

## Occurrence and distribution of bacterial indicators and pathogens in coastal waters of Orissa

Ajit Kumar Patra, Bhaskar Chandra Acharya & Anil Mohapatra  
Institute of Minerals and Materials Technology, Bhubaneswar – 751 013, India

Received 9 April 2008; revised 19 January 2009

Bacterial population in the coastal water of Orissa, up to 10 km from shoreline along 6 transects were observed during 2005-06 and 2006-07. Total viable counts (TVC), total coliforms (TC), faecal coliforms (FC), faecal streptococci (FS), presumptive *E. coli*, *Shigella*, *Salmonella*, *Proteus/Klebsiella*, total *Vibrio*, *Vibrio cholera*, *V. parahaemolyticus* and *Pseudomonas aeruginosa* as well as physico-chemical parameters are determined. Bacterial population was higher in Mahanadi, Paradip and Puri transects compared to other transects. Higher microbial population was recorded in stations close to the shore than the offshore (5 km and 10 km) stations. FC showed positive significant correlation with presumptive *E. coli*, *Shigella*, *Salmonella* and *Proteus/Klebsiella*, Total *Vibrio*, *V. cholera*, *V. parahaemolyticus* and *P. aeruginosa*. TC showed the positive significant correlation with presumptive *E. coli*, total *Vibrio*, *V. cholera* and *P. aeruginosa*. FS showed the positive significant correlation with *Salmonella*, total *Vibrio*, *V. cholera* and *V. parahaemolyticus* and *Shigella*. Correlation analysis revealed positive relationships between faecal indicators and pathogenic microorganisms. Bacterial population showed positive relationship with BOD and negative relationship with salinity indicating dominant influence human activities.

[**Keywords:** Bay of Bengal, Microbiology, Bacterial indicators]

### Introduction

Sewage effluents contain a wide range of human enteric pathogens, which may pose a health-hazard to the exposed human population when they are discharged into natural waters<sup>1,2</sup>. Therefore, microbiological criteria for water quality have been directed towards protection of consumer and bathers from possible microbial pollution, which may cause public health hazards<sup>3</sup>. In recent years, the human interference through urbanization and industrialization has grave impact on the microbial population of coastal water of the Bay of Bengal. Coastal water generally contains both pathogenic and non-pathogenic microbes derived from river run off, sewage, industrial effluents, agricultural activities, wild life and indigenous microorganisms. These pathogens can create health hazard to bathers and consumers when an infective dose of pathogen colonizes a suitable growth site in the body and leads to a disease<sup>4,5</sup>.

The present study consists the microbial population (total viable count, total coliforms and faecal coliforms, presumptive *E. coli*, *Salmonella*, *Shigella*, *Proteus/Klebsiella faecal Streptococcus*, total *Vibrio*, *V. parahaemolyticus*, *V. cholera* and *Pseudomonas aeruginosa*) from coastal water of Orissa. Attempts

have been made to establish their relationship with some physico-chemical parameters.

### Materials and Methods

The study area (Fig. 1) is a stretch along the Orissa coast, located within lat. 19° 18' 11" - 20° 46' 47" N and long. 84° 58' 01" - 86° 59' 32" E. Water samples are collected from Gopalpur, Chilika, Puri, Paradip, Mahanadi and Dhamra transects from stations at a distance of 0 km, 0.5 km, 1 km, 2 km, 5 km and 10 km from the shoreline during post-monsoon season

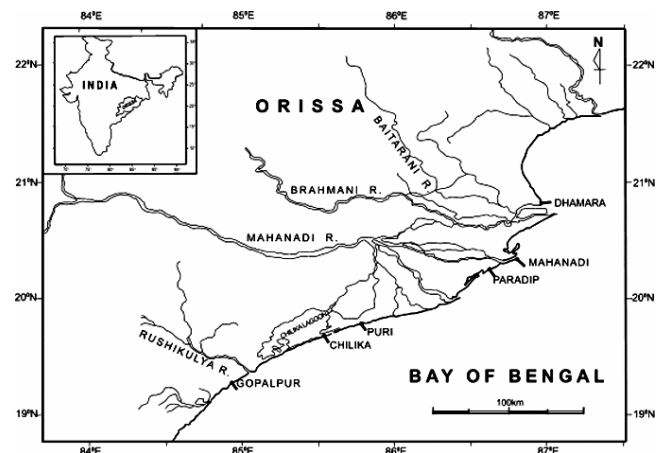


Fig. 1—Sampling location of different transects along Orissa coast

in 2005-06 and 2006-07. Water samples for microbiological analysis are collected from surface using a Niskin water sampler and aseptically transferred into sterilized glass bottles and transported in ice pack to the laboratory. Water temperature and pH are measured by using WTW Kit right in the field. Salinity, dissolved oxygen (DO), biochemical oxygen demand (BOD), suspended solids (SS) are analyzed by the standard methods<sup>6</sup>.

