

## Pollution monitoring of coastal and estuarine areas: I. Bacterial indicators along the south Gujarat coast

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A study was undertaken to assess the validity of enteric and supplemental bacterial indicators along the 115-km length of the south Gujarat region (India). Enteric organisms like *Proteus* and *Klebsiella*, *Escherichia coli*, *Salmonella* and *Shigella* were detected in 74, 25, 4 and 2 % respectively of the 47 samples. Supplemental indicators such as *Vibrio parahaemolyticus* and *Vibrio cholerae* like organisms were retrieved in 8 and 4 % of samples whereas *Pseudomonas aeruginosa* like organisms could not be detected. Total coliforms and total vibrios were retrieved in 27 and 55 % of the samples. The validity of these bacterial groups as water quality indicators is discussed with statistical relations. It is suggested that inclusion of these groups could be made mandatory in coastal and estuarine waters for the environmental impact assessment.

Coastal waters are often used for several purposes including domestic and industrial discharge of wastewaters for recreation and aquaculture. The south Gujarat coast has several perennial rivers, which flow westwardly before emptying into the Arabian Sea. In general, the rivers are narrow in the freshwater zone but widen considerably in the tidal or estuarine zone which are low lying and marshy and sporadic floods are very common. Hence, major developmental activities have taken place along the banks of rivers and estuaries several kilometers upstream. The wastewater emerging from cities and the variety of industries are discharged ( $4-130 \times 10^6 \text{ m}^3/\text{d}$ ) with minimal treatment<sup>1</sup>. Discharges of sewage effluents have a negative impact on the coastal environment and make it less attractive for recreation as well as aquaculture; the latter also is sensitive to pathogenic microorganisms and toxic compounds. Moreover, occasional pollution by effluents containing human pathogens may result in the prohibition of sale of shellfish causing economic loss.<sup>2</sup> Though the fresh water bodies of the study area provide the major source of water for domestic and industrial use, these areas lack information on the level of microbial pollution indicators. A study was conducted to ascertain the levels of bacterial indicators in these waters.

### Materials and Methods

Sixteen stations were selected, 9 in the mouth of

the major rivers and 7 in the corresponding near shore waters at about 5 m depth along 115 km of the south Gujarat coastline, covering an area of 800 km along north west coast of India (Fig. 1). Sampling at all the

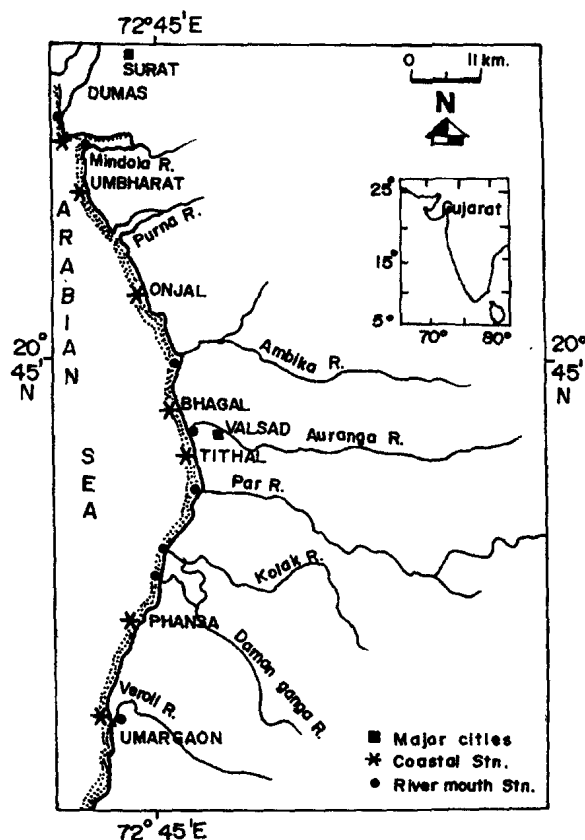


Fig. 1—Location of sampling stations

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stations normally began about one hour before the time of high tide slack and continued until the ebb commenced. Sampling was carried out during Feb. 1996.

