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STUDIES ON HETEROTROPHIC BACTERIA AND TOTAL COLIFORMS IN RELATION WITH ENVIRONMENTAL PARAMETERS OF WATER IN GURUPUR ESTUARY, OFF MANGALURU, KARNATAKA, INDIA

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ABSTRACT

The physico-chemical and microbiological characteristics of water along Gurupur estuary Mangalore, Karnataka were studied for eight months from October 2014 to May 2015. Four stations selected in the estuary are near Kuluru Bridge (Station 1), the Konkan Seva samithi bridge where sewage discharge point is located (Station 2), the Sultan battery area which receives organic waste from few seafood processing industries (Station 3) and a confluence point of Netravati and Gurupur estuary near old port (Station 4). The minimum and maximum values of Total Heterotrophic Bacteria (CFU x 10^7/mL) varied between 0.063 to 930, 0.39 to 440, 0.01 to 65 and 0.005 to 71 at Station 1, 2, 3 and 4 respectively. Total Coliforms (MPN/100 mL) in water varied from 10 to 290, 9 to >1100, 10 to 460 and 10 to >1100 respectively at Station 1, 2, 3 and 4. The Faecal Coliforms in water (MPN/100 mL) ranged from 3 to 290, 4 to >1100, 3 to 240 and 10 to >1100 at Stations 1, 2, 3 and 4. Seasonally, THB, TC and FC counts were reduced from post-monsoon (October) to pre-monsoon (May) in all the stations. Both THB and TC exhibited positive significant correlation with dissolved oxygen. However, both THB and TC showed a negative correlation with pH, salinity and water temperature. The study on heterotrophic bacteria and total coliforms provides baseline information for decision makers and resource planners and would be a useful tool for further ecological monitoring and assessment of this estuary.

KEY WORDS: Heterotrophic bacteria, Total coliforms, Gurupur estuary, India

INTRODUCTION

Estuary is one of the most productive and complex natural ecosystem as it receives the intrusion of freshwater and seawater. Human based activities have led to the destruction of many of the natural water resources including estuaries (Aslan-Yilmaz *et al*., 2004). Estimation says that globally 10,000 million gallons of sewage, 3.25 million metric tons of oil, 10 billion tons of ballast water and millions of tons of solid waste are discharged into the marine environment per annum (Ruiz-Villarreal *et al*., 2006 and UNEP, 2011). Almost 70% of industrial discharges and 85% of waste water are discharged untreated from developing countries. While in India, as per the report from Central Pollution Control Board out of 33,000 million litre of wastewater generated every day from Class-I cities (cities with population >100,000) and class -II towns (population 50,000-100,000), of which only about 30% is collected and treatment capacity exists for less than 20% (CPCB, 2007).

Aquatic microorganisms play a significant role in the process of decomposition of organic matter, nutrient cycling and carbon flux (Edlund and Jansson, 2006; Head *et al*., 2006; Ekpo *et al*., 2012).

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The heterotrophic bacterial distribution, diversity and activities are controlled by various hydrobiological factors (Ducklow and Hill, 1985). For instance coliform, a major group of bacteria in heterotrophic family is an indicator of sewage contaminated water. Enumeration of this heterotrophic bacterial load in relation with environmental parameters is highly imperative to analyse the pollution load in the particular region. Some of the reports available on physico-chemical parameters in relation with heterotrophic bacteria in Pazhayakayal estuary, Tuticorin, India (Rani *et al*., 2012), Nethravathi Gurupur estuary (Mridula *et al*., 2014), Rajakamangalam estuary (Rajee and Palaniappan, 2006), Mullipallam creek in Muthupettai mangroves (southeast coast of India) (Ashok Kumar *et al*., 2011), Cuddalore fishing harbour (Mahalakshmi *et al*., 2011) and coastal waters of southern Kerala (Robin *et al*., 2012) revealed the extent of heterotrophic bacterial pollution along west and east coasts. But there is no report available on heterotrophic group in relation with hydrological parameters particularly in Gurupur estuary. Hence a detailed study was undertaken to investigate the effect of hydrological parameters of water on the distribution of heterotrophic load (total heterotrophic bacteria, total coliforms and faecal coliforms) of Gurupur estuary from October 2014 to May, 2015.