Samples are serially diluted, surface plated in duplicate in appropriate media before microbial analysis. Total viable counts (TVC), enteric bacteria like total coliforms (TC), faecal coliform (FC), presumptive *Escherichia coli*, *Faecal streptococci* (FS), *Salmonella*, *Shigella*, *Proteus/Klebsiella*, with supplementary microorganisms like total *Vibrio*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Pseudomonas aeruginosa* are enumerated to assess the water quality. The media (HiMedia, Mumbai) used for the growth of different groups of microorganisms are Nutrient Agar for TVC, M-FC Agar for FC, MacConkey Agar for TC and presumptive *E. coli*, Thiosulphate Citrate Bile Sucrose Agar (TCBS) for total *Vibrio*, *Vibrio parahaemolyticus* and *Vibrio cholerae*<sup>7</sup>, Xylose Lysine Deoxycholate (XLD) agar for *Salmonella*, *Shigella* and *Proteus/Klebsiella*, M-Enterococcus Agar for FS and Cetrimide Agar for *Pseudomonas aeruginosa* counts. Nutrient Agar plates are incubated at 28±1°C and counted after 24 hrs and 48 hrs. M-FC plates incubated at 44.5±1°C and counted after 48hrs. All other plates are incubated at 37±1°C and counted after 48 hrs.

Pearson correlation analysis was performed on correlation matrix of rearranged data of 19 attributes (microbiological parameters and some water quality parameters) and 63 cases, with the help of SPSS-ver. 10.0 software.

**Results and Discussions**

**Microbiological parameter**

During the investigated period, TVC varied from 25 to 1000 CFU/ml, TC 10 to 200 CFU/ml, FC ND to 30 CFU/ml, FS ND to 8 CFU/ml, presumptive *E. coli* ND to 17 CFU/ml, *Shigella* ND to 12 CFU/ml, *Salmonella* ND to 17 CFU/ml, *Proteus/Klebsiella* ND to 20 CFU/ml, Total *Vibrio* ND to 23 CFU/ml, *Vibrio cholera* ND to 15 CFU/ml, and *Pseudomonas aeruginosa* ND to 10 CFU/ml (Table 1). Bacterial counts in all transects were higher in stations close to the shore than offshore stations.

The TVC are recorded higher in the Mahanadi transect than the other transects of Orissa during both the years (Fig. 2). This might be due to the discharges from the municipality sewages and fishing harbour activity near the river mouth and river runoff. TVC in the Gopalpur, Chilika, Puri and Dhamra transects were higher during the year 2006-07 in comparison to 2005-06, while in transects like Mahanadi and Paradip, the counts are decreased in 2006-07 compared to 2005-06.

