



Self-cleansing properties of Ganga during mass ritualistic bathing on Maha-Kumbh

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Abstract The deterioration of water quality of river Ganga is a huge concern for Govt. of India. Apart from various pollution sources, the religious and ritualistic activities also have a good share in deteriorating Ganga water quality. Thus, the aim of the present study was to evaluate the changes in physico-chemical properties, microbial diversity and role of bacteriophages in controlling bacterial population of Ganga water during mass ritualistic bathing on the occasion of Maha-Kumbh in 2013. The BOD, COD, hardness, TDS and level of various ions significantly increased, while DO decreased in Ganga water during Maha-Kumbh. Ganga water was more affluent in trace

elements than Yamuna and their levels further increased during Maha-Kumbh, which was correlated with decreased level of trace elements in the sediment. The bacterial diversity and evenness were increased and correlated with the number of devotees taking a dip at various events. Despite enormous increase in bacterial diversity during mass ritualistic bathing, the core bacterial species found in pre-Kumbh Ganga water were present in all the samples taken during Kumbh and post-Kumbh. In addition, the alteration in bacterial population during mass bathing was well under 2 log units which can be considered negligible. The study of bacteriophages at different bathing events revealed that Ganga was richer with the presence of bacteriophages in comparison with Yamuna against seven common bacteria found during the Maha-Kumbh. These bacteriophages have played a role in controlling bacterial growth and thus preventing putrefaction of Ganga water. Further, the abundance of trace elements in Ganga water might also be a reason for suppression of bacterial growth. Thus, the current study showed that Ganga has characteristic water quality in terms of physico-chemical property and microbial diversity that might have a role in the reported self-cleansing property of Ganga; however, the increased pollution load has surpassed its self-cleansing properties. Since water has been celebrated in all cultures, the outcome of the current study will not only be useful for the policy maker of cleaning and conservation of Ganga but also for restoration of other polluted rivers all over the world.

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Introduction

The river Ganga is considered divine in India and an integral component in all the religious and spiritual practices. It is one of the longest rivers of the Indian subcontinent, originating from the Western Himalaya in the state of Uttarakhand and flowing about 2525 km through the vast plains of Uttar Pradesh, Bihar and West Bengal, and finally amalgamates with the Bay of Bengal (Singh and Singh 2007). The plains of Ganga are among the most fertile and densely populated regions in the world (Koshal 2014). Millions of people depend on water from the holy river for their daily needs like drinking, bathing and other household activities as well as for agriculture and industry (Singh and Choudhary 2013; Amarasinghe et al. 2016).

Many scientific studies have been carried out on Ganga water, and now it has been proven that Ganga water has high levels of antimicrobial property and dissolved oxygen indicating a reasonable self-purifying capability (Nautiyal 2009; Singh et al. 2011; Bhargava 2013). In addition, some biomolecules are induced naturally and may provide preservative, curative and therapeutic strength in Ganga water (Bhargava 2013). The recent report of National Environmental Engineering and Research Institute (NEERI) also showed that Ganga water has a remarkably higher number of bacteriophages in comparison with Yamuna and Narmada Rivers. The number of bacteriophages was particularly high in the upper Himalayan stretch of Ganga. These results show higher bactericidal capacity of the river Ganga (NEERI 2017). Ganga is admired as the holy river of India, and bathing in it is considered a source of salvation; thus, mass bathing takes place at several places on the course of the Ganga (Arora et al. 2013). However, Maha-Kumbh at Prayag has a special significance (Shukla and Gupta 2015). It is held only at the banks of the river Ganga at Prayag, Allahabad, where the three rivers Ganga, Yamuna and the mythical Saraswati meet; the confluence is known as Sangam. The Maha-Kumbh 2013 was a rarest of the rare occasion, held after every 144 years, and thus it is looked upon by most Hindus as one of the greatest holy festivals. Millions of people, from all over the world, took a dip in the river at Sangam with the hope of salvation (UP Govt. 2013). However, Ganga water quality has been severely deteriorated due to various point and non-point sources (Dwivedi et al. 2018; Kamboj and Kamboj 2019). Several studies have reported altered physico-

chemical properties of water and sediment of river Ganga and the presence of pathogenic bacteria such as faecal coliform, faecal streptococci, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio fluvialis* and other bacterial species such as *Salmonella*, *Bacillus*, *Shigella*, *Pseudomonas*, *Proteus*, *Aeromonas*, *Plesiomonas* and *Salmonella* due to untreated waste water discharge (Kulshrestha and Sharma 2006; Joshi and Sati 2011; Arora et al. 2013). In recent years, the level of various toxic elements has been increased in Ganga water from anthropogenic sources (Dwivedi et al. 2018; Siddiqui and Pandey 2019). The mass ritualistic bathing and other religious activities taking place throughout the banks of river Ganga also have a good share in Ganga water pollution. The sources and current status of pollution in river Ganga have been discussed in detail in Dwivedi et al. (2018).

In the last three decades, Govt. of India has spent several million rupees for abatement of pollution in river Ganga through different Ganga cleaning programmes. For instance, the Ganga Action Plan (GAP) phase I (launched in 1986 targeting reduction of sewage discharge), GAP phase II (launched in 1993 targeting the tributaries of Ganga) and National Ganga River Basin Authority (NGRBA) launched in 2009 for cleaning and conservation of Ganga (Chaudhary and Walker 2019). However, these plans did not lead to expected results, and the cleaning of Ganga seemed to be a herculean task. Nevertheless, based on the reports and recommendations of previous plans, in 2014, Govt. of India launched the National Mission for Clean Ganga (NMCG) called the Namami Gange Programme. It is the most ambitious plan to conserve the Ganga and improve its water quality through an integrated conservation programme including immediate, medium-term and long-term activities. Several international agencies such as Japan International Cooperation Agency (JICA), German International Cooperation (GIC), International Water Management Institute and World Bank are also involved in NMCG for financial and technical support based on their experience of river management (Ministry of Water Resources 2016; The Hindu 2016; IWMI 2015). Since mass ritualistic bathing is one of the most important events occurring periodically on the banks of Ganga, it is important to study its impact on the water quality of river Ganga, and similarly the impact of water quality on the health of bathing population. Keeping in view all these facts, the present study has been designed to assess the physico-chemical,

elemental and microbial changes during Maha-Kumbh at Sangam to understand the consequences of anthropogenic activities on Ganga water quality during this great event. This information would be highly useful for the policy maker for the clean Ganga mission to include the pollution mitigation strategies during such events. The experience gained from the challenges during cleaning and restoration of Ganga would be helpful for restoration of other polluted rivers all over the world.

