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Seasonal influence, enteropathogenic microbial load and diarrhoeal enigma in the Gangetic Delta, India: Present scenario and health implications

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ABSTRACT

Background: Environmental diarrheagenic enteropathogens, effect of surrounding attributes and disease dynamicity remains far from being conclusively explored. Population flux, poor sanitation and hygienic practice poses potential health threat in diarrhoea endemic tropical countries like India. We aim to identify environmental attributes, seasonality of water-borne enteropathogens and health risk assessment off the river Ganges.

Methods: A yearlong sampling data generated from three sites on either sides of the River was analysed and implications have been reported. Immediately after sample collection, physico chemical and bacterial indices were measured at the sampling site and laboratory respectively, followed by further statistical analysis of the findings.

Results: Annual variation of physico-chemical indices viz., temperature 18 °C–36 °C, pH 7.49–8.67, conductivity 215–468 μS/cm and turbidity 25.6–593 NTU was recorded in the riverine water samples. High temperature and turbidity were recorded in the summer and monsoon at all sites. High bacterial dispersion has been positively correlated with turbidity and temperature variation ($P < 0.01$; $P < 0.1$) as we report TBC 10^3–10^5 CFU/ml, TCC 10^3–10^4 CFU/ml and CVC 4–212 CFU/ml, with higher distribution in the monsoon and reverse in the winter. This suggests that the bacterial pool proliferates at higher temperature whereas turbidity enhances their survival providing the substratum for the bacterial pool. CVC could be positively correlated with conductivity which implies that ionic content of water augments the Vibrio load. Adaptive capability of Vibrios to sustain in very low saline riverine setting seems to be assisted by turbid water coupled with nutrient rich organic matter.

Conclusion: Our present work establishes the interplay of seasonal variants on the dynamicity of enteropathogenic bacteria in flowing aquatic ecosystem. It also categorises the existing microbial threats in the Ganga River to help monitor the conventional as well as emerging diarrhoeal pathogens to reduce diarrheal recurrences.

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Introduction

Water is the most fundamental resource required to sustain life on this planet. However a sizeable number of the world's population lack access to adequate and safe drinking water supplies. As a result, waterborne diseases including diarrhea and severe gastroenteritis with consequent morbidity and mortality continues to be a worldwide burden mostly in developing countries with some sporadic cases from the developed countries as well [1,2]. Globally,diarrhea accounts for 25% of child deaths under 5 years of age, has been estimated at 1.5 million in the year 2017 which withstands the fact that it is still the 2nd most common cause of death due to infectious diseases among infants followed by pneumonia [3]. India ranks 1st amongst top five countries with highest global burden of child diarrheal deaths, followed by Nigeria, Pakistan, Democratic Republic of Congo and Angola with nearly three lakhs children dying in 2016 [3–5]. Climate change, alteration of hydrological vari-

Abbreviation: CDC, Centers for Disease Control and Prevention; NOVC, non O1 *V. cholerae*; NTU, Nephelometric Turbidity Units; TBC, Total Bacterial Count; TCC, Total Coliform Count; TEC, Total *Escherichia coli* Count; CVC, Cultivable Vibrio Count; PSU, Practical Salinity Unit; IPCC, Intergovernmental Panel on Climate Change.

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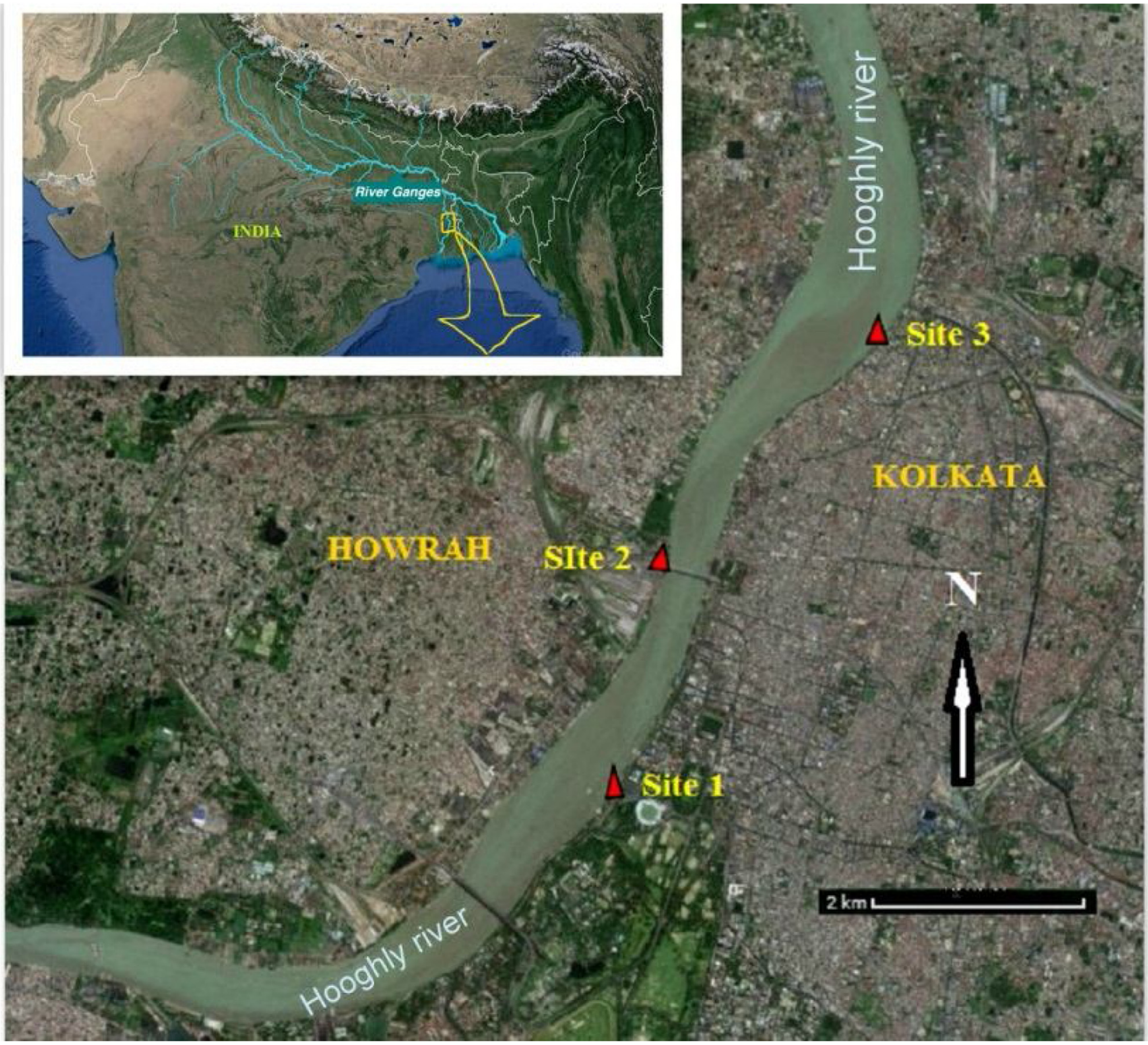

