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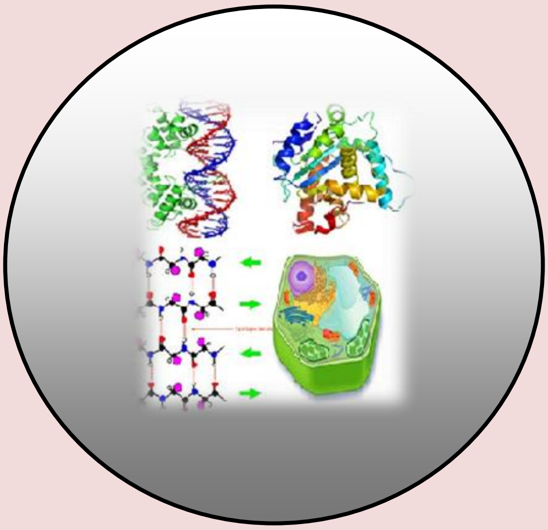

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RESEARCH PAPER

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Surveillance of Enteric Bacteria and their Spatial Pattern in Relation to Faecal Pollution in Lower Ganga at Bihar

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ABSTRACT
River Ganga is most important and complex system in its course due to dependency of millions of people, industry and wildlife for their various purposes. River Ganga has myriad issues and determinants of deteriorating its quality which adversely affect the denizens. The present study has been carried out in lower stretch of river between Munger to Katihar aiming to identify and enumerate spatiotemporal variation in the microbial load due to faecal pollution. Water samples were collected at six sampling sites and results were drawn from Total Coliforms (TC), Faecal Coliforms (FC), E. coli and Total Standard Count (TSC) in three seasons (2016-17). Overall the river showed a range of TC between 13 and 920 MPN/100 mL whereas FC ranged between 3.7 and 540 MPN/100 mL. The results on TSC and E. coli followed the similar pattern and ranged from 16-320 X 10^3 CFU/100mL and 24-370 X 10^4 CFU/100mL respectively. The cluster analysis suggests the combination of sites indicating the proper functioning of biogeochemical cycling but Bhagalpur site indicated highly polluted point depicted as outliers in whisked plots. All the water samples examined during the study period exceeded limits of WHO, BIS and CPCB for drinking and bathing water standards. The results of microbiologically evaluated water samples indicate that the river water can't be considered potable without primary treatment.
Key words: Bhagalpur, Faecal Pollution, Lower Ganga and Enteric Bacteria.

INTRODUCTION
River water pollution and degradation in surface water quality is the major concern all over the world. The river water provides provisioning, recreational, cultural and regulating services in daily life, but these are restricting due to its deteriorating quality. Identification of these natural and anthropogenic polluting sources become obligatory, as most of the drinking water supply projects in India rely on the river water. The consistency on river water for different purposes opens the channel of discrimination for the pathogenic microbial forms and chemical contaminants in human life (Basu et al. 2016). About 1.5 billion children are suffering from water borne diseases and according to WHO estimates the mortality rate has reached up to 5 million people per year. Out of these, more than 50% has been caused by the intrinsic microbial infection that accounts high percentage in developing countries (Cabral, 2010). Among the enteric infections, pathogenic microbial intrusions are occurring via ingestion of contaminated water with human and animal feces.

These contaminants are reaching to the river water through direct openings of sewerages from the nearby towns and cities including some non-point sources. The river Ganga is one of the mighty rivers in the world, regarded as the most sacred river among all the rivers in India. It sustains millions of people from 29 class I, 23 class II cities and 50 towns of India. During its course in Bihar, it flows about 405 km that adds 580 MLD (Million Liter Per Day) of waste water and untreated municipal sewage to the river, whereas the available total capacity of treatment is only 109 MLD (CPCB, 2016). Theses untreated wastes deteriorate the water quality and contrary to that during local festivals and rituals in different seasons people take holy bath and drink water irrespective to its quality. Pollution in river Ganga has been an alarming issue since 1970s but after commencement of 1^{st} phase of GAP (Ganga Action Plan) in the year 1986, it came in serious concern on both physico-chemical and microbiological account. Microorganisms especially coliforms bacteria are the best indicator to assess the water quality parameter due to its pathogenicity even in lower numbers. These forms of enteric bacteria are considered to be the potent danger to human health. A series of studies (Bilgrami and Kumar, 1998; Tiwari et al. 2005; Baghel et al. 2005; Arora et al. 2013; Basu et al. 2016; Singh et al. 2017) are available on the water quality with special reference to bacteriological studies in different places which suggests that the water has exceptionally high microbial load in comparison to the standard limits set by WHO, BIS and CPCB. But no systematic study has been conducted on microbial contamination and health issues in past few decades in the lower Ganga between Munger and Katihar districts. Therefore, the present investigation has been carried out in lower Ganga stretch at Munger, Bhagalpur and Katihar districts which are famous from ecological and spiritual point of view with an aim to identify the microbial pollution at important ghats of river Ganga in the lower stretch. The study also aimed to ascertain the suitability of water for human health consumption for drinking and bathing purposes and for developing corrective measures.

