).

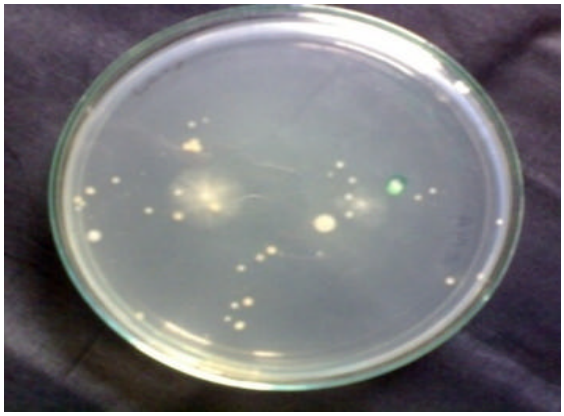

Fig. 8: The THB in Zobell's Marine Agar (Water sample)

Fig. 9: The THB in Zobell's Marine Agar (Sediment sample)

Table 1: Identification of pathogenic bacteria followed by Bergey's manual

Biochemical characteristics	*Vibrio cholerae*	*V. parahaemolyticus*	*E. coli*
Indole	+	+	+
Methyl red	+	-	+
Vogesprouskaur	±	-	-
Gramstain	-	-	-
Motility	+	+	±
Urease	-	-	-
Catalase	+	+	+
Oxidase	-	-	+

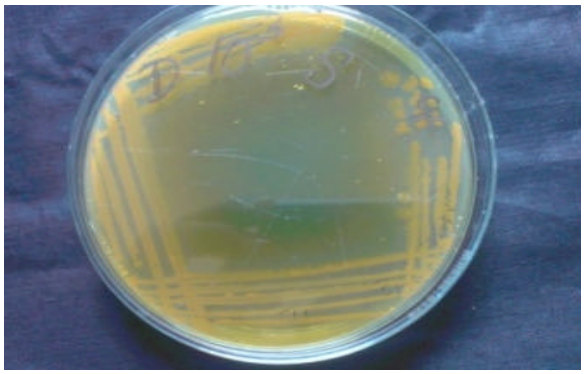

Fig. 10: The quadrant streaking of *V. cholera* (TCBS-Sediment)

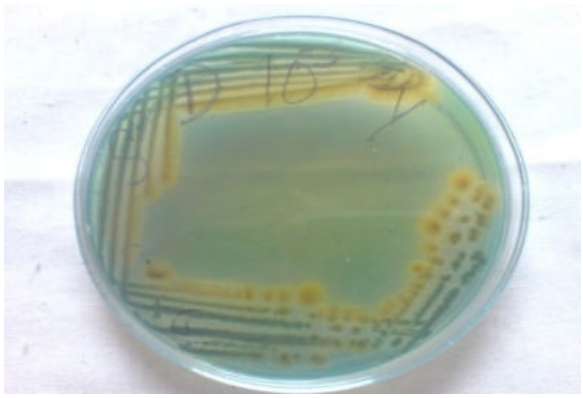

Fig. 11: The quadrant streaking *V. parahaemolyticus* (TCBS -Sediment)

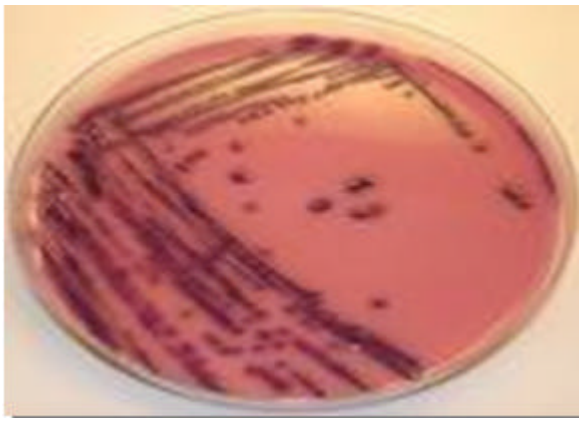

Fig. 12: The quadrant streaking of *E. coli* (EMB-Sediment)

DISCUSSION

A change in physico chemical parameters alters the microbial environment leading to alteration in microbial community. In the present study the Cuddalore fishing harbor area was affected by tsunami waves. This alteration of the environment and the microbial population of this area is identifiable from the study. Water sampled immediately after the Tsunami in 2005 showed a

reduction in TVC and in the counts of *E. coli* and *Vibrio* sp. However in the later month, the water and sediment sample showed a high in the bacterial flora (Balasubramanian, 2005). The environmental factors such as pH has low influence on the survival and proliferation of this bacterium particularly when nutrient loading is heavy in the coastal water due to discharge of sewage and industrial effluents (Velammal, 1993). Thus, the present study revealed the load of *Vibrio* was more in alkaline pH.

