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Isolation and Identification of Enteric Pathogenic Bacteria in Kadinamkulam Estuary, Kerala

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Abstract: ***Bacterial load in surface and bottom waters of, Kadinamkulam estuary, Trivandrum District was evaluated and the results are included in this paper. Bacterial components such as total viable count (TVC), total coliforms (TC), faecal coliforms (FC) and total enterobactreiacae (TE) and the presence of few potentially pathogenic forms like Vibrio cholerae (VC), Vibrio parahaemolyticus (VP), Shigella spp. (SH), Salmonella spp.(SAL), Escherichia coli (E.coli), Faecal Streptococci (FS), Pseudomonas aeruginosa (PA), Proteus and klebsiella spp.(PKL) were isolated and identified. Three regions (head, mid, end) were identified and water was collected from surface and bottom of the estuary and collections were made during all the three seasons. The microbiological analysis of both surface and bottom waters of the estuary was made. The distributional patterns of these bacteria were estimated. The results clearly revealed that Kadinamkulam estuary is highly polluted with enteric pathogenic and indicator bacteria. The highest mean TVC was (14280- 26810 cfu/ml) for mid estuary, (7985-12878 cfu/ml) for end estuary and (5201.25-6563 cfu/ml) for head estuary, were obtained for surface and bottom waters respectively, during the premonsoon season for Kadinamkulam estuary.***

Keywords: Faecal coliforms, Enteric pathogens, Pollution, Kadinamkulam estuary

1. Introduction

Microorganisms in aquatic environment affect man either positively or negatively so also directly and indirectly,in other words, these minute living forms may be beneficial or harmful to man, his activities as well as other natural resources. Aquatic ecosystem is the highest organization in the hierarchy of life. It covers individual organisms in the food chain, population, energy flow, biochemical cycles, and interaction between living, nonliving recourses as well as human impact are involved in all these activities hence man should take cognizance of microorganisms in sustainable development (Tonie Victoria *et al.*, 2002). Bacteria found in the shore and depth of the seas including the hyper saline dead sea with salinity above 35‰, these are the extremely halophilic bacteria namely *Halobacterium halobium* (Haruhico *et al.*, 1995). Some microbial pathogens in the coastal environment are indigenous to fresh water, estuary, including *Vibrios' spp*. whereas others like *Escherichia coli, Salmonella spp. and Shigella spp.* are allochthonous which introduced through agricultural, urban surface runoff, waste water discharges and from domestic animals. Most of the *Vibrios spp.* and *Salmonella spp*. are pathogenic to humans and some have fatal infections with *Vibrios' spp.* (Blake *et al.*, 1980). The current study is aimed at analyzing the total viable count of bacteria, total coliforms, total enterobacteriaceae, feacal coliforms, *Vibrio cholerae, Vibrio parahaemolyticus, Salmonella spp, Shigella spp. E.coli, Pseudomonas aeroginosa,* Feacal *Steptococci* in the water samples collected during three seasons from three regions namely head, mid and end regions of Kadinamkulam estuary. Indicator organisms are commonly used to assess the microbiological quality of surface waters and faecal coliforms (FC) are the most commonly used bacterial indicator of faecal pollution. They are found in water that is contaminated with faecal wastes of human and animal origin. Total coliforms (TC) comprise bacterial species of faecal origin as well as other bacterial groups (e.g. bacteria commonly occurring in soil and water). The coliforms are indicative of the general hygienic quality of the water and potential risk of infectious diseases from water. Infections with *Vibrios* are known to be associated with either consumption of seafood or exposure to marine environment (Raveendran *et al*., 1990).

2. Materials and Methods

Kadinamkulam estuary (Fig.1), (NL 8°35' to 8°382 and EL 76°52') is categorized under the bar-built/closed/blind estuary.This estuary is the centre for activities like fishing, aquaculture, coconut husk retting, tourism, transportation, sand mining, dumping of waste, estuarine reclamation etc. The sand bars of this estuary will be either open due to monsoonal floods or in some cases they are cut open manually and establish connection with the sea. Three different zones of the estuary such as the fresh water zone (head estuary), brackish water zone (mid estuary) and the bar mouth region (end estuary) were identified for microbial study. Water samples were collected for isolation and identification of bacterial strains from these three regions of the Kadinamkulam estuary for a period of one year from 2012-2013.