MATERIALS AND METHODS

Study area and sampling locations

The present study was conducted in the lower Ganga stretch in the state of Bihar at six major stations, i) Kastaharani Ghat (Site - I); ii) Budhi Gandak-Ganga confluence (Site - II); iii) Sultanganj (Site - III); iv) Bhagalpur (Site - IV); v) Kahalgaon (Site - V) and vi) Kosi-Ganga confluence (Site - VI). One part of selected segment (Table 1) is protected area, Vikramshila Gangetic Dolphhin Sanctuary for protection and conservation of endangered Gangetic River Dolphins, which is unique feature of the study area. The river sustains three major cities along the selected points. Apart from the direct discharge of domestic sewage, the study area receives water from the catchments including agricultural runoffs from river Budhi Gandak-Ganga confluence about 25 km downstream from the Site –I and a small inflow from Barua-Koa basin near Site – IV and a second major mixing at Kosi-Ganga Confluence near Kursela of Katihar district.

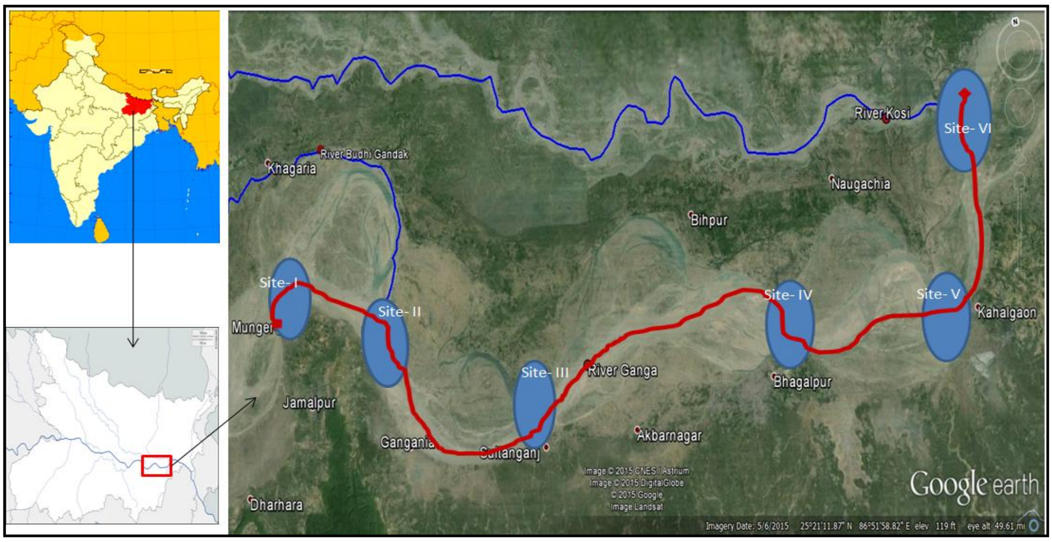

Figure 1. Location map of study area with sampling sites in lower Ganga stretch in Bihar.

Table 1. Description and location of sites in lower Ganga stretch.