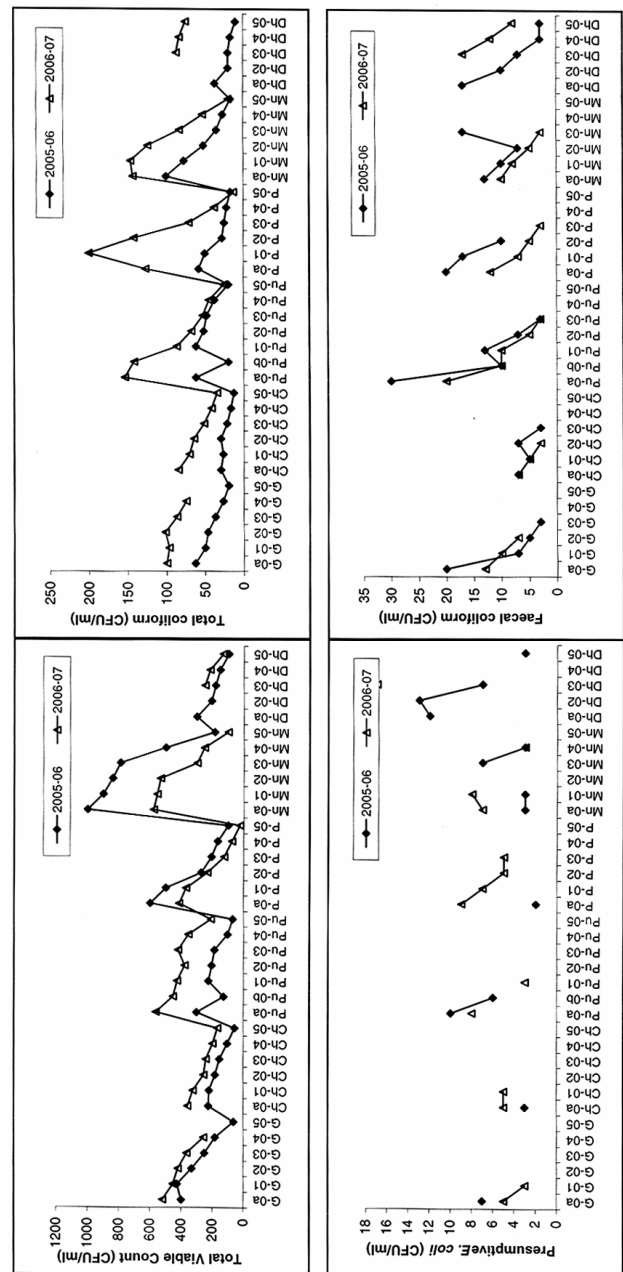


Fig. 2—Variation of TVC, TC, FC and presumptive *E. coli* population at different stations during 2005-06 and 2006-07

Table 1—Minimum, maximum and average value of bacterial population [CFU/ml at different transects in 2005-06 and 2006-07.

	Gopalpur		Chilika		Puri		Paradip		Mahanadi		Dhamra	
	Min-Max	Average	Min-Max	Average	Min-Max	Average	Min-Max	Average	Min-Max	Average	Min-Max	Average
2005-06												
TVC	63-427	275.8	58-225	157.5	70-302	174.3	100-600	308.0	187-1000	702.8	97-302	188.4
TC	20-63	40.7	13-30	23.2	20-62	43.1	17-58	33.3	17-100	51.3	10-37	20.8
FC	0-20	5.8	0-7	3.7	0-30	9.0	0-20	7.8	0-17	7.8	3-17	8
Pres EC	0-7	1.2	0-3	0.5	0-10	2.3	0-2	0.3	0-7	2.7	0-13	7
SH	0-3	1.5	0-7	3.3	0-10	5.0	0-10	3.5	0-7	3.0	0-5	1
SA	0-17	3.7	0-5	2.3	0-7	3.7	0-7	2.8	0-5	2.5	0-5	1
PK	0-3	0.5	0-7	2.5	0-7	4.1	0-20	5.5	0-10	2.8	0-0	0
TV	0-17	7.3	0-17	5.0	0-23	8.7	0-22	6.5	0-20	10.0	0-13	5.2
VP	0-10	4.2	0-7	2.2	0-15	5.1	0-15	3.5	0-10	4.7	0-8	3
VC	0-7	3.2	0-10	2.8	0-8	3.6	0-7	3.0	0-10	5.3	0-5	2.2
FS	0-5	1.3	0-2	0.3	0-3	0.4	0-0	0.0	0-3	0.8	0-3	1.2
PA	0-5	1.2	0-2	0.3	0-2	0.3	0-0	0.0	0-3	0.8	0-3	0.6
2006-07												
TVC	256-521	403.2	168-359	257.8	210-563	400.7	25-415	207.7	100-580	387.2	133-250	200.0
TC	75-102	92.2	35-85	58.3	26-154	82.1	13-200	98.5	20-146	94.8	75-87	81.7
FC	0-13	6	0-7	2.5	0-20	6.9	0-12	4.5	0-10	4.3	8-17	12.3
Pres EC	0-5	1.6	0-5	1.7	0-8	1.6	0-9	4.3	0-8	3.0	0-17	5.7
SH	0-5	1.6	0-3	1.0	0-3	1.3	0-5	3.0	0-6	1.5	0-12	6.7
SA	0-3	1.2	0-0	0.0	0-7	1.7	3-7	4.3	0-5	2.2	0-0	0.0
PK	0-5	1	0-0	0.0	0-7	1.0	3-8	5.2	0-7	1.7	0-5	2.7
TV	0-20	6.6	0-10	3.0	0-13	6.0	0-12	6.7	0-10	1.7	6-8	7.3
VP	0-5	1.6	0-3	1.0	0-7	2.6	0-7	3.2	0-3	0.5	3-5	4.3
VC	0-15	5	0-7	2.0	0-10	3.4	0-5	3.5	0-7	1.2	3-3	3.0
FS	0-7	2.4	0-0	0.0	0-7	1.9	3-8	4.2	0-3	0.5	0-5	1.7
PA	0-10	3.4	0-0	0.0	0-10	2.6	0-3	0.5	0-3	0.5	0-8	2.7

