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Sewage-pollution indicator bacteria

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11.1 Introduction

Microbiologists the world over detect sewage contamination of aquatic habitats by enumerating coliform groups of bacteria (Brock *et al*. 1994). As is universally accepted, higher the sewage contamination (either through indiscriminate, deliberate, accidental, or regular/routine disposals), higher will be the number of coliforms in environmental samples. Further, microbiologists rely on the principle that higher the incidence of sewage indicator bacteria in any environment, higher will be the chances for human pathogenic bacteria to be present (Brock *et al*. 1994). Also, bacterial metabolism is such that if a particular group, say *E. coli*, is the dominant bacterium in the sewage discharges, it can compete with and outgrow the native microflora. This can lead to increased levels of indicator bacteria in the water bodies, and the loads of human pathogenic bacteria may well exceed both ecological and human acceptable limits.

Raw sewage disposal into the Mandovi and Zuari estuaries has been a common practice throughout the history of the estuaries. Treatment of sewage from major cities like Panaji before its disposal into the estuary is a recent development. With increasing population on the banks of the estuaries, the amount of sewage dumped in the estuary has also increased. It is therefore of interest to determine what the levels of pollution indicator bacteria are owing to sewage disposal. This information would help determine if careful waste treatment and disposal procedures are needed to safeguard the natural environment.

In this chapter, we describe the spatial distribution and annual cycle of sewage-pollution- indicator and human-pathogenic bacteria in water and sediment samples in the Mandovi and Zuari estuaries. We then discuss the accepted norms for these indicators in developed countries and reflect on the measures that we need to put in place to improve the conditions in the estuaries.

11.2 OBJECTIVES AND METHODOLOGY

We monitored microbiological parameters to understand spatial and temporal variations in the abundance of sewage-pollution-indicator and human-pathogenic bacteria. For assessing bacteriological quality, samples were collected and analyzed from 12 different locations shown in Map B: M_1, M_2, M_3, M_4, M_5, M_6, and M_7 in the Mandovi, and Z_1, Z_2, Z_3, Z_4, and Z_5 in the Zuari. Sampling was carried out during three seasons representing pre-monsoon (28 April to 8 May 2002), summer monsoon (5–8 September 2002), and post-monsoon (12–15 March 2003). The schedule of observations is given in table 11.1. At each location, water samples were collected every three hours for 24 hours. The eight samples collected over a 24-hour period allowed us to examine the variability in bacterial abundance at different locations in these estuaries.

Water samples were collected using Niskin samplers. A van Veen grab was used for sediment sampling. Only one sediment sample was analyzed from each location during each of the three seasons. After collection, the samples were stored on ice and transported to the laboratory for analysis (usually) within 3 hours. Standard and established microbiological methods (American Society for Microbiology 1957) were followed for all the microbiological analyses. Water samples were analyzed using the standard membrane filtration technique and the filters were placed on specific media. Suitable dilutions of sediment samples were prepared and two replicate aliquots were spread-plated on to agar plates. All plates were incubated at 37°C, and final counts of colony forming units were recorded after 48, or in some instances, 72 hours of incubation.

The pollution-indicator bacteria enumerated from these samples are total coliforms (TC) and total fecal coliforms (TFC). The human pathogens, *Escherichia-coli*-like organisms (ECLO) and *Streptococcus faecalis* (FS), were also enumerated. The TC and ECLO were enumerated on EcoliO157 agar (Hi-Media, Mumbai). All colonies formed on this medium were counted as TC. Typical, blue, convex, entire, 2 mm diameter colonies were counted as ECLO. The FS and TFC were enumerated by using rapid enterococci agar (Hi-Media, Mumbai). All colonies appearing on this medium were counted as TFC. Bluish, entire, convex, small (less than 2 mm

Table 11.1 Sampling schedule followed for enumeration of bacterial populations during this study.

Estuary	Sampling dates	Sampling strategy
Mandovi	28–29 April 2002	Water samples collected every three hours covering a 24-hour period during spring tide
Mandovi	5–6 May 2002	Water samples collected every three hours covering a 24-hour period during neap tide
Mandovi	5–6 September 2002	Water samples collected every three hours covering a 24-hour period
Mandovi	12–13 March 2003	Water samples collected every three hours covering a 24-hour period
Zuari	30 April–1 May 2002	Water samples collected every three hours covering a 24-hour period during spring tide
Zuari	7–8 May 2002	Water samples collected every three hours covering a 24-hour period during neap tide
Zuari	7–8 September 2002	Water samples collected every three hours covering a 24-hour period
Zuari	14–15 March 2003	Water samples collected every three hours covering a 24-hour period

Table 11.2 Mean ± standard deviation of counts of bacterial populations (no ml^{-1}) during 28–29 April 2002 at sampling locations in the Mandovi (M_2–M_7) and Zuari (Z_2–Z_5) estuaries. The bacterial counts are the mean values from at least eight samples from each period of observations. See table 11.1 for the sampling schedule followed in this study. Only one sample was collected at stations denoted with "#". See Map B for station locations from where water and sediment samples were collected.