Water was collected from surface and near bottom using a Niskin water sampler and aseptically transferred into sterilized glass bottles. Sediment samples were collected using a van Veen grab and sub samples were aseptically transferred into fresh polythene bags with a sterile spatula before disturbing the sediment for other analyses. Immediately after collection both water and sediment samples were stored in ice and transported to the field laboratory. Before analysis the samples were serially diluted from  $10^2$  to  $10^{10}$  and then surface plated in replicates in appropriate media. Enteric bacteria are the main indicator organisms for water quality monitoring. Supplementary bacterial water quality indicators are proposed in pollution studies of coastal waters to ensure proper seawater quality evaluation. In the present study we evaluated the enteric bacteria like total coliforms (TC), *Escherichia coli* like organisms (ECLO), *Salmonella* like organisms (SALO), *Shigella* like organisms (SHLO), *Proteus*, *Klebsiella* like organisms (PKLO), with supplementary micro organisms like total Vibrios (TV), *Vibrio parahaemolyticus* like organisms (VPLO), *Vibrio cholerae* like organisms (VCLO), *Pseudomonas aeruginosa* like organisms (PALO) to assess the water quality.

The media used for the growth of different groups of microorganisms were nutrient agar for total viable counts (TVC), MacConkey agar for TC and ECLO, thiosulphate citrate bile sucrose agar for TV, VPLO and VCLO, xylose lysine deoxycholate agar for SALO, SHLO and PKLO and cetrinide agar for PALO. Counts were made after 48-72 hours. Since confirmatory tests have not been carried out results are expressed as like organisms (LO).

The correlation analysis was carried out to find the relation between the different groups using the computer based program Microstat (1984, Ecosoft, Inc.).

## Results and Discussion

### *Bacterial abundance*

Quantitative abundance and variations of different groups of bacteria in water and sediment are presented in Table 1. Heterotrophs were well represented at all stations. The total viable count of the coastal waters ranged from 0.1 to  $3.32 \times 10^6$  l<sup>-1</sup>. Maximum total viable population of  $3.32 \times 10^6$  l<sup>-1</sup> was

observed in the bottom waters of Phansa. Sample of Daman -Ganga showed a population of  $1.14 \times 10^6$  l<sup>-1</sup>. In river water, TVC varied from 0.07-  $2.42 \times 10^6$  l<sup>-1</sup> at Ambika and at Kolak bottom water and respective coastal stations ( Bhagal and Phansa) recorded 0.45 and  $3.32 \times 10^6$  l<sup>-1</sup>. River discharges have been ascribed as stimulants for higher rates of microbial population and heterotrophic activity in the coastal waters<sup>4</sup>. However the riverine influence to the heterotrophic population was not discernible. The population was found to be quite comparable to other areas of west coast of India, especially Gujarat coast<sup>1</sup>. TVC in sediment was higher than in overlying waters. Sediments from the mouth of Par river had the highest populations of TVC ( $106.4 \times 10^3$  g<sup>-1</sup> dry wt.) of the sediment. Higher population in the sediment is generally attributed to the rich organic content. Besides, it could also be due to the less residence time of the microorganism in the water column compared to sediment.<sup>5</sup> In general, variations in the population between riverine and the respective coastal station were not much. The noticeable exception was the coastal sediment at Phansa where the numbers were two orders less than the population in Par river (Table 1). This is perhaps due to the extensive riverine influence into the coastal area .

### *Occurrence of bacterial groups*

Contribution of total coliforms (TC) to TVC was high at coastal stations of Tithal, Phansa and the rivers Daman Ganga and Kolak. TC counts were higher in Phansa bottom waters ( $1.24 \times 10^6$  l<sup>-1</sup>) in association with a population of  $3.8 \times 10^5$  l<sup>-1</sup> of ECLO ( Table 1). As TC and ECLO exceeded the permitted standards by 2-3 orders, the hygienic quality of the water is affected ( $1.26 \times 10^3$  l<sup>-1</sup> TC and  $2.0 \times 10^3$  l<sup>-1</sup> FC of US EPA)<sup>6</sup>. Hiraishi *et al*<sup>7</sup>, opined that FC and TC are not always reliable indicators of recent fecal contamination. Further, samples with low number of fecal micro-organisms may have high number of non-enteric microorganism and vice versa, which is not surprising because of the diverse nature of the microbial population in seawater<sup>8</sup>. Besides, not many of them survive for long in seawater under certain conditions.

