

Pollution in Cochin Backwaters with Reference to Indicator Bacteria

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Quantitative abundance of indicator bacteria, viz. coliforms, *Escherichia coli* and *Streptococcus faecalis*, in sediment and water samples from 6 sewage outfall stations of Cochin backwaters is studied for 1 yr. FC/FS (faecal coliform/faecal streptococci) ratio indicates that the principal source of faecal pollution is non-human type originating from land drainage, discharge of organic waste and also from sewage discharge. Based on quantitative abundance and distribution of these organisms the 6 stations are classified as intensely polluted, fairly polluted, and slightly polluted. Consumption of edible animals obtained from the Cochin estuarine environment, as a source of health hazard is highlighted.

Data on pollution of Cochin backwaters are meagre^{1,2}. Sewage imparts considerably to the quantity of faecal microorganisms, which principally forms potential health hazard. Therefore, it is essential to understand the magnitude of faecal pollution in the environment. Information on faecal pollution of Cochin backwaters is scanty^{3,4} and hence merits a detailed investigation. In this paper, based on data collected during June 1975 to May 1976, quantitative occurrence of indicator organisms like coliforms, *Escherichia coli* and *Streptococcus faecalis* in surface water and sediment samples is presented.

Surface water and bottom sediment samples were collected at fortnightly intervals from June 1975 to May 1976 from 6 selected shallow water stations (1, 2, 4 to 7 as indicated earlier¹) with an average depth of 1½ m, from near the opening of the major canals into the Cochin backwaters. The sediment was collected using a Peterson Grab. A sample was taken from the central portion of the mud in the grab and transferred into sterile sampling petri dishes using a spatula. A plastic bucket was used for collecting water samples from the surface. The samples were held at 0°C on board and transported to the laboratory for plating within 5 hr of collection. Details of plating procedure, enumeration technique, preparation and composition of different media used were same as given earlier⁵. Standard statistical methods were employed to study variance⁶. FC/FS (faecal coliform/faecal streptococci) ratio was calculated from counts both in water and sediment samples for various stations and different months as suggested by Finstein⁷.

S. faecalis, coliforms and *E. coli* occurred regularly in considerable numbers in all stations, both in sediment and water (Table 1).

Streptococcus faecalis—They were less in number in monsoon months (May to July). In general, higher

counts were encountered in sediment samples than in water samples suggesting that they are adsorbed by the sedimentary particles and can survive long periods. Counts of *S. faecalis* ranged from 0 to 22/ml in water and 0 to 40/g in sediment. Highest counts were recorded from st. 1 both in water and in sediment.

The data were subjected to analysis of variance to study whether these organisms vary between stations and between months. Abundance of *S. faecalis* varied significantly between months and between stations at 0.1% level. The analysis showed highest incidence at st. 1 followed by st. 4. November 1975 and February to April 1976 were the peak periods of incidence for the water samples. Sediment samples showed significant variations between months at 0.1% level while between stations variations were not significant. Peak months of incidence for sediment samples were November 1975 and January to April 1976.

Escherichia coli—They showed erratic distribution both in sediment and in water and also with reference to different stations and months. High counts were recorded in June and October. The pattern of occurrence was just the reverse of that of *S. faecalis*, in that higher counts were noticed in water than in sediment. The counts ranged from 0 to 98/m³ in water and 0 to 22/g in sediment. As in *S. faecalis*, here also highest counts were encountered from st. 1 both in sediment and water.

Analysis of variance showed higher counts in st. 1 followed by st. 2, 4 and 5. June and October 1975 were the peak months of incidence in water. For sediment samples, the peak months of incidence were December 1975 to June 1976 and st. 1 followed by st. 4 recorded highest incidence. There was significant variation between stations at 1% level while between months it was significant at 0.1% level. A decline of *E. coli* and coliform counts was recorded³ in monsoon months. In the present study also higher counts were obtained in monsoon month (June). However, higher counts were recorded in postmonsoon months also. Bonde⁸, while studying the faecal coliform counts of the sediments from Oresound found that the counts decreased with depth. In the present study no such relationship with depth was noticed because the stations were shallow. Rittenberg *et al.*⁹ reported *E. coli* and coliform counts to be higher in sediment than in water from the California marine sewage outfall. This is markedly different from the present results, mainly because of the shallow nature of the sampling area.