MATERIALS AND METHODS

The Gururpur estuary situated in Dakshina Kannada district of Mangalore (Fig. 1) is formed by the confluence of Gurupur and Nethravati river at Mangalore. For the present study four stations were selected, of which first station (S_1) was near Kuluru Bridge (12°55′31.60" N and 74°49′38.06" E). Konkan Seva Samithi Bridge, a sewage discharge point (12°54′13.41" N and 74°49′17.22" E) was the second station (S_2). The third station (S_3) near the Sultan battery area of Mangaluru (12°53′24.74"N and 74°49′17.32"E) is in the vicinity of seafood processing industries which receives fish processed waste and the fourth station (S_4) was selected near old port (Bunder) is a confluence point of Nethravati and Gurupur estuary (12°51′48.22"N and 74°49′52.82").

The water samples were collected for a period of 8 months at monthly intervals from October, 2014 to May, 2015. Temperature and pH of water were recorded from all the stations using a standard mercury thermometer and digital pH meter (WTW pH320) respectively. Other parameters like dissolved oxygen (DO), biological oxygen demand (BOD), salinity, nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonia-nitrogen (NH_4-N) and phosphate-phosphorous (PO_4-P), were analyzed following Strickland and Parson (1972).

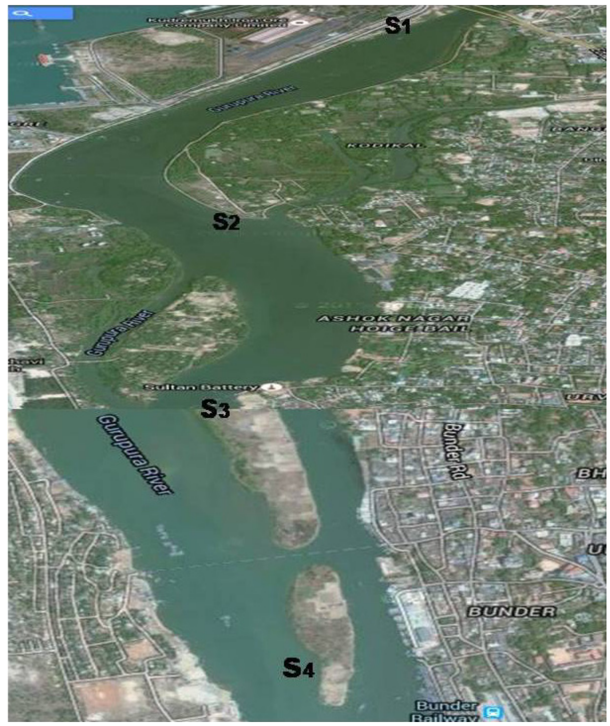

Fig. 1. Map showing the sampling stations selected at Gurupur estuary of Mangalore

The microbiological parameters such as Total Heterotrophic Bacterial Count were analyzed by using spread plate technique (Jhon and Robert, 1960) with Nutrient agar (NA) as enriched growth media and the counts were expressed as CFU/mL for water and CFU/g for sediment samples. The Total Coliforms and Faecal Coliforms of water samples were determined by the standard technique of Most Probable Number (MPN) with Lauryl Sulfate Tryptose Broth (LSTB for Total Coliforms) and *Escherichia coli* **Broth** (EC- Broth for Faecal Coliforms) as growth media and expressed as MPN/100mL and the results were compared with MPN Table.

The physico-chemical parameters and microbiological data were subjected to statistical analysis to check the relationship within and among the stations and months using (Snedecor and Cocharn, 1967).

