Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254 - 8704
CODEN: JEBIDP

Analysis of water quality parameters of River Ganga during Maha Kumbha, Haridwar, India

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Publication Info

Paper received:
22 June 2011

Revised received:
09 February 2012

Re-revised received:
30 June 2012

Accepted:
08 August 2012

Abstract

This study represents the summary of the water quality of River Ganga during mass bathing in Haridwar during Maha Kumbha of 2010 in terms of microbiological and molecular analysis. The sample was collected from River Ganga during Makar Sankranti to Shakh Poornima and assessed for fecal indicator bacteria *Escherichia coli* along with Standard Plate Count (SPC) to determine total bacterial load in the river. Of all the nine days of sample collection (mass bathing days) results on the main royal bath (Baisakhi) displayed maximum SPC (log 6.79 cfu ml^{-1}) and most probable number (210 and 150 MPN 100 ml^{-1} for total and fecal coli form, respectively). The water was extremely contaminated and not suitable for drinking on Somvati Amavasya, Maghi Poornima, Maha Shivratri and Baisakhi. The results clearly indicated that the mass bathing coupled with ritual activities performed by bathers was most probable cause of increased values of different parameters. The polymerase chain reaction analysis targeting malate dehydrogenase (mdh) gene proved to be more rapid and sensitive than classical culture techniques.

Key words

Maha Kumbha, River Ganga, *Escherichia coli*, Malate dehydrogenase, Water quality

Introduction

During festivals people take a holy dip in the river and also use it for drinking (Aachman), irrespective of its water quality (Semwal and Akolkar, 2006). The root of the problem lies in the fact that there is a conflict between the belief systems of people, and the strategies applied to de-pollute the river (Singh, 2013).

The organic matter contribution during mass bathing is normally significant, as revealed by earlier studies (Bhatnagar and Sangwa, 2009). Apart from washing with detergents, pilgrims offer milk, curd, ghee, flowers, coins, idols, ashes of departed ones, body hairs and other religious materials into the water. Many a times such offerings are brought in polythene carry bags and in the absences of a proper disposable system, these polythene bags and other non-biodegradable materials are dumped at the site of river banks which remain either floating on the water surface or cover the river bed substratum, creating a noxious environment to aquatic life (Semwal and Akolkar, 2006). Since all natural waterways contain bacteria and nutrients, almost any waste compound introduced into such waterways will initiate biochemical reactions thus enhancing the biological oxygen demand (BOD) (Kumar *et al.,* 2010). Traditionally, indicator micro-organisms have been used to suggest the presence of pathogens. Coliform bacteria are used as indicators for water quality (Ashbolt *et al*., 2001). Freedom from contamination with fecal matter is the most important parameter of water quality because it is generally considered to be a greater risk to human health as it is more likely to contain dangerous enteric pathogens (Scott *et al.,* 2003). Presence of indicator bacteria in water bodies as a sign of fecal contamination suggests the potential danger of health risk (Baghel *et al.,* 2005). The higher the level of indicator bacteria, the higher the level of fecal contamination and the greater will be the risks of water borne diseases (Pipes, 1981). A previous study made on the impact of mass bathing

on river water quality at Allahabad revealed that bacteriological parameters such as total coliforms and fecal coliform bacteria were found to be exceptionally high in comparison to their standard values (Chandra *et al*., 1996).

Present study threw light on the impact of mass population bathing on water quality of river Ganga at Har ki Pauri, Haridwar during the span of Maha Kumbha 2010 that lasted for 104 days.

Materials and Methods

The water samples were collected in triplicate from the river bank (Har Ki Pauri, Haridwar) after bathing activity on these particular days of Kumbha mela programme viz. Makar Sankranti, Surya Grahan, Basant Panchami, Maghi Poornima, Maha Shivratri, Somvati Amawasya, Naya Samwat, Baisakhi and Shakh Poornima. The labeled samples were kept in ice box and transported to the laboratory for analysis which was performed within 18 hr after collection.

Physico-chemical parameters including pH, phenolphthalein alkalinity, chlorine, total dissolved solids (TDS) and total suspended solids (TSS) were analyzed according to American Public Health Association (APHA, 2005). Microbiological analysis including quantitative enumeration of total bacteria (SPC), total coli forms and fecal coli forms (*E. coli*) was done according to Ram *et al.* (2007). MPN method was used to determine the coli form bacteria. The positive tubes from presumptive test were spreaded on eosine methylene blue agar plates in triplicate. Colonies from each plates showing metallic sheen were selected for further identification according to Bergey's Manual of Systematic Bacteriology (Garrity, 2005).

