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Distribution of potentially pathogenic enteric bacteria in coastal sea waters along the Southern Kerala coast, India

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Abstract

This study evaluated the relationship between the traditional indicators of faecal pollution, total coliforms (TC), faecal coliforms (FC) and *Faecal streptococci* (FS), and the presence of few potentially pathogenic enteric bacteria, *Vibrio cholerae* (VC), *Vibrio parahaemolyticus* (VP), *Shigella* spp. (SH) and *Salmonella* spp. (SL) in coastal sea water. The distributional statuses of these bacteria were also studied along the Southern Kerala coast. Cluster analyses were done to identify similar groups of indicator as well as enteric pathogenic bacteria. Kochi was found to be highly polluted with enteric pathogens and indicator bacteria (TC of 4700, VC of 820, FC of 920 and FS of 410 CFU ml^{-1}). Percentage incidence of VC (97.42%) was comparatively higher than the traditional indicator bacteria (TC 95.04%, FC of 63.64% and FS of 47.64%). VC found to be rather stable and showed significant relationship with all the traditional indicator bacteria ($R^2 > 0.370$), suggests that both quantitatively and qualitatively the abundance of *Vibrio cholerae* can determine faecal pollution, could be used as a faecal pollution indicator bacterium, especially in the marine environment where traditional indicator bacteria failed to survive. It would be advisable to always perform the detection of SH and VP beside the traditional indicators as no significant relationship ($R^2 \leq 0.076$, $p>0.05$) exists among them.

Key words

Faecal pollution, Pathogenic enteric bacteria, *Vibrio cholerae,* Southern Kerala coast

Introduction

Faecal contamination of coastal waters is a paramount concern in tropical developing countries (Byamukama *et al*., 2005). Worldwide coliform bacteria are used as indicators of faecal contamination (El- Naggar *et al*., 2003; El-Shenawy and Farag, 2005) and hence, the possible presence of disease causing organisms (Tyagi *et al*., 2006; Sabae, 2006; Rosenfeld *et al*., 2006). However, studies comparing survival of fecal coliforms and pathogenic bacteria in estuarine and coastal seawaters have frequently yielded conflicting results (Martha and Howard, 1988), because faecal coliforms die off rapidly in sea water (Bordalo *et al*., 2002; Hrenovic and Ivankovic, 2009). In addition, in recent years, the reliability of total coliforms as indicators of faecal pollution and pathogens has been questioned; in particular, the densities of faecal coliforms do not necessarily coincide with known sources of faecal contamination or the presence of enteric pathogens (Gabutti *et al*., 2004; Townsend, 1992). Furthermore, it has been shown that in tropical aquatic environments, classical faecal indicators are suspected to proliferate and thus be detectable at levels which do not reflect the original extent of fecal contamination (Desmarais *et al.*, 2002; Solo-Gabriele *et al.*, 2000), or even worse, they become an autochthonous part of the aquatic microbial community in the tropical waters considered (Byamukama *et al.*, 2005). Therefore, it is important to understand the potential and limitations of these indicator organisms before realistically implementing guidelines and regulations to safeguard our water resources and public health.

However, examination of water column for the presence of bacteria indicatory of the sanitary state and of pathogenic enteric bacteria may supply information on water quality in the nearest future (Albinger, 1992), that might be useful for an effective environs of this state which is currently experiencing intense aquaculture, industrial and urbanization activities. The adequacy of faecal coliforms as indicators for faecal pollution in tropical and subtropical regions has also not been properly demonstrated. Apart from two previous studies (Ouseph *et al*., 2009; Hatha *et al*., 2004), no systematic studies have been carried out on the microbial pollution of this important natural water body during the last decade. This study was carried out to document current levels of pollution indicator bacteria as well as some groups of human pathogenic bacteria in different areas in both the estuaries and offshore waters of Southern Kerala coast, India. The study also focused on understanding the possible relation between density of faecal indicators and presence of enteric pathogens.