Site No.	Site Name	Location	GPS Position	Distance from Site-I
Site-I	Kastaharani	Kastaharani Ghat, Munger	N 25°23.019′ E 86°27.562′	0 km
Site-II	Budhi Gandak-Ganga Confluence	5 km from Bariarpur, Munger	N 25°19.813′ E 86°34.881′	25 km
Site-III	Sultanganj	Sultanganj Ghat, Bhagalpur	N 25°15.285′ E 86°44.353′	45 km
Site-IV	Bhagalpur	Near Vikramshila bridge, Bhagalpur	N 25°16.189′ E 87°01.942′	75 km
Site-V	Kahalgaon	Near LCT Ghat, Kahalgaon, Bhagalpur	N 25°15.900′ E 87°13.528′	105 km
Site-VI	Kosi-Ganga Confluence	Near Kursela bridge, Katihar	N 25°24.995′ E 87°15.187′	125 km

Sample collection and processing

An array of samples were collected from all the six selected points on the northern bank of river Ganga in the three seasons i.e. Summer, Monsoon and Winter in the year 2016-17. Water samples were collected from the subsurface of 30 cm below the river surface and 5 m away from the bank of river following immersion sampling method. The samples were kept into pre-sterilized glass bottles by rinsing from the same water at the site of collection. The extra care was taken during collection and handling to avoid cross contamination of other microbial sources. The collected samples were stored in ice-box and transported to the Environmental Biology Laboratory of University Department of Botany, T. M. Bhagalpur University for further analysis.

To understand the overloading of water with organic compounds, the samples were analyzed by using standard plate count method (nutrient agar plates at 37 °C for 24 hours) to enumerate the total heterotrophic bacterial count and MPN technique for the enumeration of total coliforms by following standard methods of APHA (2005). The isolated coliforms were detected and quantified with the use of Eosin Methylene Blue (EMB) agar for total coliforms and *Escherichia coli* at 37 °C and MacConkey agar for faecal coliforms at 44 °C with monitoring in the laboratory after 24 hours of incubation period. All the counts were expressed in CFU/100 mL of the sample.

RESULTS AND DISCUSSION

The selected study stretch between Kastaharani Ghat of Munger district and Kosi-Ganga Confluence point near Kursela of Katihar districts are highly populous but less industrialized then the upper and middle stretch of river Ganga. This biologically rich stretch is the home of national aquatic animal Gangetic River Dolphins (*Platanista gangetica*). The river banks near Sultanganj, Bhagalpur and Kahalgaon towns and adjoining areas are the main center for unorganized sectors like flood zone agriculture, ritual centers, cremation ghats, local festivals etc. Majority of the population residing near to the banks in this stretch of about 125 kms in the lower Ganga use river water directly in their daily life practices for different purposes. However, the aesthetic beauty of the river and portability of water is degrading by the intrusion of untreated sewage and illegal discharge of septic tank contents through the domestic drain which directly reaches to the river (Singh et. al. 2017). The unavailability of sewage treatment plants (STPs) and other filtration systems magnify pollution in the river and deteriorate the water quality for human and animal uses.

In the present study, the river water was examined microbiologically to enumerate the bacterial load in the water to ascertain the suitability for stakeholders from local society and wildlife. The group coliforms and specially faecal coliforms were found highest in numbers at Bhagalpur site ranging from 280-920 MPN/100 mL and 130-540 MPN/100 mL respectively with the average of 516.7 MPN/100 mL and 296.7 MPN/100 mL followed by the Kahalgaon site in the downstream ranging from 94-240 MPN/100 mL and 26-94 MPN/100 mL respectively with the average of 154.7 MPN/100 mL and 66.3 MPN/100 mL.

The sampling site at Sultanganj shows the similar trend like Kahalgaon with slightly lower mean value 151 MPN/100 mL and 56.1 MPN/100 mL for TC and FC respectively. The lowest range of TC 17-94 MPN/100 mL and 3.7-33 MPN/100 mL of FC were recorded at Kastaharani site in meager among all the sites in the study area. Overall the river showed the TC in the range between 13 and 920 MPN/100 mL, FC in range between 3.7 and 540 MPN/100 mL with the mean value of 172.1 MPN/100 mL and 81.7 MPN/100 mL for TC and FC respectively.

Table 2. Average value of TC, FC, TTC and faecal coliform index in lower Ganga stretch at different sampling site during 2016-17.