Fig. 1. Study sites.

ants, anthropogenic interventions, contamination of river water, untreated sewage disposal and poor sanitary practices are primary perpetrators for south Asian countries to stand as century old diarrhea endemic milieu [6–8].

Ganga is the most significant river system in India and accounts for 25% of India's water resources. It has played a major role in the development of Indian civilization and economy [9]. Ganges–Brahmaputra delta in the eastern India is a homeland of cholera and well known for its diarrheal endemicity. *Vibrio cholerae,* the etiological agent of cholera is an inhabitant of tropical estuaries and cholera epidemics have been effectively related to changes in the physico-chemical properties of water, tidal influence and biogeochemical factors [8].

Interestingly, even in the high altitudes nearer to the origin of the river Ganges, coliform contamination has been noted [10]. Total coliforms and *Escherichia coli* are used as bio indicators to measure the degree of pollution and sanitary quality of healthy water and their presence in water also indicates fecal contamination level with the possible presence of disease-causing pathogens, such as bacteria, viruses and parasites [11]. Untreated sewage disposal from cities into the riverine environ increases the risk of direct pathogenic transmission into the community while accessing water for drinking, bathing and other household purposes [12].

A recent report by CDC (2012) showed a 43% worldwide increase in *Vibrio* induced gastric infections in humans. Resurgence of *V. cholerae* with a soaring degree of toxicity inclusive of wide range of NOVC pool (non O1 *V. cholerae*) are matters of utmost concern [13]. Although few studies were conducted to identify the favorable physico-chemical conditions for *Vibrio* survivability and growth, the microbial dynamics of this large aquatic ecosystem, characterized by high loads of organic matter and suspended sediments, flowing through a densely populated cholera hyper-endemic zone is still poorly understood [8]. Advancement of human civilization has set serious questions to the safe use of river water for drinking and other purposes [6].

The present study attempts to investigate and identify the inter-relation among environmental and hydrological factors with entero-pathogenic microbial load in the Hooghly River, one of the main distributaries of the river Ganges in India. This will help to identify the key environmental drivers and extent of anthropogenic intervention effect on abundance of entero-pathogens and their role in understanding of diarrheal disease dynamics.

