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Harnessing Bacterial Indicators along with Physicochemical Parameters to Assess Pollution in the Ganges River

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Water is an important asset for every developing country especially the river water which is the prime source for drinking water. The Ganges, largest river of Indian subcontinent is being severely polluted by mass bathing, sewage treatment plants, factory effluents and various other human activities. In the present study, a long stretch of Ganges from Devprayag to Allahabad is selected to ascertain the bacteriological quality of water. The parameters *viz.,* standard plate count, total coliforms, fecal coliforms and FC: FS ratio has been investigated to detect the changes in water quality due to presence of microorganism. The results obtained have been compared with the Bureau of Indian standards. The Most probable number (MPN) values from all the places were found to be higher. A comparatively higher FC: FS ratio was observed in stretches having high population density as compared to stretches where the population density is substantially lower. Various physicochemical parameters *viz.,* dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), and total dissolved solids (TDS) were also examined. Except DO and BOD values, all other parameters were found to be higher than the permissible limits. The percentage of Gram negative bacteria (62.8%) was found to be very high during the course of investigation. The morphological and biochemical tests highlighted the presence of *E. coli* in the maximum number from all the locations.

Key words: Pollution, Total Coliform (TC), Fecal Coliform (FC), Fecal Streptococci (FS).

The Ganges, largest river of the Indian sub continent flows about 2,510 km from Himalayas to Bay of Bengal. The Ganges flow through the most densely populated states of India. The Ganges is lifeline for approximately 400 million people, residing along its basin[1]. For this reason, it is heavily polluted bacteriologically and physiologically. The various point and non point sources of pollution include sewage[1], fertilizers, herbicides and insecticides from agricultural lands and residential areas oil, grease and toxic chemicals from urban runoff, energy production sediment from improperly managed construction sites, crop and forest lands, eroding stream banks, irrigation practices[2], acid drainage from abandoned mines, bacteria and nutrients from livestock[3], pet wastes and faulty septic systems[4]. In addition, the Ganges is heavily polluted by the mass bathing of people in accordance with the Hindu spirituals[5]. The water being oligotrophic does not support the bacterial growth for long time but it act as a vector for their transmission and is a source for gastro-intestinal diseases[6, 7]. On the other hand, water acts as a reservoir for many bacterial species providing favourable temperature and pH conditions leading to abundance and diversity of the bacterial strains[8]. The Ganges is the major source of municipal drinking water supply and masses of

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population uses it directly. Due to association of water quality with the public health, it is important to regularly check the Ganges water for the occurrence of pathogenic bacteria as well as for the harmful chemicals.

The microbial profiling of the river Ganges has revealed that it contains mostly Gram negative bacteria most of them are unexplored for their bioprospecting potential[9]. Most of the bacterial species present in the Ganges is still unidentified due to the inefficiency/no availability of the culture media[10]. These groups of bacteria are viable but unculturable (VUC). New techniques and methodologies are required for the discovery and analysis of the prevalent bacterial strains for new uses. The viable culturable (VC) bacteria are also not studied and optimised for their novel uses.

The present study focuses on the isolation of prevalent bacterial strains from the upper, middle and lower stretches of Ganges and assesses the quality of water using bacterial indicators.

MATERIALS AND METHODS

Description of study area

The Ganges was divided into three stretches *viz.,* upper, middle and lower for the ease of sample collection. Starting from Devprayag to Allahabad five sits were selected under three stretches (Table 1).

At the upper stretch in Devprayag, Bhagirathi and Alaknanda rivers confluence to form the Sangam which forms the Ganges in the downstream, following Rishikesh, Haridwar, Garhmukteshwar and Allahabad. In the lower stretch at Allahabad the Ganges and Yamuna rivers met and form Sangam which further flows as Ganges in the downstream leading to the Bay of Bengal.

Sample collection

In the upper stretch, samples were collected in sterile containers from Sangam, Bhagirathi and Alaknanda and were designated as UD1, UD2, and UD3 respectively. Similarly at the middle stretch constituting Rishikesh, Haridwar and Garhmukteshwar samples were collected in triplicates from each site in sterile containers and designated as MR1, MR2, MR3, MH1, MH2, MH3, MG1, MG2 and MG3. In the same way, the samples were collected from Allahabad in triplicates having codes LA1, LA2 and LA3.