RESULTS AND DISCUSSION

Mean and range of physico-chemical characteristics of water are presented in Table 1. Temperature is considered to be one of the most important environmental parameter affecting the growth and survival of microorganisms. During the study period the water temperature varied between 24.5°C and 32.6°C. The minimum values were recorded during postmonsoon season (Oct, 2014) and maximum values were observed during premonsoon season (May, 2015). The increasing trend in water temperature observed from post monsoon to pre-monsoon season could be due to the increased solar radiation and tidal variation (Mridula *et al.*, 2014). The lower values observed during monsoon season could be due to the strong land sea breeze and precipitation (Das *et al.*, 1997, Karuppasamy and Perumal, 2000). Similar findings were also observed by Leenagrace (2006) in Veli-lake south west coast of India and Vijaya Kumar and Vijaya (2014) in Mangrove water off Kundapur, Karnataka. pH is generally considered as an index for suitability of the environment (Rani *et al.*, 2012). During the study period the pH of water varied from 6.7 to 8.25. The pH was alkaline in all the stations with the maximum values from January to May which may be due to the uptake of carbon dioxide by photosynthesizing organisms (high biological activity) and influence of sea water penetration (Govindaswamy *et al.*, 2000; Paramshivam and Kannan, 2005). In the present study, dissolved oxygen content of water varied from a minimum of 0.1 mg/L to a maximum of 8.56 mg/L showing wide fluctuation at all the stations. Higher values of dissolved oxygen recorded during monsoon in this study might be due to the cumulative effect of higher wind velocity, rainfall and the resultant freshwater mixing (Das *et al.*, 1997). The salinity values were ranged from 0.0 PSU to 32.91 PSU. Maximum salinity was recorded during pre-monsoon (April/ May) and minimum during post monsoon (Oct).

Biochemical oxygen demand (BOD) is an indicator of the organic load and is a pollution index especially for water bodies receiving organic effluent (Vijayakumar *et al.*, 2014). BOD values ranged between 0.82 mg/L to maximum of 6.93 mg/L. The present study recorded high BOD values at St. 1 and this increase may be due to the influx of organic sewage from anthropogenic activities, wastewater discharges and/or agricultural activities.

Nutrients are considered as one of the most important parameter in the estuarine environment. It influences the growth, reproduction and metabolic activities of the living beings. Distribution of nutrients is mainly based on the seasons, tidal

Table 1. Physico-chemical characteristics of water (Mean ± SD) in Gurupur estuary from October 2014 to May 2015

Parameters	Stations			
	1	2	3	4
Temperature (°C)	27.14 ± 1.78 (25.0 – 29.7)	27.05 ± 2.02 (24.7 – 30.5)	27.44 ± 2.14 (24.5 – 31.0)	28.33 ± 1.98 (26.2 – 32.6)
pH	7.74 ± 0.43 (7.1 – 8.1)	7.71 ± 0.59 (6.7 – 8.25)	7.73 ± 0.47 (6.8 – 8.1)	7.61 ± 0.42 (6.9 – 8.05)
DO (mg/L)	6.2 ± 1.49 (4.4 – 8.56)	4.77 ± 2.22 (2.04 – 7.74)	4.80 ± 2.0 (2.82 – 8.10)	3.15 ± 2.02 (0.1 – 5.7)
Salinity (PSU)	18.94 ± 13.41 (0.0 – 32.5)	17.12 ± 12.85 (0.0 – 31.5)	21.8 ± 11.35 (1.0 – 31.6)	24.62 ± 9.45 (8.0 – 32.91)
BOD (mg/L)	5.0 ± 1.34 (3.67 – 6.93)	2.78 ± 1.45 (0.82 – 5.11)	3.28 ± 1.95 (1.2 – 6.1)	2.16 ± 1.49 (0.4 – 4.08)
Ammonia-N (µg/L)	12.03 ± 10.85 (0.35 – 32.85)	9.94 ± 8.68 (0.09 – 28.73)	4.47 ± 3.47 (0.87 – 10.20)	27.54 ± 34.9 (1.40 – 81.26)
Nitrite-N (µg/L)	0.85 ± 0.54 (0.11 – 1.37)	0.94 ± 0.74 (0.08 – 1.95)	4.94 ± 11.52 (0.08 – 33.4)	2.03 ± 2.21 (0.32 – 7.23)
Nitrate-N (µg/L)	3.93 ± 3.69 (0.24 – 11.91)	4.8 ± 2.95 (0.16 – 8.51)	10.13 ± 12.52 (0.59 – 36.3)	8.38 ± 8.22 (1.70 – 23.24)
Phosphate –P (µg/L)	1.56 ± 1.53 (0.15 – 4.6)	5.33 ± 5.96 (0.15 – 17.1)	3.69 ± 6.37 (0.15 – 19.0)	5.33 ± 5.96 (0.15 – 17.1)