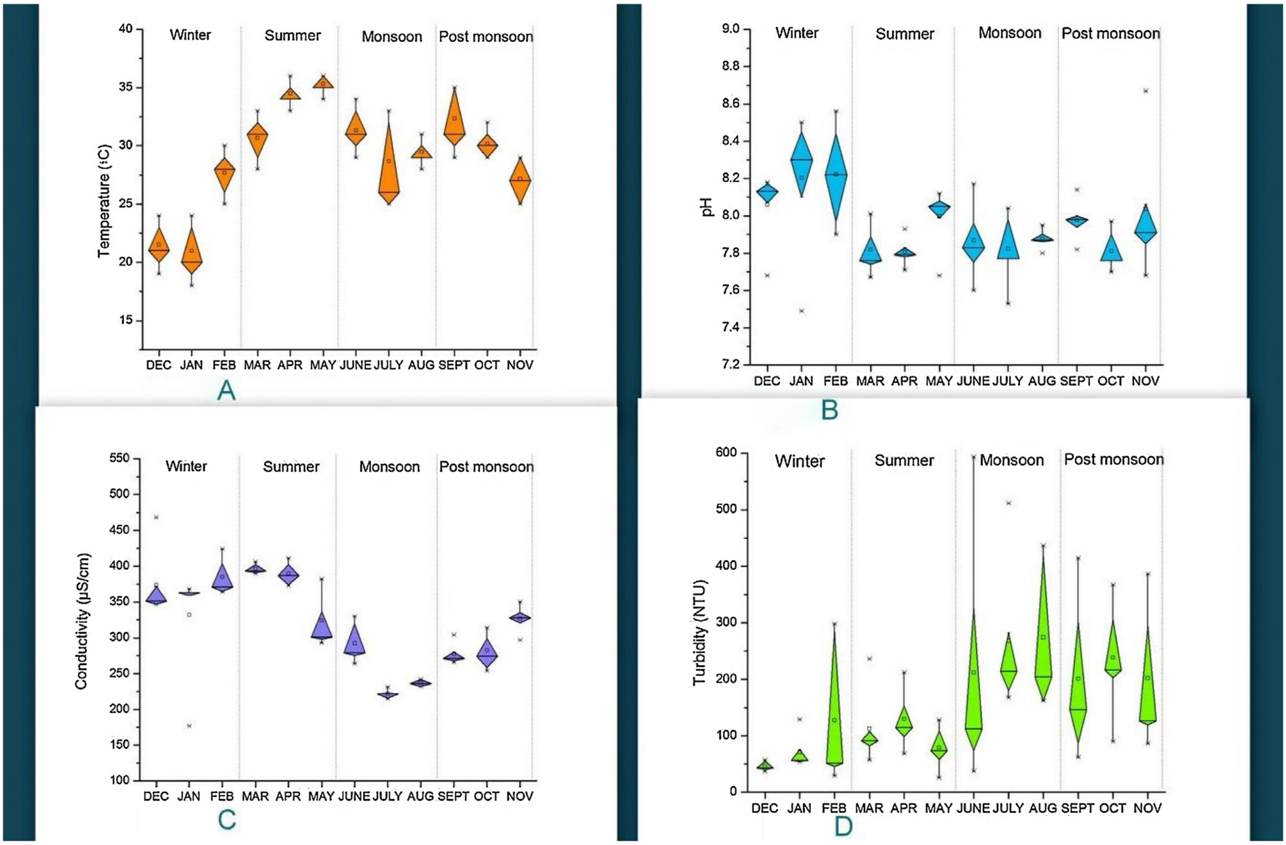

Fig. 2. (A) Seasonal variation of temperature; (B) seasonal variation of pH; (C) seasonal variation of conductivity; (D) seasonal variation of turbidity.

Material and methods

Study sites

Hooghly River (260-km-long) is a major distributary of the river Ganga (stretched approx. 2525 km from its source to its opening into the sea mouth), splitting from Murshidabad district of West Bengal near Farakka Barrage, eventually flows into the Bay of Bengal through lower deltaic districts of West Bengal including thickly populated mega cities of Kolkata and Howrah on its either side (Fig. 1) [6].

Site 1. Babughat (22°33′52.0″N 88°20′16.7″E)
This site is situated close to the center of the city of Kolkata and the river at this site receive high anthropogenic invasions regularly for various purposes viz. bathing, washing clothes, religious ceremonies etc.

Site 2. Howrah (22°34′56.4″N 88°20′39.1″E)
This site is130 km away from the sea mouth of the river and has an estuarine condition due to significant tidal oscillation of ~3 m.Here, the river flows through the densely populated region in southeast of West Bengal, as well as through many areas of industrial and agricultural belt. Mostly untreated sewage and industrial waste disposes into river water.The river water is also accessed for drinking, washing, bathing, cleaning utensils and for many religious rituals.

Site 3. Bagbazar (22°36′21.6″N 88°21′56.5″E)
The Hooghly River at this site is accessed for bathing, pursuing festive rituals and religious ceremonies all throughout the year. This site receives a great deal of organic disposal due to different religious rituals and anthropogenic intrusions.

Sample collection:

Water samples were collected from three sampling stations of the Hooghly River as described earlier (Fig. 1). Each station is about 3 kilometers away from each other and surface water samples were collected along the banks of the river. Water samples were collected twice in a month (in spring tides) and collection periods have been settled as winter (December–February), summer (March–May), monsoon (June–August) and post monsoon (September–November) to embrace the effect of seasonal changes on the physicochemical and bacteriological indices throughout the year. Water samples were taken from 50 cm below from the water surface in sterilized glass bottles marked with the respective identification name and transported to laboratory in an icebox by maintaining the temperature around 4 °C.

Physico-chemical and hydrological indices

Immediately after sample collection, temperature, pH, conductivity and salinity were measured with a Multi-meter (pH/Cond 340i WTW, Weilheim, Germany). Turbidity was measured by a portable turbidimeter TD-100 (Eurotech, Singapore) and expressed as Nephelometric Turbidity Units (NTU). Salinity is expressed as Practical Salinity Units (PSU), which for all practical purposes is almost identical to g/L or parts per thousand.

Bacteriological analysis

The samples were analyzed for Total Bacterial Count (TBC), Total Coliform Count (TCC), Total *E. coli* Count (TEC) and Cultivable *Vibrio* Count (CVC) following standard procedures [14–16]. Briefly, 0.1 ml of each sample (diluted or concentrated) were spread plated on Nutrient Agar (Becton, Dickinson, USA) for TBC; Chromocult Col-

Table 1
Correlation matrix between bacterial load with physico-chemical indices.

	pH	Temp.	Cond.	Turbidity	TBC	TEC	TCC	CVC
pH	1							
Temp.	−0.284**	1						
Cond.	0.308***	−0.093	1					
Turbidity	−0.149	0.108	−0.414***	1				
TBC	−0.229**	0.016	−0.331***	0.207*	1			
TEC	−0.181	0.102	−0.226**	0.277**	0.570***	1		
TCC	−0.175	0.072	−0.384***	0.324***	0.710***	0.792***	1	
CVC	−0.052	0.134	0.034	0.047	0.11	0.045	0.005	1

* Correlation is significant at the 0.1 level ($p < 0.1$).
** Correlation is significant at the 0.05 level ($p < 0.05$).
*** Correlation is highly significant at the 0.01 level ($p < 0.01$).

iform Agar (Merck, Darmstadt, Germany) for TCC and TEC; and Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS; Becton, Dickinson, USA) for CVC determination, respectively. This was followed by a16 to 18 h incubation at 37 °C for all determinations. All microbiological enumerations were done in triplicate. Suspected *Vibrio,* coliform and *E. coli* isolates grown on selective media were verified by biochemical tests following standard procedures, e.g. oxidase, gelatinase, motility, reactivity to Kligler's Iron Agar slant, carbohydrate fermentation assay (arabinose, glucose, mannose, sucrose, mannitol, inositol, citrate, etc.), decarboxylase reaction in amino acids (arginine, lysine, ornithine), salt tolerance tests (0%, 6.5%, 8%) etc [14,17]. Some of the biochemically identified strains belonging to pathogenic species, e.g., *V. cholerae*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* etc. were verified by PCR based molecular methods following previously established protocols [18].