Physicochemical analysis

Physicochemical parameters including dissolved oxygen (DO) and temperature were measured on site at the time of sample collection using portable instruments[11], while total dissolved solids (TDS), chemical oxygen demand (COD) and BOD were analyzed in the laboratory.

Microbiological processing of samples

The total viable count (TVC) was determined using standard plate count (SPC) method by spreading on nutrient agar (Himedia) plates in triplicates[12]. The results were enumerated after 48h of incubation at their native temperature and expressed as colony forming units (cfu/ml) per unit volume. The isolates having different cell morphology and colony characteristics were selected and stored on nutrient agar slants at 4 to -20 °C.

By using the multiple tube fermentation technique in lactose broth with bromophenol blue dye as indicator at 37 °C for 24 h, the presence of total coliforms was determined. Coliform count was enumerated by Most Probable Number (MPN) technique through the production of acid and gas. Further a loop full of culture from the tubes showing positive results in MPN were streaked on EMB agar and Endo agar for the detection of lactose and non-lactose fermenters.

For the identification of fecal coliforms and fecal streptococci, a loop full of culture from the MPN tubes were inoculated in the EC broth and glucose azide broth and kept at 44.5 °C for 24 hours. The MPN values obtained were used to calculate the FC: FS ratio[10]. Further, a loop full of culture from positive tubes were streaked on EMB agar and KF streptococcal agar and kept at 44.5 °C for 24 hours.

The isolated bacterial strains were analyzed on the basis of morphological characters and biochemical tests using Bergey's manual of determinative bacteriology[13]. The IMViC (Indole, Methyl Red, Voges-Proskauer, and Citrate) test was performed for the characterizing the bacteria on the basis of their metabolic activities. Triple sugar iron agar (TSI) detects the ability of the bacterial isolates to ferment glucose, lactose and sucrose. These characteristics helps in distinguishing various members of Enterobacteriacae family,

including *Salmonella* and *Shigella*, which are intestinal pathogens. Morphology and staining reaction of the selected culture were observed by microscopic techniques

Physicochemical parameters such as BOD, COD, TDS, and DO were calculated[14] and compared with the bureau of Indian standard 2004 specification for the drinking water quality.

RESULTS AND DISCUSSION

The standard plate counts were in the order of magnitude of 10^2 cfu ml^{-1} for all sites (Fig. 1). All samples were found to have SPC higher than those prescribed in bureau of Indian Standards[15]. It indicates that the bacteriological quality of water is not fit for drinking. The SPC count was found to increase in downstream flow which is in accordance with the work done by Sood *et al.,* 2008[9]. The SPC was exceptionally high at Allahabad which may be due to the large

Table 1. Different stretches of Ganges for the Sampling

Stretch	Site	Code
Upper	Devprayag	Dev
Middle	Rishikesh	Ris
Middle	Haridwar	Har
Middle	Garhmukteshwar	Gar
Lower	Allahabad	Ald

Table 2. Biochemical and morphological tests of the prevalent bacterial strains isolated from Ganges

S. No.	Strain name	IMViC	TSI		Gram Reaction	Shape
			Butt	Slant		
1.	JSD11	- - - -	R	R	-	Rod
2.	JSD12	- + + -	Y	Y	+	Coccus
3.	JSD13	- - + -	Y	R	-	Rod
4.	JSD14	+ + - -	R	R	-	Rod
5.	JSD15	- - - -	R	R	+	Coccus
6.	JSD21	- + + +	Y	R	+	Coccus
7.	JSD31	- - - +	R	R	-	Rod
8.	JSR11	- - - -	R	R	+	Coccus
9.	JSR12	- + - +	Y	Y	-	Rod
10.	JSR13	- - - -	R	R	+	Coccus
11.	JSR14	+ + + +	Y	R	+	Coccus
12.	JSR21	- - - +	Y	R	-	Rod
13.	JSR22	+ + - -	Y	Y	-	Rod
14.	JSR23	- - - +	R	R	-	Rod
15.	JSR24	- - - -	R	R	+	Coccus
16.	JSR31	- - - +	Y	R	-	Rod
17.	JSH11	- - - -	Y	Y	+	Coccus
18.	JSH12	- - - -	Y	R	+	Coccus
19.	JSH13	- - - -	R	R	-	Rod
20.	JSH14	- + - -	R	R	+	Coccus
21.	JSH21	- - - +	Y	R	+	Coccus
22.	JSH22	+ + - -	R	R	-	Rod
23.	JSH23	+ + - -	R	R	-	Rod
24.	JSH24	- - - +	Y	R	-	Rod
25.	JSH25	- - - -	R	R	+	Coccus
26.	JSH26	- - - -	R	R	+	Coccus
27.	JSH27	- + - +	R	R	-	Rod
28.	JSH28	- + - +	R	R	-	Rod
29.	JSH29	- + - -	R	R	+	Coccus

