

Long-term variations in abundance and distribution of sewage pollution indicator and human pathogenic bacteria along the central west coast of India

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Abstract

Safe water quality criteria on the load and types of microbial populations are important for human use from fishery, tourism and navigational viewpoints. To understand the variations in sewage pollution indicator and certain human pathogenic bacteria, data collected from various locations along central west coast of India during 2002- 2007 were analyzed. Water and sediment samples were examined for total viable counts (TVC), pollution indicator bacteria (total coliforms - TC, fecal coliforms - FC and *Escherichia coli* - EC) and potential pathogens (*Vibrio cholerae* – VC; *Shigella* - SH and, *Salmonella* spp - SA). In both Mandovi and Zuari estuaries, where fishing and tourist-related activities are sizable and long-term data collection was regular, we observed high counts of TC, FC, VC, SH and SA in particular during monsoon due to increased land runoff. Further, the abundance of TC and FC has increased significantly over the years in the water column to much above either USEPA or India permissible limits. The concentrations of *Vibrio cholerae*, and *Shigella* correlated with those of coliforms. Pathogenic bacteria were detected even 20 km and/or 25 km offshore mainly due to dumping of raw or improperly treated sewage effluents either from land, fishing trawlers and/or ships in the anchorage. Higher concentrations of fecal-coliforms and pathogenic bacteria in neretic waters signify threats to environmental and human health.

Key words: pollution indicators, pathogens, *Escherichia coli*, *Vibrio cholerae*, *Shigella* and *Salmonella* spp.

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1. Introduction

One serious consequence of the increased human settlements near the coastal locations is sewage contamination which has negative impacts on the water quality, and adds to pathogenic microorganisms and toxic compounds (Ramaiah, 2002). The main effect of such contamination is on public health, and the quality and safety of seafood. In this regard, the mandatory water testing relies on enumerating fecal bacteria, to discern both fecal contamination and presence of disease causing organisms (Tallon et al., 2005). Presence of fecal coliform bacteria, especially *E. coli* (Guan et al., 2002), has shown stronger correlation to swimming associated illnesses than total fecal coliform organisms (Uzoigwe et al., 2007).

As fecal contamination and higher incidence of pathogens are reported in literature (Fujioka, 2002; Wilkes et al., 2009), it is essential for collating the abundances of certain known human pathogenic bacteria along with the enumeration of coliforms. As Lipp et al. (2001) reported, the pathogenic bacteria could be present even when the sewage pollution indicator populations may be well within or below the permissible limits. In addition to quantifying fecal contamination indicator groups, detection of pathogens when culturable indicators do not exceed recommended standards would therefore be useful to assess the quality of water bodies.

Raw sewage disposal into many of the Indian estuaries has been a common practice throughout their history (Ramaiah et al., 2007). In order to document the variability of total and fecal coliforms as well as certain human pathogenic bacteria on a long term-basis, regular seasonal sampling surveys in the lower stretches of Mandovi and Zuari estuaries were carried out. In addition, two transects were sampled annually to determine the extent of dispersal of fecal contamination indicators into the open sea. Seawaters along the Indian coasts are classified by Central Pollution Control Board of India (CPCB) as fit for commercial fishing, contact recreation and bathing activities when the FC is ≤ 100 CFU/100 ml (Anonymous, 1993). Whereas, the USEPA sets FC limits of 200 CFU/100 ml natural water for recreational purposes (USEPA, 1986). In order for assessing the water quality of the neretic regions off Goa and Ratnagiri along the central west coast of India, fecal coliform concentrations from the present study were compared with both Indian and USEPA standards.

2. Material and Methods

2.1. Sampling Sites

The central west coast of India experiences intense rain fall during the monsoon period (June-September). During summer (March-May), both air (28-36 °C) and water (24-32 °C) temperature is far higher than they are during ‘winter’ (January-February; air: 17-26 °C; water: 21-25°C). Goa, along the central west coast of India has two major estuaries, Mandovi and Zuari. These highly productive estuaries play vital role in the local fisheries, tourism and navigation. Many major settlements in Goa are along the rivers Mandovi and Zuari. The daily sewage, mostly untreated, and other effluent discharges are estimated to be about 16 million litres (Sawkar et al., 2003).

Sampling locations selected for the study are shown in figure 1. In order to obtain the overall distribution pattern of sewage indicator bacteria in the entire stretch of Mandovi and Zuari, a seasonal sampling survey was undertaken during 2002-2003. During each season mentioned above, seven stations along Mandovi (M1 to M7) and five stations along Zuari (Z1 to Z5) were sampled in the year 2002-2003. To cover the variability of different bacterial populations, time series sampling was carried out every season at a location in the mouth of the river Mandovi from 2002 to 2007. Similarly, time series sampling was carried out once a year from a location in the mouth of Zuari. For transect studies, five locations 5, 10, 15, 20 and 25 km away from the shore were covered to collect near-shore as well as off-shore samples off Mormugao, and Ratnagiri.

2.2. Sample Collection

Surface and bottom water samples at Mandovi and Zuari were collected at an interval of three hours onboard hired trawlers using 5 litre capacity Niskin bottles (General Oceanics, FL). Water samples along transects off Mormugao and Ratnagiri were collected onboard CRV Sagar Paschmi (2002-2005) and CRV Sagar Purvi (2006-2007). Sediment samples were collected from each location during all the sampling trips using van Veen grab. After the collection, samples were kept on ice till transported to the laboratory and taken up for microbiological analyses.

2.3. Enumeration

Different populations of bacteria enumerated from these samples were: total viable counts (TVC), sewage pollution indicator (total and fecal coliforms) bacterial counts, *Escherichia coli* like organisms (ECLO), *Shigella* like organisms (SHLO), *Vibrio cholerae* like organisms (VCLO) and *Salmonella* like

organisms (SALO). Nutrient agar prepared in 10% seawater was used for enumerating TVC. All colonies grown on HiCrome coliform agar (HCA) and m-FC agar were quantified as total coliforms (TC) and fecal coliforms (FC) respectively. Typical blue colonies on HCA were enumerated as ECLO. Yellow colonies with < 2mm diameter were quantified as VCLO on thiosulphate citrate bile salts sucrose (TCBS). Pink/red colonies on xylose lysine deoxycholate agar (XLD) were enumerated as SHLO. The SALO (pink colonies) were enumerated using HiCrome Improved Salmonella agar. *Salmonella* spp was enumerated only during 2006-2007. All the media were purchased from HiMedia, Mumbai.

2.4. Biochemical Characterization

At least 200 purified isolates each of ECLO, VCLO, SHLO and SALO were subjected to a series of appropriate sets of biochemical tests (Table 1). The characteristics of 'like organisms' conforming to at least 80% similarity were used to calculate the apparent populations of EC, VC, SA and SH in the samples from which all four types of 'like organisms' were enumerated.

Two-way ANOVA was carried out on log transformed data of CFU of all populations we enumerated using Excel software to discern the variations in different indicator and pathogenic bacterial concentrations due to seasons, years and/or locations. Multiple correlation coefficient between different indicator and pathogenic bacteria was carried out using the same package.

3. Results

As was evident from biochemical characterization, over 76 % of ECLO possessed at least 80% of EC characteristics. Similarly, 72.2 % of VCLO 75 % of SHLO, and 78.2% of SALO were found to be close to VC, SH and SA respectively. These percentages were used to rework the data on 'like organisms' to obtain the numbers of EC, VC, SH and SA.

3.1. Distribution and variations in different populations of bacteria in the water

3.1.1. Seasonal variations in pollution indicator bacteria along Mandovi and Zuari

During 2002-2003, TC varied from 3 to 1900 CFU ml⁻¹ along Mandovi, and 1 to 587 CFU ml⁻¹ along Zuari estuaries (Fig. 2). Their average counts were higher during monsoon (404 CFU ml⁻¹) and post-monsoon (34 CFU ml⁻¹) along Mandovi, and during pre-monsoon (22 CFU ml⁻¹) along Zuari. Along Mandovi, FC ranged from 1 to 800 CFU ml⁻¹ and from 1 to 383 CFU ml⁻¹ along Zuari. The highest FC counts were during monsoon at M1 and Z1, both upstream locations. In general, the EC were higher in

Mandovi during all the seasons. They ranged from nil to 15 in Mandovi, and from nil to 4 CFU ml⁻¹ in Zuari.