PCR analysis was performed from DNA, extracted directly from water samples (Pitcher *et al.,* 1989). PCR for targeting *mdh* gene a house keeping enzyme of the citric acid cycle, for *E. coli* and other organisms, was selected and used for the design of *E. coli*-specific PCR primers (Hsu and Tsen, 2001) : (1) Emdh1: 5'ACTGAAAGGC AAACAGCCAAG 3' (forward); (2) Emdh2: 5'CGTTCT GTTCAAATGGCCTCAGG3' (complementary sequence). The reaction conditions and amplification reactions were performed according to Hsu and Tsen (2001) with slight modifications. The reaction mixture was formed in total volume of 25µl containing 200 mM deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 50 pmol of each primer, 5 ml of template DNA and 2.5 U of *Taq* polymerase (Promega) in 1x PCR buffer. Molecular weight of the expected PCR product was 392 bp (Hsu and Tsen, 2001). Mixture was subjected to 35 PCR using thermo cycler (Eppendorf Master Cycler Gradient) with following temperature program: 3 min at 94°C to denature the template; 35 cycles of 94°C for 20 sec 60°C for 30 sec, 72°C for 30 sec and finally 72°C for 5 min. The amplified product was electrophoreses on a 1.5% agarose gel in Tris-Borate-EDTA buffer at 80 V (Ibenyassine *et al.,* 2008).

Results and Discussion

The results for physico-chemical analysis showed that pH of river water was in between 8.1 to 8.5, within the permissible limit (pH 6.5 to 8.5) of Indian drinking water standards (2009). The pH values were generally found to be constant due to strong buffering capacity of water. Similar

Table 1 : Physico-chemical characteristics of River Ganga water during Maha Kumbha, Haridwar during 2010

Date of sample collection		Events	pH (mgl^{-1})	Chlorine (mgl^{-1})	Alkalinity (mgl^{-1})	[a]TDS (mgl^{-1})	[b]TSS
January	14th	Makar Sankranti (First date of bathing)	8.5±0.005	0.69±0.005	31.0±0.005	60.0±0.50	37.0±0.25
	15th	Surya Grahan (Solar Eclipse)	8.5±0.005	0.70±0.1	31.0±0.005	60.63± 0.55	37.73± 0.25
	20th	Basant Panchami	8.5±0.26	10.5± 0.5	31.0±0.005	79.46± 1.34	56.2±0.25
	30th	Maghi Poornima (First Royal Bath)	8.4 ±0.231	18.15± 1.0	30.0±0.85	70.63±1.12	72.63 ±0.55
February	12th	Maha Shivratri	8.2±0.025	13.09± 2.08	29.1±2.0	80.7 ±0.55	89.83 ±0.76
March	15th	Somvati Amavasya (Second Royal Bath)	8.3 ±0.032	15.03±0.28	29.6±1.12	97.63± 0.55	90.26± 0.03
	16th	Naya Samwat	8.3 ±0.020	17.56± 0.45	29.6±0.55	130.63±2.85	92.08± 0.07
April	14th	Baisakhi (Main Royal Bath)	8.1 ±0.011	23.33± 0.49	28.0±0.26	137.6 ±0.55	96.8±0.25
	28th	Shakh Purnima (Last date of bathing)	8.4±0.0057	16.83± 0.05	30.0±0.005	102.73±0.52	87.56± 0.51
		IndianStandards [ISI10500-91;1991]	6.5-8.5	250-1000	_	500	_

Values are mean of 5 replicates ± SD; [a]Total Dissolved Solids; [b]Total Suspended Solids