Material and methods

Although thousands of people stayed at the banks of river Ganga during the entire Maha-Kumbh period, the number of bathing population increased several folds during some special events. Further, the confluence of river Ganga and Yamuna was the main site of bathing because it has special significance in Hindu mythology. Therefore, these two factors were considered for the selection of dates and sites of water sample collection to compare the effect of mass bathing on Ganga water quality as detailed in the following section.

Site selection and collection of water samples during Maha-Kumbh

The confluence of river Ganga and Yamuna in Allahabad (known as Sangam) situated at 25° 28' N and 81° 54' E in Uttar Pradesh, India, was the main site of the ritualistic bathing within a stretch of 500 m. Five sampling sites around the Sangam were selected within an approximately 8-km stretch. The samples taken about 1 km upstream of river Yamuna from confluence (at Saraswati Ghat), where no bathing took place, was termed as Yamuna control (YC), and the samples collected 1 km upstream of Ganga towards the Kanpur side (near Phaphamau Bridge) was termed as Ganga control (GC). Samples collected from the confluence point of Ganga and Yamuna, the centre of the crowd during bathing events, was termed as Sangam I (S-I), the samples collected from the other side of river (towards Arail Ghat) at Sangam, where heavy bathing took place during special events, was termed Sangam II (S-II) and the sampling site about 2.5 km downstream from S-I, after the confluence, was termed after Sangam (AS) (Fig. 1). The samples were collected during six special mass ritualistic bathing events and 20 days before the start, i.e. pre-Kumbh (collected on December 26, 2012), and

20 days after the last event, i.e. post-Kumbh (collected on March 31, 2013), for the evaluation of water quality of river Ganga.

The special mass ritualistic events took place on the following dates: Makar Sankranti on January 14, 2013; Paush Purnima on January 27, 2013; Mauni Amavasya on February 10, 2013; Basant Panchami on February 15, 2013; Maghi Purnima on February 25, 2013; Maha Shivratri on March 10, 2013. The sampling dates and other details are given in Table 1. Water samples were collected from all the selected locations in three sets for microbial studies, physico-chemical parameters and bacteriophage studies. Each set was collected in triplicate by taking the samples from about 15 m distance in 1-l amber-coloured, sterilized plastic bottles. One set was acidified by adding 1 ml nitric acid (35% v/v) for elemental analysis.

Evaluation of physico-chemical characteristics

The water quality evaluation was done according to the standard protocols. A total of 22 physico-chemical parameters (dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD), pH, electrical conductivity (EC), total dissolved solids (TDS), alkalinity (measured as CaCO₃), calcium hardness, magnesium hardness, potassium, free chlorine, total chlorine, chlorine dioxide, bromine, fluoride, phosphate, phosphorus, sulphate, nitrite, nitrate and ammonia) were analysed using Ion Specific Meters on a multiparameter photometer (Hanna, USA, HI83099) and a set of sophisticated water analysis kits. The DO and pH of the water were measured on the spot using a portable dissolved oxygen meter (Hanna, USA, HI98186) and pH meter (Thermo), respectively. The other parameters were measured in the laboratory after filtration using Whatman filter paper (no. 41) to remove sand and other suspended particles except for turbidity. COD was analysed through a COD reactor (Hanna, USA, HI839800), and turbidity through a turbidimeter (Hanna, USA, HI98703). The rest of the parameters were tested on a multiparameter photometer (Hanna, USA, HI83099) after calibration by appropriate standards.

Quantification of elements

The mineral nutrients (viz. Fe, Zn, Mn, Cu, Co, Se) and toxic elements (viz. Cr, Cd, Pb, As, Hg) were analysed in the acidified samples using an inductively coupled



Fig. 1 Map showing five selected sampling sites at Sangam during Maha-Kumbh 2013 at Allahabad: the red circles indicate the sampling points

plasma mass spectrometer (ICP-MS 7500ex, Agilent Technologies, Japan). Before estimation, the water samples were filtered (0.22- μ m filter), and rhodium was added to all samples for internal standardization (Dwivedi et al. 2010). Multi-element calibration standard 2A (8500-6940) and 3 (8500-6948; Agilent

Technologies, USA) were used for calibration of the instrument, and the multi-element calibration standard 2A-HG (8500-6940-HG; Agilent Technologies, USA) was used for the estimation of mercury. The analytical precision and accuracy of the HPLC-ICP-MS were maintained per the requirements of NABL accredited

Table 1 Bathing events, dates and number of devotees taking a holy dip in Ganga during Maha-Kumbh 2013 at Allahabad

S. No.	Kumbh 2013		
	Bathing events	Sampling dates	No. of devotees/events
1	Pre-Kumbh	December 12, 2012	*8 thousand (0.07 million)
2	Makar Sankranti	January 14, 2013	110 Lac (11 million)
3	Paush Purnima	January 27, 2013	55 Lac (5.5 million)
4	Mauni Amavasya	February 10, 2013	305 Lac (30.5 million)
5	Vasant Panchami	February 15, 2013	193 Lac (19.3 million)
6	Maghi Purnima	February 25, 2013	165 Lac (16.5 million)
7	Maha Shivratri	March 10, 2013	55 Lac (5.5 million)
8	Post-Kumbh	March 31, 2013	*2 thousands (0.03 million)
		Total	883.10 Lac (88.310 million)

Source: UP Govt. website of Kumbh Mela 2013

*A rough estimate from local volunteers

lab (certificate no. T-1381) (National Accreditation Board for Testing and Calibration Laboratories). The calibration and quality assurance for each analytical batch were ensured by repeated analysis ($n = 5$) of river water samples spiked with known amounts of elements. Recovery of Fe, Zn, Mn, Cu, Co, Se, Cr, Cd, Pb and As from the water samples was found to be more than 98%. The detection limit for each element was $1 \mu\text{g l}^{-1}$.

Evaluation of microbial population

Serial dilutions of water samples from different sites and at different events were plated on the selective agar plates, viz. nutrient agar, Rose Bengal agar, HiChrome ECC agar, *Salmonella* agar and *Pseudomonas* isolation agar (from HI-MEDIA Laboratories Pvt. Ltd., Mumbai, India) for the isolation and enumeration of heterogeneous bacterial, fungal, coliform, salmonella and pseudomonas populations, respectively. After 72 h of incubation, the developed colonies were counted and transferred to nutrient agar slants and kept at 4°C (Nautiyal 2009). The identification of bacteria was done by the Biolog® system after growing on Biolog® BUG® agar (Biolog Inc., Hayward, CA, USA). In preparation for analysis, the colonies were picked from pure cultures of bacteria and were plated as a lawn of bacteria onto a BUG® agar plate, and separate plates were set up for each strain to be analysed. The Biolog Microlog® Bacterial Identification System consists of databases combined with specialized 96-well plates (test panels). A panel of 95 different substrates gives a very distinctive and repeatable pattern of purple wells for “Metabolic Fingerprint” (June et al. 2006). The Gram-positive and Gram-negative panels and databases were used in this study.

