ECOLOGY OF TOTAL HETEROTROPHIC BACTERIAL POPULATION FROM RAMESWARAM ISLAND(PALK BAY) SOUTHEAST COAST OF INDIA.

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Abstract

The pathogeneous and opportunistic marine micro organisms may causes some sudden out breaks in the coastal environments. To monitoring the marine quality the scientists were using bacterial indicators in world-wide. Marine environments are being used as universal dustbin which has led to dreadful changes in natural resources of the coastal zone system. Total heterotrophic bacteria are the major components of marine as well as estuarine ecosystems and they play an important role in the degradation of organic matter and in the recycling of nutrients in the sea and their abundance is influenced by biotic and abiotic environmental factors. In present study, Agni Theertham (station 1) reported more bacterial load than Olaikuda (station 2) because Agni Theertham is a famous south Indian holy perennial pilgrim centre of Rameswaram Island, this area was polluted by the devotes from different parts of the country through fecal contamination and dumping. Most of the hotels and house hold wastes are discharged in to the sea and runoff water during monsoon season brings the animal's waste, human wastes that contain faecal coliforms and elevated *Vibrios* population which will contaminating the sea water causing serious outbreaks.

Keywords: Bacteriological Assessment, THB Analysis

I. INTRODUCTION

The heterotrophic bacteria are the major components of marine as well as estuarine ecosystems. They play an important role in the degradation of organic matter and recycling of nutrients in the sea[1]. Seasonal changes in bacterial and fungal population are likely to be more pronounced in the coastal environment than the offshore environment[2]. The microbial growth in the marine environment is controlled by ecological factors such as salinity, dissolved oxygen, pH, light intensity, temperature and nutrients or by environmental signals.

Pathogenic bacteria which can cause diseases like typhoid, dysentery, diarrhoea and cholera etc., are found in the coastal region. Generally, polluted water contains large number of pathogens. The most common indicator organisms are a group of microbes called as coliforms, the presence of which simply indicates that the pathogens are expected to be present[3]. As coastal communities continue to expand rapidly, accurate quantification of fecal contaminants in near shore marine waters will continue to be a pressing public health and environmental concern[4]. Fecal pollution of water resources is the problem of increasing world wide concern. Human population growth, inadequate sewage treatment systems and management of animal wastes are some of the issues associated with maintenance of supplies of clean water[5].

Maintenance of the microbiological quality and safety of water system used for drinking, recreating and harvesting of seafood was imperative, as contamination of these systems can form exact high risks to human health. It resulted in significant economic looses due to closures of beaches and shellfish harvesting areas. Waters contaminated with human faeces are generally regarded as a greater risk to human health, as they are more likely to contain human-specific enteric pathogens. Water quality surveillance programmes are important for evolving regulations on coastal environmental protection[6]. Hence the present investigation was carried out to provide the basic insights into the abundance, distribution and variations of heterotrophic bacterial populations in relation to physiochemical parameters from Rameswaram (Palk Bay) Island.

Description of the study area

RAMESWARAM ISLAND (PALK BAY).

The present investigation was carried out from **Rameswaram Island.** The Rameswaram Island is nearly 88 sq. km. bounded by the Palk Bay in North and East, Gulf of Mannar in the South and connected to the mainland by Pamban Bridge. Rameswaram is an island. It has been divided a narrow sea from the main land.For the present investigation samples were collected from Statiom-1:Agni Theertham :Statiuon 1(N.Lat.09°17'.330” and E Long. 079°19'.247”) and Station-2: Olaikuda (N.Lat.09°18.300' and E Long. 079°20.096')

For the sake of convenience and interpretation, a calendar year of study was divided into four seasons. This kind of grouping viz.

Monsoon (October – December, **Postmonsoon** (January - March)

Summer (April – June, **Premonsoon** (July – September).

II. MATERIALS AND METHODS

Collection of samples:

Collection of samples of water and sediments were made every month for a period of one year (July 2002 to June 2003) from two different stations (Agni Theertham and Olaikuda) of Rameswaram Island. For bacteriological assessment, water samples were collected in 100 ml sterile screw capped bottles just below the water surface leaving enough air space in the bottles to allow thorough mixing. Precautionary measures were taken to minimize the contamination through handling. Water samples for the estimation of physico-chemical parameters were also collected from the same sampling site.