The variations in abundance of different bacterial groups from Mandovi mouth monitored for five years are presented in figure 3a and 3b. The TVC were the highest during monsoon 2005 with averages of 49456 in surface, and 58992 CFU ml⁻¹ in bottom waters (Table 2). Average counts of TC were the highest during monsoons of 2002 (1094 CFU ml⁻¹ in surface water) and 2006 (5933 CFU ml⁻¹ in bottom water). Counts of FC were the highest during monsoon 2005 in both surface (av. 3992 CFU ml⁻¹) and bottom (av. 4926 CFU ml⁻¹) samples. Although EC was occasionally detected, its average counts were high during monsoon 2004 in surface (av. 20 CFU ml⁻¹) and post-monsoon 2006 in bottom waters (68 CFU ml⁻¹). In surface waters, VC (av. 755 CFU ml⁻¹) and SH (av. 168 CFU ml⁻¹) were high during post-monsoon 2005. In the bottom water their counts were far more during post-monsoon 2006 (VC: 743 CFU ml⁻¹ and, SH: 1057 CFU ml⁻¹). The counts of SA, enumerated only during 2006-2007, were the highest during monsoon in surface (av. 306 CFU ml⁻¹) and bottom (av. 533 CFU ml⁻¹) samples. Overall, the counts were higher in the bottom samples *vis a vis* the ones collected from surface.

The time series data collected once a year during 2002-2007 from Zuari mouth suggests that the TVC show an increase over the years (Fig. 4a and 4b)). The TC in surface samples ranged from 9 to 4800 CFU ml⁻¹ while in bottom samples, from 1 to 9120 CFU ml⁻¹. Abundance of FC ranged from none to 4920 CFU ml⁻¹ and, none to 3480 CFU ml⁻¹ in surface and bottom samples, respectively. During 2006, the numbers of EC ranged between none and 54 CFU ml⁻¹. In surface water samples VC, SH and SA ranged from none to 3260 CFU ml⁻¹, 16880 CFU ml⁻¹ and 3480 CFU ml⁻¹, respectively. While in bottom samples VC, SH and SA ranged from none 1680 CFU ml⁻¹, 1615 CFU ml⁻¹ and 935 CFU ml⁻¹ respectively.

3.1.2. Seaward abundance and distribution of different bacterial populations

Abundance of different groups of bacteria up to ~25 km off Mormugao (Fig. 5a and 5b) and Ratnagiri (Fig. 6a and 6b) during different years indicates gradual to rapid decreases seaward. Off Mormugao, the TVC were higher up to 10 km from the shore (#1 and #2) and the TC ranged from none to 12160 CFU ml⁻¹ in surface and from none to 11680 CFU ml⁻¹ in bottom water samples. Along the transect FC were detectable only during 2006 ranging from none to 520 CFU ml⁻¹. The EC were lower both in surface (none to 8 CFU ml⁻¹) and bottom (none to 69 CFU ml⁻¹) samples. Counts of VC were quite high during

2005 and 2006. The SH were quite high in the surface (none to 9300 CFU ml⁻¹) and bottom (none to 4080 CFU ml⁻¹) samples. Higher counts of SA were observed in the surface samples.

3.1.3. Statistical Analysis

The correlation coefficients (r) calculated using log transformed CFU data to understand the relationship between coliforms and pathogenic bacteria suggest that at Mandovi mouth location, the r was statistically highly significant between TC and SH ($r = 0.2568$; $n = 60$); FC and VC ($r = 0.4764$); FC and SH ($r = 0.5089$) and EC and VC ($r = 0.4667$) during premonsoon. Significant correlation was also observed during monsoon, between TC and VC ($r = 0.2890$); TC and SH ($r = 0.251$); FC and VC ($r = 0.356$); FC and SH ($r = 0.307$), as well as EC and SH ($r = 0.2460$). During postmonsoon, correlations were significant between TC and VC ($r = 0.3025$); FC and SH ($r = 0.3803$); EC and VC ($r = 0.3882$); and EC and SH ($r = 0.2206$). In the Zuari mouth location, the TC correlated strongly with SH ($r = 0.4805$; $n = 60$); FC with SH ($r = 0.4620$) and EC with VC ($r = 0.2153$). Along the transect off Mormugao, the correlations were statistically highly significant between TC and SH ($r = 0.6255$; $n = 50$) as well as between EC and VC ($r = 0.2895$). Similarly, along the transect off RTN, TC bore strong correlation with VC ($r = 0.2104$; $n = 50$); FC with VC ($r = 0.2931$) while EC correlated strongly with VC ($r = 0.4954$) and SH ($r = 0.3131$).

From the two-way analysis of variance (ANOVA), it is clear that there were significant seasonal differences in the abundances of TC, FC, VC, and SH at the Mandovi mouth location. Between the years, significant differences were mostly in the TC and FC concentrations. This is suggestive of the fact that over the years TC and FC have increased as also seen from the numerical data. At the Zuari mouth location, the annual differences in all of the examined groups were also statistically significant. From the annual data from different locations in both Mandovi and Zuari, it was discernible that season-wise variations in TC, FC and EC concentrations were statistically significant in Mandovi locations. In Zuari locations, such differences were significant only in those of TC and FC. Further, location-wise differences in the concentrations of these groups in the Mandovi estuary were not significant. This was largely true for Zuari as well excepting EC, the location-wise concentrations of which were significantly different. Along transects off MRG and RTN, the differences in the concentrations of all the examined groups between locations were few. However, between the years, there were statistically significant differences in the concentrations of TC, FC, SH and VC off MRG and only in the TC and FC off RTN.

3.2. Distribution and variations in different populations of bacteria in the sediments

The counts (CFU X 10³ g⁻¹ dry wt.) of different bacterial groups enumerated from the sediment samples (Table 3) reveal that TC and FC were present in most of the samples in higher numbers during premonsoon in both Mandovi and Zuari, and their counts were higher at M4, M5, Z3 and Z4, the midstream locations. The EC was detected only occasionally during premonsoon and monsoon. Bacterial counts in sediment samples collected for over five years from Mandovi mouth were higher than those in the overlying water (Supplementary Table 1). However, there is a decreasing trend in the counts of TVC, TC, FC and VC over the years which is generally in contrast to that seen in water samples. The EC was detected only during the 2004 premonsoon (av. 4 CFU X 10³g⁻¹). Counts of SH decreased over the years but those of SA were the highest during the 2006 monsoon.

In the sediment samples from Zuari mouth, the counts of TVC and TC were high during 2002 and show a decrease in later years (Supplementary Table 2). In the overall, their counts were higher in sediment than in the overlying water column. Counts of FC were highest (300 CFU X 10³ g⁻¹) during 2007. EC was in non-detectable levels. Though detected during all the years counts of VC were more during 2002 and 2007. In general similar trend was observed in the abundance of SH and SA.

In the sediment samples collected off Mormugao (MRG) and Ratnagiri (RTN), the abundances of different bacterial populations (Supplementary Table 3) suggest that off MRG, both TVC and TC were high during 2002 and FC, during 2006. EC was in non-detectable range. Occasionally, counts of VC were in high numbers up to 25 km and, SH ranged from none to 325 CFU X 10³ g⁻¹ along this stretch. SA was present in the far offshore location though its counts were the highest in the close-to-coast station (622 CFU x 10³ g⁻¹). Off RTN, TVC and TC were high during 2002 in the near-coastal location. FC was detected only during 2005-2006 with the highest counts of 254 CFU X 10³ g⁻¹ at location 2 during 2006. EC was rare at all locations. VC was detected at most stations with its highest counts (1352 CFU X 10³ g⁻¹) during 2002 at location 1. SH was detected at most locations, and in 2006, even at the sampling location 25 km offshore. SA, though sporadic, was also detected at the far offshore location. Overall, the bacterial counts off RTN were higher at the first two coastal locations, and off MRG, at offshore locations.