Data processing and statistical analysis

Statistical analyses and graphics were carried out using 'Origin software' (V.08; Origin Lab Corporation) and MS Excel 2013. Single or median values were used to describe the temporal sequences. In diamond box plots, the bottom and top of the diamond box represent the 25th and 75th percentile, respectively. Horizontal lines in the box indicate the median values, while vertical bars show the standard deviations. Maximum and minimum values indicated by highest and lowest points on diamond box plot and Small Square represents mean value.

Interrelation between physicochemical attributes and bacteriological markers have been demonstrated by the correlation matrix (Table 1) and exponential regression model (Figs. 5 and 6). Required statistical enumeration and regression line construction was executed using MS Excel 2013.

Results

The yearlong (December, 2016–November, 2017) sampling campaigns were carried out from the three selected sites (Site1, Site2 and Site3) of the either side Hooghly River and a total of 72 (seventy two) surface water samples were collected from these sites.

Physico-chemical parameters

The sampling was initiated from winter, followed by summer, monsoon and post monsoon periods respectively, encompassing all the prevalent season in the tropical region. Minimum surface water temperature was recorded to be 18 °C in January and increased up to 36 °C in the month of May and again declinedto 25 °C in November (Fig. 2A). Similar temperature variations were observed at all three study sites. Conductivity varied between 215 μS/cm to 468 μS/cm throughout the year. Maximum and minimum values were recorded in winter and monsoon respectively (Fig. 2C). Turbidity level fluctuates between 25.6 NTU to 298 NTU during the winter and summer season respectively and drastically increased up to 593 NTU in rainy season and again settled down in post monsoon at an average of 214 NTU (Fig. 2D). During the entire study period, salinity levels were too low to be expressed in PSU. Values of pH ranged between 7.49 and 8.67throughout the year with higher values obtained during the winter season (Fig. 2B).

Bacteriological analysis

Total bacterial count (TBC) varied between $\sim 10^3$ CFU/ml in winter and $\sim 10^4$ CFU/ml summer season respectively. It depicts an increasing trend from the end of summer and remain high ($\sim 10^5$ CFU/ml) throughout the monsoon and post monsoon period (Fig. 3C) before dipping in the winter. TCC varied 1×10^3 to 3×10^4 CFU/ml throughout the year with lowest and highest values recorded in December and October respectively. Higher prevalence of total coliforms was detected in monsoon (2.2×10^3 to 2.0×10^4 CFU/ml) and post monsoon (2.8×10^3–3.0×10^4 CFU/ml) seasons (Fig. 3A). Substantial *E.coli* load has been noted all over the year which varies between 2.1×10^2 CFU/ml to 1.4×10^4 CFU/ml. Maximum *E. coli* load have been found in June and minimum load observed in the month of January (Fig. 3B). Vibrios pool was observed very little in winter season (Avg. 14 CFU/ml) and attain a rapid upsurge in summer (Avg. 46 CFU/ml), after that reached its highest density (Avg. 67 CFU/ml) in monsoon and again started to diminish from post monsoon (Avg. 34 CFU/ml). Highest vibrio load (212 CFU/ml) was recorded in August and lowest (4 CFU/ml) in the month of January (Fig. 3D).

Correlation between different bacteriological and physiochemical variants

Correlation between seasonal changes of physico-chemical parameters and microbial abundance has been established in the present study. Turbidity quotient have a distinct positive correlation with all bacterial pool including TBC, TCC, TEC and CVC, although correlation matrix shows that values of the correlation coefficient of TCC and TEC ($p < 0.01$ and $p < 0.05$ respectively) are more statistically significant than the rest. Interestingly conductivity exhibits positive correlation with cultivable Vibrios (CVC) but shows negative correlation with other bacterial organisms viz. TBC, TCC and TEC. However, all the investigated bacterial counts show positive correlation with temperature and negative correlation with pH (Table 1).

Regression line analysis between physiochemical and bacteriological variables

Exponential regression model has been established to identify the relationship between the two sets of variables. The regression model which has been developed in the present study will assist in