ranges of pH were also obtained by other works on river Ganga (Sood *et al.,* 2008; Sati *et al.,* 2011). No considerable change was observed with phenolphthalein alkalinity and the range of observed mean values were found to be 28.0 to 31.0 mgl^{-1}. Although no significant conclusion could be established but the mild fluctuations in values may be due to the activities of bathers such as use of soap, may be responsible for this (Sinha *et al.,* 1991). The range of chlorine concentration was in between 0.7-23.33 mgl^{-1}, that was within permissible range. Maximum concentration of chlorine was on Baisakhi (23.33 mgl^{-1}). The increase in values during bathing may be due to dirt and sweat of bathers (Sinha *et al.,* 1991). TDS (60.63-137.6 mgl^{-1}) and TSS values (37.7-96.81 mgl^{-1}) were within permissible range. TDS (137.6 mgl^{-1}) and TSS (96.2 mgl^{-1}) were found to be maximum on Baisakhi. The increase in the values of TSS may be due to resuspension of sand and clay particles due to the discharge of wastes (Daphne *et al*., 2011) (Table 1). The results of microbiological analysis showed that range of total coliforms count was 0-210 MPN/100 ml and that of fecal coliforms 0-150 MPN/100 ml. The values compared with Indian Standards (not defined for fecal coliforms) confirm that the water quality can be designated as class B i.e. although fit for bathing but not for drinking particularly on Baisakhi (Table 2). A probable reason may be mixing of sewage contaminants due to various activities of huge gathering of bathers during the peak bathing dates particularly on Baisakhi around 14.5 million people took dip in the river. Values of the fecal coliforms were recorded zero on initial bathing days; Makar Sankranti, Surya Grahan, Basant Panchami, and again on last bathing day (Shakh Poornima) indicating either they were absent or too diluted to be detected with the traditional culture techniques. It is generally believed that more is the MPN of coliforms, higher is the extent of pollution in a given sample (Kulshrestha and Sharma, 2006) indicating heavy bacterial load and contamination (Bhadra *et al.,* 2003).

SPC value was found to be maximum (log 6.79 cfu ml^{-1}) on Baisakhi and minimum (log 4 cfu ml^{-1}) on Makar Sankranti supporting the fact that on Baisakhi, Haridwar was hosting the biggest gathering of Maha Kumbha with millions of people taking the holy dip making the water unfit for drinking, supporting the earlier findings (Jamwal *et al.,* 2008; Sood *et al.,* 2008). Coliforms were present in five water samples (Maghi Poornima, Maha Shivratri, Somvati Amawasya, Naya Samwat, Baisakhi). Isolates from these samples were Gram-negative rods with regular margin and convex elevation. Biochemical analysis of isolates showed clear characteristics of *E. coli* (Table 3). Physiological analysis showed that all the isolates maintained survivability on a range of temperature (10 to 50°C), pH (4.4 to 9) and bile salt up to 2%. Boke *et al.* (2010) reported low pH and bile secretions reduce the survival of pathogenic bacteria in gastrointestinal tract. But Tambekar and Bhutada, (2010) proved that acid tolerance and resistance to bile salt are important parameters of *E. coli* for the ability to colonize gastrointestinal region and cause pathogenesis and outbreaks. The presence of *E. coli* indicates the possibility of water borne diseases and their association with such festivals. Earlier reports have also reported that mass bathing cause significant deterioration and changes in the quality of water by enhancing fecal coliform load (Kumarswami and Vignesh 2009; Marale *et al.,* 2010).

As far as PCR amplification of *mdh* gene was concerned (specific for *E.coli*) it was expected to be positive for the five samples, which showed the presence of *E.coli* by traditional culture technique. However, apart from these five samples, three other water samples also revealed the presence of *E.coli* by PCR assay (Fig. 1). The water samples of Surya Grahan, Basant Panchmi and Shakh Purnima also proved to be positive for *E. coli* by the PCR assay while same were not showing the presence of the bacteria by traditional culture techniques. Vaughan *et al.*

Table 2 : Values of microbiological parameters in River Ganga water during Maha Kumbha, Haridwar of 2010

Date of sample collection		Events	SPC (cfu $ml^{-1} \times 10^4$)	MPN/100ml Total coliforms	MPN /100 ml Fecal coliforms
January	14th	Makar Sankranti (First date of bathing)	1.0	0	0
	15th	Surya Grahan (Solar eclipse)	6.75	0	0
	20th	Basant Panchami	4.7	0	0
	30th	Maghi Poornima (First Royal Bath)	43.5	75	64
February	12th	Maha Shivratri	162	70	55
March	15th	Somvati Amavasya (Second Royal Bath)	325	93	75
	16th	Naya Samwat	341	39	23
April	14th	Baisakhi (Main Royal Bath)	622	210	150
	28th	Shakh Purnima (Last date of bathing)	299	26	0
		IndianStandards [ISI10500-91;1991]		a.50 b.500	