Present investigation highlights the ubiquitous distribution of the THB and pathogenic bacteria in the water and sediment samples collected from the Uppanar estuary. THB and pathogenic bacterial densities were higher in sediments than in water samples. Wollast (1991) reported that the coastal and shelf sediments play a significant role in the demineralization of organic matter which supports the growth of microbes. Anon (1997) also reported the higher bacterial population density in the sediments than water in generally due to the rich organic content of the former and the lesser residence time of the microorganisms in the water column than the sediments. Total heterotrophic bacterial population various from seasons to season. The high bacterial population during monsoon may be due to the rain water flow which brings huge quantities of nutrients (Martin, 1981; Sathiyamurthy *et al.*,1992). During summer the population level was maintained at low in water (Velammal, 1993; Natarajan *et al.*, 1980) also observed very low levels of pathogens in estuarine and marine waters during summer season. In addition to temperature and salinity, nutrients availability also plays a major role in the distribution of marine bacteria (Carlucci, 1974). Pathogenic bacteria such as *V. cholerae, V. parahaemolyticus* and *E. coli* have been recorded. Population density of *Vibrio* sp. in the marine environment is usually more because *Vibrios* can occur in a wide range of aquatic environments including estuaries, marine and coastal waters and sediments (Urakawa *et al.*, 2000; Thompson *et al.*, 2004). In the present investigation *Vibrios* contributed only 72% and *E. coli* contribution only 15% contribution in the coastal areas of the Uppanar estuary, which incase higher microbial population due to *Vibrio* in the coastal environment of the Uppanar estuary.

The greatest enhancement of total heterotrophic bacteria was seen in the present study when compared with the counts of total heterotrophic bacteria like *Vibrio* spp. and *E coli* in Uppanar and Vellar estuary. Jayalakshmi (1992) recorded 3.6×10^{9} cfu mL^{-1} in water and 1.5×10^{7} cfu g^{-1} in sediment during monsoon and also reported *Vibrio* sp. in the month of monsoon 6.4×10^{1} cfu mL^{-1} in water and 8.3×10^{1} cfu g^{-1} in sediment. *E. coli* also isolated in monsoon season like 8.7×10^{4} cfu mL^{-1} in water and 9.6×10^{4} cfu g^{-1} in sediment. Premalatha (2001) recorded *Vibrio* sp. 3.8×10^{4} cfu mL^{-1} in water and 3.4×10^{5} cfu g^{-1} in sediment, during monsoon season and distribution of THB in Uppanar estuary during the month of monsoon season, the higher population was observed like 8.7×10^{6} cfu mL^{-1} in water and 9.4×10^{7} cfu g^{-1} m in sediment. The early reports supports to the result obtained as and in the sediments showed it's lower of 7×10^{3} cfu g^{-1} in (summer) and higher of 46.5×10^{3} cfu g^{-1} in (Monsoon). This trend could be the rescan of Tsunami water (Swaranakumar *et al.*, 2008). In areas where there is not a lot of sunlight, bacteria thrive and maintain a good population. In the presence of sunlight the bacteria become inactive and eventually die. Visual light may be the cause rather than the ultra violet for the effect and decrease in the bacteria population (Fujioka *et al.*, 1981).

Whereas, the present study shows similar values of *V. cholerae, V. paraheamolyticus* and less values of *E. coli* when compared to them. However, the sediment was found to record higher values in the investigated areas compared to the water column. The microbial population was found to have only marginal differences between sediment and water column after tsunami (Table 2).

Table 2: Comparison of bacterial load with the earlier studies

Vibrio cholerae		*V. parahaemolyticus*		*E. coli*		
Water (cfu mL^{-1})	Sediment (cfu g^{-1})	Water (cfu mL^{-1})	Sediment (cfu g^{-1})	Water (cfu mL^{-1})	Sediment (cfu g^{-1})	Reference
8.4×10^{1}	6.3×10^{1}	7.8×10^{1}	6.3×10^{2}	8.7×10^{4}	9.6×10^{4}	Jayalakshmi (1992)
8.7×10^{6}	9.4×10^{7}	5.2×10^{3}	5.6×10^{4}	-	-	Premalatha (2001)
6.3×10^{4}	5.7×10^{4}	6.2×10^{5}	7.3×10^{4}	5.9×10^{4}	4.7×10^{4}	2007 (Present)

CONCLUSION

The present observation on the THB of Uppanar estuary revealed higher level of population in the sediment and the water column. In the present study, an increase in population density was noticed during the monsoon season (11.3×10^{4} cfu g^{-1}) this was due to the addition of terrigenous material through land run off carrying high bacterial population. This could have been achieved by eluting a large number of soil bound bacteria and transporting them into the study area.

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