Evaluation of microbial diversity using the carbon source utilization pattern

Biolog EcoPlates (Biolog, Inc.) were used to determine microbial diversity and evenness based on carbon source utilization pattern of water samples. Inoculation of water samples was carried out as described earlier (Nautiyal et al. 2007). Data were recorded for days 1–7 at 590 nm. Microbial activity in each microplate, expressed as average well colour development (AWCD), was determined as described by Garland (1996). Diversity and evenness indices were calculated as described by Staddon et al. (1997). Principal

component analysis (PCA) was performed on data derived by the AWCD as described by Garland and Mills (1991). Formulas used for diversity calculations were described by Staddon et al. (1997); data collected after day 5 were used. At least three independent experiments were conducted for each treatment. Statistical analyses were performed using SPSS 16.0 and STATISTICA 8.0.

Isolation and characterization of bacteriophages

For the isolation and characterization of bacteriophage diversity from the rivers Ganga and Yamuna, water samples were processed directly by using the double agar overlay method. Briefly, the water samples were filtered through $0.45\text{-}\mu\text{m}$ membrane filters (Merck Millipore, Billerica, MA) and 1 ml of filtered water samples was added to 0.7% top agar containing 100 μl of overnight grown bacteria of interest and poured on to the agar plates until the appearance of lytic zones. The plaque forming units were counted for enumeration. In samples having higher abundance of phages, the samples were serially diluted (1:10 in water) and plated as described above until the appearance of countable lytic zones. In samples where the concentration of phages was low, the phages were isolated by following the method of Carvalho et al. (2010) with slight modifications. One hundred millilitres of the Ganga/Yamuna water sample was added to 100 ml of double strength TSB and bacteria of interest at the exponential phase and incubation at 30°C . After 12 h, the cultures were centrifuged (3500g, 20 min, 4°C), and the supernatants were filtrated using $0.45\text{-}\mu\text{m}$ filters (Merck Millipore, Billerica, MA). For the detection of phages, filtrates were spotted on a lawn of bacteria of interest made using the double agar overlay method and incubated at 37°C for 24 h. Clear zones were picked and suspended in 1 ml of SM buffer (100 mM NaCl, 8 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50 mM Tris-HCl and 0.01% gelatin, pH 7.5), and single plaques were formed using the double agar overlay method. This procedure was repeated at least three to five times for obtaining purified phages.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 and WindowState 7.5. Correlation matrices were made between the different physico-chemical parameters; bacterial diversity was also correlated with population of different events. PCA, Duncan’s multiple range test

(DMRT) and analysis of variance (ANOVA) were performed to know the significant changes in water quality and microbial and bacteriophage diversity due to mass bathing and anthropogenic disturbance.

Results and discussion

Physico-chemical characteristics of Kumbh water

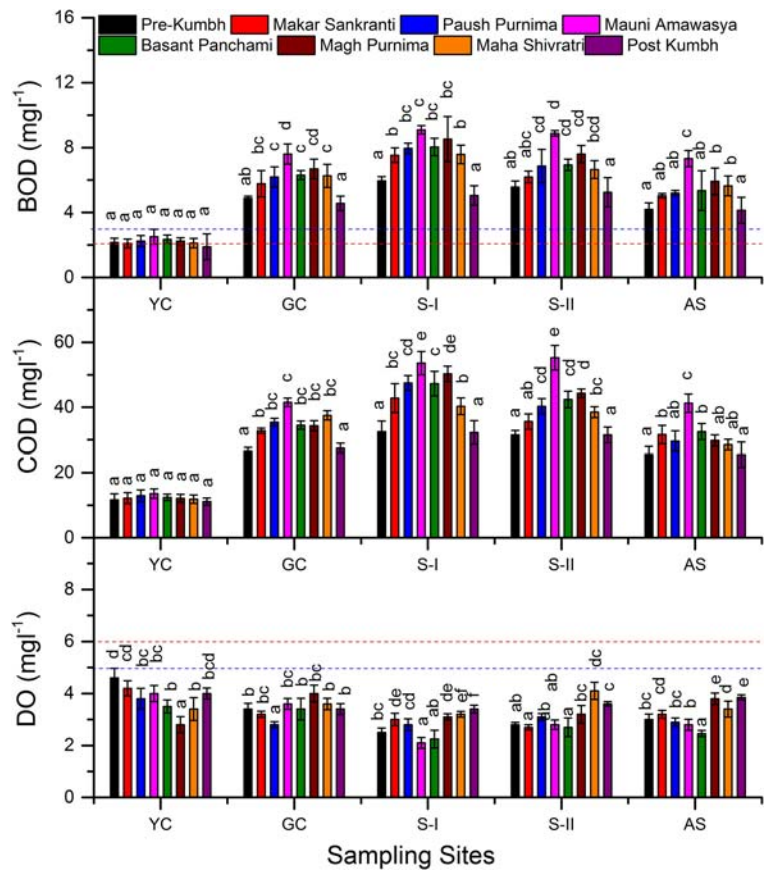
During the Maha-Kumbh in 2013, one of the world's biggest mass gathering took place at the banks of the river Ganga in Allahabad where over 88 million people took a bath at various events within 2 months (January 14 to March 10): over 30.5 million in one day (Table 1), and thousands stayed at the banks of river Ganga during the entire period (UP Govt. 2013).

