Eco-restoration of River Ganga water quality during COVID-19 lockdown period using Total Coliform (TC) as proxy

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Abstract

In the recent past, rapid urbanization and industrialization induced environmental contamination especially in the water bodies including river waters has posed a severe challenge to the aquatic fauna as well as to the rural and urban populace whose life depends on these river ecosystems. However, owing to the Coronavirus induced global pandemic and the concomitant lockdown in nearly all parts of the World, the situation has changed significantly. The present study is a time series analysis of the coliform bacterial load at two sites (2nd Hooghly Bridge and Babughat) in the River Ganges along the city of Kolkata, the capital of the state of West Bengal, India. The river water samples were collected during the pre-monsoon season from April 2008 – April 2020 for analyzing the Total Coliform (TC) load in the selected sites. A significant spatial difference was observed in the TC matrix of the study site with relatively higher value in Babughat (10,271.92 ± 3050.35 MPN/100 ml) compared to second Hooghly Bridge (8,485.58 ± 2589.40 MPN/100 ml), with a p value of < 0.1. Interestingly, it was also observed that there was an abrupt decrease in TC values during the COVID-19 lockdown period (in the month of April 2020) irrespective of the study site (i.e., from 10,942.50 ± 1942.50 to 2,225 ± 754.43; p < 0.01 in case of Babughat and from 9,045.00 ± 1695.97 to 1,772.50 ± 477.87; p < 0.01 in case of second Hooghly Bridge). This sudden drop in coliform bacterial load may be due to non-functioning of the industrial units, tourism, traffic movements together with reduced waste disposal and fishing activities, absence of bathing activities and religious rituals along the bank of the River Ganges amidst the COVID-19 induced lockdown phase.

Keywords: Total Coliform, Multiple-tube fermentation technique, Most Probable Number, River Ganges water, COVID-19 lockdown period

Introduction

Like many other metropolitan cities, the city of Kolkata (the former capital of British India and the capital of the state of West Bengal, India) is located on the bank of the River Ganges or Ganga. This 2,601 km long river primarily originates in the Gangotri Glacier situated in the western Himalayas of Uttarakhand, India and flows south-east along the Gangetic Plains of India and Bangladesh, and eventually empties into the Bay of Bengal. The

River Ganges is a lifeline to millions of people living along its course of flow. It is considered as a sacred river by the Hindus and worshiped as the goddess *Ganga* in Hinduism. However, over the past several decades the Ganges is threatened by severe pollution mostly owing to the rapid industrialization, urbanization and various other increased anthropogenic actions including but not limited to tourism and fishing. Various rituals (such as cremation, religious offerings of burnt ashes of the dead bodies, fruits and flowers, immersion of mud idols of the Hindu Gods and Goddesses), and bathing activities are performed round the year by thousands of worshippers along its banks, which together with laundry (by the domestic and launderer's community), fishing activities (by the fishermen), tourism ventures, ferry and freight services contaminate the river water with numerous organic and inorganic wastes including detergents, heavy metals and hydrocarbons like polycyclic aromatic hydrocarbons (PAHs) (Goswami and Mazumdar, 2016; Rakshit and Sarkar, 2018). Moreover, the various industries including jute mills, brick kilns, tanneries, battery industries, fertilizers and soap factories, oil refineries, thermal power plants, fishery and shrimp farming units etc. located on the fringes of this river along with the sewage canals connected with this river (and untreated sewage discharge) are the additional point sources of coliform bacteria, heavy metals and hydrocarbons (Mitra, 1998; Mitra, 2013). Together, these river water pollutants pose a danger not only to humans, but also to endemic faunal community as the Ganges is home to ~140 species of fishes and ~90 species of amphibians. The river also harbors reptiles and mammals, including the critically endangered species like the gharials and the South Asian river dolphins. In addition, the environmental health especially that of river water has significant influence on the microbial flora since the microbes derive their nutrition from the ambient water and pollutants that stimulate microbial growth and proliferation. The levels of fecal coliform bacteria from human wastes in the river in specific locations are hundred times higher than the Indian government's official permissible limit. The Ganga Action Plan, an environmental initiative to clean up the river, is yet to see the light of success, due to various reasons including poor technical expertise and environmental planning, and a lack of awareness amongst the mass on the ecosystem services of this mighty river of the sub-continent.