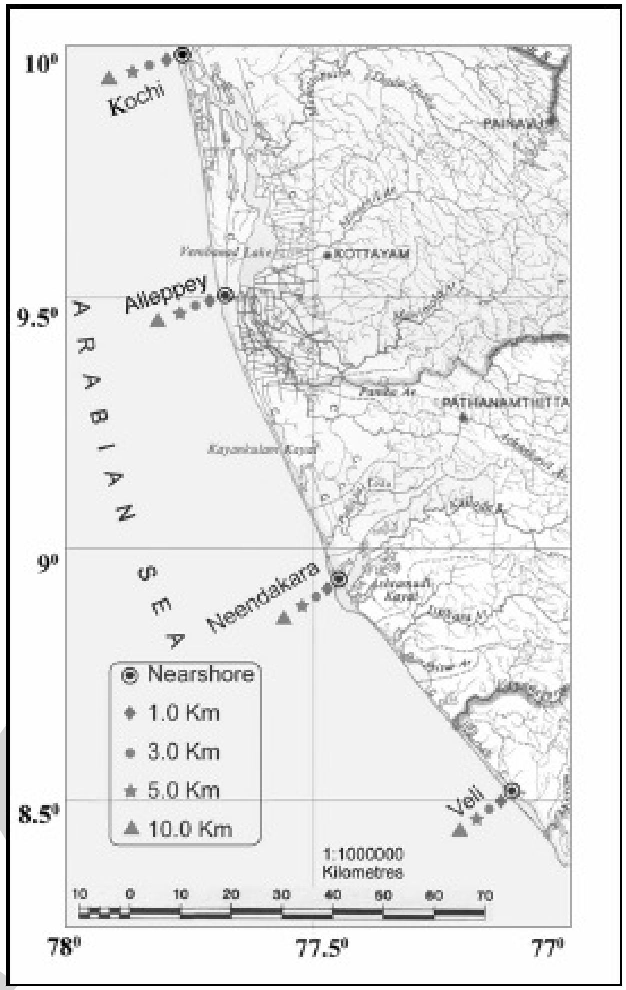

Fig. 1: Study sites along the Southern Kerala coast, India

Materials and Methods

Study area, sample collection and identification of bacteria: Four stations (Fig. 1) were selected *viz.* Veli, Neendakara, Alleppey and Kochi. Sampling was carried out at 0 km (near shore), 1, 3, 5 and 10 km from shore, for a period of 1 year (2008-2009) during the cruises of *Sagar Purvi*, the coastal research vessel of Ministry of - Earth Science, Government of India. One hundred twenty nine water samples were made from depths varying 3 to 50 m (<m-surface; 3 or <10 m-both surface and bottom; 10 or <10m-surface, mid and bottom) using Niskin water sampler and aseptically transferred into sterilized glass bottles. Duplicate sampling and analysis were performed for each depth and station.

Readymade media (Himedia) were used for the isolation of different faecal indicator organisms *viz.*, total coliforms (TC), faecal coliforms (FC) and *Faecal streptococci* (FS) were carried out using the membrane filtration method (MF): this is based on the filtering, under negative pressure, of the water sample through a cellulose acetate membrane with a porosity of 0.45 µm and connecting the inox steel filtration unit to an aspiring pump. 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) and rosolic acid, incubated for 24 hr at 44.5±0.2°C, characteristic colonies had blue colour. The medium m-Enterococcus agar (M081) was used for the investigation of FS. After the filtration and the incubation of the plates at 37°C for 48 hr, the pink-red colonies were considered as positive.

Spread plate technique was adopted for all the pathogenic bacteria. Various aliquots ranging from 0.2 to 1.0 ml were 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) and *Shigella* spp. (SH). Characteristic red colour colonies were selected as SH and red colonies with black centre were SL. Characterization of each group was determined by APHA (1995) method and all indicator bacterial groups after identification expressed as Colony forming unit ml^{-1} (CFU ml^{-1})

Statistical analysis: The data were elaborated statistically using the soft ware package SPSS 18. In cases in which bacterial levels were below the detectable limit a value of zero was assigned for statistical analysis. The mean and standard deviation were calculated, and regression analyses were performed to analyze significant relationship. Analysis of variance (ANOVA) was carried out to determine if there were differences in pattern of bacterial population between stations. Cluster analyses were done to identify similarity.