To examine the changes in water quality due to mass gathering and bathing, the water samples 20 days before the start of bathing events (pre-Kumbh) and 20 days after the end of events (post-Kumbh) were collected along with six mass bathing events (Table 1). The water samples from three major bathing sites, Sangam-I (S-I), Sangam-II (S-II) and after Sangam (AS), were collected for the analysis, and the water samples before the confluence of Ganga and Yamuna were collected as control for comparison on the individual water quality of the rivers (Fig. 1). The physico-chemical properties of pre-Kumbh water samples from control sites showed that Yamuna water was nearly neutral (pH 7.24), slightly hard (within the range 17.1 to 60 mg l⁻¹) and has higher electrical conductivity in comparison with Ganga water which was alkaline (pH 8.61), relatively harder (within the range > 60–< 180 mg l⁻¹) and has lower EC (Supplementary Table S1). Though the level of total dissolved solids (TDSs) was several times higher in Ganga water than in Yamuna, the alkalinity of both rivers were almost similar at control sites. The levels of various ions such as Mg²⁺, K⁺, SO₄⁻, Br⁻, NO₃⁻, NO₂⁻ and PO₄⁻ were generally higher in Yamuna control than in Ganga control except for the level of NH₄⁺. In contrast, the levels of chlorine dioxide and free chlorine were higher in Ganga control than Yamuna (Supplementary Table S1). BOD was below the maximum permissible limit (3 mg l⁻¹) set by WHO for bathing water in Yamuna but it was higher in Ganga (4.89 mg l⁻¹) (Fig. 2). COD was also higher for Ganga control than Yamuna. DO was higher in Yamuna than in Ganga control though it was significantly lower than the

minimum WHO limit for bathing water (5 mg l⁻¹) in both Ganga and Yamuna. During Maha-Kumbh, the level of BOD and COD further enhanced reaching to around 5-fold higher than the permissible limit for bathing, while DO reduced to lower than half the bathing water limit (Fig. 2). The EC and TDS of Yamuna water did not change significantly during the Kumbh, but pH increased to > 8, and hardness also increased though it remained lower than 60 mg l⁻¹, the limit for slightly hard water, while the water from various bathing sites after confluence as well as Ganga control exhibited high increase in EC, TDS and hardness during the Kumbh. The TDS and hardness were 983 and 255 mg l⁻¹, respectively, at the main bathing site S-I on Mauni Amavasya, which was the biggest mass bathing day. The level of various ions also increased during the bathing events with high increase in NO₃⁻, NO₂⁻, PO₄⁻, total P and NH₄⁺. The increases in various parameters were higher at main bathing sites such as S-I and S-II and on the major bathing events such as Makar Sankranti, Paush Purnima, Mauni Amavasya and Vasant Panchami (Supplementary Table S1) though a few parameters (hardness, alkalinity, NH₄⁺, total P, SO₄⁻) tended to decline after Mauni Amavasya.

From the above, it is evident that the deterioration in water quality was related to the bathing population at different events and bathing sites with water being most deteriorated at site S-I having the biggest crowd, followed by S-II, and the change in water quality was more on Paush Purnima (27 January 2013) and Mauni Amavasya (10 February 2013). During the Kumbh, every day, tens of thousands of people were taking baths, and the numbers were exceeding in orders of magnitude during the special events. Some preventive measures, discussed below, were also taken by the government and non-government agencies to manage the quantity and quality of water to facilitate the religious bathing during Kumbh. Therefore, the net impact on water quality at different sampling sites and time points was an outcome of several factors such as the pre-Kumbh ban on discharge of industrial and sewage effluent to river Ganga and Yamuna, and release of extra water from Tehri and Narora dams. An estimate of around 2000 million litres of waste water is discharged in Ganga every day from Kanpur to Allahabad stretch (about 874 million litres per day (MLD) sewage and 1200 MLD industrial waste (CPCB 2014)), which was banned by the government 1 month before the start of Kumbh to revive the water for bathing. More than 12,000 cusecs of water was

Fig. 2 Impact of mass ritualistic bathing on the level of trace elements in Ganga water during Maha-Kumbh 2013 at Allahabad. Study includes five sampling sites and eight special ritualistic bathing events. YC, Yamuna control; GC, Ganga control; S-I, Sangam-I; S-II, Sangam-II; AS, after Sangam. Red dashed line, WHO limit for drinking water; blue dashed line, WHO limit for bathing water



released from Narora and Tehri dams time to time during the Kumbh. Further, the addition of Yamuna water at confluence, use of disinfectants and bathing and other activities of millions would have affected the quality of water in various ways. The positive correlation between some physico-chemical parameters (BOD, COD, level of ions, EC, TDS and hardness) and mass gathering clearly indicated that it deteriorated the water quality because people carelessly use soaps, shampoos and detergents, and throw polythene and discarded clothes, as well as throw food, flower, leaves, milk, curd, ghee, coins, etc., as an offering to river Ganga. An increase in BOD, COD, total and faecal coliform, ammoniacal nitrogen (NH₄-N) and phosphate (PO₄) and decreased DO in river Ganga have been found during mass ritualistic bathing at various places where religious Kumbh gatherings were organized in different years (Arora et al. 2013; Tyagi et al. 2013). Srivastava et al. (2013) evaluated the water quality during Maha-Kumbh 2013, in Allahabad, using the fuzzy environmental model for physico-chemical and bacteriological parameters

of water at different locations of rivers Ganga and Yamuna. The data analysed during three main ritualistic mass bathing events (from 10 to 25 February, 2013) revealed that the water quality was poor to very poor on all three bathing events, at Sangam. A recent report on water quality index of Ganga also showed that anthropogenic activities deteriorated its quality (Kamboj and Kamboj 2019). In the current study, though some dilution effect was clearly evident due to the release of water before anticipated large-crowd events, the quality of water, however, was not adequate for bathing and drinking. The pH was on the higher side of the tolerance limit (6.5–8.5) of bathing, often exceeding the limit, while BOD and COD were also beyond the respective permissible limits. The use of cleaning substances and desorption of Ca and Mg from sediment due to agitation of water may increase the pH and alkalinity, while the food and organic matter would cause increase in BOD and COD and reduction in DO. Disturbances of water led to increased turbidity and TDS. During the Maha-Kumbh period (55 days), thousands of people stayed at

the banks of river Ganga including Saints belonging to different sects, devotees, tourists and administrative personnel. The temporary settlements and temporary hospitals set up during Maha-Kumbh and the pets owned by different sects of saints generated huge amounts of wastes including untreated sewage. Though there were several temporary toilets installed, still a significant amount of waste was unmanaged (Rani et al. 2014) which could have contaminated the river leading to increase in various ions.