The number of coastal and riverine samples tested and the percentage of positive water quality indicators in 47 samples (Table 2) showed that among the enteric groups, TC and ECLO occurred in 88.2 and 41.1% of the riverine (17) water samples and 71.4 and 21.4% of the coastal (14) water samples. *Proteus* and

Table 1—Bacterial population in various samples.

River	Surface water										Bottom water										Sediment									
	TVC	TC	TV	VPLO	VCLO	SALO	PKLO	TVC	TC	ECLO	TV	VPLO	SHLO	PKLO	TVC	TC	ECLO	TV	VPLO	TV	ECLO	TC	ECLO	TV	VPLO	PKLO				
Veroli	1.36	0.10	2.02	1.45	0.57	0.02	nd	ns	ns	ns	ns	ns	ns	ns	27.23	nd	0.41	nd	ns	ns	ns	ns	0.41	nd	1.22					
Daman	0.85	0.17	0.07	0.01	nd	nd	0.25	1.14	0.17	nd	0.27	0.05	nd	nd	1.21	0.04	nd	0.10	nd	nd	nd	0.10	0.31	nd	0.31					
Kolak	1.08	0.2	0.21	0.02	nd	nd	0.85	2.42	0.24	0.02	0.05	0.04	0.04	nd	2.05	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd					
Par	1.74	0.33	0.03	nd	nd	nd	0.02	0.36	0.2	0.01	0.04	0.04	0.04	nd	106.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd					
Auranga	0.15	0.15	nd	nd	nd	nd	0.01	1.7	0.03	0.01	0.05	0.01	0.01	nd	0.99	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.012					
Ambika	1.36	0.03	0.04	0.02	nd	nd	0.05	0.07	0.03	0.01	0.04	0.02	0.02	nd	1.17	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.004					
Purna	0.24	0.01	nd	nd	nd	nd	0.02	1.03	0.08	nd	0.03	nd	nd	nd	0.12	nd	nd	0.01	0.12	nd	nd	nd	nd	0.01	0.22					
Mindola	0.47	0.01	0.01	nd	nd	nd	0.58	0.3	nd	nd	nd	nd	nd	nd	0.03	nd	nd	nd	0.03	nd	nd	nd	nd	nd	nd					
Tapi	0.39	nd	0.03	0.02	nd	nd	0.20	0.3	0.01	nd	nd	nd	nd	nd	0.04	nd	nd	nd	0.04	nd	nd	nd	nd	nd	nd					
Coastal																														
	Surface water										Bottom water										Sediment									
	TVC	TC	TV	VPLO	VCLO	TVC	TC	ECLO	TV	VPLO	SHLO	PKLO	TVC	TC	ECLO	TV	VPLO	TV	VPLO	TV	ECLO	TC	ECLO	TV	VPLO	PKLO				
Umargaon	0.79	0.01	0.06	0.02	0.04	0.1	0.02	nd	0.02	0.01	nd	0.07	21.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.55					
Phansa	0.23	0.15	0.02	nd	nd	3.32	1.24	0.38	0.02	nd	nd	nd	1.21	0.04	nd	0.10	nd	0.10	nd	nd	0.10	nd	nd	0.31	0.31					
Tithal	1.21	nd	0.01	nd	nd	2.58	0.13	nd	nd	nd	nd	0.65	6.46	nd	0.25	0.01	nd	0.37	0.01	nd	0.25	0.01	nd	0.37	0.37					
Bhagal	0.3	nd	nd	nd	nd	0.45	0.02	0.01	nd	nd	nd	0.07	0.48	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.01	nd	nd					
Orijal	0.46	nd	0.02	nd	nd	0.47	0.03	nd	0.03	nd	0.05	0.11	0.89	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.03	0.03					
Umbharat	0.77	0.25	0.11	0.11	nd	0.82	0.28	nd	0.37	nd	nd	0.11	7.67	0.01	nd	nd	nd	nd	0.01	nd	nd	nd	nd	nd	nd					
Dumas	0.28	0.01	nd	Nd	nd	0.19	nd	0.01	nd	nd	nd	0.13	0.10	nd	nd	nd	nd	nd	0.10	nd	nd	nd	nd	nd	nd					

