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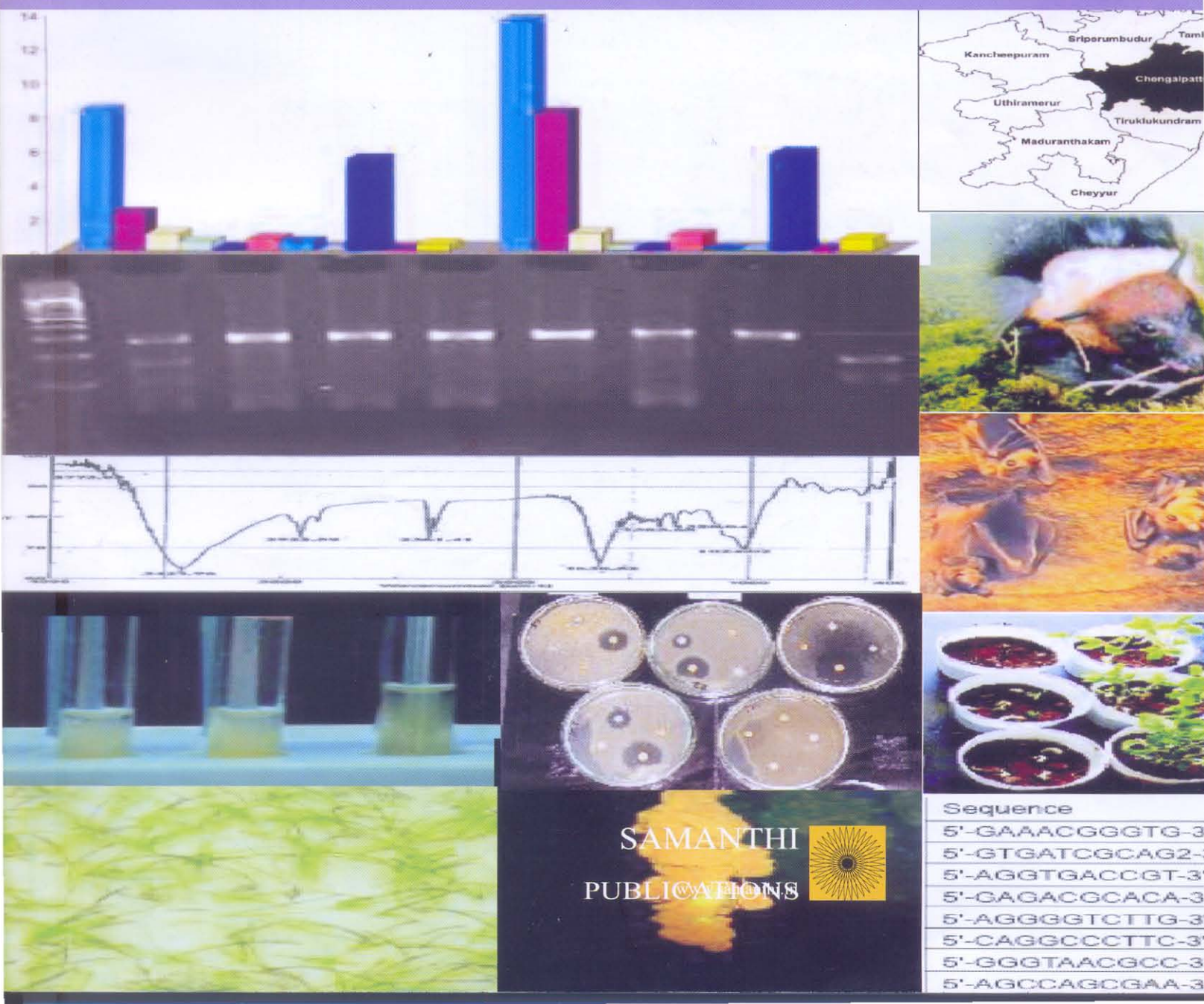

SAMANTHI PUBLICATIONS

Seasonal Variations and Identification of Pathogenic Pollution Indicators from Royapuram Backwaters