TC counts were comparatively higher in 2006-07 than 2005-06. The TC population increased during 2006-07 in stations close to the shore water (Fig. 2). It indicates the increase in the human induced activities in the near coastal zone and riverine discharge sources. TC population was recorded higher in Puri, Paradip and Mahanadi transects compared to other transects.

The FC population was very high in Puri transect due to human interference through settlements and mixing of untreated municipality sewage at (PU-0a) station. In all transects except Dhamra, the FC population was higher during 2005-06 (Fig. 2). FC counts in most of the stations close to the shore crossed the permissible limit for Class SW-II w (for bathing, contact water sports and commercial fishing: 100 CFU/100ml given by Environment Protection Rules, New Delhi, 1998). FC population was not recorded from the farther stations of most of transects except Dhamra. Several authors have reported loss of

recovery of faecal coliform in seawater due to microbial die-off and entry of microorganisms in to a viable but non-culturable state<sup>1,8-9</sup>. The presence of FC in the offshore station of Dhamra is localized in nature and may be due to contamination from fishing boats. The TC counts are always higher than FC counts, since total coliform can originate from non-faecal sources such as plants and soils<sup>10</sup>. Coliforms are generally accepted as an indicator for sewage pollution. The coliform bacteria were higher in stations close to the shore at Puri, Mahanadi and Paradip transects.

The presumptive *E. coli* counts were almost absent in stations beyond 3 km from shoreline of Gopalpur, Chilika and Puri transects. The EC population was very high in Dhamra transect and Puri close to the shore stations (Fig. 2). Coastal water close to shore contained more presumptive *E. coli* counts, mainly due to faecal contamination from the human settlement along the coastal stretch and municipal

sewage mixing. *E. coli* is normally found in human and animal intestines and is the most reliable indicator of faecal contamination in water, which indicate the possible presence of pathogens<sup>11</sup>.

Higher FS counts are found at Paradip close to the shore during 2006-07. More counts are recorded at Puri and Paradip close to the shore (Fig. 3). The population has crossed the limit 100 CFU/100ml. FS are considered by many authors to be a good indicator

of faecal pollution because they are more resistant than coliforms to environmental stress and its relationship with gastro-intestinal disturbances<sup>1, 12-13</sup>.

*Salmonella* and *Shigella* are pathogens that are distributed world wide and transmitted mainly through food and water ingestion. Their presence in water including recreational water, render that water unfit for human use<sup>5</sup>. The higher counts of *Shigella* are found at Dhamra (Dh-03) during 2006-07, and at Puri and Paradip during 2005-06 (Fig. 3). Higher counts of *Salmonella* are found at Gopalpur close to the shore in 2005-06.

*Vibrio* includes several species, the most of which is *V. cholera* and *V. parahemolyticus*. Higher total *Vibrio* counts are found at Puri, Paradip and Mahanadi transects in 2005-06. Water close to the shore contains higher total *Vibrio* counts than the offshore. Puri, Paradip and Mahanadi transects contain more counts of *Vibrio parahemolyticus* than the other transects during both the years. Higher *Vibrio parahemolyticus* counts are found during 2005-06. Higher *Vibrio cholera* was found at Gopalpur close to the shore in the year 2006-07 (Fig. 4). Coastal water close to shore contains more counts than the off shore. The higher influence of the marine environment provokes bacterial inactivation of the allochthonous microbiota from sewage discharges, except for the halotolerant microorganisms *Vibrio* and staphylococci<sup>1, 14-15</sup>.

Higher number of *Proteus/Klebsiella* counts are found at Paradip (P-01) during 2005-06. In both the year, Paradip water close to the shore contained more counts of *Proteus/Klebsiella* than offshore water. *Proteus/Klebsiella* are recorded in water close to the shore (Fig. 3) other than Dhamra transect.