Please refer text for abbreviations

Populations

in water cfu x 10<sup>6</sup> l<sup>-1</sup>; nd= non-detectable level

in sediment cfu x 10<sup>3</sup> g<sup>-1</sup> ns=no -sampling

*Klebsiella* like organisms (PKLO) were recovered in 94.1% in the riverine and in 71.4% of the coastal samples. PKLO was noticeable in 55.5 % of the riverine, 57.1 % of the coastal sediments (Table 2). Their presence in high numbers could have resulted from the output of the industrial and sewage waste<sup>1,9</sup> and also their successful survival in coastal waters. In sediment, TC and ECLO were detected in 11.1% each in the riverine stations and 28.5 and 14.2% in coastal stations respectively. Pathogenic bacteria such as SALO and SHLO were absent in most of the stations. PALO is a potential pathogen which was in non-detectable levels in riverine and coastal samples. The low recovery of PALO could be due to its poor survival in the seawater. Vibrios are an important group of autochthonous microflora in the marine environment, Their presence implies that there is an

active biodegradable process by the native microflora<sup>10,11</sup>. The incidence of *V. parahaemolyticus* in the east coast inshore water and west coast offshore is documented<sup>12,13</sup>. They occurred at 76.4% in riverine water samples and 64.2% in coastal water samples. and the highest population was observed in the mouth of Veroli ( $2.02 \times 10^6 \text{ l}^{-1}$ ). VPLO and VCLO occurred at 47 and 5.8 % respectively in riverine water samples. However VPLO were present in water and sediment samples of the coastal regions at 21.4 and 14.2%. These numbers were 3 to 4 orders higher than the offshore waters of Arabian Sea<sup>14,15</sup>.

#### Bacterial group and frequency of occurrence

Both enteric and supplementary groups occurred at high frequencies in low densities (Fig. 2). However the enteric organism PKLO occurred 17 times while VPLO among the supplementary group was recovered nearly 10 times. Hence it is indicated that sewage, effluents from pulp, paper and textile finishing factories persist and remain undiluted over a long period of time.

Group	Riverine		Coastal	
	Water(17)	Sediments(9)	Water(14)	Sediment(7)
TC	88.2*	11.1	71.4	28.5
ECLO	41.1	11.1	21.4	14.2
TV	76.4	22.2	64.2	28.5
VPLO	47	0	21.4	14.2
VCLO	5.8	0	7.1	0
SALO	11.7	0	0	0
SHLO	0	0	7.1	0
PKLO	94.1	55.5	71.4	57.14

\* Value arrived as percentage =  $(15/17 \times 100)$

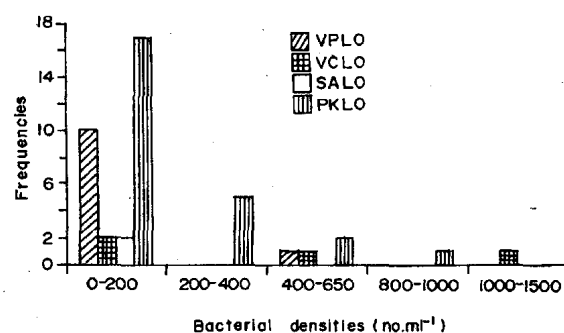


Fig. 2—Pathogenic bacterial density and their frequencies

	TVC	TC	ECLO	TV	VPLO	VCLO	SALO	SHLO	PKLO
TVC	1.0000								
TC	-0.717	1.0000							
ECLO	0.1416	0.4992	1.0000						
TV	-0.0547	0.0701	-0.0659	1.0000					
VPLO	-0.0349	0.0213	-0.0492	0.9704	1.0000				
VCLO	-0.0304	0.0074	-0.0452	0.9681	0.9948	1.0000			
SALO	-0.0281	0.0115	-0.0423	0.9697	0.9965	0.9975	1.0000		
SHLO	-0.0331	0.1498	-0.0423	1.445	-0.0261	-0.0232	-0.0217	1.0000	
PKLO	0.1745	-0.0720	0.0729	-0.0545	-0.0504	-0.0487	-0.0459	-0.0289	1.0000