Coliform bacteria are a group of aerobic and facultative anaerobic, Gram-negative, non-spore forming, rod-shaped, motile or non-motile microorganisms, which ferment lactose with the production of acid and gas within 48 hours when kept under 35°C – 37°C (Li and Liu, 2018). Besides being present in the environment, these special groups of bacteria typically comprise the microflora of the fecal matter of all warm-blooded animals including humans (Martin et al., 2016). Although, the coliforms generally do not cause serious illness or disease, however, their presence either in drinking water (ground and surface water) or food is a strong indicator of the presence of pathogenic organisms of fecal origin in the system under study (Li and Liu, 2018). The complete coliform bacterial population is termed Total Coliform (TC) out of which a subset is referred to as the Fecal Coliform (FC) and *E. coli* bacteria comprise a fraction of this FC population (Brackett et al., 1993). Presence of TC in water is indicative of environmental contamination while presence of FC and *E. coli* indicate fecal contamination. However, determination of the adverse impacts of anthropogenic activities on the environmental health is not always easy unless one carries out a comparative analysis of the same under two contrasting conditions *i.e.*, both in presence and absence of human activities to fully understand or pin point the parameters of eco-restoration of river water quality. The Coronavirus induced global pandemic (https://www.coronavirus.gov/) and subsequent lockdown phase for maintaining adequate social distancing measures provided the perfect opportunity to study the comparative health of the River Ganges flowing through this densely-populated city of Kolkata with industries. Therefore, the overall objective of the present study is to compare the Total Coliform (TC) load of the River Ganges at two sites of the Kolkata metropolis prior to global COVID-19 pandemic and during COVID-19 induced lockdown period through a time-series analysis.

Materials and Methods

Sampling of the river water

Water samples from the River Ganges were collected aseptically in sterilized glass container (autoclaved) on a yearly basis with utmost care from two selected study sites namely second Hooghly Bridge (22°33'31.4"N; 88°19'38.5"E) and Babughat (22°34'10.3"N; 88°20'28.5"E) located in Kolkata district of West Bengal, India for a period of 12 years during 2008-2019 in the pre-monsoon seasons prior to the Coronavirus pandemic. However, during the phase of COVID-19 induced lockdown, weekly water samples were collected during April 2020 for a period of one month. The collected samples were immediately transferred in ice-box and brought to the laboratory for further analysis.

Determination of Total Coliform (TC) from water

The total coliform of water was determined by multiple-tube fermentation technique (APHA, 1998). The multiple-tube fermentation technique is a three-stage procedure in which the results are statistically expressed in terms of the Most Probable Number (MPN) (https://www.epa.gov/sites/production/files/2015-12/documents/9131.pdf).

The technique involves inoculating the sampled water in a liquid medium of lauryl tryptose broth, a selective media used for the detection of coliaerogenes bacteria in water (Corry et al., 2003). After completion of the incubation period, the tubes were examined for growth, acid and gas production by the coliform organisms. This test is known as presumptive test. Since the organisms other than the coliform may also produce this reaction, the positive tubes from the presumptive test were subjected to a confirmatory test. The density of bacteria was calculated based on positive and negative combination of the tubes. The results were expressed in MPN/100 ml (APHA, 1998). The total coliform was determined using lauryl tryptose broth and Brilliant Green Lactose Bile (BGLB) broth.

Preparation of the lauryl tryptose broth for presumptive test

To prepare lauryl tryptose broth (tryptose - 20.0 g/l; lactose - 5.0 g/l; K_2HP0_4 - 2.75 g/l; KH_2PO_4 - 2.75 g/l; NaCl - 5.0 g/l; sodium lauryl sulfate - 0.1 g/l), at first the required amounts of dehydrated ingredients for single strength (SS) and double strength (DS) were dissolved separately in each 1 l of sterilized distilled water and it was thoroughly mixed and slightly heated by proper swirling. The pH was adjusted to 6.8±0.2. After that, it was distributed as required (10 m1 DS and 10 ml SS) in test tubes containing inverted Durham's tubes and then placed in the autoclave for sterilization at 121℃ and 15 lb for 15 minutes.

Preparation of the Brilliant Green Lactose Bile broth for confirmed test

For preparation of Brilliant Green Lactose Bile broth (peptone - 10.0 g/l; lactose - 10.0 g/l; oxgall - 20.0 g/l; brilliant green - 0.0133 g/l), firstly the required amounts of dehydrated ingredients were dissolved in 1 litre of sterilized distilled water, which was thoroughly mixed and slightly heated by proper swirling and then pH was adjusted to 6.8± 0.2. After that, the broth was distributed in the test tubes (10 ml each) containing inverted Durham's tubes and then placed in the autoclave for sterilization at 121°C and 15 lb for 15 minutes.