Sediment samples were collected by employing an alcohol rinsed and air- dried small Petterson grab. The central portion of the collected sample was aseptically transferred into new sterile polyethylene bags using a sterile spatula for bacteriological analysis.

III. ESTIMATION OF PHYSICO-CHEMICAL PARAMETERS

Rainfall data of Rameswaram Island (Palk Bay) was obtained from the Meteorological Department of Pampan (Rameswaram Island). Physico-chemical parameters studied in the present investigation included air temperature, surface water temperature, salinity, pH, dissolved oxygen, inorganic phosphate, nitrate, nitrite, and silicate. Air and surface water temperature were measured in the field itself using a good grade mercury filled centigrade thermometer. Salinity was estimated with the help of a Salinometer (model E-2) and pH was measured using an Elico pH meter (model LI-120). For the estimation of dissolved oxygen, Winkler's titration method was followed[7]. Dissolved inorganic phosphate, nitrite, nitrate and reactive silicate were estimated by adopting the methods given by Strickland and Parsons (1972) (Table 1 & 2).

W-water, S-sediment

PREPARATION OF SAMPLES FOR BACTERIOLOGICAL ANALYSES From Sediment samples

A known weight of sediment (1g) was aseptically weighed and transferred to a Stoppard (150 ml) conical flask containing 99 ml sterile diluent. The sediment diluent mixture was agitated by means of a mechanical shaker for about 5-10 minutes and later subjected to bacteriological examination. Identification of the pure culture up to generic level was done with the schemes of Buchanan and Gibbons (1975).

IV. RESULT & DISCUSSION

TOTAL HETEROTROPHIC BACTERIAL (THB) POPULATION DENSITY

Total heterotrophic bacterial population density in water varied from 4.2 to 13.2 x10^6 CFUml$^{-1.}$ At station 1, the minimum population density of THB (5.4 x10^6 CFUml^{-1}) was recorded during the premonsoon season (August) and the maximum (13.2 x10^6 CFUml^{-1}) was recorded during the monsoon season (November). At station 2, the minimum density (4.2 x10^6 CFUml^{-1}) was recorded during the premonsoon season in the month of September and the maximum density (11.6 CFUml^{-1}) during the monsoon season in the month of November.

Seasonal mean population density of total heterotrophic bacteria in water

was worked out At station 1, the minimum density (5.9 x10^6 CFUml^{-1}) was recorded during the premonsoon season and the maximum density (11.3 x10^6 CFUml^{-1}), was noticed during the monsoon season. At station 2, the minimum density (5.5 x10^6 CFUml^{-1}) was recorded during the premonsoon season and the maximum density (8.53 x10^6 CFUml^{-1}), was observed during the monsoon season. In general, primary peak was observed during the monsoon season at both the stations. When compared to Olaikuda, Aghni theertham registered higher bacterial density (Table 1 & 2).

Table 1. Physio-chemical , nutrients and THB analysis data from station -1: Agnitheertham (Palk Bay) N.Lat.09°17'.330" and E Long. 079°19'.247"(2002 July- 2003June)

Parameter →	Temperature (°C)		pH	Salinity (ppt)	D.O. (mg/L)	NO_2 (µg/L)	NO_3 (µg/L)	SiO_3 (µg/)	In org. PO_4 (µg/L)	THB-W	THB-S
July 2002	32.0	30.5	8.53	30.67	6.98	0.19	2.76	12.40	0.21	165.7	118
August	31.5	31.0	8.45	32.45	7.10	0.23	3.14	11.12	0.16	165	123
September	32.0	30.6	8.55	32.64	6.72	0.24	2.17	8.07	0.17	154.9	93.1
October	30.5	29.5	8.30	28.87	4.78	0.14	1.54	8.60	0.16	192	259
November	28.5	28.0	8.26	27.90	5.26	0.18	1.91	10.20	0.36	296.2	331
December	27.0	26.4	8.10	28.32	5.88	0.23	1.59	13.60	0.24	184.3	282
January 2003	30.5	29.0	8.45	30.44	6.11	0.21	1.78	12.50	0.23	105.3	107
February	31.5	30.5	8.40	32.54	6.78	0.15	1.41	10.2	0.21	181.7	169
March	30.0	30.0	8.75	33.20	7.83	0.11	0.81	8.70	0.23	128.5	113
April	32.7	31.5	8.82	33.52	8.22	0.12	0. 98	7.62	0.18	234.4	134
May	33.5	33.0	8.50	34.79	8.75	0.14	1.47	6.03	0.16	201.8	181
June	31.5	30.6	8.40	32.86	8.48	0.21	2.34	10.09	0.19	257.1	146