Coliforms—Quantitative occurrence of coliforms were more in water than in sediment as in the case of *E. coli*. Higher incidence was recorded in premonsoon (February) and monsoon (May and June). As in the previous cases st. 1 recorded highest counts both in water and in sediment. The counts ranged from 5×10^3

Table 1—Incidence of Coliforms, *E. coli* and *S. faecalis* at Different Stations
 [Results are expressed per ml for water samples (W) and per gram for mud samples (M)]

Month	Coliforms ($\times 10^3$)		<i>E. coli</i>		<i>S. faecalis</i>		Coliforms ($\times 10^3$)		<i>E. coli</i>		<i>S. faecalis</i>		Coliforms ($\times 10^3$)		<i>E. coli</i>		<i>S. faecalis</i>		
	W	M	W	M	W	M	W	M	W	M	W	M	W	M	W	M	W	M	
June '75	210	11	98	19	8	9	118	16	33	10	3	nil	120	9	21	1	2	nil	
July '75	42	5	32	6	1	6	22	3	nil	nil	nil	2	17	2	1	4	1	2	
Aug. '75	8	5	10	4	6	nil	32	12	2	nil	nil	3	11	16	2	7	1	2	
Sept. '75	12	24	4	6	3	6	15	18	5	8	2	3	28	20	4	5	2	3	
Oct. '75	20	28	37	nil	3	2	24	20	46	nil	4	3	26	18	7	nil	2	3	
Nov. '75	124	68	22	10	22	19	62	12	12	1	12	11	140	69	14	9	16	17	
Dec. '75	210	122	41	22	16	21	110	28	8	8	10	13	118	89	12	5	7	11	
Jan. '76	96	48	12	16	12	26	42	20	8	3	3	11	78	42	11	9	8	17	
Feb. '76	260	136	36	10	22	40	112	21	12	6	10	30	218	39	12	8	12	20	
March '76	128	31	17	14	18	28	58	22	8	4	8	12	90	30	11	3	10	21	
April '76	112	56	16	17	14	18	75	48	11	10	8	7	93	42	7	5	12	18	
May '76	131	72	14	nil	20	20	118	28	5	nil	10	22	118	74	nil	nil	nil	nil	
Station 4																			
June '75	172	10	20	2	2	nil	235	11	30	4	nil	nil	108	10	28	nil	nil	nil	
July '75	13	9	4	3	1	2	7	8	nil	nil	nil	nil	10	12	nil	3	nil	nil	
Aug. '75	23	9	3	10	3	2	5	12	2	4	nil	4	8	11	nil	1	nil	3	
Sept. '75	20	12	4	5	3	2	10	11	2	3	1	3	15	8	3	5	1	3	
Oct. '75	21	11	33	nil	3	2	8	12	66	nil	1	4	12	10	34	nil	1	3	
Nov. '75	73	22	8	4	15	17	11	12	8	2	6	13	14	14	1	1	6	8	
Dec. '75	120	61	9	7	9	11	20	112	10	12	8	10	70	15	7	12	2	3	
Jan. '76	71	35	8	11	11	15	92	30	6	9	5	17	24	21	2	8	2	8	
Feb. '76	205	37	14	5	11	30	104	31	10	5	12	25	91	24	8	3	8	20	
March '76	110	72	15	8	16	13	123	35	11	9	14	22	81	23	18	8	8	12	
April '76	82	63	13	7	14	15	102	53	10	15	11	8	62	38	9	10	9	7	
May '76	102	88	5	4	12	20	82	72	nil	nil	nil	nil	73	36	nil	nil	nil	nil	
Station 5																			
Station 6																			

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Table 2—FC/FS Ratio of Water (W) and Mud (M) in Cochin Backwaters from June 1975 to May 1976