SPC- Standard plate count, MPN- Most probable number; a - drinking, b - bathing

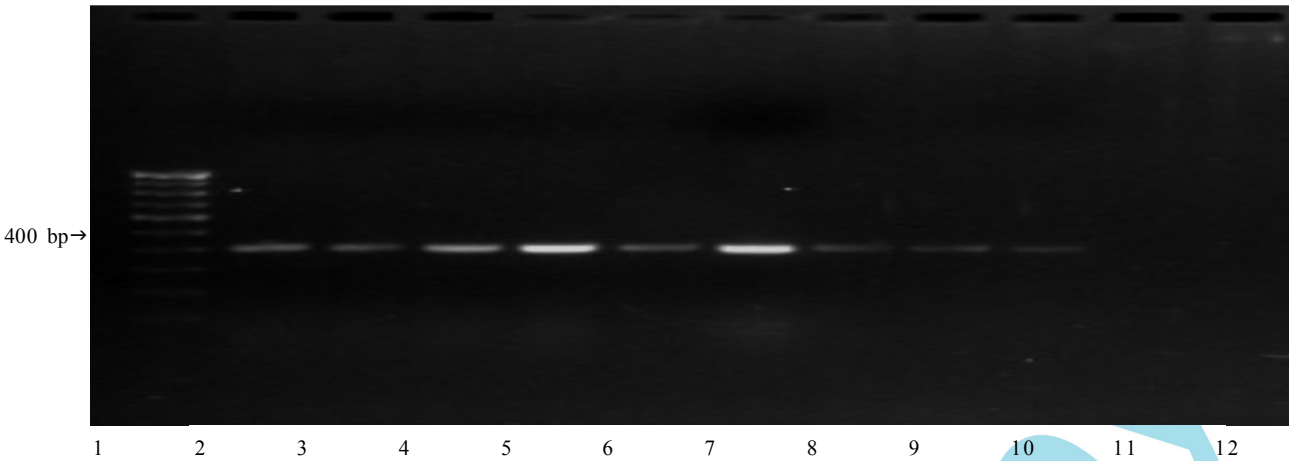

Fig. 1 : Gel picture showing results of PCR for *mdh* gene of *E. coli ;* Lane 1:100bp DNA Ladder (Banglore Genei); Lane 2: Positive Control (*E. coli* MTCC 40); Lane 3: [a]WS Shakh Purnima; Lane 4: WS Baisakhi; Lane 5: WS Naya samwat; Lane 6: WS Somvati amavasya; Lane 7: WS Maha Shivratri; Lane 8: WS Magh Poornima; Lane 9: WS Basant Panchami; Lane10: WS Surya Grahan; Lane 11: WS Makar Sankranti; Lane 12: Negative control; [a]WS: Water Sample

Table 3 : Characteristic properties of the strains isolated on EMB agar plates

Biochemical tests	Percentage (%) of isolates showing positive results
Indole	100
Methyl red	100
Voges-Proskauer	-
Simmons citrate	-
H_2S	-
Urease	-
Phenyl alanine	-
Lysine decarboxylase	90
Arginine dihydrolase	-
Ornitine decarboxylase	80
Motility	100
Gelatinase	-
Esculin	40
Acetate utilization	100
Lipase	-
Nitrate reduction	100
Oxidase	-
Catalase	100
ONPG	100
D-Glucose fermentation	100
D- Arabinose	100
D-Mannitol	100
D-Mannose	100
Lactose	100
Raffinose	60
Rhamnose	80
Mucate	100
Salicin	50
Sucrose	60
Trehalose	100
Xylose	100

ONPG: O-Nitrophenyl-β-D-galactopyranoside; (-) : None of the isolates gave positive results

(2003) and Alhamlan *et al.* (2013) also reported the rapidity, sensitivity and specificity of PCR technique in comparison to complex traditional techniques for checking the microbes in water samples.

On the basis of physio-chemical and microbiological analysis it is stated that the quality of river water deteriorated with the number of people taking dip. On the basis of present findings it is concluded that mass bathing during Maha Kumbha mela – 2010, altered the physico-chemical and microbial nature of river Ganga by decreasing its pH, increasing chlorine, TDS, TSS, SPC and MPN (total and fecal coli form). The most badly affected scenario was on main royal bath of Baisakhi, on which highest number of people took bath in the river and caused greatest increase in TDS, TSS, SPC and total coliform. Results clearly indicate that organic matter and untreated sewage were mixing in the river during the period of Maha Kumbha.

Acknowledgments

Thanks are due to UGC, New Delhi, India for providing financial assistance for the study. Authors are thankful to Mr. R.P. Arora and Vivek Arora for helping in collection and transportation of water samples and Vice Chancellor, BBA University for providing the facilities.

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