More counts of *Pseudomonas aeruginosa* are found at Gopalpur and Puri close to the shore during 2006-07 (Fig. 4). *P. aeruginosa* in recreational waters is harmful because it caused ear infections among bathers<sup>16-18</sup>.

**Physico-chemical parameter**

The water temperature during sample collection varied from 21.5°C to 29.3°C in 2005-06 and from 20.5°C to 26.7°C in 2006-07. pH of coastal water samples vary between 7.96 to 8.70 and salinity from 9.30 to 31.09 PSU (Table 2). Comparatively salinity was lower in Mahanadi, Paradip and Dhamra

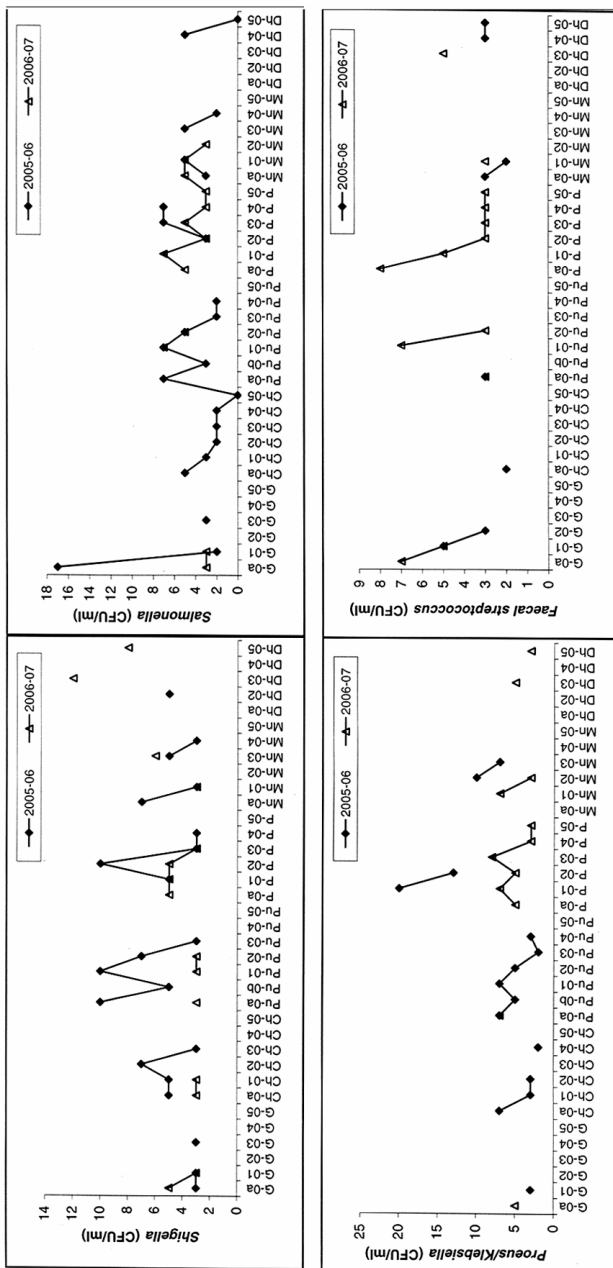


Fig. 3—Variation of Shigella, Salmonella, Proteus/Klebsiella, Faecal streptococcus population at different stations during 2005-06 and 2006-07