H- head M- mid E-end

Figure 1: Kadinamkulam Estuary

The surface and bottom water samples were collected from the backwater in sterile screw capped bottles for bacteriological assessment. All samples were brought to the laboratory in portable icebox within two hours of collection. The samples were analyzed for T VC (total viable count), TC (total coli forms), FC (faecal coli forms), FS (faecal *Streptococci*), TE (total enterobacteriaceae), *E.coli, Proteus and klebsiella spp. Shigella spp. Pseudomonas aeroginosa spp. Salmonella spp. Vibrio cholera* and *Vibrio parahaemolyticus.*

Readymade media (Himedia) were used for the isolation of different bactesrial strains for total viable count (TVC) total coliforms (TC), faecal coliforms (FC) and Faecal streptococci (FS). Total coliforms were screened using MacConkey agar (M081), and the plates were incubated for 24 hrs at a temperature of 37°C; the count was performed considering the pink-red colonies. Faecal coliforms were identified using M-FC agar (M1122) with rosolic acid, incubated for 24 hr at 44.5±0.2°C, characteristic colonies had blue colour. The medium M-Enterococcus agar (M1108) used for the identification of pink to red colour colonies of faecal *Streptococci*. Cetrimide agar M1742 was used to identify *Pseudomonas aueroginosa*, Tergitol 7 M616 was used for *E.coli*. Total enterobacteriacae were identified by using the agar violet red bile glucose agar MH581. Nutrient agar Nm011 was used for total viable count. After the filtration and the incubation of the plates at 37°C for 48 hr, the pink-red colonied plates, plated in duplicate, in order not to miss the lower limits. *Vibrio cholerae* (VC) and *Vibrio parahaemolyticus* (VP) were identified using thiosulphate citrate bile sucrose agar (M189). After incubation yellow and green colour colonies were enumerated respectively as VC and VP. The medium xylose lysine deoxycholate agar (M031) was used for the identification of *Salmonella spp*. (SL), *Shigella spp*. (SH) *Proteus and Klebsiella spp.* (PKL *).*Characteristic red colour colonies were selected as *Shigella spp.*, red colonies with black centre were *Salmonella spp*. and yellow coloured colonies were *Proteus and Klebsiella spp*. Characterization of each group was determined by APHA (1995) and APHA (1995) methods and all indicator bacterial groups after identification was used as colony forming unit (cfu/ml).