Month	Station 1		Station 2		Station 3		Station 4		Station 5		Station 6	
	W	M	W	M	W	M	W	M	W	M	W	M
	1975											
June	12.2 ^a	2.1 ^a	11 ^a	10 ^a	10.5 ^a	1 ^b	10 ^a	2 ^a	30 ^a	4 ^a	28 ^a	0
July	32 ^a	1 ^b	—	0	1 ^b	2 ^b	4 ^a	1.5 ^b	0	—	—	3 ^a
Aug.	1.66 ^b	4 ^a	2 ^a	0	1 ^b	3.5 ^a	1 ^b	5 ^a	2 ^a	1 ^b	—	0.33 ^b
Sept.	1.33 ^b	1 ^b	2.5 ^a	2.6 ^a	2 ^a	1.6 ^b	1.33 ^b	2.5 ^a	2 ^a	1 ^b	3 ^a	1.6 ^b
Oct.	12.3 ^b	0	11.5 ^a	0	3.5 ^a	0	11 ^a	0	66 ^a	0	34 ^a	0
Nov.	1 ^b	0.5 ^b	1 ^b	0.09 ^b	0.8 ^b	0.5 ^b	11 ^a	0	2 ^a	1 ^b	3 ^a	1.66 ^b
Dec.	2.56 ^a	1.04 ^b	0.8 ^b	0.06 ^b	1.7 ^b	0.45 ^b	1 ^b	0.63 ^b	1.2 ^b	1.7 ^b	3.5 ^a	4 ^a
	1976											
Jan.	1 ^b	0.6 ^b	2.6 ^a	0.27 ^b	1.37 ^b	0.5 ^b	0.7 ^b	0.73 ^b	1.2 ^b	0.52 ^b	1 ^b	1 ^b
Feb.	1.6 ^b	0.25 ^b	1.2 ^b	0.2 ^b	1 ^b	0.4 ^b	1.3 ^b	0.16 ^b	0.83 ^b	0.2 ^b	1 ^b	0.15 ^b
Mar.	0.94 ^b	0.5 ^b	1.0 ^b	0.3 ^b	1.1 ^b	0.14 ^b	0.93 ^b	0.6 ^b	0.7 ^b	0.8 ^b	1.5 ^b	0.66 ^b
Apr.	1.14 ^b	0.8 ^b	1.37 ^b	1.4 ^b	0.57 ^b	0.27 ^b	0.9 ^b	0.47 ^b	0.9 ^b	1.8 ^b	1 ^b	1.4 ^b
May	0.7 ^b	0.1 ^b	0.6 ^b	0	—	—	0.4 ^b	0.2 ^b	—	—	—	—

a = human and b = non-human

to 260×10^3 /ml in water and 2×10^3 to 136×10^3 /g in sediment samples.

Analysis of variance showed a significant variation between stations at 0.1 % level. The highest incidence was recorded in st. 1 followed by st. 3, 4 and 2 while the peak months of incidence were June 1975, February and May 1976 in the water samples. In sediment samples, between stations variation was not significant at 5% level. Peak months of occurrence were December 1975, April and May 1976. A declining trend of coliform incidence during monsoon and higher counts during premonsoon months were reported³ and the latter observation agrees with the present.

FC/FS ratio—FC/FS (Table 2) ratio revealed no regular pattern from month to month or from station to station. Human faecal pollution was dominant (ratio > 2), both in sediment and in water during June 1975 only, while in rest of the months it was non-human type (ratio < 2) in sediment samples except in August. Human faecal type pollution was recorded in water during September to November 1975, while rest of the months it was non-human type. Highest (66) ratio was recorded in October 1975, in st. 5 in water.

The importance of FC/FS ratio in interpreting the faecal pollution of a given watermass was suggested by several authors¹⁰⁻¹². Geldreich and Clarke¹³ while giving preference to FC/FS ratio in assessing and indicating human and non-human faecal pollution emphasised the importance of collection of samples from the nearest location of sewage outfall to formulate the ratio. In the present investigation also FC/FS ratio was calculated as per Geldreich and Clarke. Based on the quantitative occurrence of indicator group of

bacteria (Table 1) the 6 stations could be classified into intensely polluted (st. 1), fairly polluted (st. 5, 4 and 3) and slightly polluted (st. 2 and 6) areas, which agrees with the earlier observations^{1,2}.

Occurrence of faecal coliform in water and sediment, is regarded as a single important indicator of public health hazard. These organisms get ingested into the body of filter-feeding organisms, bottom dwelling fish, prawns, bivalves, etc., rendering them potential carriers and making them unfit for human consumption. Geldreich and Clarke¹³ reported that the occurrence of coliforms in water or mud signifies the potential presence of other enteric pathogens in the same environment. It is, therefore, obvious that the extent of occurrence of *E. coli*, coliforms and *S. faecalis* in the Cochin backwaters warrants thorough bacteriological quality control of edible animals marketed.