Results and Discussion

Similarity groups among the indicator as well enteric pathogenic bacteria were achieved by cluster analysis, resulted a dendrogram (Fig. 2), grouping all the seven bacterial parameters into three statistically meaningful clusters. Generally microbial parameters in the water column followed a decreasing trend in values from onshore to offshore, as might be expected. However, reduction in population dynamics towards offshore varied from species to species. Of all the indicator bacteria, TC (Fig. 3) reported their numerical superiority especially towards onshore than rest of the indicator bacteria and form the first cluster. For the parameter TC, the maximum value was found from Kochi estuary (4700 CFU ml^{-1}), highest medium value was also reported from the same site. They showed a mean variation of 140 CFU ml^{-1} (Veli) to 655 CFU ml^{-1} (Kochi) with an overall mean of 355 CFU ml^{-1} throughout the study. It indicated the increase in the human induced activities in the near coastal zone and riverine discharge sources (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 got narrowed down to the faecal coliforms and the faecal streptococci (Kistemann *et al*., 2002; Pathak and Gopal, 2001; Harwood *et al*., 2001; Vaidya *et al*., 2001). Cluster 2 includes VC and VP (Fig. 4A) which produced a moderate population throughout the study.

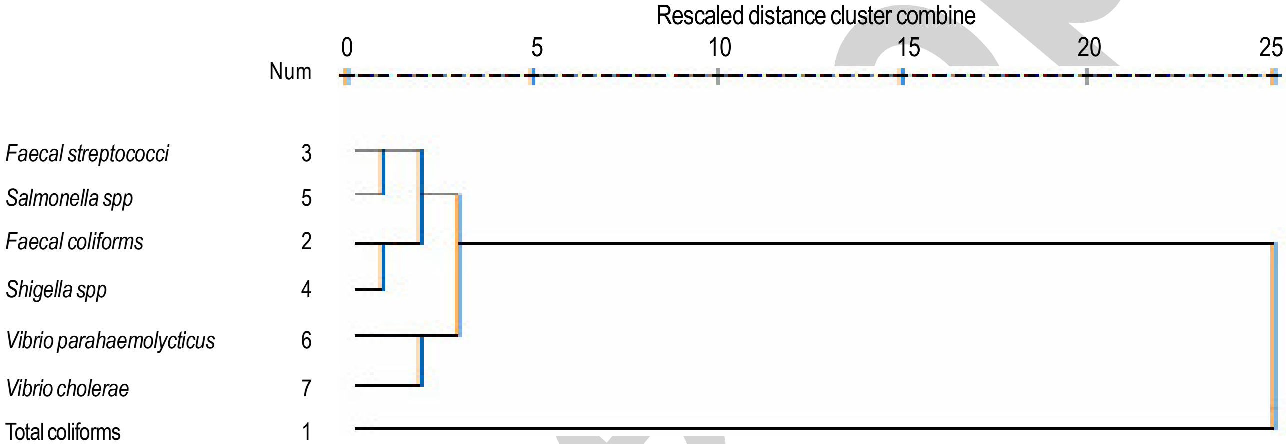

Fig. 2: Dendrograph showing similarity group clusters for faecal indicator bacteria and enteric pathogens