30.	JSH210	- - - +	Y	Y	-	Rod
31.	JSH211	- - - +	Y	R	-	Rod
32.	JSH212	+ + - -	Y	Y	-	Rod
33.	JSH213	- - - +	R	R	-	Rod
34.	JSH214	- + - +	Y	Y	-	Rod
35.	JSH215	+ + - -	Y	Y	-	Rod
36.	JSH31	- - - -	R	R	+	Coccus
37.	JSH32	- - - -	R	R	+	Coccus
38.	JSH33	- - - -	R	R	+	Coccus
39.	JSG11	- - - -	R	R	-	Rod
40.	JSG12	- - - -	R	R	-	Rod
41.	JSG13	- - - +	R	R	+	coccus
42.	JSG14	- + - -	Y	Y	-	Rod
43.	JSG15	- + + -	Y	R	+	Coccus
44.	JSG16	- - + -	R	R	+	Coccus
45.	JSG17	- + + -	Y	R	+	Coccus
46.	JSG18	- - + +	Y	R	-	Rod
47.	JSG21	- - - -	Y	R	-	Rod
48.	JSG22	- - - +	Y	R	+	Coccus
49.	JSG23	- + + -	Y	R	-	Rod
50.	JSG24	+ + - -	Y	R	-	Rod
51.	JSG25	- - - +	R	R	+	Coccus
52.	JSG26	- - - -	R	R	-	Rod
53.	JSG27	- - - +	Y	R	+	Coccus
54.	JSG28	- - - +	R	R	-	Rod
55.	JSG31	- + - -	R	R	+	Coccus
56.	JSG32	- + - -	Y	Y	+	Coccus
57.	JSG33	- + - -	Y	Y	+	Coccus
58.	JSG34	- - - +	R	R	-	Rod
59.	JSG35	- - - -	Y	R	+	Coccus
60.	JSG36	- - - +	Y	R	+	Coccus
61.	JSG37	- + - -	R	R	-	Rod
62.	JSG38	+ + - -	R	R	-	Rod
63.	JSG39	+ + - -	R	R	-	Rod
64.	JSA1	- + - +	Y	R	-	Rod
65.	JSA2	- + - +	Y	R	-	Rod
66.	JSA3	- + - +	Y	Y	-	Rod
67.	JSA4	- + - +	Y	Y	-	Rod
68.	JSA5	- - - +	R	R	-	Rod
69.	JSA6	- + - +	Y	R	-	Rod
70.	JSA7	- + - +	Y	R	-	Rod
71.	JSA8	- + - +	Y	Y	-	Rod
72.	JSA9	- + - +	Y	Y	-	Rod
73.	JSA10	- - - +	R	R	-	Rod
74.	JSA11	- + - +	R	R	-	Rod
75.	JSA12	+ + - -	Y	Y	-	Rod
76.	JSA13	- + - +	Y	R	-	Rod
77.	JSA14	+ + - -	Y	Y	-	Rod
78.	JSA15	+ + - -	Y	Y	-	Rod

Y: yellow; R: red; '-' : Gram negative; '+': Gram positive

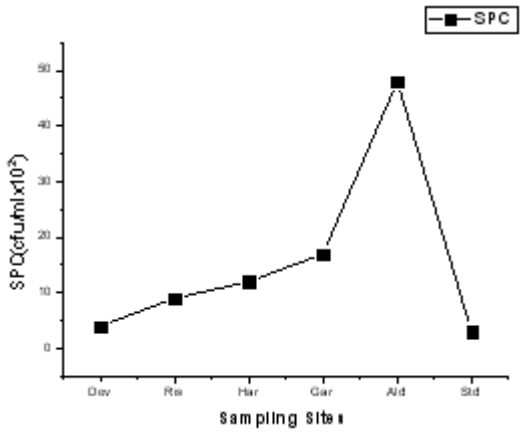

Fig. 1: SPC at different stretches of river Ganges. (Std* is BIS standard for SPC)