4. Discussion

Mandovi and Zuari estuarine systems are typical of the Indian west coast estuaries. They are influenced by the seawater inflow up to a considerable distance inland, and receive large volume of freshwater during the south-west monsoon season (Shetye et al., 2007). Seasonal sampling in 2002-2003 indicated higher coliform counts during monsoon which may be attributed to increased surface runoff into these estuaries carrying, among others, the fecal matter from the land. La Rosa et al. (2001) also suggested that high pollution during monsoon may be a consequence of heavy surface runoff providing inputs of pathogenic microorganisms into the water column. We observed higher CFUs of TC in sampling locations in the mouths of these estuaries. However, FC and EC were higher upstream. The sampling locations upstream being riverine and low saline, FC, as observed, can get adapted to fluctuating salinities and survive for extended periods leading to higher counts.

Both seasonal and annual time-series observations from Mandovi and Zuari also suggest that CFUs of TC are high during monsoon. Higher bacterial populations during heavy rainfall have been reported (Crowther et al., 2001; Lipp et al., 2001; Ramaiah et al., 2001). Further, an increase in FC with time was observed in both the estuaries. As also observed during this study, Mohandass and Loka Bharathi (2003) reported higher bacterial numbers at 10- to 15- m depth than at a 5- m depth in coastal water off Nagore on the east coast of India. Similar to our observations of significant correlations between coliform group and certain pathogenic bacteria, Nagvenkar and Ramaiah (2009) reported strong relations between abundance of TC, FC, VC and SH from these estuaries.

The lower stretches of both Mandovi and Zuari are being used for recreation, fishing/shell-fishing and navigation. Presence of potentially pathogenic organisms such as *E. coli*, *V. cholerae*, *Salmonella* and *Shigella* spp in high numbers in the water column is of serious concern. High coliform numbers and pathogenic bacteria have been reported in waters used for dumping of sewage (Adingra and Arfi, 1998). Overall, the bacterial concentration in Mandovi estuary is high compared to Zuari. Mandovi has many tributaries and receives a greater runoff than Zuari (Qasim, 2003) hence the input of bacterial load may be higher in Mandovi. There is an increasing trend in the abundance of fecal coliforms in the estuaries of Mandovi and Zuari. The common practice is that settlements along the estuaries discharge raw waste including the septic waste directly into the estuaries. Apart from such daily discharges of untreated sewage effluents deliberately and/or incidentally at various locations, the seaward flows during low tide-times increase the amounts of effluents leading to higher concentrations of sewage pollution indicator

bacteria. Thus, the non-point sources are major causes of the deterioration of water quality. Our observations are similar to those of Lipp et al. (2001) who reported highest levels of pollution indicator organisms in Phillippi Creek. The higher counts of FC in the water column of both estuaries can be considered to be due to fresh/recent sewage contamination as also suggested by Craig et al. (2002).

Transect studies along MRG and RTN covering the coastal and offshore locations indicated varying levels of bacterial contamination. The abundance, especially of TC, was higher up to 10 km from the shore in both water and sediment. Thus, it can be inferred that the effect of sewage is up to, and/or occasionally beyond 10km offshore. This is particularly true for FC which was detected in high numbers at the 25 km location off MRG and 20 km off RTN. Both of these locations possess medium sized ports and, experience shipping related activities. In general, indicator bacterial counts off MRG were higher. This can be attributed to sewage effluent releases by the ships in the outer anchorage. Presence of VC, SH and SA at offshore sites in high numbers indicates allochthonous bacterial contamination in the offshore waters. Overall, whenever present, the FC was beyond the CPCB permissible limits.

The abundance of all groups of bacteria was, in general, more in the sediment than in the water column which was also reported in other studies (Nagvenkar and Ramaiah, 2009; Haller et al., 2009). High bacterial load in sediments may be due to enhanced survival by low/no exposure to stressors, such as sunlight and predation, or by increased availability of nutrients (La Rosa et al., 2001; Craig et al., 2002). Desmarais et al. (2002) reported extended proliferation and regrowth of *E. coli* and enterococci in sediment environment with high organic content. Hence, in coastal waters there may be increased risks of infection to humans due to the re-suspension of potentially pathogenic micro-organisms from the surface sediment layer during recreational activities.

5. Conclusion

In the present study, seasonal sampling during 2002-2003, seasonal and annual time-series observations from Mandovi and Zuari suggest that the concentrations of TC are high during monsoon. High CFUs of coliforms were observed in bottom water samples. Bacterial concentrations in Mandovi estuary are higher than those from Zuari. There is an increasing trend in the abundance of fecal coliforms in these estuaries. Prevalence of EC, VC, SA and SH in the lower stretches of the estuaries are of serious health concern since these areas are used for recreational, fishing and shell-fishing purpose. Coliforms as well as other potentially pathogenic bacteria were detected in high numbers at offshore locations off

Marmugao and Ratnagiri. Besides highlighting significant correlations between the coliform group and certain pathogenic bacteria at many locations, this study is useful to recognize statistically significant season-wise and yearly variations in the abundances of coliform.

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References:

Adingra, A.A., Arfi, R., 1998. Organic and bacterial pollution in the Ebrie lagoon, Cote d'Ivoire. *Mar. Pollut. Bull.* 36 (9), 689-695.

Anonymous, 1993. Criteria for classification and zoning of coastal waters (sea waters) – A coastal pollution control series: COPOCS/6/1993-CPCB, New Delhi.

Craig, D.L., Fallowfield, H.J., Cromar, N.J., 2002. Enumeration of fecal coliforms from recreational coastal sites: evaluation of techniques for the separation of bacteria from sediments. *J. Appl. Microbiol.* 93, 557-565.

Crowther, J., Kay, D., Wyer, M.D., 2001. Relationships between microbial water quality and environmental conditions in recreational water: the Fylde coast, UK. *Water Res.* 35, 4029-4038.

Desmarais, T.R., Solo-Gabriele, H.M., Palmer, C.J., 2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl. Environ. Microb.* 1165-1172.

Fujioka, R., 2002. Microbial indicators of marine recreational water quality, in: Hurst, C.J., Crawford, R.L., Knudsen, G., McIneny, M.J., Stetzenbach, L.D. (Eds), *Manual of environmental microbiology*, Washington DC: American Society for Microbiology Press, 2nd ed., 234-243.

Guang, S., Xu, R., Chen, S., Odumeru, J., Gyles, C., 2002. Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. *Appl. Environ. Microb.* 68, 2690-2698.

Haller, L. Pote, J., Loizeau, J.L., Wildi, W., 2009. Distribution and survival of faecal indicator bacteria in the sediment of the Bay of Vidy, Lake Geneva, Switzerland. *Ecol. Indic.* 9 (3), 540-547.

La Rosa, T., Mirto, S., Marino, A., Alonzo, V., Maugeri, T.L., Mazzola, A., 2001. Heterotrophic bacteria community and pollution indicators of mussels-farm impact in the Gulf of Gaeta (Tyrrhenian Sea). *Mar. Environ. Res.* 52 (4), 301-321.

Lipp, E.K., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S.R., Rose, J.B., 2001. The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. *Estuaries* 24, 266-276.