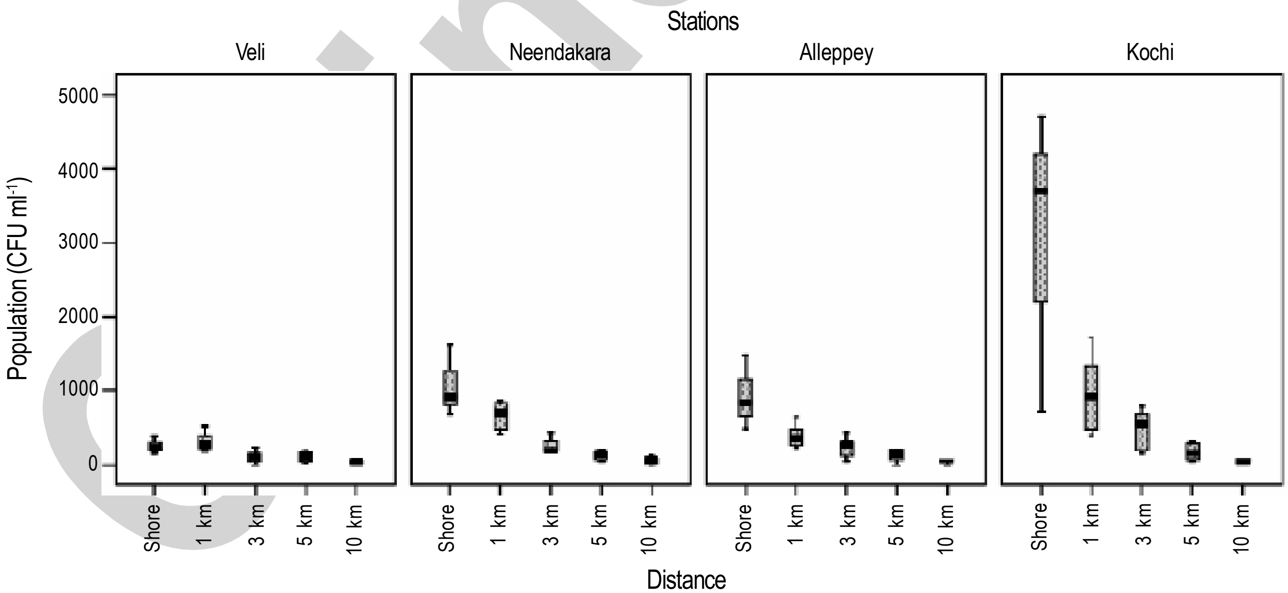

Fig. 3: Box plot showing the variation of total coliforms along the Southern Kerala coast