Presumptive test for Total Coliform of water

For the presumptive total coliform test, Lauryl Tryptose Broth was used as culture medium. For analysis of water, five test tubes each of 10 ml, 1 ml and 0.1 ml sample portion were used for the presumptive test. The first set containing five numbers of 10 ml DS broth tubes. Second and third sets containing ten numbers of 10 ml SS broth tubes. Each tube in a set of five containing 10 ml, 1 ml and 0.1 ml of water samples were inoculated in the first, second and third sets of media tubes respectively and mixed thoroughly. In each case, a control set was also run parallel. The inoculated test tubes were incubated at 36±1°C. After 24 hours examined for growth, gas and acidic reaction. If there was no gas and acid production then the tubes were incubated and examined again at the end of 48 hours. Within each tube, Durham's tube was placed in an inverted position to show the bacterial growth with emission of gas. Production of gas bubbles and acids with growth was shown in the tubes within 48 hours contributes a presumptive reaction. After the incubation period of 48 hours, the numbers of positive tubes were counted and proceeded for confirmatory test.

Confirmatory test for Total Coliform of water

For confirmatory test for Total Coliform, culture medium used was Brilliant Green Lactose Bile broth. The positive presumptive tubes were gently shaken and with a sterile loop (3.0 - 3.5 mm in diameter), one or two loop full of culture was transferred to a test tube containing 10 ml Brilliant Green Lactose Bile broth with an inverted Durham's tube. The inoculated Brilliant Green Lactose Bile broth tubes were incubated at 36±1°C. Formation of any gas with growth within 48 hours constituted the confirmed test. The results were obtained in MPN/100 ml by comparing with standard MPN table.

Statistical analysis

Analysis of statistical significance was performed using One-Way ANOVA. All statistical analyses were done using SPSS 12.0 for Windows (SPSS Inc., USA).

Results

The river water samples were collected from two different study sites namely 2nd Hooghly Bridge (site A) and Babughat (site B) during the pre-monsoon season from April 2008 – April 2020 for comparative analysis of Total Coliform (TC) load in the River Ganges flowing through the metropolitan city of Kolkata and the result is represented in Fig. 1. For site A, the TC values in the river water sample ranged from 1.773×10^3 MPN/100 ml to 11.940×10^3 MPN/100 ml whereas in case of site B, the TC values ranged from 2.225×10^3 MPN/100 ml to 14.810×10^3 MPN/100 ml (Fig. 1).

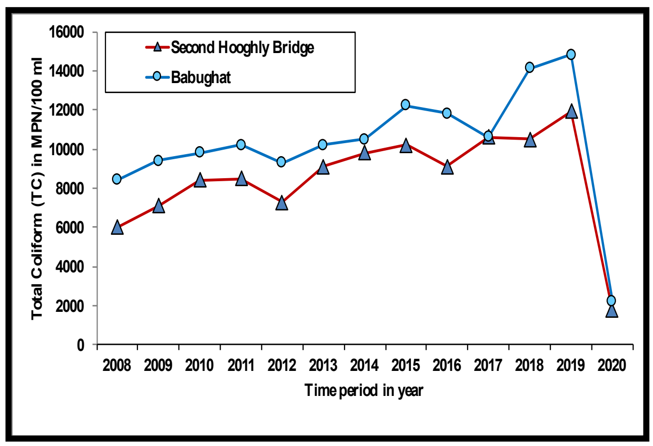

Fig. 1. A comparative time-series analysis of Total Coliform (TC) in the water of the River Ganges at two different sites (2nd Hooghly Bridge, closed triangles; Babughat, closed circles) collected during pre-monsoon season. Each symbol represents the mean of triplicate values

A significant spatial difference is noticed in the TC matrix of the study site's river water samples with relatively higher mean MPN value in Babughat (10,271.92 ± 3050.35 MPN/100 ml) compared to second Hooghly Bridge (8,485.58 ± 2589.40 MPN/100 ml) with a p value of < 0.1. Interestingly, it is also observed that there is a drastic decrease in average TC values (MPN/100 ml) during the

COVID-19 lockdown period (in the month of April 2020) irrespective of the study site (*i.e.*, from 10,942.50 ± 1942.50 to 2,225 ± 754.43; $p < 0.01$ in case of Babughat and from 9,045.00 ± 1695.97 to 1,772.50 ± 477.87; $p < 0.01$ in case of Second Hooghly Bridge) compared to pre-COVID-19 period (Fig. 2).