Table 2. Physio-chemical , nutrients and THB analysis data from station -2:Olaikuda (Palk Bay) N.Lat.09°18.300' and E Long. 079°20.096' . (2002 July- 2003June)

Parameter →	Temperature (°C)		pH	Salinity (ppt)	D.O. (mg/L)	NO_2 (µg/L)	NO_3 (µg/)	SiO_3 (µg/L)	In org. PO_4(µg/L)	THB-W	THB-S
July 02	32.5	32.0	8.40	32.15	7.82	0.17	2.09	12.32	0.18	142	94
August	31.6	31.2	8.30	32.40	7.63	0.21	3.02	10.08	0.11	138.3	81.1
September	31.5	30.0	8.46	32.55	6.52	0.22	2.18	8.01	0.12	129.4	86
October	30.5	29.5	8.10	27.68	4.88	0.13	1.34	8.54	0.13	260	189
November	28.5	28.0	8.00	28.01	5.19	0.15	1.41	12.14	0.31	176.6	188
December	26.0	25.5	7.88	29.48	5.68	0.20	1.29	13.52	0.19	158.9	150
January 03	32.0	31.5	8.54	30.34	6.76	0.18	1.61	10.43	0.18	95.4	96.7
February	31.5	30.5	8.50	32.45	6.88	0.12	1.01	10.11	0.17	123.5	102
March	30.5	30.0	8.58	33.10	7.63	0.10	0.61	8.62	0.20	114	111
April	32.0	31.2	8.77	33.46	8.32	0.11	0.90	7.32	0.16	206	150
May	33.0	32.5	8.48	34.04	8.76	0.14	1.40	6.01	0.12	164.3	114
June	31.5	30.0	8.20	33.80	8.85	0.20	1.63	10.02	0.14	194	169

Table 3. Total Number of isolates (Gram positive and Gram Negative bacteria) from Water and Sediment samples from Station 1 & 2.

Genera	Water		Sediments		Total
	Station:1	Station:2	Station:1	Station:2	
Gram '+' ve					
Bacillus sp.	12	8	14	6	40
Staphylococcus sp.	4	3	6	6	19
Micrococcus sp.	3	1	-	1	5
Total	**19**	**12**	**20**	**13**	**64**
Gram '-' ve					
Pseudomonas sp.	14	12	16	13	55
E.coli	10	6	11	4	31
Vibrio sp.	13	8	16	9	47
Aeromonas sp.	6	2	6	-	14
Flavobacterium	-	1	-	2	3
Enterobacter	3	1	3	2	9
Salmonella sp.	1	-	3	1	5
Shigella sp.	3	-	1	1	5
Klebsiella sp.	1	-	2	1	4
Total	52	30	58	33	173
Grand Total	71	42	78	46	237

Table 4. Percentage(%) of identified Microbial flora from station 1 & 2.

Genera	Station-1	Station-2
Pseudomonas sp.	20.13	28.40
E.coli	14.09	11.36
Vibrio sp.	19.46	19.31
Aeromonas sp.	8.05	2.27
Flavobacterium	-	3.40
Enterobacter	4.03	3.40
Salmonella sp.	2.68	1.14
Shigella sp.	2.68	1.14
Klebsiella sp.	2.01	1.14
Bacillus sp.	17.44	15.90
Staphylococcus sp.	11.36	10.22
Micrococcus sp.	3.4	2.27