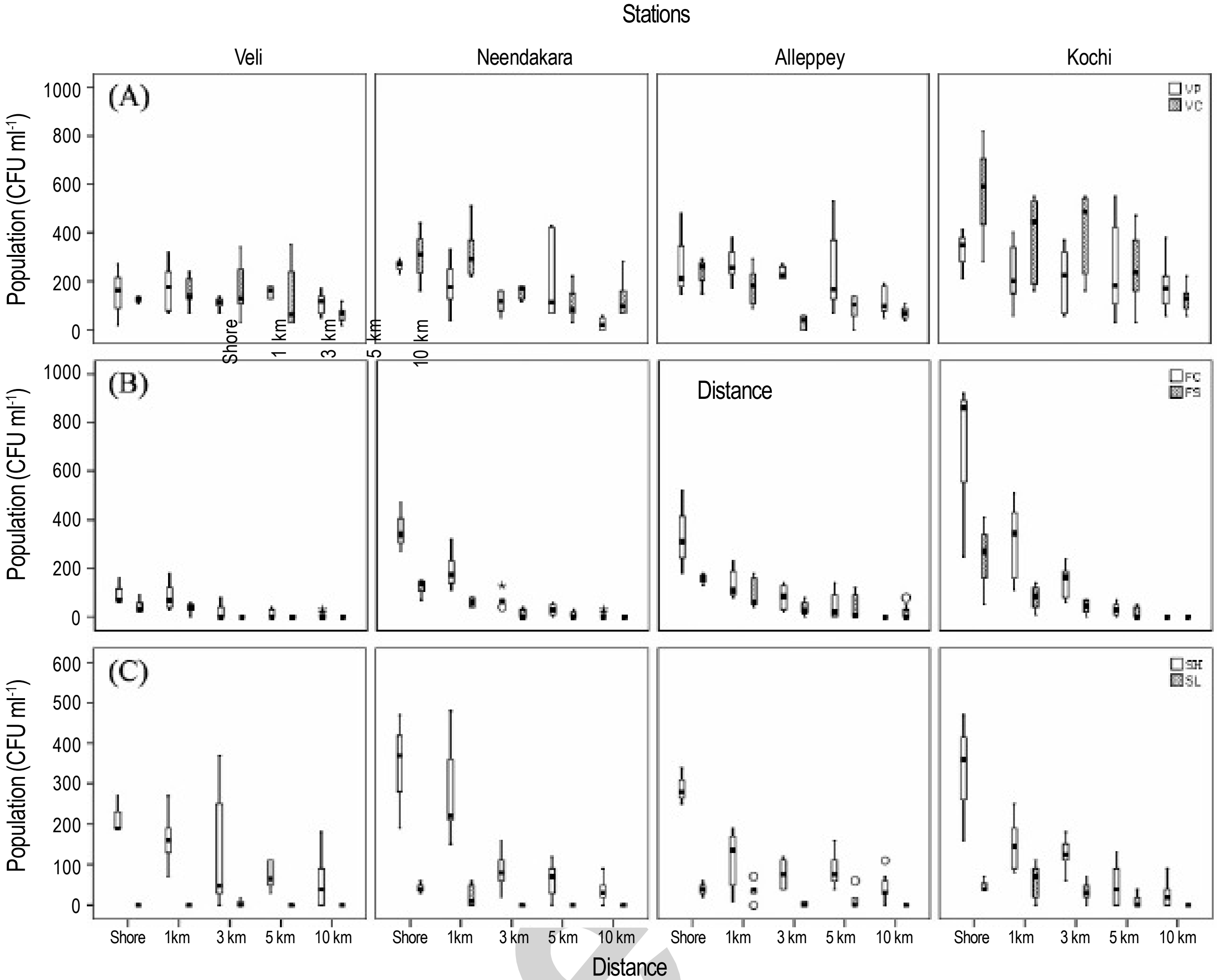

Fig. 4: Box plot showing the variation of different indicator bacteria and enteric pathogens along the Southern Kerala coast. (A) *Vibrio cholerae* and *Vibrio parahaemolyticus*, (B) Faecal coliforms and *Faecal streptococci*, (C) *Shigella* spp. and *Salmonella* spp.)

Although VP reported their maximum value of 610 CFU ml^{-1} in Alleppey (10 km), showed the mean high value in Kochi (225 CFU ml^{-1}). In the case of VC, in addition to the high median value (310 CFU ml^{-1}), the maximum value of 820 CFU ml^{-1} (Estuary) was also reported from Kochi. It had been observed that Vibrios were preferential in waters rich in organic nutrients (Sudhanandh *et al*., 2010a), such as might be expected in areas heavily impacted by land runoff and wastewater discharges and the same is true in the case of Kochi. The FC, FS, SHLO and SLO (Fig. 4B,C) were comparatively absent towards offshore especially 5 and 10 km from shore and showed a low population, forming cluster 3. May be the fact that as the salinity rises making the environment more and more marine the organisms get destroyed resulting in low counts (Bordalo *et al*., 2002). The maximum values of FC and FS were also reported from Kochi respectively of 920 and 410 CFU ml^{-1} (estuary), showed their mean high values in the same station respectively of 165 and 50 CFU ml^{-1}. Counts were significantly higher for both indicators across sites located close to the shoreline, which are known to receive faecal pollution from variable point sources including in and around harbor sites. In accordance with the FC/FS ratio, *i.e.* FC/FS ratio in excess of 2 probably represents primarily human faecal pollution while ratio of 1 or less may be due to the non-human sources, Kochi might be principally polluted with possible human sources. The same was also supported by the fact that Kochi is subjected to severe human interferences (bathing, ships berth). The SH and SL reported their maximum values of 510 CFU ml^{-1} at 5 km and 110 CFU ml at 1 km in Veli and Kochi with an average of 115 and 15 CFU ml^{-1} throughout the study. Generally indicator bacterial groups were comparatively high in onshore surface water samples, were replaced by bottom towards offshore. The same was also reported by Nandini and Somashekar (1999), who stated that sedimentation and adsorption of the microorganisms to sand and clay particles culminated in the increase in the density of bacteria at the bottom zone

Regardless of the environmental conditions, the survival of enteric pathogens especially VC and VP were always higher than