Profile of trace and toxic elements during Maha-Kumbh

In the pre-Kumbh samples, the control Ganga water and sediment exhibited higher levels of trace elements such as Fe, Zn, Mn, Cu, Co and Se than Yamuna water and sediment. After commencement of the month-long festival of Kumbh, the level of trace elements further increased in Ganga control and at various bathing sites, whereas their levels decreased in Yamuna control (Fig. 3). The level of trace elements in sediments of both Ganga and Yamuna was decreased during the Kumbh (Supplementary Table S2). In all water samples, the level of trace elements was in the order of Fe > Zn > Mn > Cu > Co > Se and their level increased at bathing events chronologically up to Mauni Amavasya with the maximum level at the main bathing site S-I. After Mauni

Amavasya, the concentration of trace elements declined gradually reaching the levels similar to pre-Kumbh on Maha Shivratri (Fig. 3). The level of toxic elements (As, Cd, Cr and Pb) was also higher in Ganga water and sediment than Yamuna control, except the level of arsenic in Ganga water and Pb in Ganga sediment (Fig. 4, Supplementary Table S3). After the start of Kumbh, the level of toxic elements slightly increased in the water, while they decreased in the sediments. The increase was more in the level of As and Cd than Pb and Cr in Ganga water. Mercury was below the detection limit in all the samples of water and sediments. The level of toxic elements in Ganga water increased up to Paush Purnima then declined already at Mauni Amavasya and at later events. It was noticeable that Cd and Pb were found below the detection limit on some auspicious events, i.e. Mauni Amavasya, Basant Panchami, Maghi Purnima and Maha Shivratri (Fig. 4).

During the Kumbh, two major factors could have affected the concentration of elements at a given time point and place: these are dissolution of elements due to agitation of water by bathing population and dilution due to the ban of industrial effluent into Ganga and Yamuna and release of extra water from Narora and Tehri dams upstream of Ganga. The different trends of trace and toxic elements (i.e. more effect of dilution on toxic elements than the trace elements) indicate their

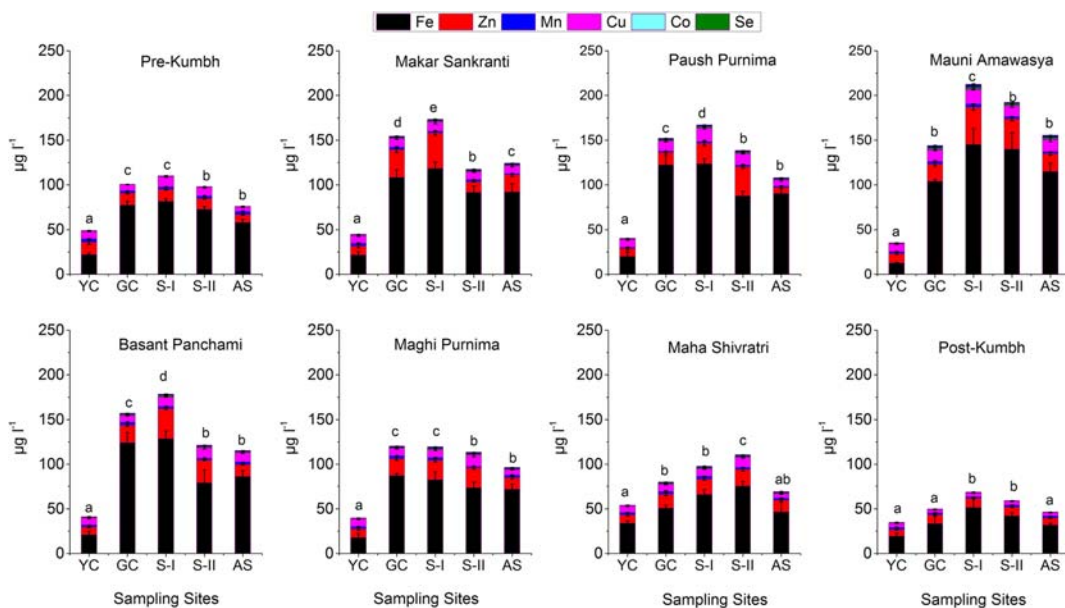


Fig. 3 Impact of mass ritualistic bathing on the level of trace elements in Ganga water during Maha-Kumbh 2013 at Allahabad. Study includes five sampling sites and eight special ritualistic

bathing events. YC, Yamuna control; GC, Ganga control; S-I, Sangam-I; S-II, Sangam-II; AS, after Sangam

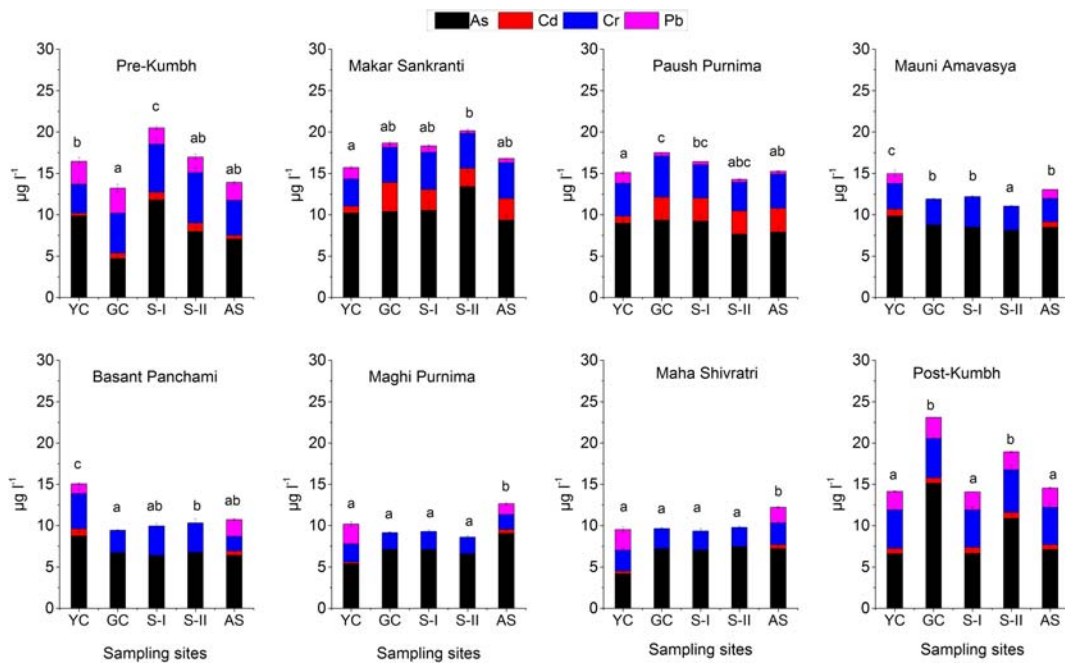


Fig. 4 Impact of mass ritualistic bathing on the level of toxic elements in Ganga water during Maha-Kumbh 2013 at Allahabad. Study includes five sampling sites and eight special ritualistic

bathing events. YC, Yamuna control; GC, Ganga control; S-I, Sangam-I; S-II, Sangam-II; AS, after Sangam