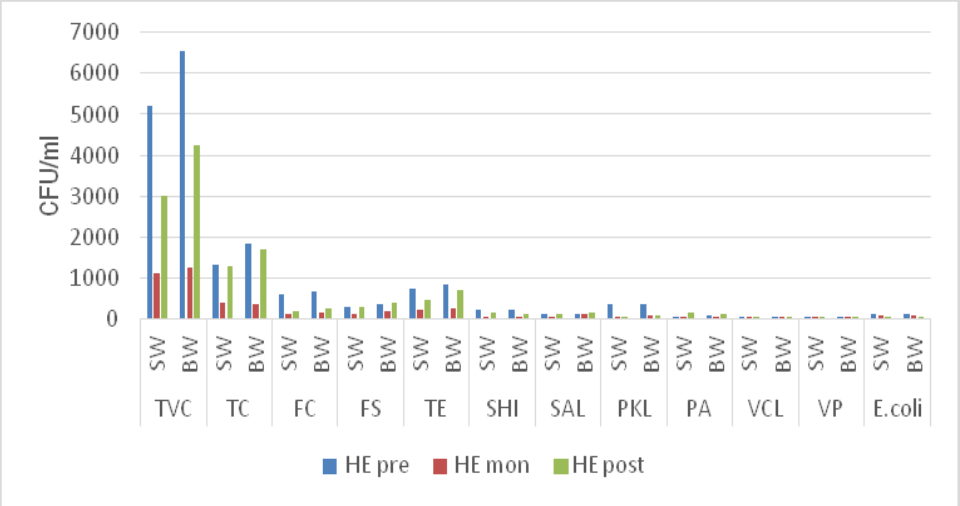
3. Results and Discussion

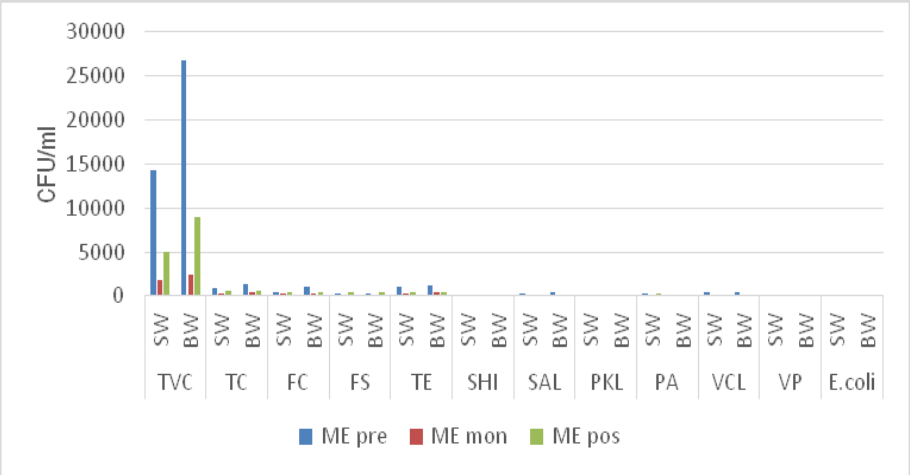
Bacterial load of Kadinamkulam estuary is studied. Seasonal variation of bacterial load in water was analyzed. The estuary is divided into three zones namely the head estuary (olygohaline region), mid estuary (brackish water region) and end estuary (barmouth region) for the bacterial contaminants evaluation and the sampling was done for 12 months of three seasons. The results clearly revealed that of the three zones examined, the mid estuary had the highest bacterial load and this may be attributed to the retting activity, so also the different discharges and untreated sewage enter into the estuary due to anthropogenic interferences. The data on the isolation and identification of enteric pathogenic bacteria present in the three regions of Kadinamkulam estuary to three regions and three seasons are presented in Table 1 and Fig. 2.

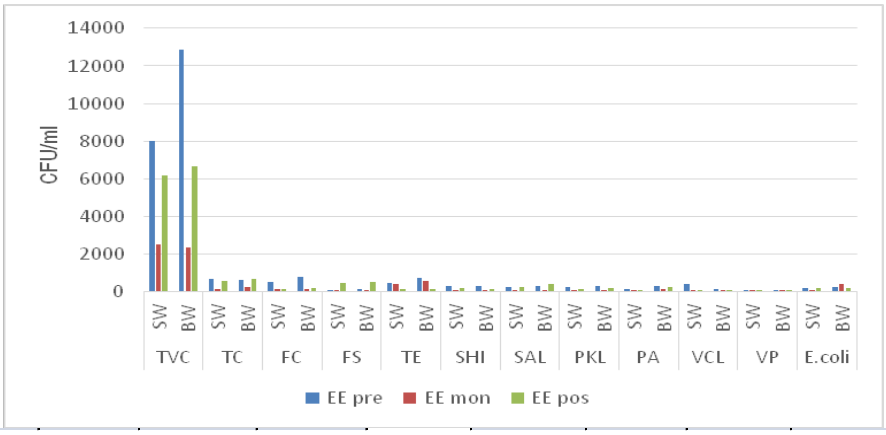
The highest mean value of total viable count (TVC) was noted in premonsoon season for both surface and bottom waters and the values ranged from 1112 cfu/ml to14280 cfu/ml and 1241.75 cfu/ml to 26810 cfu/ml. When TVC reaches 1X 10 ^{6}cfu/gm or milliliter of product the product is presumed to be at or nearing spoilage. Vanderzant *et al.* (1971) noted a bacterial count of 5- 5.5 X 10^{-6} cfu/gm from pond and their values are much higher than that recorded during the present study, so also the studies of Chandrika and Nair (1944). The total coli form count for Cochin backwaters was much higher than that noted during the present study.