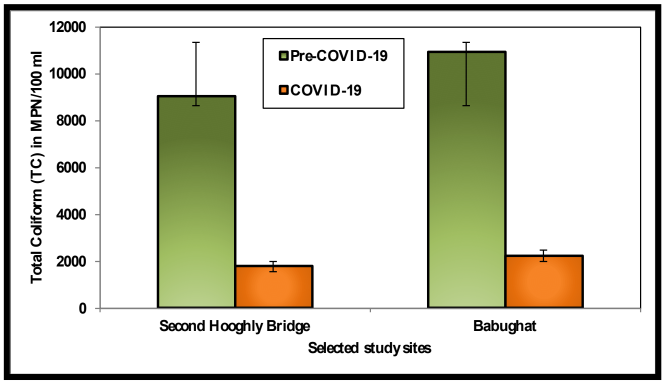

Fig. 2. A comparative analysis of Total Coliform (TC) load in the water of the River Ganges at two selected sites (Second Hooghly Bridge and Babughat) during pre-COVID-19 phase versus COVID-19 induced lockdown phase. The error bars represent the standard deviation from mean of twelve values and four values for pre-COVID-19 phase and COVID-19 induced lockdown phase respectively irrespective of the study site

Moreover, the weekly data set from both site A and B for the month of April, 2020 during the COVID-19 lockdown phase showed that the TC values in the water samples ranged from 1.25×10^3 MPN/100 ml to 2.40×10^3 MPN/100 ml and 1.50×10^3 MPN/100 ml to 3.10×10^3 MPN/100 ml for site A and site B respectively with highest value in the first week of April 2020 and lowest value in the last week of April 2020 irrespective of the study sites (Fig. 3).

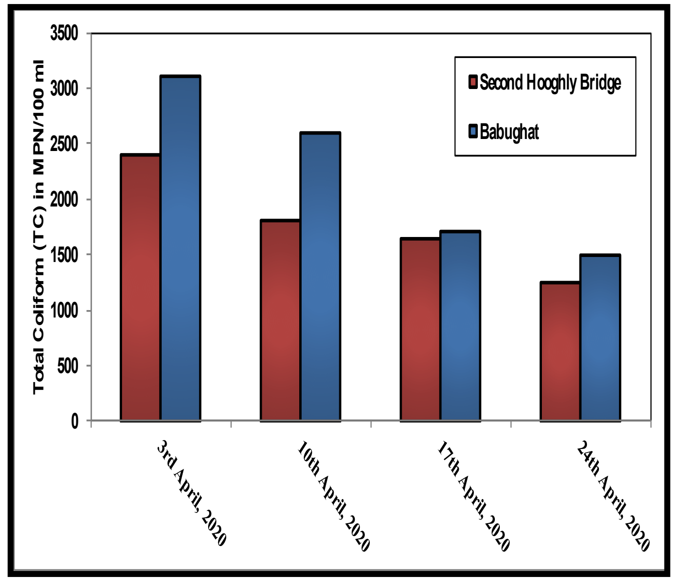

Fig. 3. A weekly comparative analysis of Total Coliform (TC) load in the River Ganges water at two different sites (Second Hooghly Bridge and Babughat) for the month of April during COVID-19 induced lockdown phase. Each column bar represents the mean of triplicate values

Discussion

The River Ganges flowing through this capital city of the Indian state of West Bengal is an integral part of the city's urban life and many industries and domestic lives are dependent on its water. At the same time, various industrial and anthropogenic activities are the constant sources of the river water contamination including coliform bacteria (Mitra, 2019).

Although, the rise of industries and human interventions over a period of time has an adverse impact on environmental health, it is congenial for the growth and multiplication of microbes as the latter obtain various nutrition from these industrial effluents and sewage wastes. In developing countries like India where discharge of sewage water is not always adequately controlled, the river water receiving the untreated discharges from the sewage canals connected with the river are often threatened with the problem of fecal contamination (Strauss, 1996). Reports of the multiplication of microbes in the scenario of increasing pollution is available throughout the World (https://www.coronavirus.gov/) and the present study area with a huge quantum of solid and liquid effluents is an ideal premise for rapid proliferation of the microbes (especially the coliform bacteria).

The Coronavirus mediated global pandemic has resulted in lockdown throughout the World. The metropolis of India such as Kolkata is no exception. Interestingly, the global pandemic mediated lockdown improves river water quality to a great extent. The sharp decline in the TC load during the lockdown phase may be attributed to non-functioning of the industrial units, traffic movements, tourism together with reduction in waste disposal and fishing maneuvers, lack of community bathing activities and various religious rituals along the banks of the River Ganges. The results of the present study shed light on the importance of lockdown induced reduction of human intervention of the surrounding environments (Ganges in this case), thereby drastically improving the microbiological quality of the River Ganges water.

Few mitigating measures that could be undertaken to control the contamination of the River Ganges water include a) controlled industrial waste disposal, b) treatment of sewage wastes prior to their discharge in the river water, c) prohibition of community bathing, d) control of religious rituals, e) controlled anthropogenic ventures, f) periodic monitoring of water quality, g) routine microbial analysis (coliform load) of river water, and h) introduction of probiotic strains or coliphages (bacteriophages that could kill coliforms such as *E. coli*) with proper EIA.

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