- Mohandass C., Loka Bharathi P.A., 2003. Representation, dispersion and variation of bacterial indicators in the coastal waters of Nagore (east coast of India). *Water Environ. Res.* 75 (1), 66-72.
- Nagvenkar, G.S., Ramaiah, N., 2009. Abundance of sewage-pollution indicator and human pathogenic bacteria in a tropical estuarine complex. *Environ. Monit. Assess.* 155, 245-256.
- Qasim, S.Z., 2003. *Indian Estuaries*, Allied Publishers Private Limited, pp. 450.
- Ramaiah, N., Kenkre, V.D., Verlekar, X.N., 2001. Marine environmental pollution stress detection through direct viable counts of bacteria. *Water Res.* 39 (9), 2383-2393.
- Ramaiah, N., 2002. Coliform and human pathogenic bacteria in tourism affected water bodies in North Goa. *Coastin* 7, 13-16.
- Ramaiah, N., Rodrigues, V., Alvares, E., Rodrigues, C., Baksh, R., Jaya, S., Mohandas, C., 2007. Sewage-pollution indicator bacteria, in: Shetye, S.R., Kumar, M.D., Shankar, D. (Eds.), *The Mandovi and Zuari Estuaries*, Research Publishing Services, Chennai, India., pp. 115-120.
- Sawkar, K., Vethamony, P., Babu, M.T., Dias, C., Mesquita, A., Fernandes, B., Moses, S., Padmavati, M., Naik, S., 2003. Measuring, modeling and grading the health of water bodies, in: Noronha, L., Lourenco, N., Lobo-Fereira, J. P., Lleopart, A., Feoli, E., Sawkar, K., Chachadi, A., (Eds.), *Coastal tourism, environment and sustainable local development*, Tata Energy Research Institute (TERI), New Delhi, India., pp. 179-210.
- Shetye, S.R., Shankar, D., Neetu, S., Suprit, K., Michael, G.S., Chandramohan, P., 2007. The environment that conditions the Mandovi and Zuari estuaries, in: Shetye, S.R., Kumar, M.D., Shankar, D. (Eds.), *The Mandovi and Zuari Estuaries*, Research Publishing Services, Chennai, India., pp. 3-27.
- Tallon, P., Magajna, B., Lofranco, C., Leung, K.T., 2005. Microbial indicators of fecal contamination in water: A current perspective. *Water Air Soil Poll.* 166, 139-166.
- United States Environmental Protection Agency, 1986. Ambient water quality criteria for bacteria EPA-440/5-84-002. U.S. Environmental Protection Agency, Washington, D.C.
- Uzoigwe, J.C., O'Brien, E.H., Brown, E.J., 2007. Using nutrient utilization patterns to determine the source of *Escherichia coli* found in surface water. *Afr. J. Environ. Sci. Tech.* 1 (1), 007-013.
- Wilkes, G., Edge, T., Gannon, V., Jokinen, C., Lyautey, E., Medeiros, D., Neumann, N., Ruecker, N., Topp, E., Lapen, D., 2009. Seasonal relationships among indicator bacteria, pathogenic bacteria, *Cryptosporidium* oocysts, *Giardia* cysts and hydrological indices for surface waters within an agricultural landscape. *Water Res.* 43 (8), 2209-2223.

Table 1: List of biochemical tests done to confirm the identity of like organisms

Biochemical Tests	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Shigella</i> sp.	<i>Salmonella</i> sp.
Gram staining	- ^a	-	-	-
Motility	+ ^b	+	-	+
Catalase	+	+	+	+
Oxidase	-	+	-	-
Indole	+	ND ^c	-	-
Growth at 37°C	+	+	+	+
Growth w/o ^d NaCl	ND	-	ND	ND
H ₂ S	-	ND	-	+
MR ^e	+	ND	+	+
VP ^f	-	ND	-	-
Gas from glucose	+	ND	-	+
Citrate utilization	-	ND	-	+

^anegative, ^bpositive, ^cnot done, ^dwithout, ^emethyl red, ^fvoges Proskauer

Table 2: Overall range (CFU ml⁻¹) of various bacterial groups in water samples during 2002-2007 at Mandovi mouth

Bacterial population	Source	Premonsoon	Monsoon	Postmonsoon
TVC ^a	surface	80-3280	198-64320	110-34080
	bottom	10-11520	327-77280	130-50240
TC ^b	surface	ND ^h -760	ND-1492	ND-1975
	bottom	ND-9600	ND-29120	ND-1738
FC ^c	surface	ND-11200	ND-8980	ND-1525
	bottom	ND-5440	ND-11180	ND-22080
EC ^d	surface	ND-20	ND-35	ND-53
	bottom	ND-30	ND-50	ND-280
VC ^e	surface	ND-25	ND-188	ND-1440
	bottom	ND-11200	ND-720	ND-2260
SH ^f	surface	ND-100	ND-443	ND-260
	bottom	ND-1760	ND-4640	ND-453
SA ^g	surface	ND-52	10-840	ND-330
	bottom	ND-310	80-2160	65-130

^atotal viable counts, ^btotal coliforms, ^cfecal coliforms, ^d*Escherichia coli*, ^e*Vibrio cholerae*, ^f*Shigella* spp., ^g*Salmonella* spp., ^hnone detected

Table 3: Seasonal variation in pollution indicator bacteria in sediment (CFU X 10³ g⁻¹ dry wt.) samples along Mandovi and Zuari estuary collected during 2002-2003

Stations	TC ^a			FC ^b			EC ^c		
	prem ^d	mon ^e	post ^f	prem	mon	post	prem	mon	post
Mandovi									
M1	143	34	102	ND	730	7	ND	ND	ND
M2	ND ^g	ND	ND	ND	ND	38	ND	ND	ND
M3	154	64	292	ND	240	26	ND	91	ND
M4	21900	2	ND	53	25	ND	ND	114	ND
M5	18300	0.1	541	2350	370	1150	ND	ND	ND
M6	729	10	209	2910	56	2090	ND	ND	ND
M7	1760	390	ND	742	480	ND	1345	ND	ND
Zuari									
Z1	959	3	2100	ND	89	60	ND	702	ND
Z2	ND	ND	11	ND	ND	7	ND	ND	ND
Z3	8710	280	ND	484	10	ND	ND	ND	ND
Z4	4770	ND	ND	1590	2	ND	485	ND	ND
Z5	3170	34	6	2010	2	7000	ND	ND	ND

^atotal coliforms, ^bfecal coliforms, ^c*Escherichia coli*, ^dpremonsoon, ^emonsoon, ^fpostmonsoon, ^gnone detected

Figure Captions

Figure 1: Sampling locations along Central West coast of India: M1, M2, M3, M4, M5, M6, M7 - sites along Mandovi estuary; MM – Mandovi mouth; Z1, Z2, Z3, Z4, Z5 - sites along Zuari estuary; ZM – Zuari mouth; MRG 1, MRG 2, MRG 3, MRG 4 and MRG 5 - transect sites off Mormugao; RTN 1, RTN 2, RTN 3, RTN 4 and RTN 5 - transect sites off Ratnagiri.

Figure 2: Distribution of different populations of coliforms (CFU ml⁻¹) in surface water along Mandovi (A-C) and Zuari (D-F) respectively during premonsoon, monsoon and post monsoon months. (—□— Total coliforms, —◆— Fecal coliforms, —▲— *Escherichia coli*).

Figure 3a: Seasonal distribution of different bacterial populations in surface water (CFU ml⁻¹) at a regularly monitored location in the mouth of Mandovi estuary during 2002-2007. (—◆— premonsoon, —▲— monsoon, —□— postmonsoon).

Figure 3b: Seasonal distribution of different bacterial populations in bottom water (CFU ml⁻¹) at a regularly monitored location in the mouth of Mandovi estuary during 2002-2007. (—◆— premonsoon, —▲— monsoon, —□— postmonsoon).

Figure 4: Abundance of different bacterial groups in water (CFU ml⁻¹) at a location in Zuari mouth during 2002-2007 (—◆— surface, —▲— bottom).

Figure 5a: Distribution of different bacterial groups in surface water (CFU ml⁻¹) samples along a transect off Mormugao during 2002-2007 (■ 2002, ■ 2003, ■ 2005, ■ 2006 & □ 2007)

Figure 5b: Distribution of different bacterial groups in bottom water (CFU ml⁻¹) samples along a transect off Mormugao during 2002-2007 (■ 2002, ■ 2003, ■ 2005, ■ 2006 & □ 2007)

Figure 6a: Distribution of different bacterial groups in surface water (CFU ml⁻¹) samples along a transect off Ratnagiri during 2002-2007 (■ 2002, ■ 2003, ■ 2005, ■ 2006 & □ 2007)

Figure 6b: Distribution of different bacterial groups in bottom water (CFU ml⁻¹) samples along a transect off Ratnagiri during 2002-2007 (■ 2002, ■ 2003, ■ 2005, ■ 2006 & □ 2007).