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Isolation & Identification of Luminous Bacteria from Porto Novo Estuarine Environs

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A survey on the occurrence of luminous bacteria was carried out in the Vellar estuary for over 4 months and 105 strains of bacteria were isolated and identified using diagnostic tests. Ninetyeight strains were identified as *Beneckeia harveyi*, 2 as *Photobacterium fischeri* and 5 as *P. leiognathi*. *B. harveyi* was isolated from all samples while *P. fischeri* and *P. leiognathi* showed restricted distribution.

Luminous bacteria occupy a variety of niches in the marine milieu. The functional significance of symbiotic luminescent bacteria is well documented¹⁻³ but the role of luminescence in 'free living' bacteria requires elucidation. Since the proposal of Beijerinck⁴ that all luminous bacteria should be placed in one genus, *Photobacterium*, there has been a lot of confusion regarding their taxonomic status. However, in recent years extensive taxonomical studies⁵⁻⁷ have stabilised this conflicting situation. Currently their identification is based on few simple diagnostic tests. Information on luminescent bacteria from Indian waters is scarce. Jayabalan *et al.*⁸ have recorded the presence of luminescent bacteria from the light organs of 4 species of silver bellies (family: Leiognathidae) from Porto Novo. Results of a survey on 'free living', saprophytic and enteric luminescent bacteria from Porto Novo estuarine environs are presented here.

Sampling and isolation—Twenty four samples of estuarine surface water were collected at high tide between 8 May and 26 August 1978, at weekly intervals from the Vellar estuary, Porto Novo (11°29'N; 79°49'E). In the earlier part of this study, isolation of luminescent bacteria was facilitated by direct plating of water samples in salt water yeast extract medium containing (%) peptone, 1; yeast extract, 0.3; NaCl, 2.4; MgSO₄·7H₂O, 0.7; MgCl₂, 0.53;

KCl, 0.07 and agar-Difco, 1.5; pH 7.4 ± 0.2. Later, isolation technique described by Ruby and Nealson⁹ was followed with some modifications. This involves the use of a complex sea water medium (SWC) containing 750 ml sea water, 250 ml distilled water, 3 ml glycerol, 5 g bacto-peptone and 3 g yeast extract. For a soft agar SWC 7g/litre and for solid SWC medium 12 g/litre Difco agar was added. Approximately 25 ml of water sample was collected in sterile conical flask, of which 2 ml was mixed with 4 ml molten soft agar SWC (40°C), kept in sterilized 10 ml tubes and the mixture immediately spread plated on solid SWC agar plates. Six replicates were plated for each sample and after solidification of the overlay, one set (3 plates) was incubated at 25° ± 1°C and the other at 35° ± 1°C. After 36 hr incubation, plates were examined for the presence of luminous colonies in dark. Luminous colonies were marked on the outer surface of the lower dish of plates with glass marking felt pens. The plates were observed again after 48 hr for additional luminous colonies. The marked colonies were picked out using sterile toothpicks and streaked on SWC agar plates using the flat, broad end of the toothpick. Single bright luminous colonies so obtained were subcultured on SWC slants and maintained at room temperature for further characterization. Fish and shellfish (only crabs and prawns), caught from the estuary using a cast net, were immediately transferred into sterile polythene bags and transported to the laboratory in an insulated container. All specimens were examined within 4 hr of collection by swabbing the gills, external surface and intestinal contents (only for fish) using sterile cotton tipped applicator sticks and streaking on SWC agar plates. For each region of the sample, 2 plates were prepared, one incubated at 25° ± 1°C and the other at 35° ± 1°C. After incubation (36 and 48 hr) luminous colonies were isolated using the same procedure and maintained in SWC slants for further characterization.

Identification of isolates—A total of 16 tests were performed for 105 luminescent isolates obtained (Table 1). The ability to use 8 organic compounds as sole source of carbon, production of extracellular enzymes and the other tests were performed as described by Nealson¹⁰. The nomenclature established by Reichelt and Baumann⁶ was followed.

Incubation period of 36 hr was ideal in the present study for isolation of luminous bacteria with no additional luminescent colonies appearing on reobservation at 48 hr. This concurs with the findings of Ruby and Nealson⁹. Recovery of luminescent bacteria from water samples was enhanced when the isolation technique of Ruby and Nealson⁹ was used and the method was more suitable than the direct plating technique.