Population density of total heterotrophic bacteria in sediments fluctuated between 1.9 and 8.3 $x10^8$ $CFUg^{-1}$. At station 1, the minimum population density (2.3 $x10^8$ $CFUg^{-1}$) was recoded during the premonsoon season in the month of September and the maximum density (8.3 $x10^8$ $CFUg^{-1}$) was observed during the monsoon season in the month of November. At station 2, the minimum population density (1.9 $x10^8$ $CFUg^{-1}$) was noticed during the premonsoon season in the month of August and the maximum density (5.9 $x10^8$ $CFUg^{-1}$) during the monsoon season in the month of November. Seasonal mean population density of total heterotrophic bacterial in sediments was also worked out (Fig.15). It ranged between 2.1 and 7.6 $x10^8$ $CFUg^{-1.}$ At station 1, the minimum density (2.7 $x10^8$ $CFUg^{-1}$) was recorded during the premonsoon season and the maximum (7.6$x10^8$ $CFUg^{-1}$) was registered during the monsoon season. At station 2, the minimum density (2.1 $x10^8$ $CFUg^{-1}$) was recorded during the premonsoon season and maximum density (5.5 $x10^8$ $CFUg^{-1}$) was recorded during the monsoon season. In general, station 1 recorded higher THB population density than station 2. The primary peak of THB population density in sediments was observed during the monsoon season and the secondary peak was noticed during summer season.

Generic composition

A total of 237 strains were isolated from water and sediment samples collected from station 1 and 2 and identified fewer than 12 genera *i.e.; Pseudomonas* sp., *E. coli, Vibrio* sp., *Aeromonas* sp., *Flavobacterium, Enterobacter, Salmonella* sp., *Shigella* sp., *Klebsiella* sp., *Bacillus* sp., *Staphylococcus* sp. and *Micrococcus* sp. The Gram-negative group (69.20 and 68.61 % at stations 1 and 2 respectively) was more when compared to the Gram-positive group (30.8 and 31.4 % at stations 1 and 2 respectively (Table 3&4). At station 1 & 2, the maximum number of isolates belonged to the genus *Pseudomonas* (Gram-negative) and the Gram-positive bacteria *Bacillus* sp. was the predominant strain. The Gram-negative bacteria *Flavobacterium* was not recorded from station 2. In general, the Gram-negative bacteria showed a higher proportion (68.91%) than the Gram-positive bacteria (31.1%). Station 1 recorded more number of Gram-negative genera than station 2.

The marine environment is a complex system mainly influenced by a variety of physical, chemical and biological processes. One of the basic goals of ecology is to understand the factors which play their role in the distribution pattern of organisms[8]. Environmental conditions also play an important role in promoting the occurrence and abundance of commercially exploitable marine resources[9]. In the present study the microbial communities of the coastal waters were examined. It was found to be very diverse and complex. However the ecological factors such as salinity, pH, dissolved oxygen, temperature, rainfall and nutrients may influence the growth of all microorganisms. The interwoven factors for the physico-chemical parameters are involved in determining the distribution of living components of an ecosystem. Knowledge on the environment is of utmost importance to understand the distribution and colonization of marine microorganisms.

Atmospheric temperature varied between 26 and 33.5 °C at both the stations. The maximum atmospheric temperature (33.5 °C) was recorded during the summer season and the minimum atmospheric temperature (26.0 °C) was recorded during monsoon season. In general, atmospheric temperature increased during the summer and premonsoon seasons and decreased during the other seasons. This could be attributed to ocean's large thermal inertia which causes a lag between the absorption and the subsequent release of solar energy to the atmosphere[10] during the summer season. Surface water temperature was largely determined by the amount of incoming solar radiation. Water temperature variation seems to be influenced by many factors such as freshwater influx, solar radiation, warming, evaporation and cooling and mix up with the ebb flow from the adjoining neritic waters[11]. It ranged between 25.5 and 33.0 °C.

Hydrogen-ion concentration in water remained alkaline throughout the study period. Monsoon season recorded low pH while summer and premonsoon seasons recorded high pH. Station 1, recorded relatively higher pH than station 2. It may be due to the removal of CO_2 by photosynthesis, and the low pH, due to the dilution of sea water by fresh water flow during the monsoon season[12]. The pH varied between 7.8 and 8.8. The values obtained in the present study are comparable with the values reported by earlier workers[13].

Salinity influence the distribution of bacteria in tropical estuaries, bays and coastal ecosystems., since these water bodies are characterized by distinct short term (diurnal) and long term (seasonal) fluctuations.. At station 1 and 2, minimum salinity was recorded during the monsoon season and the maximum during the summer season. It varied between 27.68 and 34.79 ppt. During the monsoon period almost the rain water were added to the sea, and it reduces the salinity concentration. Mridula *et al.*[14] (2002) studied the physico-chemical parameters of Mangalore waters and reported that the salinity values were observed lower during the monsoon season. This may be due to the influence of southwest monsoon.