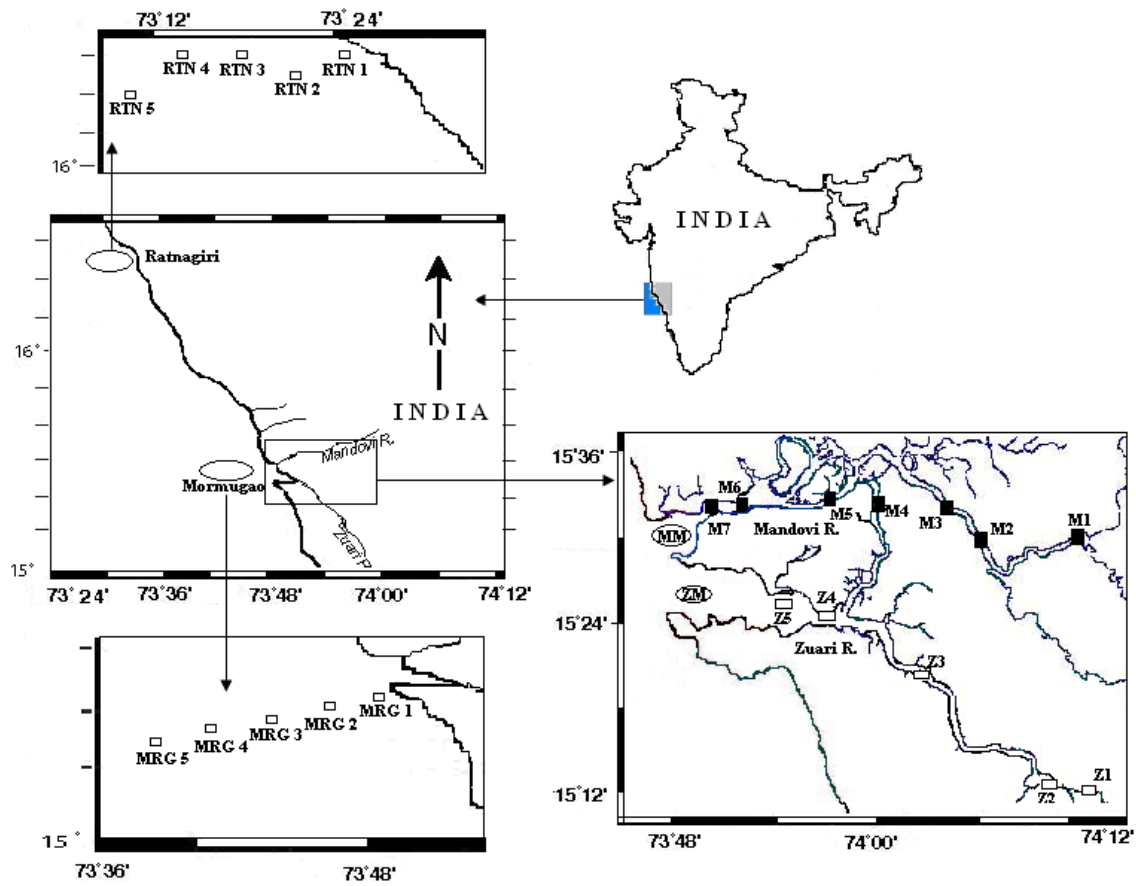


Figure 1 Rodrigues et al.

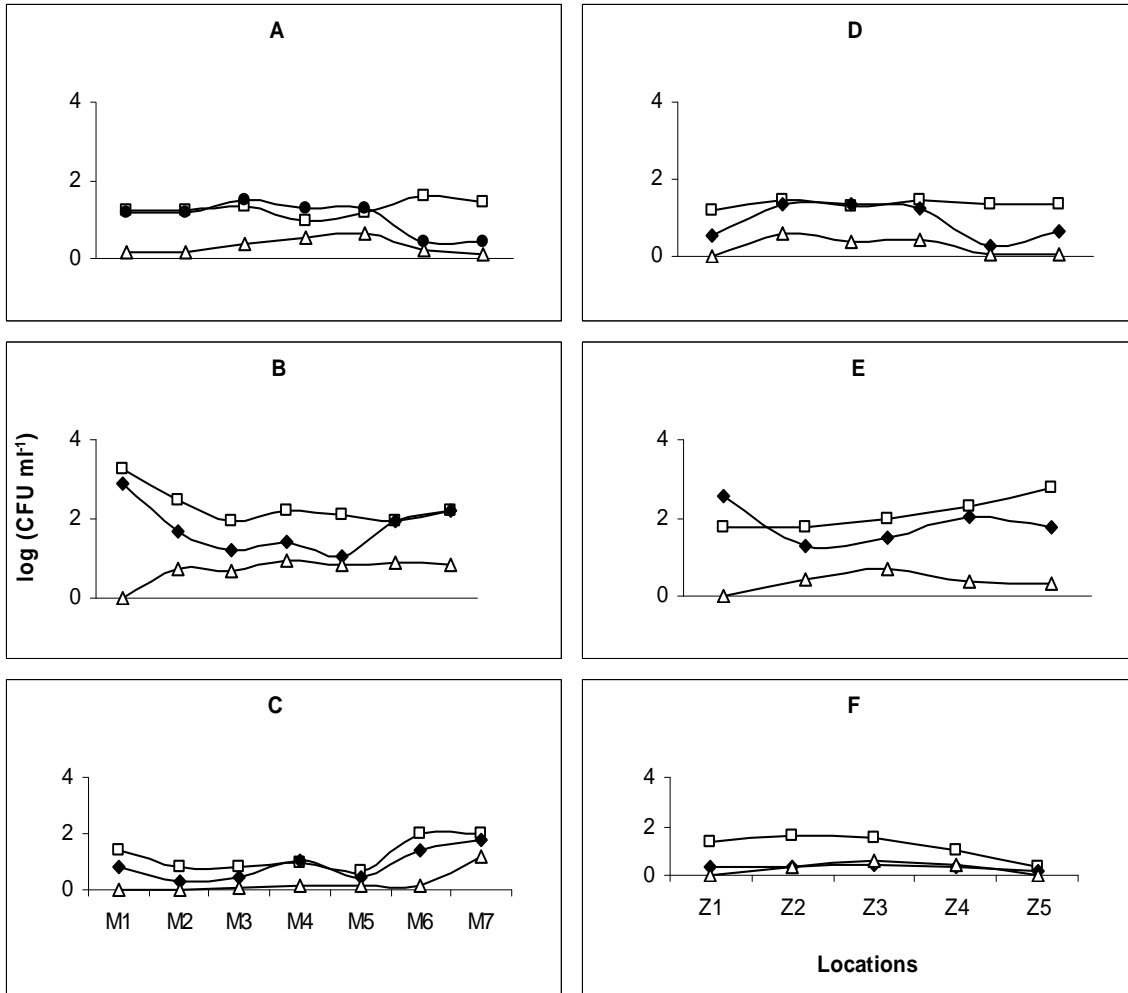


Figure 2 Rodrigues et al.