* Values in parenthesis indicates range

condition and fresh water inflow from land sources. The ammonia-nitrogen values fluctuated from a minimum of 0.09 to a maximum of 81.26 μg/L. Seasonally, the ammonia-nitrogen values showed a clear temporal variation with maximum values in summer at all the stations. Minimum values observed in the month of November, 2014. Some stations have recorded irregular pattern in the distribution of ammonia values. This irregular pattern of distribution of ammonia-nitrogen may be due to the excretion by organisms, organic accumulates and oxidation of ammonia by bacterial or by phytoplankton (Leena, 2014). Nitrite content varied between 0.08 μg/L and 7.23 μg/L. NO_2-N concentration was higher in post monsoon, which could be due to the influx of municipal sewage from Kuluru sewage treatment plant, and rich organic load from port area, river water discharge and agricultural run-off. The low value during summer might be due to lack of oxygen which prevented the nitrification of ammonia and organic nitrogen (Karuppiah *et al*., 2011). Similar findings were reported in the Mangrove ecosystem of Mahanadi estuary off Odisha (Pravat *et al*., 2013), Gurupur estuary, Mangalore (Bhattacharya (1991), Adimalthura estuary southwest coast of India (Anilakumary *et al*., 2007). Nitrate is the most stable and perhaps important source for autotrophic forms. During the present investigation, nitrate values varied from and 0.16 (April) to 23.24 μg/L (Nov). The higher values recorded during the postmonsoon season (Nov) at station S_4 could be due to its location at Port area (S_4) where lot of anthropogenic activity takes place. Similar observations were also made by Muthukumaravel *et al.* (2012) from Arasalar estuary Karaikal, Damotharan *et al.* (2010) from Calimare coastal waters. The Phosphate-phosphorus values varied from 0.15 to 17.1 μg/L. The higher values were recorded at S_2 (Table 1) and it was mainly due to the influence of domestic sewage and industrial effluents in the vicinity of this station. The values recorded in this study are comparable with the studies of Neetu *et al*. (2015) in northern coastal waters of Mumbai, India, Anantharaj *et al*. (2013) in Kattumavadi coastal region, Southeast coast of India and Dayala (2014) in Cochin estuarine waters South west coast of India.

MICROBIOLOGICAL PARAMETERS

Microbiological examination of estuarine waters have a special status in pollution studies, as it is a direct measurement of deleterious effect of coastal pollution on human health through food chain. It is essential to monitor microbial population to ensure the safety of water. Microorganisms in water include several harmful bacteria which are capable of causing diseases such as diarrhoea and cholera and make potential threat to human health Reeves *et al*., (2004). These pathogenic organisms are present in the coastal waters which are contaminated by domestic sewage and other organic waste materials. The data on microbial counts in water are given in Table 2.

The total heterotrophic bacteria include all the bacteria which utilize organic carbon as their major source of energy. The THB fluctuated between 0.005 $\times 10^7$ (May) and 930 $\times 10^7$ CFU/mL (Nov). THB load was high during postmonsoon season (Nov - Dec) compared to other seasons. On the other hand, the bacterial load was low during summer season

Table 2. Microbiological parameters in water at different stations at Gururpur estuary

Stations	Parameters	Oct14	Nov	Dec	Jan'15	Feb	Mar	Apr	May
1	Heterotrophic Bacteria(CFU X 10^7/mL)	44	11.1	930	870	56	0.84	0.063	76
	Total Coliforms (MPN/100 mL)	290	210	150	95	28	43	10	23
	Faecal Coliforms (MPN/100 mL)	290	93	75	39	15	3	3	4
2	Heterotrophic Bacteria (CFU X 10^7/mL)	71	440	76	79	6.3	5.2	0.39	0.6
	Total Coliforms (MPN/100 mL)	>1100	1100	1100	460	1100	210	23	9
	Faecal Coliforms (MPN/100 mL)	>1100	1100	1100	240	210	75	4	3
3	Heterotrophic Bacteria (CFU X 10^7/mL)	0.55	65	6.9	44	4.7	0.01	0.03	0.61
	Total Coliforms (MPN/100 mL)	460	460	290	64	93	39	3	23
	Faecal Coliforms (MPN/100 mL)	240	150	42	23	43	10	3	3
4	Heterotrophic Bacteria (CFU X 10^7/mL)	0.48	7.5	8.4	71	8.4	0.007	0.06	0.005
	Total Coliforms (MPN/100 mL)	>1100	1100	460	210	120	43	3	1100
	Faecal Coliforms (MPN/100 mL)	>1100	1100	460	210	120	43	3	1100