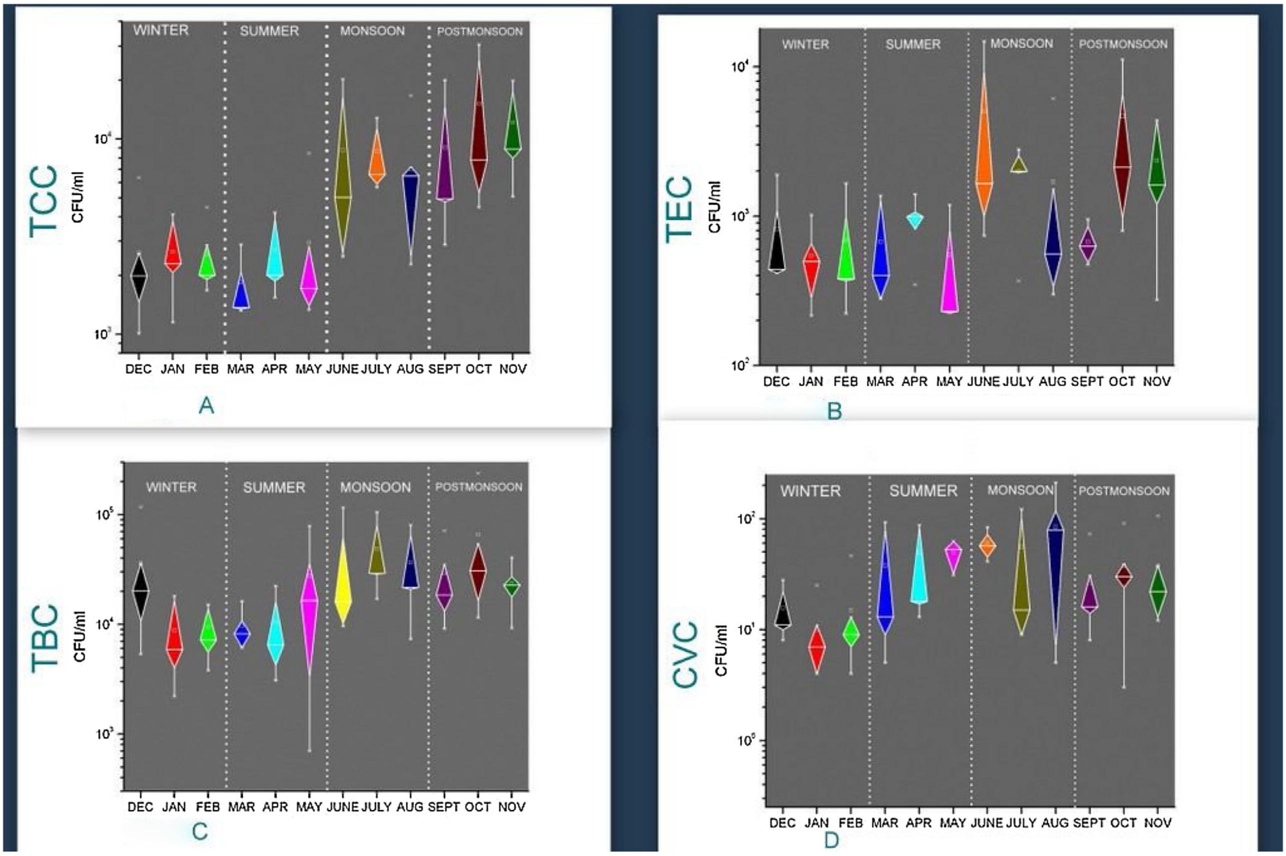

Fig. 3. (A) Seasonal variation of TCC; (B) seasonal variation of TEC; (C) seasonal variation of TBC; (D) seasonal variation of CVC.

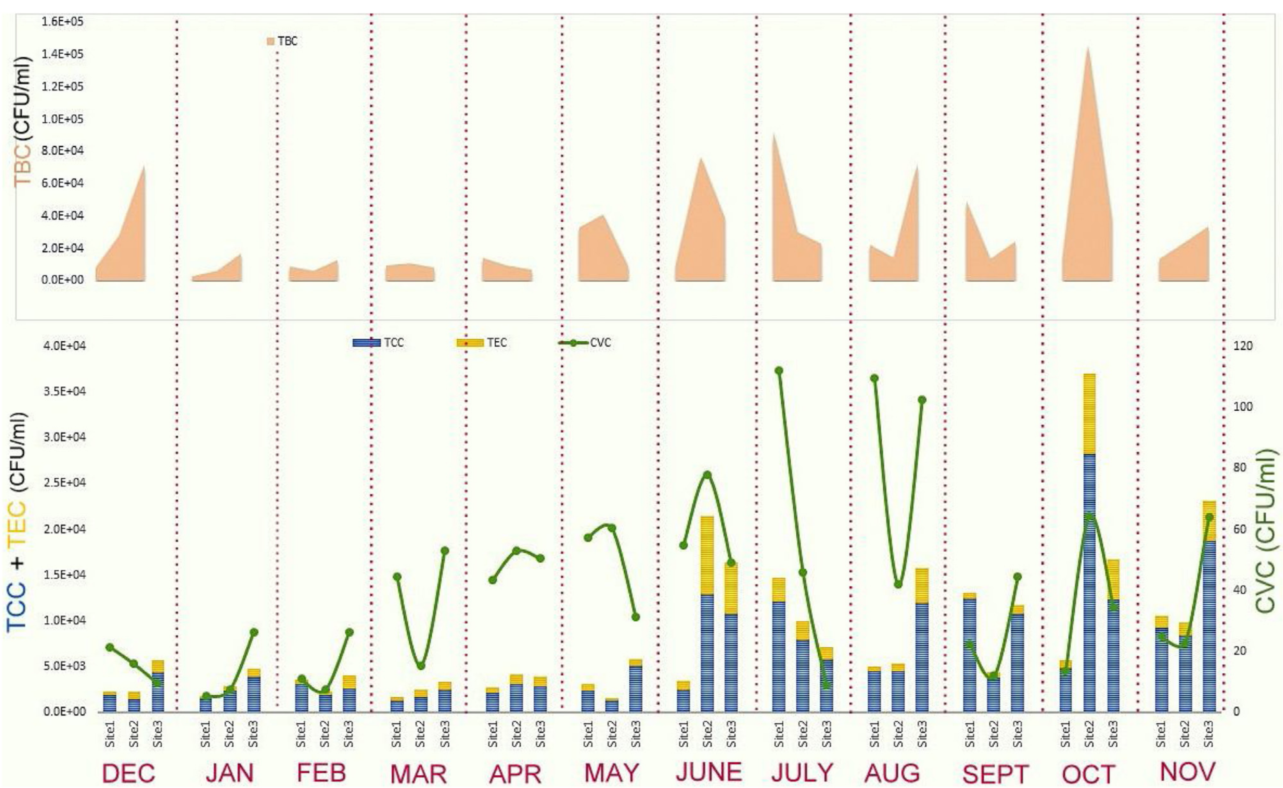

Fig. 4. Site-wise variation of bacteriological counts.