Parameters →	Total Coliforms (MPN/100 mL)		Faecal Coliforms (MPN/100 mL)		% of FC in TC		TSC X 10^4 (CFU/100 mL)		*E. coli* X 10^3 (CFU/100 mL)	
↓ Sites	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Kastaharani	17-94	48.3	3.7-33	16.9	21.8-41.2	32.7	58-370	163.3	26-110	72.0
BG-Ganga Confluence	33-110	71	6.8-27	20.3	20.6-38.6	27.9	37-136	72.7	16-144	70.0
Sultanganj	33-280	151	9.2-110	56.1	27.9-39.3	34.1	41-152	86.0	22-189	85.3
Bhagalpur	280-920	516.7	130-540	296.7	46.4-62.9	56.0	82-282	165.3	47-320	164.7
Kahalgaon	94-240	154.7	26-94	66.3	27.7-60.8	42.5	56-259	129.7	38-285	159.3
Kosi-Ganga Confluence	13-130	91	04-63	33.7	26.1-48.5	35.1	28-109	71.0	24-234	65.7
Overall River stretch	13-920	172.1	3.7-540	81.7	20.6-62.9	38.05	16-320	103.7	24-370	113.8
BIS Std. for bathing	≤ 500		100		<20		NA		NA	

The results on TSC and *E. coli* followed the similar pattern and ranged from 16-320 X 10^3 CFU/100mL with the mean value of 103.7 X 10^3 CFU/100mL and 24-370 X 10^4 CFU/100mL with the mean value of 113.8 X 10^4 CFU/100mL respectively. The presence of *E. coli* in such a high number indicates the presence of other associated pathogenic forms of bacteria like *Klebsiella*, *Pseudomonas*, *Salmonella* and *Shigella* similar to results obtained by Tiwari et al. (2005), Sood et al. (2008) and Basu et al. (2016).

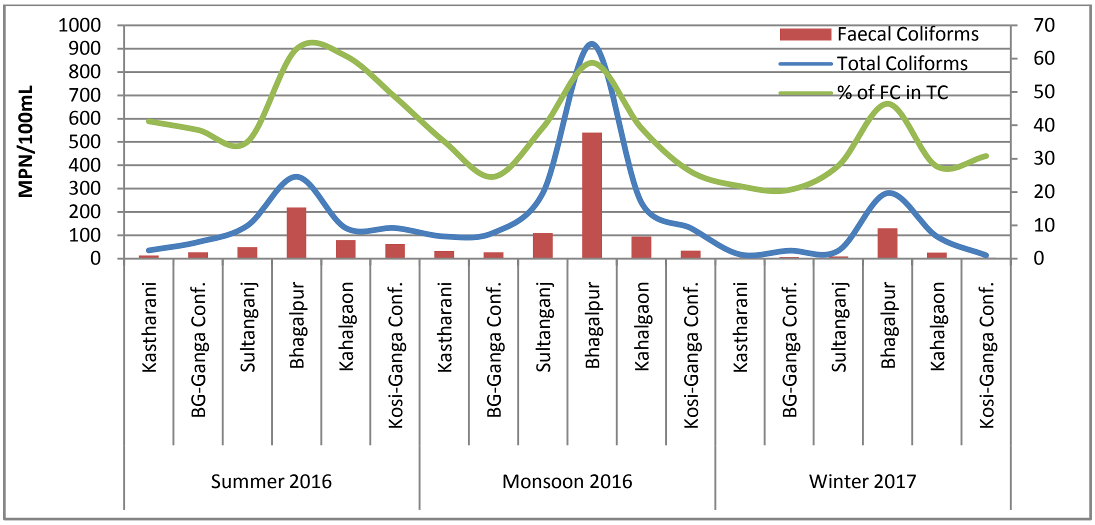

Figure 2. Concentration of TC and FC in river Ganga water at different sampling sites during 2016-17.

As per BIS, there is no relaxation in presence of coliforms and faecal coliforms in case of drinking water but the relaxation has been given up to the 500 MPN/ 100 mL and 100 MPN/100 mL respectively for the bathing and recreational purposes. But the lower Ganga stretch in the present study shows much higher values than the BIS standards for the drinking water in all around the year (Table 2). Cauvery river in southern part of India has also facing the similar conditions due to the anthropogenic load deteriorating the quality of water irrespective to its sacredness, mythological and ecological importance (Kumaraswami and Vignesh, 2009; Rajiv et al. 2012). The lack of safe drinking water in the developing countries can be attributed to disease like Cholera, Typhoid, Hepatitis, Poliomyelitis etc. BIS 10500: 2012 second revision stated that approximately 10 million cases of diarrhoea, more than 7.2 lakh typhoid cases and 1.5 lakh viral hepatitis cases occur every year a majority of which are contributed by unclean water supply and poor sanitation (BIS, 2015).