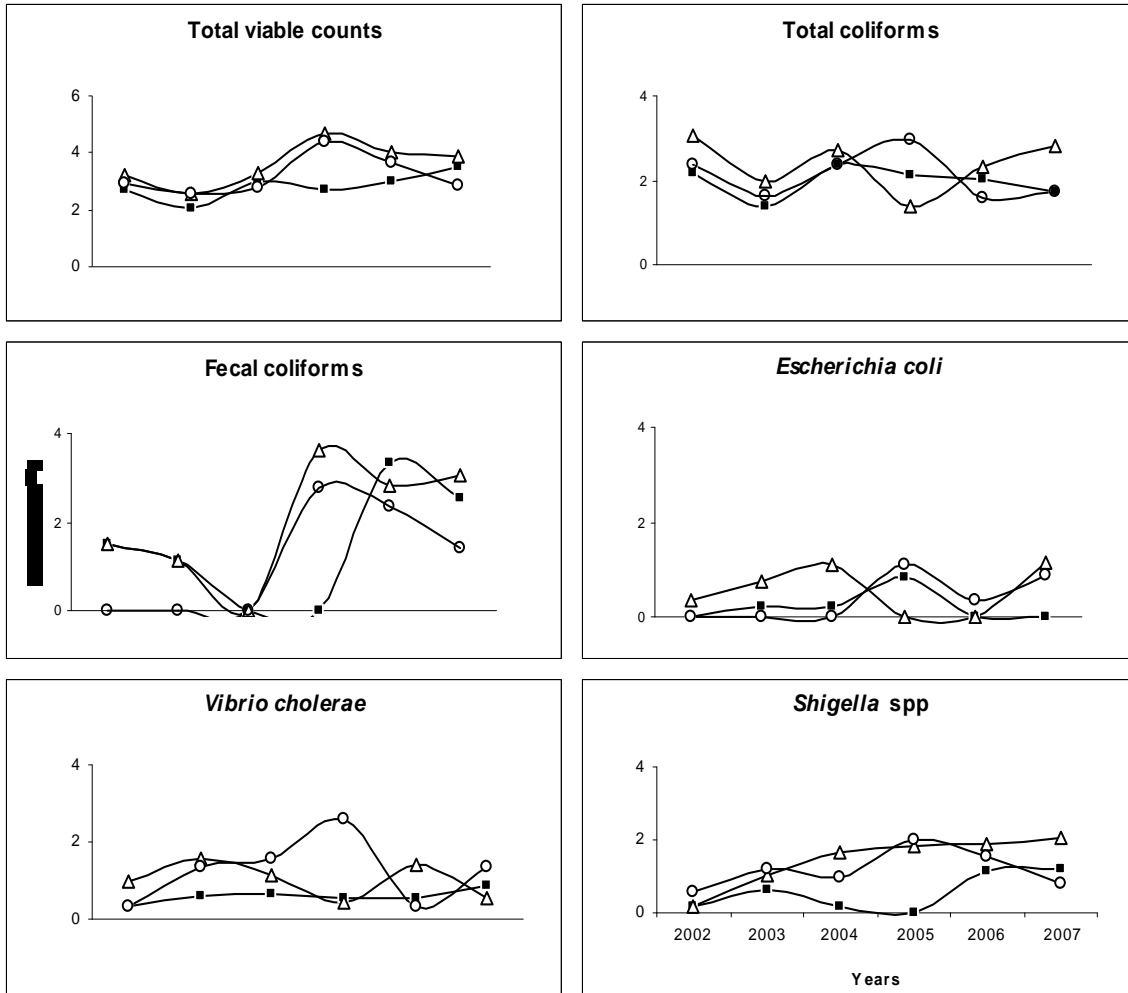


Figure 3a Rodrigues et al.

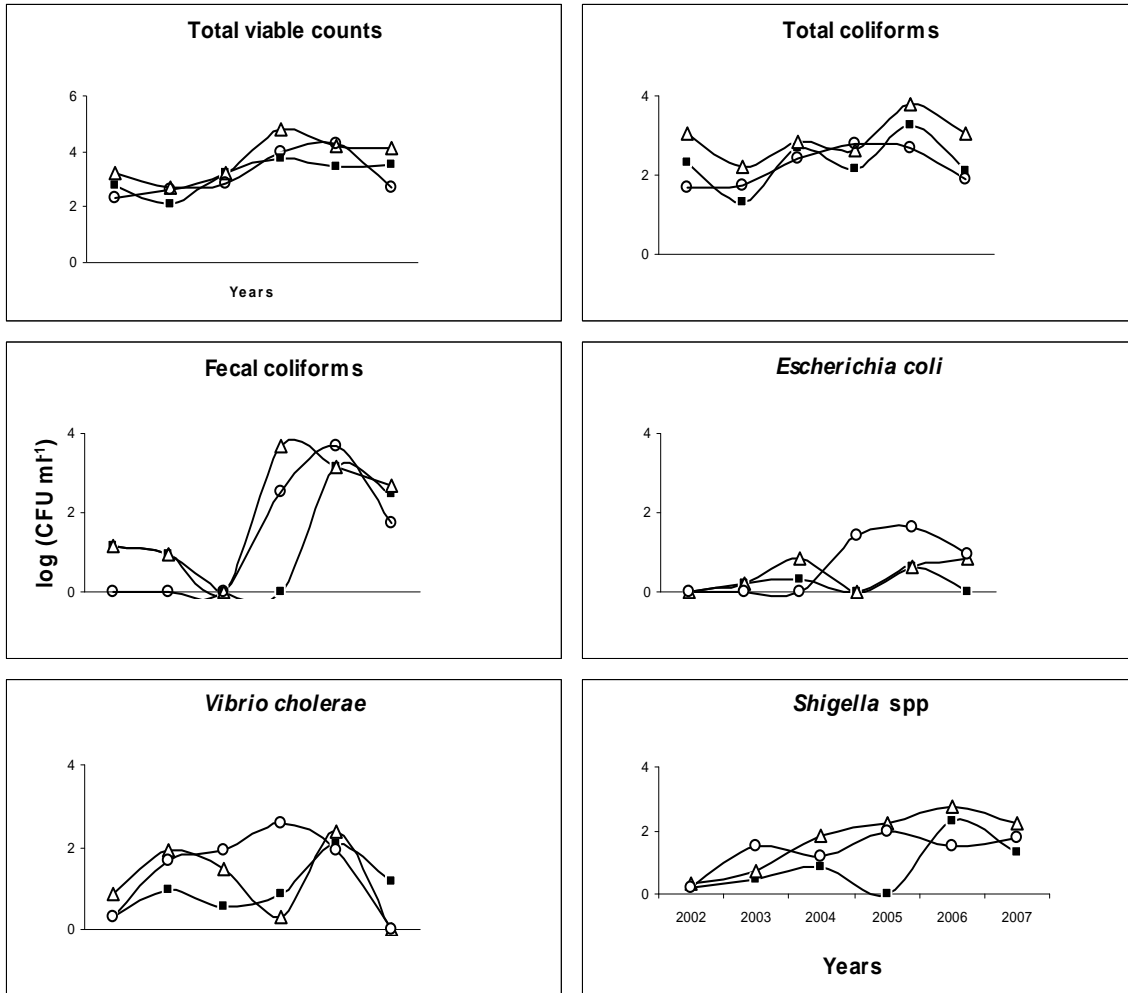


Figure 3b Rodrigues et al.

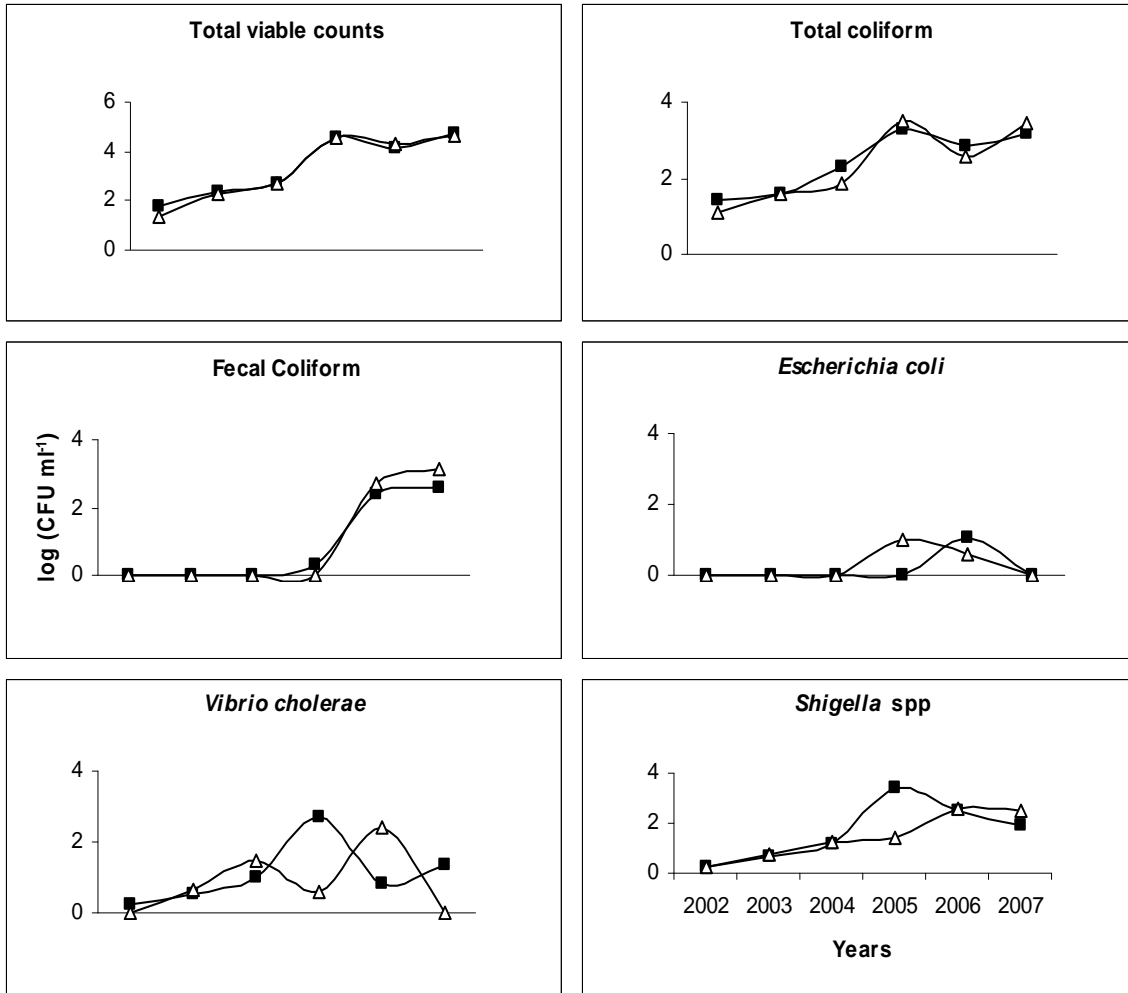


Figure 4 Rodrigues et al.