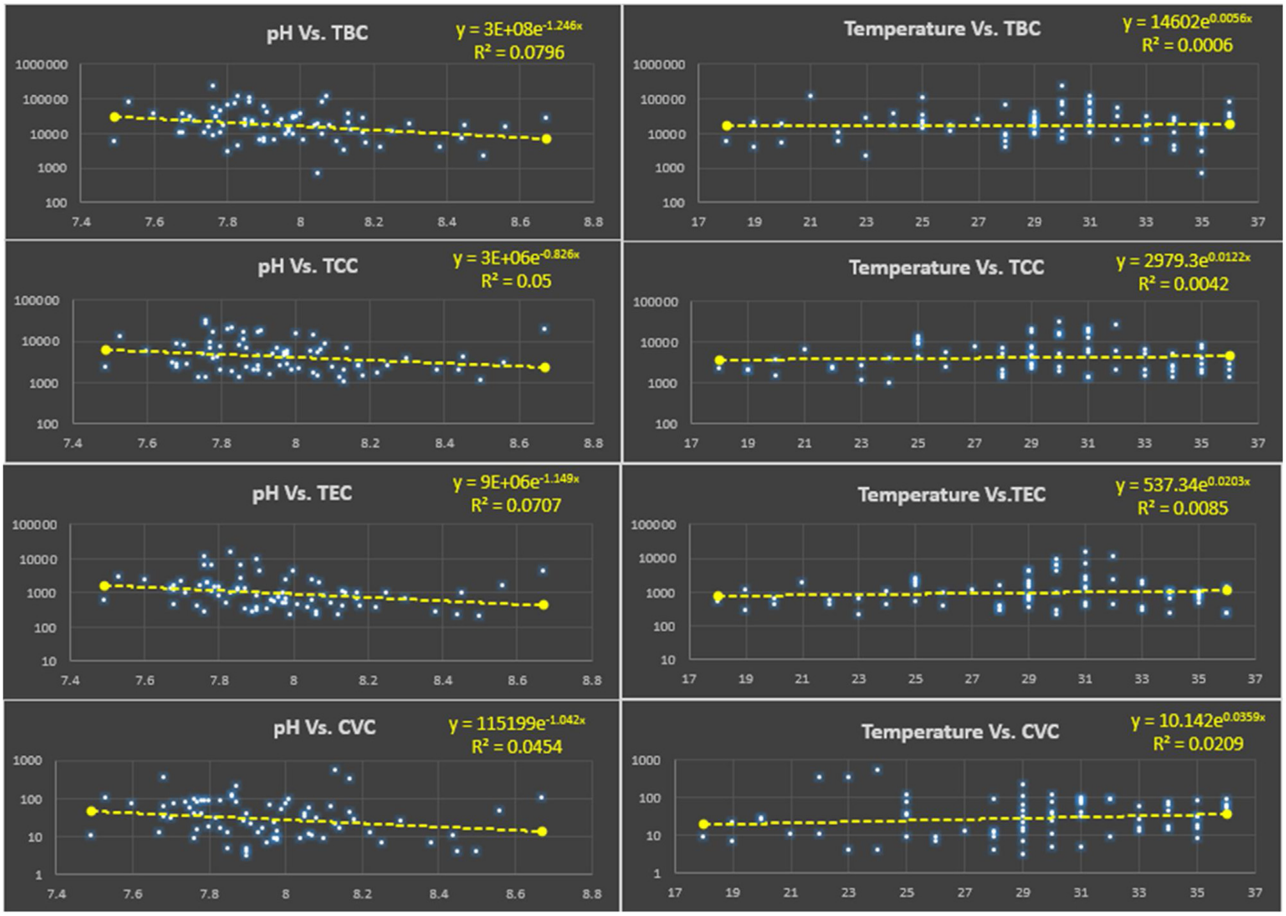

Fig. 5. Exponential regression model with best fitted straight line depicting interrelation among pH and temperature (°C) with TBC, TEC, TCC and CVC (CFU/mL). Independent variables (pH and temperature) were plotted in X axis and dependent variables (TBC, TEC, TCC and CVC) were plotted in Y axis. Regression line equation of exponential functions ($Y = a^*e^{bX}$; a = Y intersect of the line, b = slope or regression co-efficient) and Co-efficient of determination (R^2) have been inserted on the image

predicting and estimating the bacteriological load with variations in physicochemical indices. Our regression analysis implies that there is a linear relationship between physiochemical and bacteriological variables. Temperature and turbidity is directly proportional with TBC, TCC, TEC and CVC (Fig. 5), whereas conductivity and pH is inversely proportional to these attributes (Fig. 6).

Discussion

Temporal variations of physico-chemical attributes

Seasonal variations observed round the year are reflected throughout the study period in terms of the fluctuations in physico-chemical parameters. Surface water temperatures fluctuated between 18 °C to 36 °C with high values in the summer/monsoon and lowest temperatures in the winter (Fig. 2A), which aligns with the tropical climate. The pH values remain more or less same showing a slightly alkaline nature (7.49–8.67) at all the study sites round the year (Fig. 2B). Similar trends in pH were also noted by other study groups on river Ganga [10,19]. The higher mean pH value was recorded in winter (pH-8.16) and lower mean pH value obtained in Monsoon (pH-7.85). These results contradict with the earlier findings on Ganga, where higher pH recorded in summer and lowest in winter [20,21]. This may be due the effect of varying precipitation and changes in the pollution level of river and indicating local disposal of industrial effluents which may alter the pH of riverine water.

Turbidity level was recorded low in winter and summer months but drastically increased with the onset of monsoon (Fig2D). The low values of turbidity in the winter season (Avg. 81 NTU) could be due to reduced circulation velocity of water prompting the suspended particles to settle down in the river bed. Monsoons recorded significantly higher values of turbidity (251 NTU) because of sediment re-suspension from the river bed due to enhanced precipitation and it remains persistently higher during the post monsoon period (Fig. 2D).