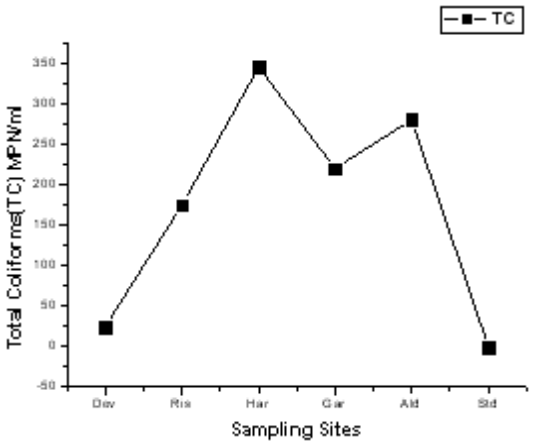

Fig. 2. TC at different stretches of river Ganges. (Std* is BIS standard for TC)

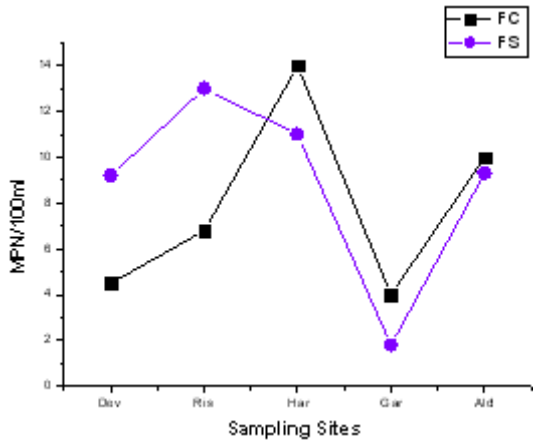

Fig. 3. FC and FS (MPN/100ml) at different stretches of river Ganges. (Std* is BIS standard for MPN/100ml)

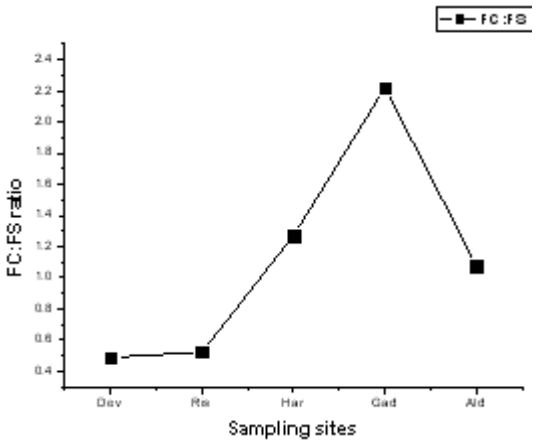

Fig. 4. FC: FS ratio at different stretches of river Ganges

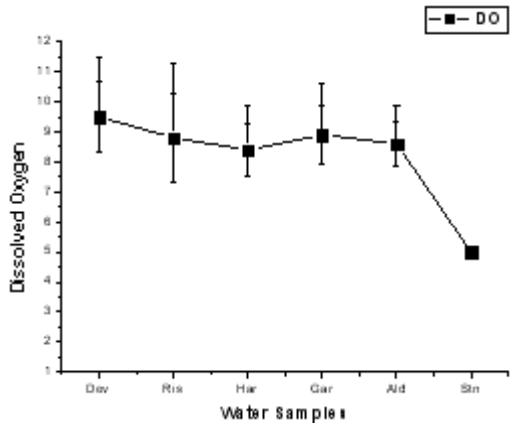

Fig. 5. DO at different stretches of river Ganges. (Std* is BIS standard for DO)

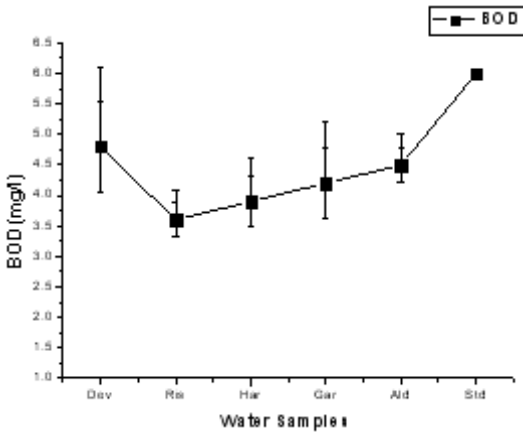

Fig. 6. BOD at different stretches of river Ganges. (Std* is BIS standard for BOD)