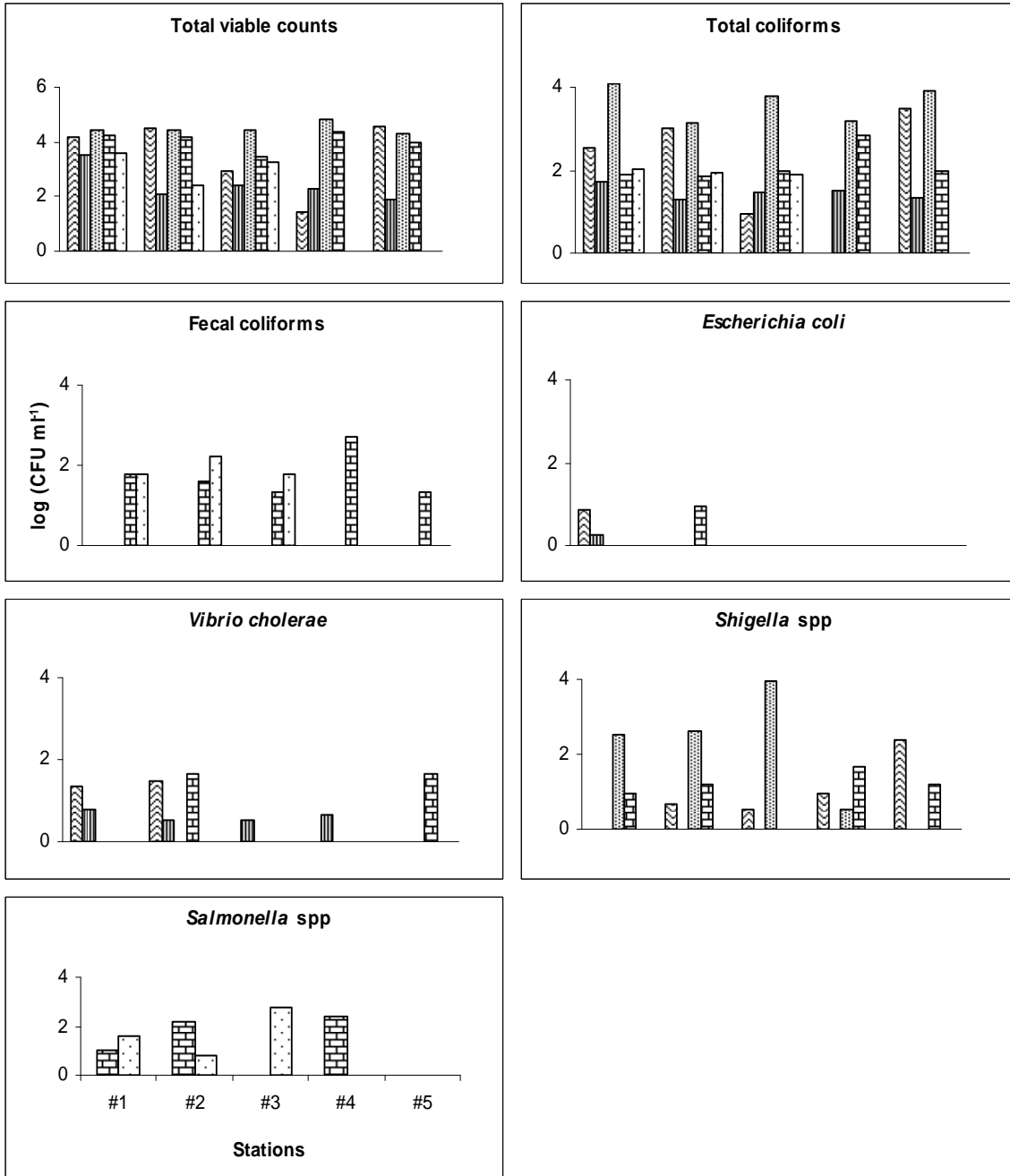


Figure 5a Rodrigues et al.

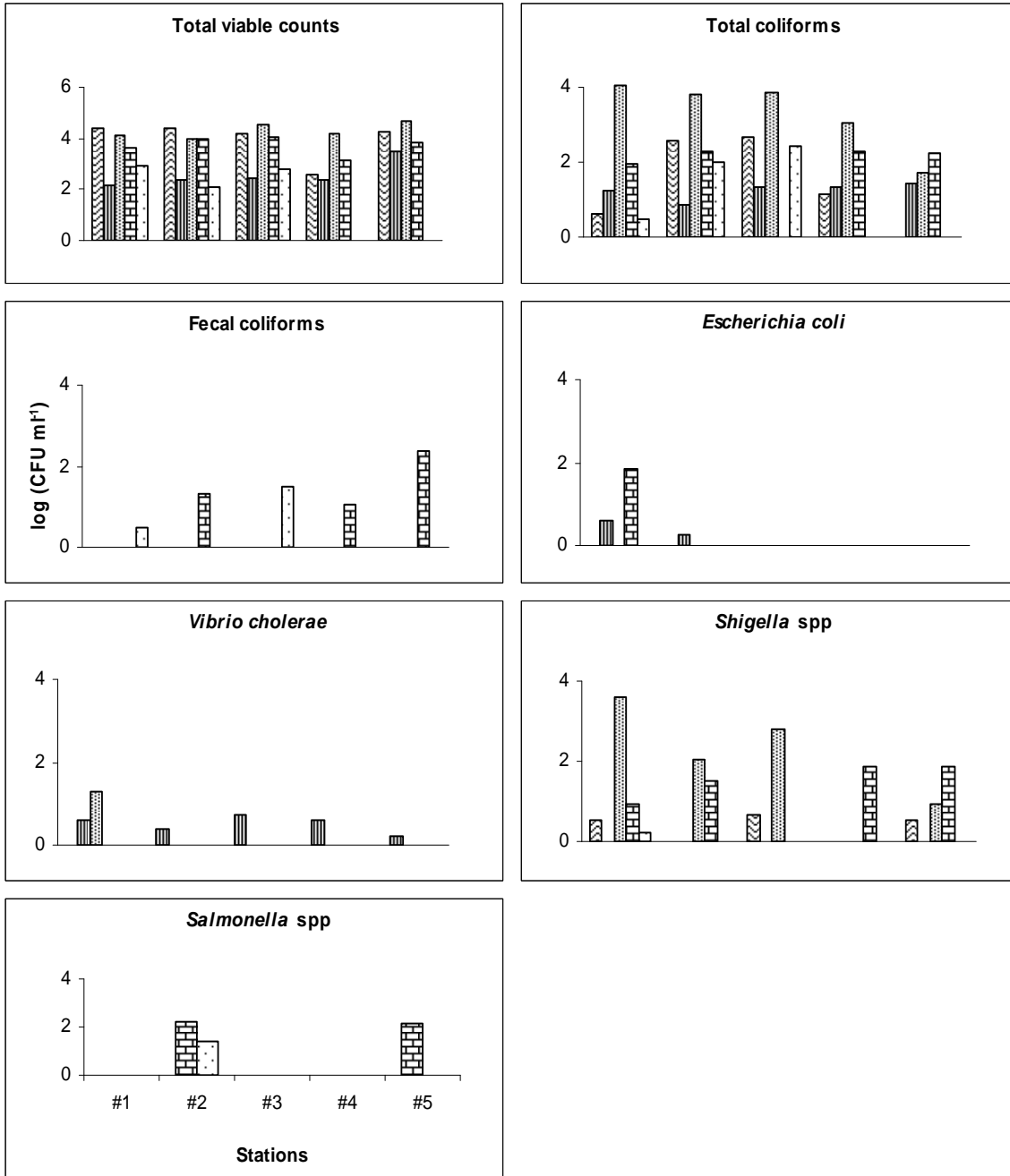


Figure 5b Rodrigues et al.