Salinity was unnoticeable (ca. 0.0) throughout all the seasons amongst the study sites because of their distant position (~130 km away) from the sea mouth. Our previous studies established that salinity could be detected up to Diamond harbor which is 50 km downstream along the Hooghly river from the present study sites [22]. Despite the absence of salinity, conductivity ranged between 215 μS/cm to 468 μS/cm (Fig. 2C). Conductivity of river is a function of the geology of the area, dependent on the ion concentration in the water which is also reflected by seasonal effect [23–25]. Maximum conductivity was recorded in winter and gradually declines with the onset of monsoon period. This is because of the heavy precipitation which is accompanied with dilution of ion concentration of riverine water by mixing with rain water. It was observed to increase during the post monsoon season with decrease in rainfall rate (Fig. 2C).

Climatic and anthropogenic influence on microbial abundance

With a gradual increase of water temperature from late winter (February) there was an overall increasing trend in TBC, TEC, TCC and CVC up to the summer months. Higher load of the bacterial counts were recorded in the monsoon and post monsoon

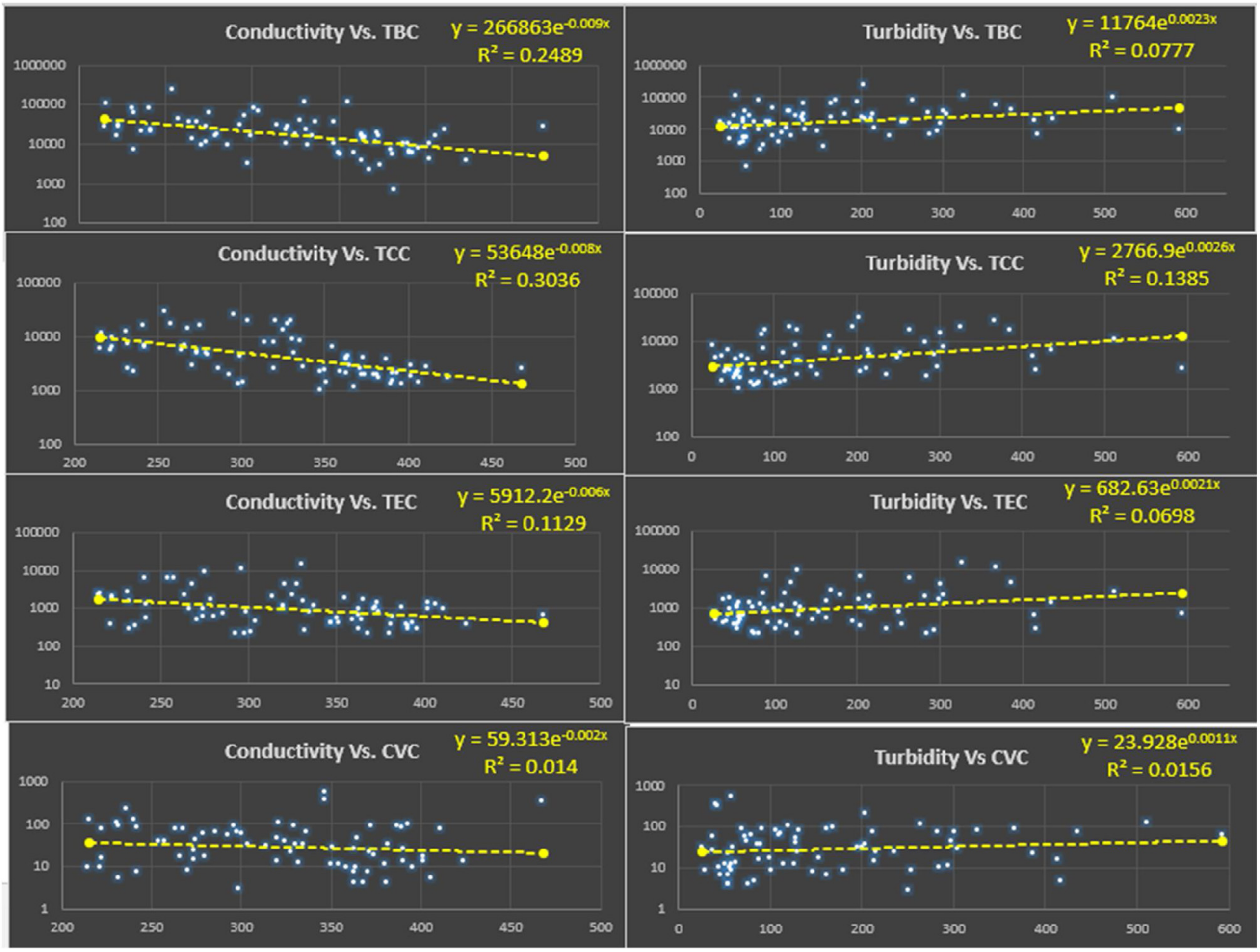

Fig. 6. Exponential regression model with best fitted straight line depicting interrelation among conductivity (μS/cm) and turbidity (NTU) with TBC, TEC, TCC and CVC (CFU/mL).
Independent variables (conductivity and turbidity) were plotted in X axis and dependent variables (TBC, TEC, TCC and CVC) were plotted in Y axis. Regression line equation of exponential functions ($Y = a^{*}e^{bX}$; a = Y intersect of the line, b = slope or regression co-efficient) and Co-efficient of determination (R^2) have been inserted on the image.

periods (Fig. 2A, Fig. 3). This trend was established by Correlation Matrix (Table 1) and regression model (Fig. 5) which displays positive interrelation between temperature and all bacterial pool in natural water bodies.