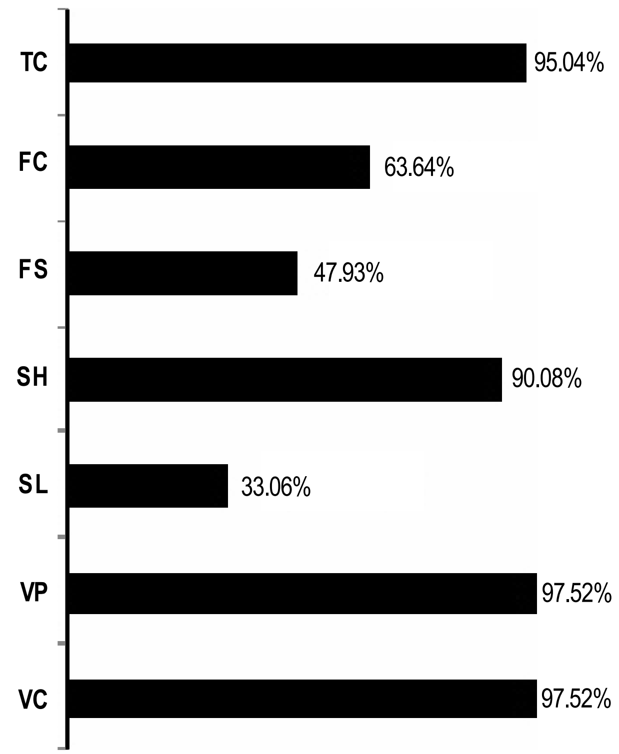

Fig. 5: Percent wise incidence of faecal indicator bacteria and enteric pathogens along the Southern Kerala coast

that of tradition bacterial indicators (FC, FS and TC), although the initial numbers of the TC were comparatively higher in the onshore region, as expected. However TC, FC and FS were unable to make their presents towards offshore, even in the presence of VC and VP. This clearly showed the inability of indicator bacteria of faecal origin to survive on comparatively saline tropical estuarine environments. Both *Vibrio parahaemolyticus* and *Vibrio cholerae* showed their maximum percentage incidence of 97.52% each (Fig. 5). In addition, VC also showed significant positive correlation with all the tested indicator bacteria (Fig. 6A). These finding suggest that VC may be used as a better sanitary indicator for monitoring faecal pollution in marine waters than traditional indicator bacterium as the former provide lower rate of dwindle owing to salinity and other environmental conditions typically prevailed in the southern Kerala coastal water, giving a more satisfactory indication of the pathogenic potential of these waters. Of the indicator bacteria abundance of FS was of the same order of magnitude as FC, both showed significant positive correlation with SL (Fig. 6B,C), however correlation were more significant between FC and SL, giving a more satisfactory indication of the pathogenic potential of these waters which is also supported by the percentage incidence FC (63.64%) and FS (47.93%). However, the lower survival of both FC and FS in water column suggested their unsuitability as a role of faecal pollution indicator bacteria in marine environment, though presents of FC and FS guarantee the presence of SL. In our study, *Salmonella* spp. were detected from 33.06% of the samples. These occurrence were line with those reported by Polo *et al*., 1998, reported greater than 30% incidence of water containing *Salmonella*