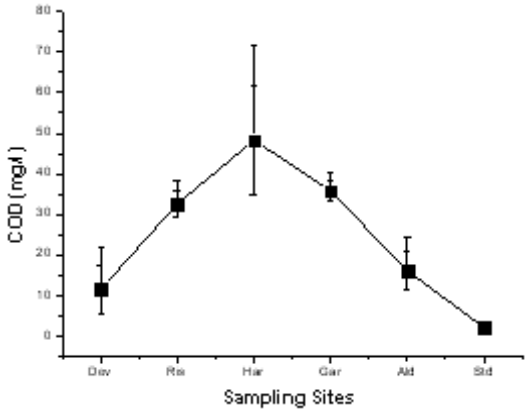

Fig. 7. COD at different stretches of river Ganges. (Std* is CPCB standard for COD)

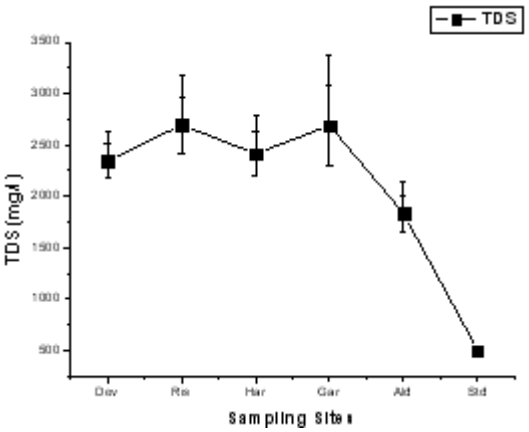

Fig. 8. TDS at different stretches of river Ganges. (Std* is BIS drinking water standard for TDS)

population size and mass bathing at the time of sample collection[16]. Total coliform count was relatively higher in Haridwar and Allahabad. It may be due to mass bathing at the time of sampling in accordance with the Hindu rituals of bathing at these two places. Except Devprayag, all sites were having TC count higher than BIS standards[15] for drinking water purposes (Fig 2).

Fecal coliform and fecal streptococci were also high at all the sites. FC count was highest at Haridwar and lowest at Garhmukteshwar. FS count was recorded highest at Rishikesh and lowest at Garhmukteshwar (Fig 3). FC: FS ratio greater than one (Fig 4) revealed the presence of human origin of FC and FS at Haridwar, Garhmukteshwar and Allahabad[17]. The FC: FS ratio was highest at Garhmukteshwar, while the FC: FS at Devprayag and Rishikesh were less than one indicating presence of coliforms of animal origin of [10, 18].

DO of the samples were within the minimum permissible limits and all values were in the range of 8-9 mg/l (Fig 5) i.e., safely above the limit (6 mg/l) set by BIS 2004. The BOD of all the sites was found to be within the permissible limits ranging between 3-5mgl^{-1}. BOD at Devprayag was noticed to be exceptionally higher than the other sites (Fig 6). COD was found higher at Rishikesh, Haridwar and Garhmukteshwar than the minimum prescribed limits of central pollution control board (CPCB)[19]. COD is within the minimum limits of CPCB[19] at Devprayag and Allahabad (Fig 7). The TDS was found to be much above the prescribed limits; ranging between 1700-2700 mg/l as compared to BIS standards 2004 for 500mg/l. Among the five stretches the TDS was recorded higher at Rishikesh and Garhmukteshwar (Fig 8).

Biochemical tests like IMViC, [12] and triple sugar iron agar (TSI) were performed on the isolated strains from the selected sites (Table 2). The tests showed the prevalence of coliforms specially the *Enterobacteriaceae* in the Ganges waters. The IMViC reaction ++- - and - +- - mostly represents the presence of *E.coli.* as in accordance with the work done by Kulshrestha and Sharma[16]. The +--- and --++ combinations show the fecal origin of the strains isolated from different stretches. There may be some chances of presence of soil bacteria in the samples collected, which showed the combination of --+-- and ---+ for IMViC test. The combinations -+-+, ++-+, -- --, and ++++ represents the intermediate group of bacterial isolates.

In the TSI analysis shown in table 2, the only glucose fermenting bacteria showed the formation of a yellow butt and red slant, while the lactose/sucrose bacteria formed yellow butt and yellow slant. The non fermenters were represented by red butt and red slant. The *enterobacteriaceae* were identified by a Y/Y or Y/R combination in TSI test, with an occurrence percentage of 58.97%. Most of the strains isolated were found to be Gram negative bacilli with an occurrence percentage of 62.8.

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