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Abstract

Sewage and industrial effluents from Ennore , Chennai harbour and surrounding area have a greater impact on the Royapuram back water affecting the aquatic animals and in turn the human population. These waste carry enormous number of microbial pathogens and other heavy metals resulting in greater economic loss.The current study is aimed at analyzing the total viable count of bacteria and pathogenic bacterial species in the water and sediment samples taken from different places of Royapuram fishing area, from where many kind of fishes and molluscans are taken for human consumption .The samples were collected in pre monsoon, monsoon and post monsoon seasons. Water analysis was done by multiple tube tests to assess the MPN values. The bacteria were isolated using Zobell's agar medium,selective and non selective medias and identified using biochemical test. The organisms were identified to genus, level according to Bergey's manual. Results of field investigations showed a definite association between the levels pollution and microbial population. Results showed a higher distribution of pathogenic and non pathogenic bacteria in the sediment than in the water sample both in monsoon and post monsoon seasons. Analysis showed the prevalence of human pathogens and fecal indicator organisms like Escherichia coli, Salmonella sp., Vibrio sp.,Shigella sp. in water and in the sediment samples.

Introduction

Water quality, is the combination of properties that are manifested in relation to human, other living creatures, items and substances. Coastal water bodies are contaminated by the sewage and industrial effluents which ultimately affects the plant ,animals and human population. 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 andindustrial effluents. 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).

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 are always present in water and also in sediment. The pathogenic ones were often and are still the cause of serious epidemics. Bacteriological analysis of water is a rather difficult and prolonged process. Basically water analysis is done for seeing the presence of feacal indicator bacteria which might indicate the presence of other human pathogenic bacterial population of intestinal origin. Marine bivalves accumulate large number of bacteria from the immediate environment due to its filter feeding nature, including gram negative *Achromobacter, Aeromonas, Alcaligenes, Flavobacteium, Pseudomonas* and *Vibrio* organisms and gram positive Bacillus , *Corynebacterium* and Micrococcus organisms. (Jan A. Olafsen *et al* 1993) The mechanism of uptake of coli form bacteria by various bivalves is well established but the effect of environmental factors and physiological activity of the shell fish to retain aquatic microorganism is not known (Cabelli & Heffernan 1970). Filter feeding shellfish such as scallops and oysters tent to concentrate bacteria from the over lying water (Slanetz. L. W. *et.al* 1968). Land drainages , domestic sewage outfalls , and other discharges alter the abundance and type of both autochthonous and allochthonous microbial populations in the near shore environments (Marchand 1986 ; Patti *et al* .1987).

The current study is made in Royapuram which is located near chennai in Tamil nadu at 13° 6' 26" North, 80° 17' 43" East. Sewage and industrial

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effluents from Ennore, Chennai harbour and surrounding area have a greater impact on the Royapuram back water affecting the aquatic animals and in turn the human population. These waste carry enormous number of microbial pathogens and other heavy metals resulting in greater economic loss.The current study is aimed at analyzing the total viable count of bacteria and identification of predominating pathogenic bacterial species in the water and sediment samples taken from different places of Royapuram fishing area, from where many kind of fishes and molluscans are taken for human consumption .

Materials and Methods

Sampling was done during premonsoon ,monsoon and post monsoon seasons of 2010. The surface water sample was collected from sterile screw capped bottles for bacteriological assessment and the sediment sample was collected and aseptically transferred into sterile polyethylene bags using a sterile spatula. All samples were brought to the laboratory in portable icebox with in 2 hours.

Multiple Tube Test

Presumptive test

Most probable number test was done with three dilutions of the sample. The dilutions used were 10ml, 1 ml and 0.1ml . Each dilution requires five Macconky broth media tubes, thus the sample is inoculated in five replicas of each dilution. In the presumptive test, the samples are inoculated, each in 10ml of double strength broth and two sets of single strength tubes. After 24-48 hours of incubation at 37°C and the results are noted based on acid production and/or gas production in the tubes.