Critical value (1-Tail, .05) = + or - .24306 Number of cases = 47

Critical value (2-Tail, .05) = + or - .28723 Number of variables = 10

The correlation matrix between different groups of bacteria (Table 3) shows that significant correlations were found between ECLO and TC (0.4992), VPLO, VCLO, SALO with TV (0.9704, 0.9681, 0.9697 respectively) and VCLO and SALO with VPLO (0.9948, 0.9965). The population of TC or FC has no impact on the occurrence of *Salmonella*.<sup>16</sup> TC was not correlated to SALO. Correlation of SALO with Vibrio groups could be attributed to the longevity of *Salmonella*. It is suggested that the source of all the pathogens are probably the same. Incidentally, the correlation between Vibrio groups and SALO could perhaps indicate the surviving ability of the latter. Moreover the simultaneous presence of the various groups suggest that the pathogens are not mutually exclusive. In conclusion our study gives an indication of the extent of microbial pollution, any further addition of wastes may deteriorate the existing hygienic quality in the area. It is recommended that regular microbiological studies including supplemental bacterial indicators should form an integral part of coastal pollution monitoring programs. As retrieved numbers are 2-3 orders less than the actual numbers the use of taxonomic probes in the future could give timely and realistic estimates of the water quality.

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#### References

- 1 Anon, *Marine environmental quality of coastal water of India*. Tech Rep ( Nat. Inst.Oceanography, Goa .India) 1993, pp.101.
- 2 Liv F, Monique P, Marie-Paule C & Ingum M, *Appl Envir Microbiol*, 60 (1994 ) 581.
- 3 APHA-AWWA-WPCF. *Standard methods for the examination of water and waste water*, 16<sup>th</sup> edn. [American Public Health Association (APHA), Washington DC] 1985, pp. 1628.
- 4 Albright L J , *Mar Ecol Prog Ser*, 12 (1983) 107.
- 5 Anon, *Ecobiological, toxicological and environmental impact assessment studies of the effluent discharge from MRL-CBR in marine environs of Nagapattinam. Tamilnadu*, Tech Rep. no. NIO/SP-12/97 (Nat. Inst.Oceanography, Goa, India) 1997, pp.86.
- 6 Cannon J J & Busse M K, *Water Res*, 23 ( 1989) 1167.
- 7 Hiraishi A, Saheki K Horie S , *Bull Jap Soci Sci & Fish*, 50 (1984)991.
- 8 Neelam R, Ramaiah N, Chandramohan D & Nair V R, *Estuar Coast Shelf Sci*, 40 (1995) 45.
- 9 Ferguson C M, Coote B G, Ashbolt N J & Stevenson I M , *Water Res*, 30 (1996) 2045.
- 10 Anon, *Oceanographical studies for the disposal of effluents for Orchid Chemicals and Pharmaceuticals Ltd, Chennai*, Tech Rep no. NIO/SP-5/96.( Nat.Inst. Oceanography.Goa, India) 1996, pp.63.
- 11 Ramaiah N, Raghukumar C, Sheelu G & Chandramohan D, *Indian J Mar Sci*, 25 (1996) 234.
- 12 Nair G B, Abraham M & Natarajan R, *Mahasagar- Bull Natn Inst Oceanogr*, 13 (1980) 285.
- 13 Pradeep R & Lakshmanaperumalsamy P, *Indian J Mar Sci*, 13(1984)113.
- 14 Lokabharathi P A, Ramaiah N & Chandramohan D, *Indian J Mar-Sci*,15(1986)96.
- 15 Lokabharathi P A, Nair S & Chandramohan D, *Mahasagar-Bull Natn Inst Oceanogr*, 20 (1987) 45
- 16 Venkateswaran K & Natarajan R *Indian J Mar Sci* ,16 (1987) 51.