The quantity of dissolved oxygen in the sea water was a main factor that influences the microbial growth. Dissolved oxygen concentration was high during the summer season probably due to the photosynthetic activity of the organisms[15]. The DO ranged between 4.78 and 8.85 ml 1^{-1}. At station 1 and 2, the minimum DO recorded during the monsoon season and the maximum during the summer season. Lower dissolved oxygen concentration recorded during the monsoon season was due to the low rate of photosynthesis, high rate of oxidation of detritus and respiration of bottom communities along with the slow diffusion of dissolved gases[16] .The primary production was a main factor that controls the level of dissolved oxygen. Bhattathiri[17] (1992) reported that the primary production in most of the estuaries was less during the monsoon period due to low light intensity and dissolved oxygen.

Availability of nutrients is one of the primary factors regulating the growth, reproduction and biochemistry of all marine organisms[12]. Nutrients showed, an increasing trend from surface to the bottom particularly at farthest stations in the coastal region, which was due to their uptake at surface and demineralization in the water column[18]. In the present study the nutrients like nitrate, nitrite, inorganic phosphate and reactive silicate were analysed. Dissolved inorganic phosphorus concentration varied between 0.11 and 0.36 µg/L at both the stations. High concentration of inorganic phosphate observed during the monsoon and post monsoon seasons were due to the monsoonal flow of fresh water and land runoff. This was followed by a sudden decrease in nutrient concentration during the summer season, probably due to the utilization by micro and macro phytobenthic communities[19]. Present study nitrate concentration varied from 0.61 to 3.14 µM. At both the stations, the minimum nitrate was observed during the summer season and the maximum were recorded during the monsoon and pre monsoon respectively. Nitrite concentration in water fluctuated between 0.10 and 0.24 µM. The minimum concentration of nitrite was recorded during the summer season and the maximum concentration during the monsoon season at both the stations. Gopinath *et al.*[20] (2000) reported that nitrate concentrations were the highest and comparatively higher concentrations were observed in the bottom water of sea due to the decomposition of organic matter resulting in the release of the thermodynamically stable nitrogen species,

In the present investigation silicate concentration varied from 6.01 to 13.60 µg/L at both the stations. The minimum concentration of reactive silicate was recorded during the summer season in the month of May and the maximum concentration of reactive silicate was recorded during monsoon season in the month of December. Kannan and Kannan[12] (1996) reported that high silicate concentration was due to the addition of silica material by land run-off, which was caused by flood during the monsoon and subsequent post monsoon seasons. During the summer season, reactive silicate concentration was poor due to the sizeable reduction in the fresh water input and greater utilization of this nutrient by the marine fauna and flora for their biological activity.

In general total heterotrophic bacterial population showed an ascending trend in counts in surface waters were recorded between July and December (Premonsoon and Monsoon).This could be ascribed to southwest and northeast monsoon rains, resulting in land drainage which gets discharged into the coastal environment. This is in concordance with the findings reported by Goyal *et al.*[21] *(*1977) and Matson *et al*.[22] (1978) at various situations. They also concluded that storm water runoff bring more indicators and other bacteria, with factors accentuating their survival[3].

Pathogenic bacteria and viruses are discharged in large numbers into the sea through sewage outfalls, posing a potential health risk to consumers of shellfish bed in adjacent waters or to bathers at contaminated beaches. Although enteric bacterial concentration is rapidly reduced in the sea as a result of dilution and other factors, it has been demonstrated that the pathogens can survive long enough in sufficiently high concentration to lead to disease transmission via bathers swimming at contaminated beaches. In present study, Agni Theertham reported more bacterial load than Olaikuda because Agni Theertham is a famous south Indian holy perennial pilgrim centre of Rameswaram Island, this area was polluted by the devotes from different parts of the country through fecal contamination and dumping. Most of the hotels and house hold wastes are discharged in to the sea and runoff water during monsoon season brings the animal's waste, human wastes that contain faecal coliforms and elevated *Vibrios* population.

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