Acid production during the fermentation was noted by the change in the dye color indicating the pH change. Small tubes, called Durham's tubes, was used to collect the gas bubbles formed during the fermentation. The medium in the inverted Durham's tubes within the test tubes are replaced by the gas produced, thus enabling the observation and the vaules were compared with MPN standard chart Growth from the presumptive test tubes. The presence of fecal indicator organism *Echerichia Coli* is also checked by inoculating in to two tubes of brilliant green lactose bile broth and incubated one tube at 44.5° C and another tube was incubated at 37° C for 24 hrs.

Confirmed test

The presence of fecal indicator Coli form, *Escherichia coli* is further confirmed by inoculating in EMB from the Positive tube of MacConkey broth and followed by completed test

Completed test

The isolated bacteria from EMB were taken to nutrient agar slant and Lactose broth with durham's tube for acid and gas production. Gram's staining was done with the growth on the nuritient agar slant

The water and sediment samples were also serially diluted , plated in Zobells agar media. Nutrient agar with different composition of NaCl and some selective media 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 48 h.

Predominant 4-5 organisms were isolated and identified. From the positive presumptive tube samples were taken to Macconky agar media for other Coliforms identifications.

Isolation and Identifiction

Individual colonies from ZoBell's agar plates ,Nutrient agar media and Macconky agar plates were randomly selected and subcultured. After purification the identification of bacteria present in all the samples was carried out using classic microbiological techniques starting with Grams reaction, followed by motility, type of flagella (Leifson *et.al.,* 1964) and IMVIC. Oxidative or fermentative metabolism was determined with methods of Hugh & Leifson (1953). The fermentation of sucrose, lactose and mannitol was done by a standard method. (Martin & Washington 1980) The organisms were identified to generic or group level according to Bergey's manual (Buchunan and Gibbons 1974). Selective and non Selective medias were also used for identification.

Results and Discussion

The quantitative result from Table 3 shows that the number of bacteria in Sediment is more than that of the water sample from Royapuram. The reason is that during monsoon the flow of water and the mixing up the domestic sewage in to the estuary water is more. During monsoon total heterotrophic bacteria in water is highest, whereas in other seasons it is comparatively less. Total heterotrophic bacteria 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 varies 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 pre monsoon the population level was maintained at low in water Natarajan *et al.*, (1980) also observed very low levels of pathogens in estuarine and marine waters during summer season.

Table 1 and 2 shows more numbers of most propable numbers of total coliforms ,which is much higher than the permissible numbers. Most of the pathogenic Coliforms and *Escherichia coli* were present in all the samples analyzed. *Flavobacteriun* Sp. and *Enterobacter* sp. are considered to have little sanitary significance and are common in surface run off *Klebsiella* sp. is ubiquitous and may be found in waters receiving carbohydrate rich effluents.

The presumptive test shows more number of total coliforms. Presence of bottle green colour colonies with metallic sheen on EMB in the

S No.	Sample 1			Sample 2			Sample 3		
	1,10ml	2 ,1ml	3,.1ml	1,10ml	2 ,1ml	3,.1ml	1,10ml	2 ,1ml	3,.1ml
1	+	-	-	+	+	+	+	+	+
2	+	+	-	+	+	-	+	+	+
3	+	+	-	+	+	-	+	+	-
4	+	+	+	+	+	-	+	+	-
5	+	+	+	+	+	+	+	+	+
MPN values	552= 540*			554=1600*			553 = 920*		

Table :1 . Most probable number water sample
*Most propable number of Coliforms in 100 ml of water.
Sample 1 Pre monsoon, Sample 2 Monsoon, Sample 3 Post Monsoon
+ Acid and Gas produced, - No Acid and Gas produced

S No.	Sample 1			Sample 2			Sample 3		
	1,10ml	2,1ml	3,1ml	1,10ml	2,1ml	3,1ml	1,10ml	2,1ml	3,1ml
1	+	-	-	+	-	-	+	-	-
2	+	-	-	+	-	+	+	-	+
3	+	-	-	+	-	-	+	+	-
4	+	+	-	+	+	+	+	+	-
5	+	+	-	+	+	-	+	+	-
MPN values	551= 350*			552 = 540*			545 =430*		