Station	Total coliforms	Total fecal coliform	*Escherichia coli*	*Streptococcus faecalis*
$M_1^{\#}$	15	5	9	2
M_2	14 ± 10	12 ± 10	0.7 ± 0.8	9 ± 7
M_3	20 ± 17	15 ± 13	0.6 ± 0.6	13 ± 13
M_4	12 ± 6	37 ± 12	2 ± 2	27 ± 15
M_5	7± 5	20 ± 10	1 ± 1	11 ± 7
M_6	10 ± 7	20 ± 13	1 ± 1	16 ± 14
M_7	24 ± 27	1 ± 0.7	1 ± 1	0.4 ± 0.6
$Z_1^{\#}$	15	3	0	0
Z_2	28 ± 14	32 ± 21	2 ± 2	30 ± 22
Z_3	18 ± 13	30 ± 22	2 ± 2	28 ± 24
Z_4	15 ± 7	24 ± 18	3 ± 3	23 ± 20
Z_5	8 ± 6	2 ± 2	0.1 ± 0.2	0.6 ± 1

Table 11.3 Same as table 11.2, except during 5–6 May 2002.

Station	Total coliforms	Total fecal coliform	*Escherichia coli*	*Streptococcus faecalis*
M_2	17 ± 8	16 ± 13	0.6 ± 0.8	10 ± 10
M_3	12 ± 6	14 ± 9	0.4 ± 0.5	8 ± 6
M_4	30 ± 48	23 ± 21	2 ± 3	3 ± 6
M_5	9 ± 10	17 ± 19	5 ± 7	9 ± 14
M_6	20 ± 25	15 ± 12	8 ± 11	9 ± 7
M_7	50 ± 62	2 ± 7	1 ± 1	0.2 ± 0.6
Z_2	25 ± 7	12 ± 7	6 ± 5	3 ± 3
Z_3	21 ± 18	14 ± 5	2 ± 2	6 ± 4
$Z_4^{\#}$	43	8	2	7
Z_5	35 ± 10	0.1 ± 0.2	0.1 ± 0.2	0.04 ± 0.1

Table 11.4 Same as table 11.2, except during 5–6 September 2002.

Station	Total coliforms	Total fecal coliforms	*Escherichia coli*	*Streptococcus faecalis*
$M_1^{\#}$	1900	800	0	1
M_2	299 ± 242	46 ± 76	6 ± 4	36 ± 60
M_3	85 ± 61	14 ± 15	5 ± 3	6 ± 2
M_4	165 ± 112	24 ± 9	11 ± 5	14 ± 7
M_5	130 ± 73	10 ± 9	7 ± 5	4 ± 4
M_6	92 ± 38	84 ± 100	9 ± 5	55 ± 82
M_7	160 ± 190	165 ± 180	8 ± 7	161 ± 180
$Z_1^{\#}$	57	383	0	358
Z_2	59 ± 29	18 ± 12	2 ± 3	8 ± 5
Z_3	94 ± 63	30 ± 42	5 ± 3	10 ± 8
Z_4	188 ± 296	1000 ± 94	2 ± 3	42 ± 56
Z_5	587 ± 1460	59 ± 34	2 ± 2	14 ± 13

diameter) colonies were counted as FS. Based on a recent study (Samant 2006), we have sorted out the uncertainty of ‘like organisms’ by employing an array of biochemical tests. By subjecting over 500 isolates designated as LO to a set of the 12 most relevant biochemical tests, we found that 72.2% of ECLO are EC and 76% of FSLO are FS. Using this result, “nearly true” percentages of EC and FS from the data on ECLO and FSLO have been presented in this chapter. Tables 11.2–11.5 summarize the mean counts of different populations of bacteria in the water samples. Table 11.6 gives their counts in sediments collected from different locations during this study.

Table 11.5 Same as table 11.2, except during 12–13 March 2003.