Table 1.Bacterial componants in Kadinamkulam estuary										
BACTERIA		**HE pre**	**HE mon**	**HE post**	**ME pre**	**ME mon**	**ME pos**	**EE pre**	**EE mon**	**EE pos**
TVC	SW	5201.25	1112	3012.75	14280	1747.25	5057.75	7985	2507.5	6174
	BW	6563	1241.75	4248.75	26810	2381	8957.75	12878	2325	6657.5
TC	SW	1297.75	387.5	1296	885	298	528.25	691	111.75	541.5
	BW	1831.25	339.5	1704.75	1297.75	465.25	616.75	602.75	214.75	654.5
FC	SW	594.25	99.75	167.75	501.5	257.5	498	492.5	108.5	140
	BW	651.25	160.25	238.5	991.5	308.75	510	764	156.25	176.5
FS	SW	287.75	103	270.5	225	115.75	448.25	89.75	61	453
	BW	350.5	184.5	399.25	229.25	131	391.75	149.25	44.25	534.75
TE	SW	743.25	229.5	464.25	1041.75	358	482.5	465.5	402	127.75
	BW	830	249.25	709	1193.5	445	493.75	749.5	545.75	148.5
SHI	SW	204.5	25	152.5	31.5	51.5	56	272	10.5	171
	BW	196.25	56.75	107.5	63.75	92.5	92.5	271.25	17	153.5
SAL	SW	95.75	50	119.75	361	155.5	78.25	261.5	17.5	247
	BW	122.5	123.5	156.75	457.5	216.5	78.75	268.5	61	416.75
PKL	SW	343.25	38.5	44.25	18	40.5	109.25	218.25	45	143.75
	BW	356.25	59.75	69	55	78.5	87.5	272	43.75	158
PA	SW	39.5	37.25	129.25	307.25	99.25	297	150	93.5	101
	BW	78	41.25	114.75	189	76.5	194.25	294	141.5	234.5
VCL	SW	33.5	16.25	12.5	475.5	15.75	12.5	386	7	49.75
	BW	41.75	26	9.5	486	38.75	9	117.75	7.75	57.5
VP	SW	13	8.5	13.75	71.5	27.25	7	23.75	9.5	33.25
	BW	13.5	12.25	7	12.5	31	7	29.25	14.5	34.75
E.coli	SW	117.5	83.5	22.25	148.25	88.5	21.25	158	65.75	199.5
	BW	119.75	60.25	24.75	200	106.5	28.75	215	374	202

TVC- Total viable count,TC - Total coliform,FC - Faecal coli form,FS- Faecal *streptococci* ,TE- Total enterobacteriaceae,SHI- *Shijella* ,SAL- *Salmonella*
PKL - *Proteus* and *Klebsiella* ,PA- *Pseudomonas auerginosa* ,VCL - *Vibrio cholera* , VP- *Vibrio parahaemolyticus* , E.coli- *Escherichia coli*
Sw- Surface water, BW - Bottom water, HE - Head estuary,ME- Mid estuary,EE - End estuary,PRE- Pre monsoon,MON- Monsoon,POST- Post monsoon







TVC- Total viable count,TC - Total coliform,FC - Faecal coli form,FS- Faecal *streptococci*,TE- Total enterobacteriaceae,SHI- *Shijella* ,SAL- *Salmonella*
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Sw- Surface water, BW - Bottom water, HE - Head estuary,ME- Mid estuary,EE - End estuary,PRE- Pre monsoon,MON- Monsoon,POST- Post monsoon

Figure 2: Bacterial contaminants in Kadinamkulam Estuary

In the case of total coliforms (TC) for both surface and bottom waters the highest values were 1297.75 and 1831.25 cfu/ml and was noticed from the head region of Kadinamkulam estuary during the premonsoon season whereas, the lowest values were 111.75 and 214.75 cfu/ml for both surface and bottom waters recorded from the end region during the postmonsoon period. In the case of faecal coliforms (FC) the highest values were 594.25 and 651.25 cfu/ml for both surface and bottom waters and was noticed from the head region of Kadinamkulam estuary during the premonsoon season whereas, the lowest readings were 99.75 and 160.25 cfu/ml for both surface and bottom waters noticed from the head region during the monsoon period. In the case of faecal *Streptococci* (FS), both surface and bottom waters had the highest values of 453 and 534.75 cfu/ml and was noticed from the end region of Kadinamkulam estuary during the postmonsoon season whereas, the lowest values were 89.75 and 149.25 cfu/ml for both surface and bottom waters recorded from the end region during the premonsoon period. In the case of total enterobacteriaceae (TE) for surface and bottom waters the highest values were 1041.75 and 1193.5 cfu/ml and was noticed from the mid region of Kadinamkulam estuary during the premonsoon season whereas, the lowest values noticed were 127.75 and 148.5 cfu/ml for both surface and bottom waters obtained from the end region during the postmonsoon period. In the case of *Shigella spp.* (SHI) for both surface and bottom waters the highest values were 272 and 271.25 cfu/ml and was noticed from the end region of Kadinamkulam estuary during the premonsoon season whereas, the lowest valuess were 10.5 and 17 cfu/ml for both surface and bottom waters recorded from the end region during the monsoon period.