Table: 2. Most Probable Number Sediment sample
*Most propable number of Coliforms in 100 ml of water.
Sample 1 Pre monsoon, Sample 2 Monsoon, Sample 3 Post Monsoon
+ Acid and Gas produced , - No Acid and Gas produced

Sample	Colony forming Units of bacteria per ml of the sample - premonsoon	Colony forming Units of bacteria per ml of the sample - monsoon	Colony forming Units of bacteria per ml of the sample - post monsoon
Water Sample	48×10^6	89×10^8	$59x\ 10^7$
Sediment Sample	56×10^7	59×10^8	64×10^7

Table : 3. The quantitative results of the number of bacteria in water and sediment sample in pre monsoon, monsoon and post monsoon seasons

All figures represent an average of 10 samples.

confirmed test and Gram negative bacteria in the completed test reveals the presence of feacal coliforms mainly *Echerichia coli* which indicates feacal contamination during all the seasons. Sewage contamination of aquatic habitats is detected by enumerating the coliform groups of bacteria (Fujioka, 2002).

Shell fishes like mussels, oysters, crabs and other fishes that are sold in the Chennai market are taken mostly from Ennore and Royapuram backwaters where considerable fresh water dilution occurs with high nutrient load. The heterotrophic bacterial species in estuarines are characterized by the mixture of marine, fresh water and soil which are adapted to organic rich environment (Kueh and chan 1975)

Mussels, oysters and other shell fishes that are harvested from these areas are often eaten raw, or smoked with minimal cooking, which emphasize on the fact that less sterilsation of the animal is done. Microbiological analysis is therefore necessary on a continuous basis for realizing the impact of sewage and effluent discharge affecting these animals.

Microbiologist rely on the principle that higher the incidence of sewage indicator bacteria in any environment, higher would be the chances for human pathogenic bacteria to be present (Brock *et al.*1994: Fujioka 2002). As is universally accepted ,higher sewage contamination would lead to increase number of coliform in natural water body and also in the parts of animal living in these water bodies.

The present study shows the presence of bacterial species listed in table 4. If the fishes and shell fishes consumed from theses area is not properly cooked or sterilized, the presence of, *Shigella* sp. may lead to food poisoning. Presence of *Streptococcus* sp. may lead to meningitis and skin infections. *Aeromonas* sp. may give rise to septicemic conditions. *E.coli* contamination may lead to gastric disorder.

Consumption of raw oyster infected with *Vibrio* sp. may lead to severe gastroenteritis with abdominal cramps, vomiting, fever and diarrhea. Consumption of *Vibrio* contaminated oyster, crabs and mussells may lead to primary *septicemia, cellulites, cholera* etc and may even be fatal. Predominance of *Vibrio* and *Aeromonas* sp. have been observed in the digestive tract of oysters and fishes (Okuzumi & Horie 1968; Sera *et al.* 1974).

It is well under stood from the bacteriological analysis of water and sediment, that Royapuram water is mixed heavily with domestic sewage, faecal contaminants and industrial waste regularly. This study would be useful in managing the water pollution occurring in the area. So maximum effort has to be enforced by the government to stop or to reduce this kind of pollution resulting in the welfare of aquatic animals and in turn to human beings.

	Pre Monsoon		Monsoon		Post Monsoon	
Bacterial species	Water	Sediment	Water	Sediment	Water	Sediment
Shigella Sp.	+	-	+	+	-	_
Escherchia coli	+	+	+	+	+	+
Vibrio Sp.	_	+	_	+	_	-
Bacillus Sp.	_	+	_	+	-	+
Aeromonas Sp.	+	+	+	+	+	-
Salmonella Sp.	+	_	+	_	+	_
Flavobacrerium Sp.	+	-	+	_	+	_
Alcaligens Sp.	+	+	+	+	+	+
Pseudomonas Sp.	+	+	+	+	+	+
Streptococci.Sp.	+	-	+	+	+	+
Micrococcus Sp.	+	+	+	+	+	+
Proteus Sp.	+	+	+	+	+	+
Klebsiella Sp.	-	-	+	+	+	+

Table :3 . The quantitative results of the number of bacteria in water and sediment sample in pre monsoon, monsoon and post monsoon seasons

All figures represent an average of 10 samples.

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