different sources; the toxic elements are probably mostly added by industrial effluent, while other minerals are primarily sourced by Ganga itself during its paths from rocks. There is a negative correlation between the levels of various elements in water and sediments before the start of Kumbh and during Kumbh points towards their release from sediment to water during Kumbh. Near the confluence at Allahabad, Ganga is more shallow and turbid than Yamuna (4 ft versus 40 ft). The agitation of water during mass bathing has resulted in further increase in suspended particle and turbidity. The suspended sediment may contain a high amount of metals (Subramanian et al. 1987). Therefore, the release of trace elements from sediment to water may be related to the change in the pH of water during Kumbh. During Kumbh, the pH of Ganga water slightly decreased in comparison with pre-Kumbh control, which may have increased the solubility of elements from sediments. Further, the suspension of sediment in water during sampling dates, i.e. on bathing events during Kumbh due to agitation by mass bathing, may be a big reason for increase in trace elements as sample preservation (acidification) may dissolve the adsorbed elements from suspended sediments (Subramanian et al. 1987). However, the similar effect will be in the acidic environment

of the stomach if water is ingested unintentionally during bathing or intentionally as a part of religious rites or drinking and cooking during the stay of pilgrims at the banks of Ganga. The level of As was higher than the permissible limit of drinking water ($10 \mu\text{g l}^{-1}$, WHO 1993) in many of samples collected during Kumbh. The level of Cd was also close to the permissible limit ($3 \mu\text{g l}^{-1}$), while Cr and Pb remained lower than the respective permissible limits (50 and $10 \mu\text{g l}^{-1}$, respectively). The area from Kannauj to Varanasi is particularly responsible for most of the industrial wastes into the Ganga. Nearly 55% of the industrial waste in Ganga basin is generated in Uttar Pradesh; Kanpur (tanneries) and Allahabad (engineering) are the major industrial cities in Uttar Pradesh. Tannery and other industrial effluents contain high levels of Cr and other toxic metals such as Cd and Pb (Dwivedi et al. 2006, 2010; Dixit et al. 2015). The level of carcinogenic elements like As, Cd, Cr and Pb was exceeding the WHO permissible limits for potable water at many sites of Ganga river in different seasons (Aktar et al. 2010; Pandey et al. 2010; Siddiqui and Pandey 2019). The decrease of trace elements and increase of toxic elements in post-Kumbh samples also point towards their different sources. Due to less water flow in the beginning of summer, the trace

element, which might primarily originate from the rocks during the path of Ganga, has decreased, while due to restart of waste water discharge after Kumbh, the level of toxic elements increased.

Change of microbial population, diversity and community structure during Kumbh

The changes in microbial population and diversity in Ganga water were investigated during Kumbh to observe the impact of mass ritualistic bathing. The microbial community *Pseudomonas*, coliforms, fungi, *Salmonella* and heterogeneous bacterial population were evaluated to determine their titre at different bathing sites and events. Among the different microbial communities, the heterogeneous bacterial population was highest, while fungal population was lowest at all the sites. Results clearly demonstrated that microbial population increased during the bathing events compared with the pre-Kumbh. Though the maximum increase observed was not beyond 1.5-fold, it was significant in comparison with the pre-Kumbh samples (Supplementary Table S4).

In recent years, the Biolog technique has been extensively used for ecological studies.

The advantages of this technique include its ability to differentiate between microbial communities, relative ease of use and reproducibility and production of large amount of data describing metabolic characteristics of the microbial communities. In the present study, the Biolog technique was used for the investigation of bacterial population and diversity in river Ganga. The identification of most discriminant bacterial populations by means of GN2 and GP2 Biolog databases revealed that different bacterial populations dominated in Ganga water during different sampling periods, i.e. pre-Kumbh, during Kumbh and post-Kumbh. Selected discriminant bacterial isolates in pre-Kumbh samples exhibited a total of 40 species belonging to 27 genera. Interestingly, these 40 species were present throughout the bathing events until the post-Kumbh (Fig. 5a and b, Supplementary Table S5). However, during bathing events, a total of eighty-eight bacterial species belonging to forty-three genera were observed. Seventy-six species of bacteria belonging to thirty-five genera were also encountered in post-Kumbh samples. *Corynebacterium*, *Pseudomonas* and *Vibrio* were the dominant genera present in pre-Kumbh Ganga water. The species belonging to these genera were further increased during Kumbh. Additionally, the species belonging to *Brevibacterium*, *Burkholderia*, *Enterobacter* and

Rhodococcus were also increased during the Kumbh and post-Kumbh samples (Supplementary Table S6). Furthermore, a significant change in bacterial community during Maha-Kumbh was observed with an addition of 39 species belonging to 16 genera in comparison with the pre-Kumbh samples. Though most of these disappeared within 20 days after the last bathing event, 22 bacterial species, which were not present in pre-Kumbh or during Kumbh, were identified in post-Kumbh Ganga water. The overall functional diversity and evenness indices, based on carbon source utilization pattern, showed increase during bathing events compared with pre-Kumbh samples. However, the changes were not more than 2 log units among bathing events as well as among the selected sampling sites and can be considered negligible (Küng 2011) (Supplementary Table S6). Despite the ban on industrial and sewage effluent discharge before the start of Kumbh, the presence of a significant number of bacterial species in pre-Kumbh samples indicates their source from the upper stretch of Ganga, as a total of 43 different bacterial species were recorded in the upper stretch of river Ganga by Sood et al. (2010). In this region, *Escherichia coli* presented the highest diversity index (0.883), followed by *Enterobacter* (0.718), *Streptococcus faecalis* (0.681) and *Pseudomonas* sp. (0.583). Even at Gangotri Glacier, the origin of Ganga, bacterial contamination has been found (Baghel et al. 2005). The increased bacterial species found in post-Kumbh water may be due to discharge of sewage and industrial effluent after the Kumbh. According to Zhang et al. (2017), the microbial community structure could be a good indicator for revealing the severity of heavy metal contamination. For instance, *Firmicutes* and *Bacteroidetes* are the main phyla in Cr-contaminated environments. The middle and lower stretches of Ganga have been reported to have high microbial and heavy metal contamination (Dwivedi et al. 2018). Further, some bacterial species which are present in Ganga water, e.g. *Bacillus cereus*, *B. thuringiensis* and *B. subtilis*, are known to reduce hexavalent chromium (Cr^{VI}), a toxic and carcinogenic form, to the non-toxic trivalent chromium (Cr^{III}) (Upadhyay et al. 2017). The presence of such bacteria may further indicate the curative properties of Ganga water because Cr^{III} is an essential nutrient that promotes metabolism of glucose, fat and protein by facilitating the action of insulin in humans and animals. Cr^{III} deficiency can cause impaired glucose metabolism and increased risk of cardiovascular disease (Hummel et al. 2007).