Increase in TBC with an increase in water temperature has been noted in summer, which flourished further in monsoon and gradually decreased from post-monsoon to winter. The increasing trend of TBC in summer results due to intrusion of marine water into river which in turn helps the halophilic bacterial community to migrate inland. An average temperature of 31 °C ± 3.0 was recorded during the warmer months (March–November) of our study period. This range of temperature favors natural growth and proliferation of all microbes including Vibrios, whereas at low temperature in winter season Vibrio count decreases from free floating form and becomes attached with solid substratum and other chitinous organisms for biofilm formation to survive in adverse climatic condition [26–28].

Enteropathogenic bacterial pool prefers an alkaline pH of 8.00 ± 0.5 for their survival and proliferation [8]. There is a little fluctuation of pH recorded in our study round the year. It remains less alkaline round the year and alkalinity increases a little more in winter (Fig. 2B), which facilitates higher microbial prevalence throughout the year except for the winter months.

Salinity is the key factor which regulates abundance and growth of halophilic bacteria [29]. However in the present study very low salinity recorded from all the study sites, favors preponderance of other non-halophilic microbes including coliforms and *E. coli.* In spite of non-detectable salinity level, a CVC surge has been persistent throughout the year along with TEC and TCC. This can be explained by the higher adaptability and inherent osmo-regulatory capability of Vibrios which assists in its survival in unfamiliar ecosystem.

Despite absence of any detectable salinity level, a conductivity gradient of 215–411 μS/cm had been obtained throughout the year. CVC is positively correlated with conductivity gradient; on the contrary negative correlation exists between conductivity and TBC, TCC, TEC (Table 1, Fig. 6). This interesting outcome substantiates the fact that, although a little ionic concentration hinders the proliferation capability of non-halophilic microbes, this factor helps to sustain halophilic Vibrio abundance in low saline water. Thus the low saline or sweet river water is the home for the different bacterial pool comprising of the *E. coli*, coliforms and even the halophilic Vibrio pool. This makes the sweet river water a potent vector for the bacterial pathogens when the community comes in contact with it.

Significant positive correlation has been established between turbidity and the bacterial counts (Table 1). Their highest count was obtained in monsoon with the subsequent turbidity maxima (Figs. 2D, 3, 6). It can be attributed to the influx of suspended sediment such as silt or clay, inorganic materials, organic matter including algae, plankton and decaying material. This implies that under nutrient rich conditions, Vibrios and other bacterial pool become active and proliferates at a much higher rate during monsoon increasing manifold the chance of diarrheal disease occurrence in the community settings coming in direct contact with the riverine waters during the monsoon of just after it [30]. In the low saline aquatic environment, turbidity plays a significant role in increasing their adaptive efficacy of diarrheal bacterial pool.

Variation of bacterial preponderance between the individual sites was observed due to some local influencing factors unique to the sites. In the month of June from the onset of monsoon higher TBC, TCC and TEC count was observed in Site 2 and Site 3 due to huge anthropogenic influence as compared to Site 1 (Fig. 4). Site 2 experiences a drastic increase of all bacterial pool in post monsoon, than other sites which may be due to massive disposal of industrial waste and other organic pollutants due to heavier water traffic at this site.

Microbial risk assessment and impact on community health

In our study, the obtained levels of TBC, TCC and TEC from all the riverine sampling sites exceeded the internationally recommended permissible threshold limits for domestic and recreational water [31]. Due to the influx of industrial waste, untreated sewage disposal system and enormous anthropogenic intervention, there is a substantial increase in fecal and organic contamination in the river. CVC level determines total Vibrio load in river water inclusive of its pathogenic pool. CVC was found to be considerably higher throughout the year, which results in annual periodic diarrheal recurrences in monsoon and post monsoon seasons in South Bengal. Rapid urbanization and industrialization along the river banks of Ganga River exert potential threat to public health by causing microbial and chemical contamination. Our observation coalesce with IPCC report (2014) which states that anthropogenic interferences and large scale industrialization are the influential contributors for global climate change including aquatic environs which leads to prevalence of pathogenic load and sporadic outbreak of infectious diseases [32].

Conclusion

Our present work establishes thereby that bacterial dynamics in river water is highly influenced by seasonal changes of climatic and hydrological factors. Turbidity and conductivity have been found to be the major regulatory factors for seasonal preponderance of bacterial pool in low saline riverine system. Free living Vibrio becomes predominant in monsoon in the most turbid phase of river water. Conductivity assists substantial preponderance of halophilic Vibrios in low saline setting but other bacterial pathogens including coliforms and *E.coli* prefer less conductance of water for their survival and proliferation.

Considering our findings, impact of the physicochemical attributes on microbial load of the lower Gangetic delta and given the tropical climate of India, continuous environmental monitoring and disease surveillance ought to be given utmost importance to prevent any threat of explosive diarrheal outbreaks. The potential bio-monitoring and re-modelling of community health including KAP (Knowledge, Attitudes, and Practices) survey, increase of local interference of public health engineering divisions, upgraded sewage disposal methods and public awareness program might be of paramount importance for reducing pollution level of the river Ganga to meet sustainable public health demands.

Spontaneous invasion of potent infectious agents into community level and its fatal effect on human health needs to be addressed regularly and substantial precautionary measures ought to be introduced by health planners for prevention and prophylaxis. Various government sponsored intervention programs target particular pathogenic microbes to exert control at regional level to reduce mortality and its effect on public health. In the course of this practice to focus on known pathogenic bacteria, other nonpathogenic opportunistic bacterium flourishes in favorable environment and acquires new pathogenic characteristics that may cause more severe infections. Therefore, we strongly suggest that, along with conventionally known pathogens causing diarrhoea other neglected pathogenic microbes should definitely be taken into consideration for their sustained monitoring and disease prevention to reduce mortality as well as morbidity.

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Competing interests

None declared.

Ethical approval

Not required.

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