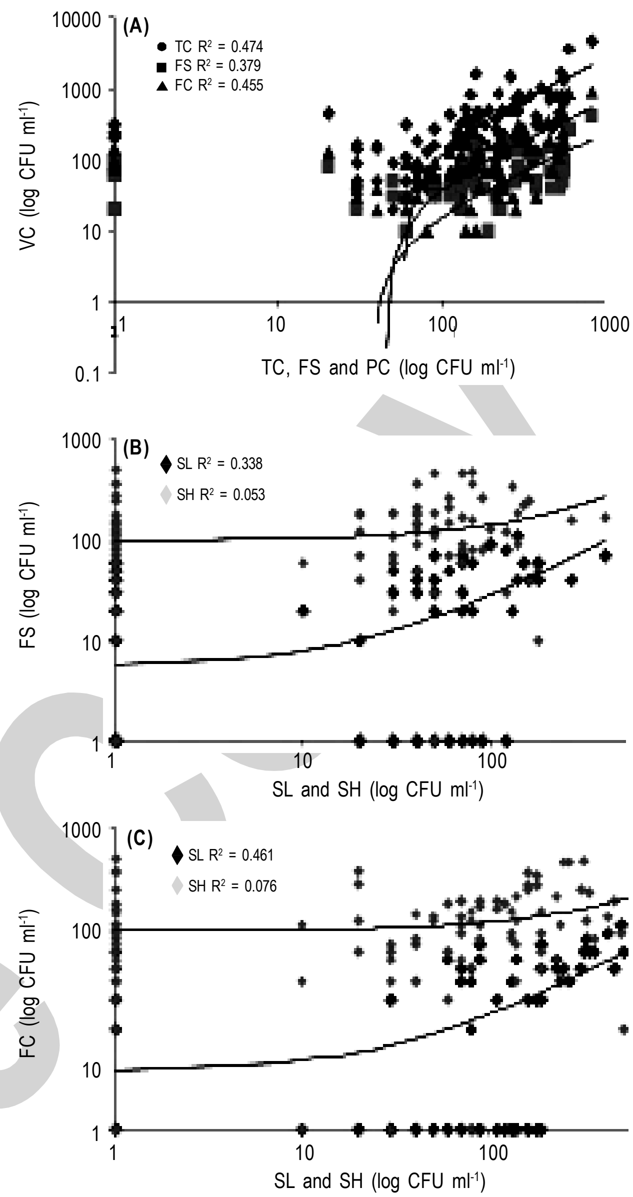

Fig. 6: Regression analysis showing the interrelationship of different indicator bacterial population and enteric pathogens. A - *Vibrio* cholerae with total coliforms, *Faecal streptococci* and faecal coliforms, B - Faecal *streptococci* with *Salmonella spp* and *Shigella spp*, C - Faecal coliform with *Salmonella* spp. and *Shigella* spp.

spp., whereas they are higher than those reported by other authors (Gabutti *et al*., 2004; Ouseph *et al*., 2009). No significant correlation were observed between indicator bacteria with VP and SH, supported by the fact that *V. parahaemolyticus* levels were more confined in the areas subject to sewage pollution (Watkins and Caballi, 1985).

Based on the quantitative occurrence of indicator group of bacteria the 4 stations could be classified into intensely polluted (Kochi, Neendakara), fairly polluted (Alleppey) and slightly polluted areas (Veli). The one-way ANOVA tests suggest significant differences observed in bacterial population between Kochi and Veli (P=0.001) for all the tested indicator bacteria and also between Kochi and Alleppey (P=0.002).On the other hand, no significant relationship was observed between Kochi and Neendakra, which indicated that the estuaries were much polluted. In general, owing to large outfalls of sewage and a variety of industrial effluents from Kochi metropolis, the Kochi harbor-dredging – creeks confluence supported high microbial activity, and abundance (both pathogenic and other allochthonous) and ecologically versatile bacterial groups. In Kochi, sea food industry also contributed microbial pollution, as the waste water from many factories, which was rich in organic matter end up in the estuary. Microbial population was comparatively high in Neendakara estuary also. In this respect it resembled Kochi estuary revealing a more or less similar bacteriological status. Broadly, Cochin reported with higher concentration of nutrients, may probably due to the release of nutrients from the interstitial sediments owing to prevalent dredging activities (Joseph and Ouseph, 2009; Sudhanandh *et al*., 2010b) and also owing to the unabated domestic waste disposal (Sudhanandh *et al*., 2010a) and this might affect the ecosystem drastically. Sudhanandh *et al.* (2010b) suggested that dredging promotes the resuspension of sediment bound bacteria causing ever increased bacterial population in the area. The higher population of enteric pathogens could have deleterious long term impact on public health, local fisheries and in tourism potential if not adequately addressed. The base line data generated on bacteriological water quality of the Southern Kerala coast may serve as biomonitoring standard and comparisons for other coastal studies and may be useful for all scientists, decision makers and resource managers working with environmental planning and management of such areas.

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