The PCA was performed on the data derived by the AWCD on Biolog EcoPlates based on carbon source

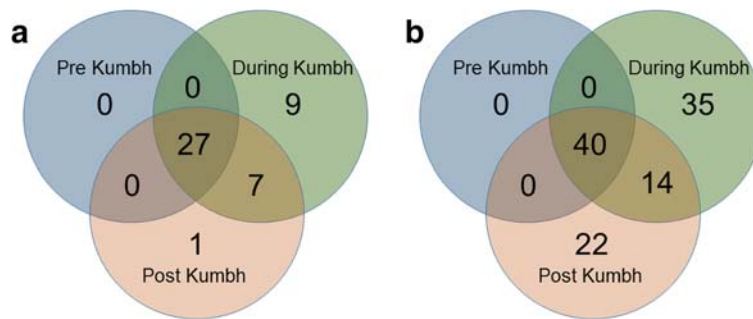


Fig. 5 Venn diagram showing change in bacterial diversity in Ganga water due to mass bathing events during Maha-Kumbh 2013 at Allahabad. **a** Changes in bacterial genera. **b** Changes in bacterial species. Study includes five sampling site. Pre-Kumbh,

20 days before the start of first mass bathing event; during Kumbh, eight special ritualistic bathing events occurred during 55 days of Maha-Kumbh; post-Kumbh, 20 days after the end of Maha-Kumbh. Exact sampling dates are given in Supplementary Table 1

utilization. The PCA of average of different bathing events revealed that the microbial functional diversity of Ganga (GC) was distinct from Yamuna (YC), while it is similar for the main bathing sites, i.e. Sangam-I and II (S-I and S-II) and after Sangam (AS), as they were overlapped on the PC1 and PC2 (Fig. 6a). It is also evident that the microbial diversity of different bathing sites was more similar to Ganga control than Yamuna. The PCA among different events (taking the average of various bathing sites) showed that all the bathing events grouped together except Mauni Amavasya and pre-Kumbh. This showed that the microbial diversities on different bathing events were completely distinct in comparison with pre-Kumbh. The microbial diversity on Mauni Amavasya, when the biggest mass gathering and bathing occurred, was also distinct from other bathing events, showing increased bacterial population on Mauni Amavasya (Fig. 6b). The increased number of *Salmonella typhi* after the biggest mass bathing during Maha-Kumbh 2013 was also reported by Rani et al. (2014). Nevertheless, the lower changes in the bacterial functional diversity and population, within bathing events, based on carbon source utilization in the present study may be due to the presence of bacteriophage which would have controlled the microbial population.

Bacteriophage diversity

The presence and abundance of lytic bacteriophages against seven most commonly found bacteria were studied during Kumbh (Supplementary Fig. S1; Table 2). The host-specific bacteriophages against *Escherichia coli* (*E. coli B* and *E. coli K12*), *Vibrio cholerae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* were

analysed at all the selected bathing sites during each event. Results depicted that Ganga was richer in bacteriophages than Yamuna (Table 2). Abundance of phages associated with *E. coli B* and *V. cholerae* bacteria increased at the different bathing sites during the main bathing events, in comparison with GC samples. The phages associated with *E. faecalis* and *S. typhimurium* were not detected in the control, pre-Kumbh and post-Kumbh samples, but these were present in the Ganga water during the main bathing events, detected by enrichment method. Interestingly, *S. aureus* bacteriophage unlike other bacteriophages was not detectable in the main bathing events (Mauni Amavasya and Basant Panchami), but it was present in the water samples of pre-Kumbh and other bathing events. This suggests susceptibility of *S. aureus* bacteriophages to increasing pollutant levels. The increasing population of lytic viruses indicates the higher availability of the host. The presence of phages against *S. typhimurium* and *E. faecalis* on major bathing events, viz. Mauni Amavasya, Basant Panchami and Maghi Purnima, indicates the increased population of hosts on these specific events.

Thus, the water quality of river Ganga was severely deteriorated during the Maha-Kumbh with a high increase in organic and inorganic substances as evident by the increased BOD, COD and various ions. The microbial populations and bacterial diversity were also increased significantly. Still, the increase in bacterial population was not as steep as observed in the other water quality parameters also in the view of high mass of diverse populations taking a dip in Ganga. Bacteriophages have long been considered to be associated with the self-purifying and healing power of the river Ganga (Nautiyal 2009). However, not only Ganga, but it is now

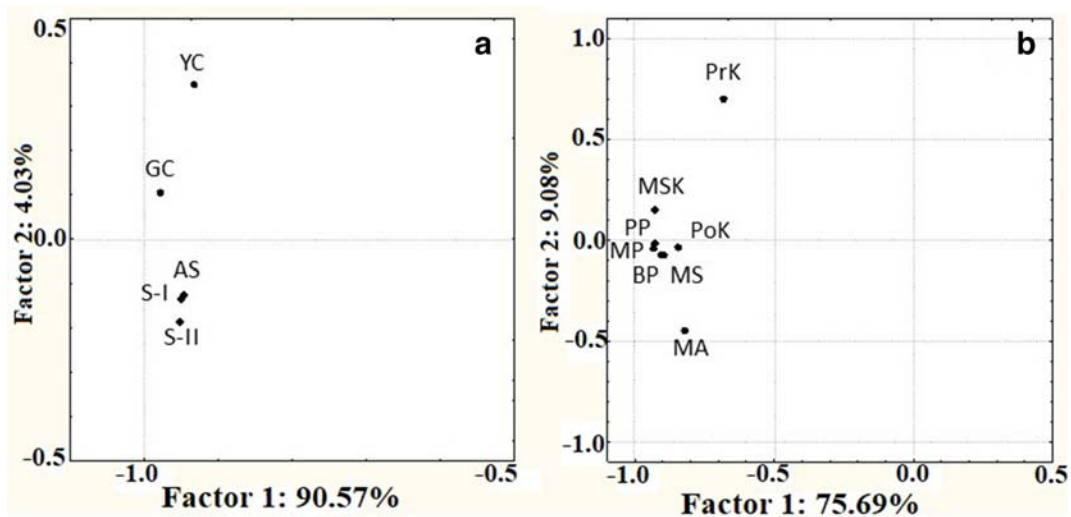


Fig. 6 Principal component analysis depicting the overall impact of mass ritualistic bathing on microbial diversity of Ganga based on carbon source utilization pattern. **a** Microbial diversity at selected sites; data were average of different bathing events. YC, Yamuna control; GC, Ganga control; S-I, Sangam-I; S-II, Sangam-