(May). In the presence of high sunlight the bacteria become inactive and eventually die. Spatially, the THB load was high in station S_1 and S_2 while in S_3 and S_4 the bacterial load was significantly less. This could be due to the presence of high salinity near the bar mouth where only the salt tolerant bacteria can survive and proliferate (Rani *et al*., 2012 and Mridula *et al.* 2014).

Coliform bacteria are commonly used as a bacterial indicator of sanitary quality of foods and water. During the present study, coliforms ranged between 9 to >1100 MPN/100mL of water. Total coliforms were relatively higher during the premonsoon season compared to the postmonsoon season. At station S_2, higher counts of total coliforms were observed throughout the study period. This could be due to the reason that this station receives the sewage effluents from nearby sewage treatment plant which could have increased the level of total coliforms as sewage treatment plants have the capacity to treat less than 20% of the collected sewage and the rest is released raw (CPCB, 2007). Station S_2 harbours the highest distribution of total coliforms compared to S_1, S_3 and S_4. Station S_4, a confluence point of Nethravati and Gurupur estuary recorded lowest distribution of total coliforms. The reason being coliforms would not have survived in high salinities particularly under the sunlight (Ashok Kumar *et al*., 2011). Namaihira *et al* (2003) also evaluated poor water quality of Huayamilpas due to the occurrence of total coliforms, faecal coliforms and faecal Streptococci because of sporadic drainage of the domestic waste water.

Faecal coliform bacteria are the typical genera which belong to the coliform groups. These faecal coliforms are the best indicators of sewage pollution, because they originate in the intestinal tract of warm blooded animals and enter the ecosystem in the form of faeces. The faecal coliforms in the water samples varied from a minimum of 3 to a maximum of >1100 MPN/ 100 mL. Among different stations, the higher and lower faecal coliform counts were recorded at S_2 and S_4 respectively (Table 2). The higher counts at S_2 indicate that it receives maximum sewage effluents throughout the study period as a sewage treatment plant is located in the vicinity of this station. Lesser counts in station S_4 provides an indication of high salinity in which bacteria fails to proliferate and quickly die in marine waters (Anderson *et al*., 1979). Season wise, the maximum counts were recorded in post monsoon and it might be due to the release of enormous quantity of nutrient rich agricultural runoff by river discharge. These results are well compared with La Rosa, *et al*. (2001) gulf of Gaeta (Tyrrhenian Sea), Sunil *et al*. (2014) in Tapi estuary along west coast of India. The lower values in summer might be linked to the higher solar radiation which destroys more than 90% of the faecal coliform bacteria on a sunny day within the first four hours after discharge into estuarine waters.

Correlation between the Physico-Chemical Parameters and Microbial Population

Temperature and pH showed negative correlation with microbial populations as both are limiting factors for the survival of bacteria in the environment. Salinity shows negative relationship with microbial populations as salinity plays a key role in the biological processes (Ajithkumar *et al*., 2006). Correlation matrix showed a strong relationship of DO and BOD with microbial populations. 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 factors (Craig *et al*., 2004; Ortega *et al*., 2009; Borade *et al*., 2014). Fluctuations in the estuary water temperature depend on the season, geographic location, sampling time and temperature of effluent entering the stream.

CONCLUSION

Among the four stations studied, Station 2 (S_2) showed the abundance of TC, FC and THB counts thereby indicating the influence of human activities and release of domestic untreated sewage into the estuarine environment. This study provides baseline information and would be a useful tool for the decision makers and resource planners working with environmental planning and management of coastal areas in order to implement proper treatment procedure for waste water and effluents before discharging into the estuarine waters and to avoid various human disease outbreaks.

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