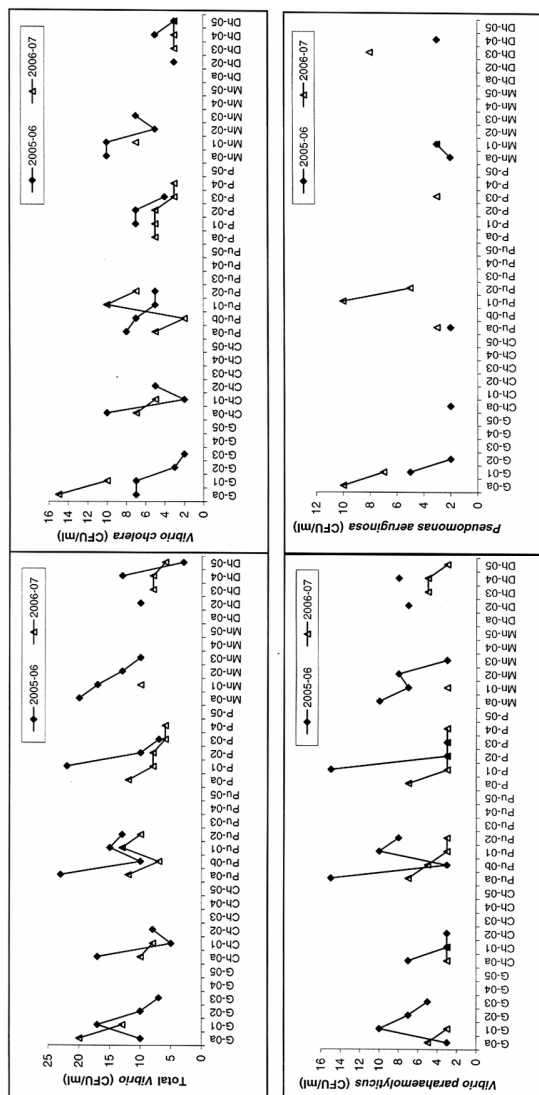


Fig. 4—Variation of total Vibrio, *V. cholera*, *V. parahaemolyticus* and *P. aeruginosa* population at different stations during 2005-06 and 2006-07

transects due to mixing of river water. The suspended solid concentration varied from 2.12 mg/l to 51.00 mg/l and higher concentrations recorded in Dhamra and Mahanadi transects. DO varied from 6.16 mg/l to 8.15 mg/l in 2005-06 and 5.24 mg/l to 8.50 mg/l in 2006-07. BOD varied from 0.14 mg/l to 2.94 mg/l in 2005-06 and 0.49 mg/l to 2.99 mg/l in 2006-07. Higher BOD value (~3.0 mg/l) was recorded from Gopalpur close to the shore station. The BOD values were higher in stations close to the shore, which may indicate the influence of human settlements and mixing of untreated sewage.

**Correlation of environmental variables and the microbial population**

The correlation among the microbial population and the environmental variables is presented in Table 3. Correlation matrix showed a very good positive relationship of BOD, with most of the bacterial counts (TVC, TC, FC, presumptive *E. coli*, FS, *Salmonella*, total vibrio, *V. cholera*, *V. parahaemolyticus*, *P. aeruginosa*). This clearly infers that the microbial population in the coastal water contributes the BOD. A negative relationship of salinity exists with the bacterial populations except FS and *P. aeruginosa*. Hence the source of bacterial population is riverine. *P. aeruginosa* is a ubiquitous organism. Suspended solid in water exhibit significant positive correlation with FC and pres. EC. This is due to their association with the former through organic debris, sewage etc. DO shows negative correlation with TC. Water temperature and pH do not exhibit any significant relationship with the bacterial population.

Table 2—Minimum, maximum and average value of physico-chemical parameter at different transects in 2005-06 and 2006-07.

	Gopalpur		Chilika		Puri		Paradip		Mahanadi		Dhamra	
	Min-Max	Average	Min-Max	Average	Min-Max	Average	Min-Max	Average	Min-Max	Average	Min-Max	Average
2005-06												
WTEMP	22.7-26.2	23.6	22.6-26.0	23.4	23.5-25.5	24.2	22.0-23.5	22.4	21.5-22.9	22.5	23.1-29.3	24.5
SSC	5.77-10.00	8.08	7.71-13.00	9.52	7.69-13.54	10.27	7.24-10.50	8.88	7.80-15.70	11.82	14.90-44.20	28.27
pH	8.15-8.23	8.21	8.21-8.40	8.25	8.13-8.70	8.29	8.06-8.24	8.20	8.22-8.23	8.23	7.96-8.15	8.10
SALIN	27.80-29.33	28.29	25.52-27.24	26.37	26.66-28.16	27.01	24.14-26.86	25.03	9.30-26.00	22.17	16.06-25.69	23.00
DO	6.86-7.11	6.99	6.62-6.90	6.77	6.16-7.88	6.76	6.69-7.91	7.61	7.48-8.15	7.86	7.6-8.12	7.782
BOD	0.77-2.94	1.61	0.20-1.17	0.57	0.14-1.35	0.57	0.58-2.18	1.34	0.67-2.48	1.30	1.08-2.23	1.8
2006-07												
WTEMP	25.7-26.4	26.1	24.8-25.7	25.3	25.3-26.7	25.8	25.3-25.7	25.4	24.0-25.1	24.3	20.5-20.9	20.8
SSC	2.12-3.40	2.98	2.25-10.20	6.80	6.60-8.64	7.56	4.51-9.98	7.22	5.77-8.38	7.14	14.08-31.9	23.29
pH	8.17-8.28	8.22	8.03-8.25	8.18	8.13-8.39	8.31	8.40-8.46	8.43	8.13-8.41	8.28	8.36-8.38	8.37
SALIN	30.71-31.09	30.90	30.17-31.09	30.67	27.11-30.75	29.53	25.97-30.66	29.19	28.01-30.24	29.17	27.93-28.54	28.24
DO	6.83-8.50	7.78	6.45-8.06	7.36	6.97-7.90	7.51	5.24-8.05	7.08	6.20-8.44	7.03	7.64-7.90	7.79
BOD	0.49-2.99	1.64	0.68-1.38	1.00	0.90-2.11	1.53	0.65-1.79	1.50	0.91-2.80	1.56	1.36-1.93	1.66