Station	Total coliforms	Total fecal coliforms	*Escherichia coli*	*Streptococcus faecalis*
$M_1^{\#}$	23	5	0	1
M_2	5 ± 3	2 ± 2	0.1 ± 0.3	0.7 ± 0.7
M_3	6 ± 5	2 ± 3	0.3 ± 0.7	0.3 ± 0.5
M_4	8 ± 5	10 ± 9	0.6 ± 0.7	6 ± 5
M_5	4 ± 3	2 ± 2	0.5 ± 1	1 ± 1
M_6	93 ± 175	26 ± 68	0.6 ± 2	1 ± 2
M_7	36 ± 105	46 ± 136	1 ± 3	0.9 ± 3
$Z_1^{\#}$	23	1	2	0.8
Z_2	37 ± 19	1 ± 1	1.56 ± 2.19	0.8 ± 0.8
Z_3	30 ± 22	2 ± 1	4 ± 4	0.7 ± 0.6
Z_4	11 ± 2	1 ± 1	3 ± 4	0.5 ± 0.7
Z_5	1 ± 2	0.6 ± 1	0	0

Table 11.6 Abundance (no $\times$ 10^5 g^{-1} dried sediment) of pollution-indicator and human-pathogenic bacteria in sediment samples collected from various locations in the Mandovi (M_1–M_7) and Zuari (Z_1–Z_5) during different seasons. See Map B for station locations.

	Total coliforms			Total fecal coliforms			*Escherichia coli*		*Streptococcus faecalis*		
Station	1	2	3	1	2	3	1	2	1	2	3
M_1	1.4	0.3	1	–	7.3	0.1	–	–	–	–	–
M_2	–	–	–	–	–	0.4	–	–	–	–	–
M_3	1.5	1.2	2.9	–	2.4	0.3	–	0.6	–	–	0.1
M_4	219	1.5	–	0.5	0.3	–	–	0.03	–	3	–
M_5	183	–	5.4	23.5	3.7	11.5	–	–	–	–	0.8
M_6	7.3	0.1	2.1	291	0.6	20.9	–	–	–	–	–
M_7	17.6	0.4	10.7	7.4	4.8	87.5	–	–	–	1.9	1.8
Z_1	9.6	9.2	21	–	0.9	0.6	–	0.03	–	0.5	–
Z_2	–	–	0.1	–	–	0.1	–	–	–	–	0.1
Z_3	–	–	–	–	–	–	–	–	–	–	–
Z_4	47.7	–	–	15.9	0.02	–	6.4	–	1.3	0.1	–
Z_5	31.7	0.3	0.1	20.1	0.01	70	–	–	5.1	0.2	–

1: April–May 2002; 2: September 2002; 3: March 2003. *Escherichia coli* were not detected in any of the samples collected during March 2003. '–' implies that the given bacterial group was not detected.

11.3 DISCUSSION AND CONCLUSIONS

Indian standards categorizing natural water bodies for safe uses are not available. There are two well-known standards that have been used extensively in Europe and in the United States of America. The European standard, known as the "European

Blue Flag Beach Criteria" (Anonymous 2002), recommends that coliform counts in excess of 5 per ml in natural water are unsafe for bathing, but the standard goes on to recommend that 5 to 100 coliform per ml are "allowed a few times during the season". In essence, total coliform counts exceeding 5 per ml have serious implications for bathers and fishers of the region. Except for gardening and use in flushing of toilets, freshwater with counts higher than 5 is unacceptable for domestic uses such as washing utensils, feeding farm animals, poultry, etc. The standard in the United States of America has been defined by the US Environmental Protection Agency (USEPA). USEPA sets limits of 2 fecal or 10 total coliforms per ml of seawater (Dufour 1984; Fujioka 2002).

As can be noted from tables 11.2–11.5, by both standards listed above, the waters of the Mandovi and Zuari are unfit for bathing. During September 2002 (table 11.4), the counts of TC exceeded 100 per ml at many locations. The TFC, EC, and FS were all higher during this period of observation than those observed during other sampling periods. This is primarily due to excessive land run-off containing raw sewage and fecal debris that support the proliferation of coliform bacteria examined. During the other observations too, there were hardly any samples that had counts of bacteria that would be considered safe.

Every effort leading to reduction in sewage-pollution-indicating bacteria and pathogenic microbes has to be promoted and implemented. Installation of sewage treatment plants at all the domestic settlements, avoidance of indiscriminate disposal of other organic wastes, and effective waste treatment measures are required. This will not only safeguard the interests of tourism-related uses (such as swimming, recreational fishing, surfing, water-scooter riding, etc.), but also help maintain healthy natural ecosystems in these estuaries. Steps must be put in place to control the flux of raw sewage and related pollutants in these estuaries.

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