In the case of *Salmonella spp*. (SAL) the highest values were 261.5 and 268.5 cfu/ml evaluated for both surface and bottom waters and was noticed from the end region of Kadinamkulam estuary during the premonsoon season whereas, the lowest readings were 17.5 and 61 cfu/ml for both surface and bottom waters recorded from the end region during the monsoon period. In the case of *Proteus and Klebsiella spp*. (PKL) for both surface and bottom waters the highest values were 343.25 and 356.25 cfu/ml and was noticed from the head zone of Kadinamkulam estuary during the premonsoon season whereas, the lowest values were 18 and 55 cfu/ml for both surface and bottom waters recorded from the mid estuary during the premonsoon period. For both surface and bottom waters, *Pseudomonas aeuroginosa* (PA) had the highest values 307.25 and 189 cfu/ml and was noticed from the mid region of Kadinamkulam estuary during the premonsoon season on the other hand, the lowest values were 39.5 and 78 cfu/ml for both surface and bottom waters recorded from the head estuary during the pre monsoon period. In the case of *Vibrio cholerae* (VCL) for both surface and bottom waters sthe highest values were 475.5 and 486 cfu/ml and was noticed from the mid region of Kadinamkulam estuary during the premonsoon season whereas, the lowest readings were 7.0 and 7.75 cfu/ml for both surface and bottom waters noticed from the end region during the monsoon period. In the case of *Vibrio parahaemolyticus* (VP) the highest values were 71.5 and 12.5 cfu/ml and were noticed from the mid region of Kadinamkulam estuary during the premonsoon season for both surface and bottom waters however, the lowest values were 7.0 and 7.0 cfu/ml for both surface and bottom waters evaluated from the mid region during the postmonsoon period. In the case of *E.coli* for both surface and bottom waters the highest values were 199.5 and 202 cfu/ml and was noticed from the end zone of Kadinamkulam estuary during the postmonsoon season whereas, the lowest values were 21.25 and 28.75 cfu/ml for both surface and bottom waters recorded from the mid region during the postmonsoon period.

Microbial load was lower during monsoon season and it may be attributed to the river run off and the influence of sea water. According to (Viera *et al.*, 2003) two groups of bacteria are of public health interest *i.e.* bacteria naturally present in the environment such as *Aeromonas spp.* and enterobacteriaceae such as *Salmonella spp., Shigella spp,* and *Escherichia coli, Vibrio spp.* which originates from contamination of the water with human residue. It is a well known fact that bacterial load is very much high in the bottom substratum than in the overlying water. Number of organisms in the water both for surface and bottom varied from species to species. Of all the indicator bacteria; TVC reported their mean value superiority. The maximum value

for total count was found from mid region of Kadinamkulam estuary (14280 cfu/ml premonsoon) for surface water and (26810 cfu/ml) bottom water also during premonsoon season for the mid region. The high occurrence of these bacterial componants indicated the increase in the human induced activities in the near coastal zone and riverine discharge sources. The present results are in good agreement with that of Sudhanandh *et al*. (2010b) and (Patra *et al*., 2009). The higher incidence of TC than FC and FS was not surprising, since TC can originate from non faecal sources and they can be reduced down to the faecal coliforms and the faecal *Streptococci* (Kistemann *et al*., 2002; Pathak and Gopal, 2001). In the case of VC, in addition to the high mean value of 310 cfu/ ml, the maximum value of 820 cfu/ml was also reported from Cochin estuary. It was observed that Vibrios were preferential in waters rich in organic nutrients (Sudhanandh *et al*., 2010a), and it is expected in areas heavily impacted by land runoff and wastewater discharges and the same is true in the case of Kochi, according to the authors. The FC, FS, SHI and SAL were comparatively absent towards offshore especially 5 and 10 km from shore and showed a low population, forming cluster as per the studies of Sudhanandh *et al.* (2010a). It may also be suggested that as the salinity increases which makes the environment more and more marine the organisms tolerant to low salinities become destroyed resulting in low counts (Sudhanath *et al.*, 2012). Counts were significantly high for both FS and FC in surface and bottom waters during the present study which are known to receive faecal pollution from various point sources including in and around, Kadinamkulam estuary. It is also known that Kadinamkulam is subjected to severe human activities in the form of bathing, fishing, coir mill, coconut husk retting and other such anthropogenic interferences. The high enteric pathogens will exert deleterious long term impact on public health, local fisheries and tourism potential, of this precious estuary, if not adequately addressed.

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