Table 3—Correlation matrix of different water quality parameters with the bacterial population

	TVC	TC	FC	Pres EC	SH	SA	PK	TV	VP	VC	FS	PA	pH	SALIN	DO	BOD
TVC	1.000															
TC	0.508**	1.000														
FC	0.496**	0.373**	1.000													
Pres EC	0.220	0.336**	0.616**	1.000												
SH	0.133	0.186	0.543**	0.398**	1.000											
SA	0.156	0.180	0.336**	0.206	0.360**	1.000										
PK	0.188	0.179	0.424**	0.171	0.521**	0.173	1.000									
TV	0.455**	0.269*	0.645**	0.294*	0.613**	0.414**	0.548**	1.000								
VP	0.364**	0.192	0.620**	0.236	0.565**	0.283*	0.571**	0.916**	1.000							
VC	0.469**	0.3*	0.558**	0.303*	0.555**	0.476**	0.428**	0.912**	0.670**	1.000						
SF	0.207	0.430**	0.291*	0.382**	0.278*	0.295**	0.187	0.527**	0.370**	0.595**	1.000					
PA	0.259*	0.261*	0.303*	0.295*	0.276*	0.198	0.075	0.498**	0.291*	0.624**	0.753**	1.000				
pH	-0.182	0.069	-0.016	0.026	0.220	0.153	0.206	0.130	0.109	0.130	0.288*	0.094	1.000			
SALIN	-0.348**	0.148	-0.332**	-0.220	-0.233	-0.076	-0.103	-0.256*	-0.314**	-0.152	0.061	0.123	0.288*	1.000		
DO	0.066	-0.294*	-0.025	-0.021	-0.062	-0.150	-0.039	0.056	-0.012	0.117	-0.043	0.198	0.176	-0.026	1.000	
BOD	0.493**	0.463**	0.526**	0.383**	0.102	0.257*	0.116	0.479**	0.371**	0.505**	0.521**	0.469**	-0.002	-0.097	0.243*	1.000

NB: \* - Significant at 0.05 level, \*\* - Significant at 0.01 level

## Conclusions

Indicator microorganisms (such as TC, FC, FS) have been used as models for the potential presence of pathogenic microorganisms in water samples. It showed positive significant correlation with the pathogenic microorganisms tested. TC showed significant correlation with presumptive *E. coli* and with *total Vibrio*, *V. cholera* and *P. aeruginosa*. FC showed positive significant correlation with presumptive *E. coli*, *Shigella*, *Salmonella*, *Proteus/Klebsiella*, *total Vibrio*, *V. cholera*, *V. parahaemolyticus* and with *P. aeruginosa*. FS showed the significant correlation with presumptive *E. coli*, *Salmonella*, *total Vibrio*, *V. cholera* and *V. parahaemolyticus* and with *Shigella*. Positive correlation of TC with FC and FS showed that the most of the bacterial population are from faecal contamination.

## Acknowledgements

The authors are thankful to Prof. B. K. Mishra, Director, Institute of Minerals and Material Technology, Bhubaneswar for kind permission to publish this paper. Thanks are due to the team members of COMAPS project for their help during the sample collection and analysis. Financial grant received from the Ministry of Earth Sciences, Govt. of India in the form of a project is gratefully acknowledged.

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