II; AS, after Sangam. **b** Microbial diversity at different bathing events; data were average of selected sites. PrK, pre-Kumbh; MS, Makar Sankranti; PP, Paush Purnima; MA, Mauni Amavasya; BP, Basant Panchami; MP, Maghi Purnima; MS, Maha Shivratri; PoK, post-Kumbh

widely accepted that bacteriophages are the main cause of bacterial morbidity in aquatic systems leading to the prevention of bacterial species (Suttle 2005). Nevertheless, the higher prevalence of phages in river Ganga in comparison with Yamuna in the current study points towards some factors conducive for the growth of phages. A recent report by National Environmental Engineering Research Institute (NEERI 2017) also demonstrated that Ganga contained around 1100 types of bacteriophage, in comparison with less than 200 species detected in Yamuna and Narmada. Ganga water exhibited certain distinct qualities in terms of higher alkalinity and pH and abundance of trace elements in comparison with Yamuna. These characteristics may have a role in self-purificatory and incorruptible properties of river Ganga by promoting growth of bacteriophages. It has long been reported that phages require divalent metal ions, such as Ca, Mg, Zn, Fe and Co, for their growth. The divalent metals may be involved in phage growth by various manners, for instance, some phages require metal ions for stability, and others may require attaching to the host cell for the entry into the nucleic acid and for multiplication (Rakhuba et al. 2010). Thus, the higher level of several divalent metal ions observed in the current study may have played a role in bacteriophage growth and inhibition of bacterial population in Ganga water (Ma et al. 2018).

Conclusion

From the current study, it could be concluded that the water quality of river Ganga deteriorated during Maha-Kumbh in terms of physico-chemical parameters, and it was correlated with the size of masses taking a dip at various events. The present study demonstrated that river Ganga has a core microbial community structure, i.e. 40 bacterial species belonging to 27 genera. Though the bacterial diversity increased during Maha-Kumbh (112 species of bacteria belonging to 43 genera), the microbial population did not change significantly, and the core bacterial diversity found in pre-Kumbh Ganga water was present in all samples during Kumbh and post-Kumbh. Our results indicate that the presence of bacteriophages, distinctive physico-chemical properties (like higher pH and alkalinity) and elemental profile of Ganga might have a role in suppressing the growth of pathogens. Further, the presence of bacterial species like *Bacillus cereus*, *B. thuringiensis* and *B. subtilis* in Ganga water has huge implications. These species are known to reduce Cr^{VI} to Cr^{III} . Thus, these native microbes can be used for the treatment of Cr-containing tannery effluent before discharging it into Ganga. This bio-remediation approach will not only reduce the toxicity of Cr^{VI} in Ganga water, but the Cr^{III} -containing Ganga water may prove beneficial for human health.

Table 2 Bacteriophage diversity and abundance in Ganga during Maha-Kumbh 2013 at Allahabad

Sites		Sampling events during Maha-Kumbh							
	Pre-Kumbh	Makar Sankranti	Paush Purnima	Mauni Amavasya	Basant Panchami	Maghi Purnima	Maha Shivratri	Post-Kumbh	
<i>Escherichia coli (B)</i>									
YC	E	E	E	E	E	E	E	E	
GC	E	E	E	10 ⁴	10 ³	E	E	E	
S-I	E	10 ³	10 ⁴	10 ⁶	10 ⁶	10 ⁴	10 ²	E	
S-II	E	10 ³	10 ⁴	10 ⁶	10 ⁶	10 ⁴	10 ²	E	
AS	E	E	10 ³	10 ⁴	10 ³	E	E	E	
<i>Escherichia coli (K12)</i>									
YC	–	–	–	–	–	–	–	–	
GC	–	–	–	–	–	–	–	–	
S-I	–	–	–	E	E	E	E	–	
S-II	–	–	–	E	E	–	–	–	
AS	–	–	–	–	–	–	–	–	
<i>Vibrio cholerae</i>									
YC	–	–	E	–	–	–	–	–	
GC	–	E	E	E	E	E	E	E	
S-I	E	10 ³	10 ⁴	10 ⁴	10 ⁴	E	E	E	
S-II	E	10 ³	10 ⁴	10 ⁴	10 ⁴	E	E	E	
AS	E	E	E	10 ⁴	10 ⁴	E	E	E	
<i>Enterococcus faecalis</i>									
YC	–	–	–	–	–	–	–	–	
GC	–	–	–	–	–	–	–	–	
S-I	–	–	–	E	E	–	E	–	
S-II	–	–	–	E	E	–	–	–	
AS	–	–	–	–	–	–	–	–	
<i>Staphylococcus aureus</i>									
YC	–	–	–	–	–	–	–	–	
GC	–	E	–	–	–	–	E	–	
S-I	E	E	E	–	–	E	E	E	
S-II	E	E	E	–	–	E	E	E	
AS	–	E	E	–	–	–	E	–	
<i>Salmonella typhimurium</i>									
YC	–	–	–	–	–	–	–	–	
GC	–	–	–	–	–	–	–	–	
S-I	–	–	–	E	E	–	–	–	
S-II	–	–	–	E	E	–	–	–	
AS	–	–	–	–	–	E	–	–	
<i>Pseudomonas aeruginosa</i>									
YC	–	–	–	–	–	–	–	–	
GC	–	–	–	–	–	–	–	–	
S-I	–	–	–	E	E	–	–	–	
S-II	–	–	–	E	E	–	–	–	
AS	–	–	–	–	–	E	–	–	

E, phages detected after enrichment; Phages concentration ml⁻¹ water; YC, Yamuna control; GC, Ganga control; S-I, Sangam-I; S-II, Sangam-II; AS, after Sangam

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Author contributions Site and event-wise water sample collection: SD, RDT, SM¹, AK, PKS and AM. Trace and toxic metal analysis and first draft preparation of the MS: SD, SM². Microbial diversity assessment: PSC and SY. Bacteriophage study: MK. Physico-chemical characteristic measurement: RC and SA. Statistical and Graphics: SM² and AK. Interpretation of the data SM². Critical revision of the article for important intellectual content: SM², SD. Logistic support: SKT, SKO, RDT and CSN. Conception, and administrative and final approval of the article: CSN. (SM¹, Shekhar Mallick; SM², Seema Mishra)

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