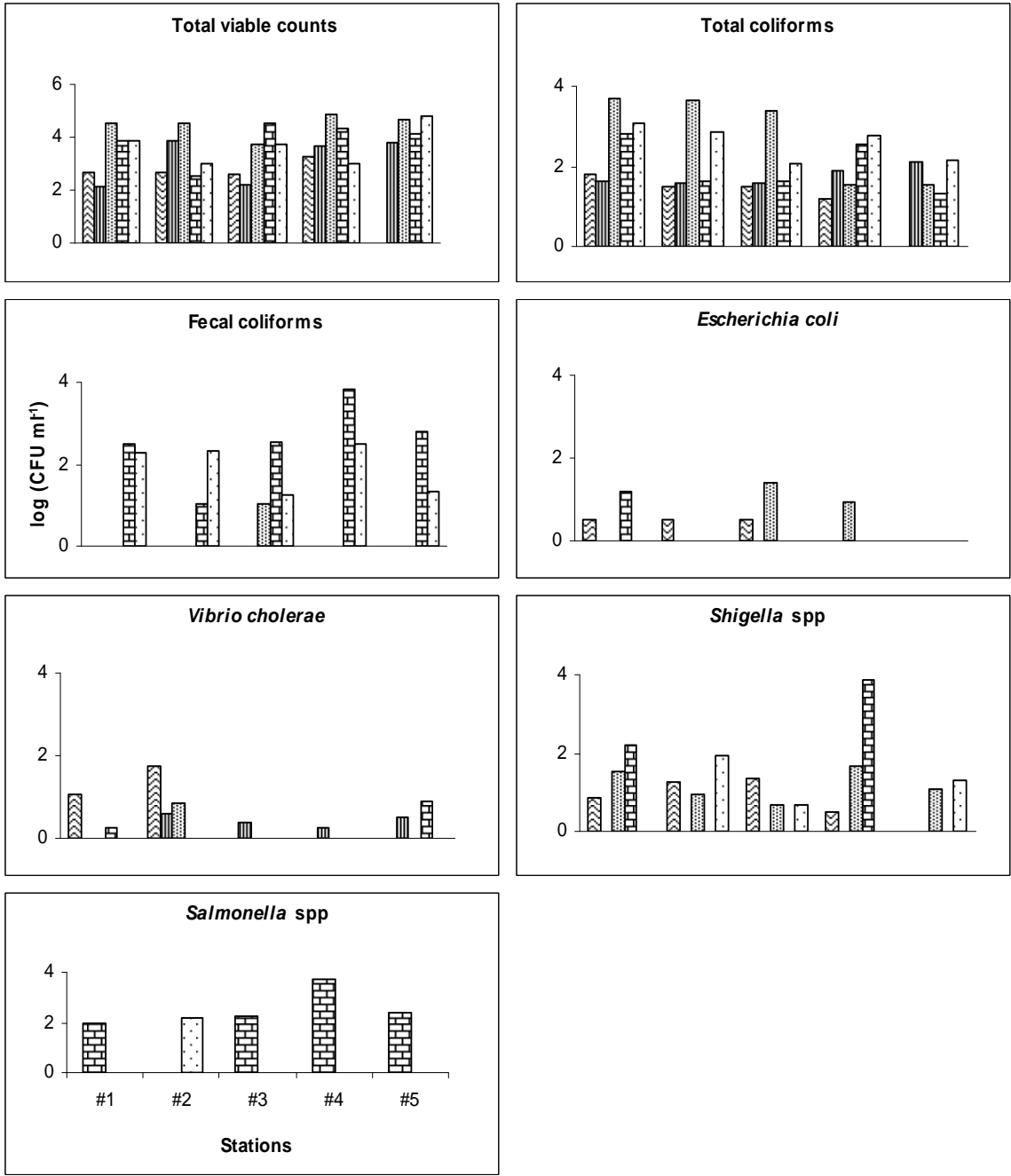


Figure 6a Rodrigues et al.

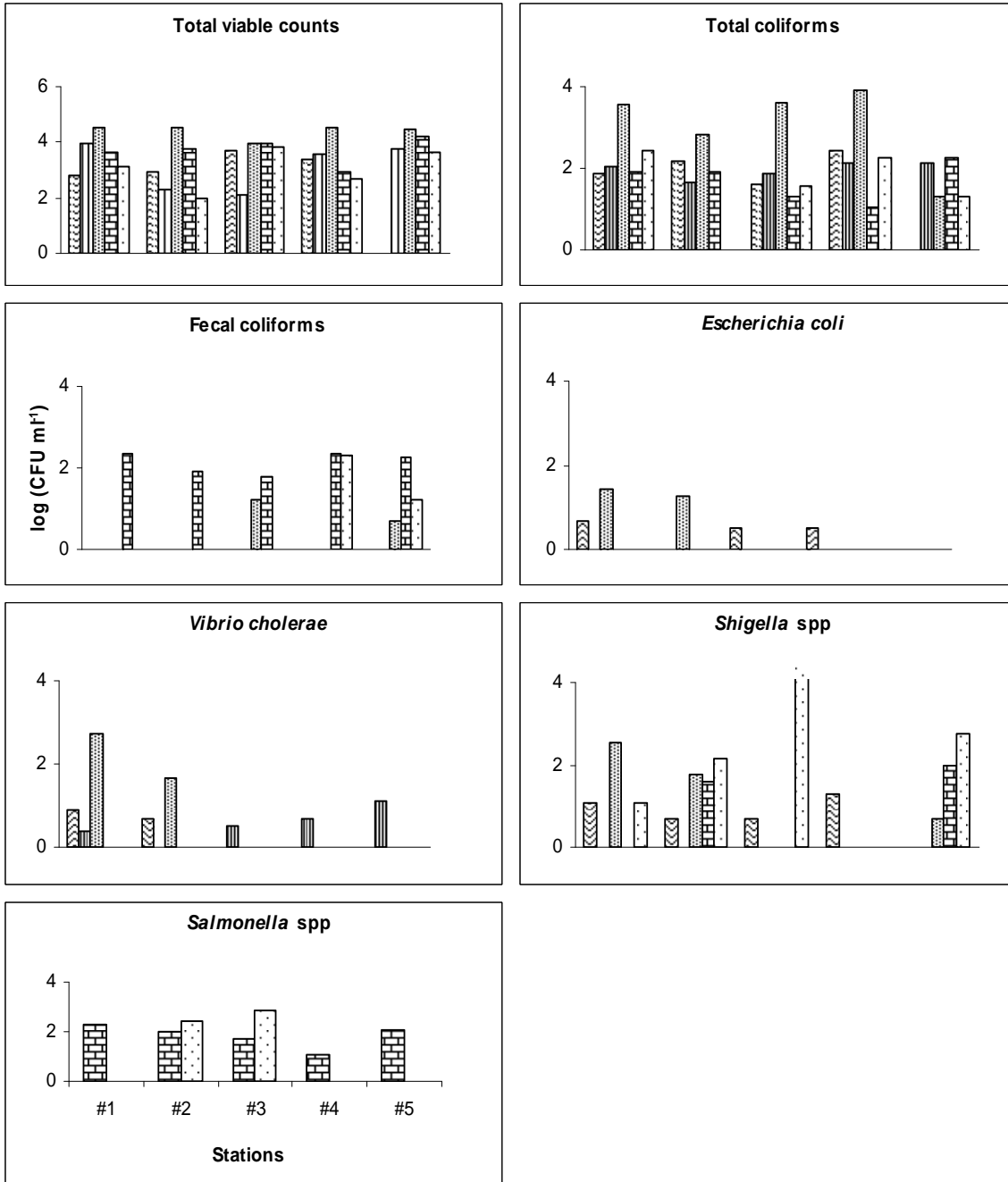


Figure 6b Rodrigues et al.