Spatial and temporal variability

The river is dynamic in nature and changes its flow and direction relative to time and space. Studies on flowing pattern of river revealed that the present stretch of river is shifting towards north and water flow reduces during lean seasons. The spatial variability was evaluated through composition of faecal pollution representing microorganisms at different sampling sites in the river. The selective groups of saprophytes were indicative of degree of organic pollution among which coliforms were chief and reliable indicators of fecal pollution. Total and fecal coliforms concentration in the water with the percent constituents are plotted in graph (Figure-2) for different sites which shown progression as river flows down from Kastaharani in Munger and reaches highest at Bhagalpur. The rate of decomposition, biogeochemical cycling and reduction in anthropogenic load may reduce the microbial concentration in downstream from Bhagalpur and least was recorded at Kosi-Ganga confluence at Kursela Katihar (Figure-3). Sampling site Sultanganj was identified as a centre of pilgrimage that adds organic load to a great extent during seasonal festivals and Hindu rituals. The fluctuations in range of both TC and FC in Sultanganj clearly indicate the magnification of pollution loads in down flows that extend the effect up to Bhagalpur (Bilgrami and Kumar, 1998; Baghel et al. 2005).

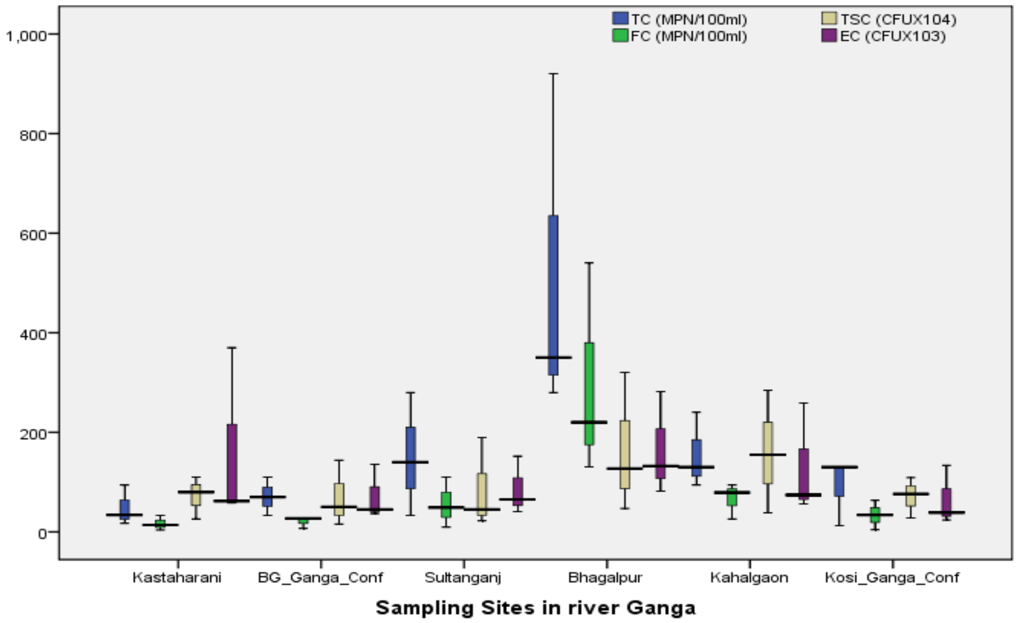

Figure 3. Spatial variation in concentration of TC, FC, TSC and EC in lower stretch of river Ganga water during 2016-17.

The box and whisker plot and clustered correlation matrix plot were plotted for the overall stretch and that shows clear seasonal changes in concentrations of both TC and FC spatially and temporally. During monsoon season the concentrations were high for TSC, TC, FC and E. coli counts due to the continuous addition of domestic sewage to the river, ambient temperature that favors the microorganisms to grow rapidly in water columns and turbidity (Figure-4).

The point Bhagalpur shows as outliers in all the three seasons due to the presence of high numbers of pathogenic microbial forms in the river water and that may be correlated to the discharge of untreated sewage that may cause serious threats to the human health (NMCGNEERI Ganga Report, 2018). The results clearly reveal that the high population density and bigger settlements have higher value of microbial contamination which cause significant deterioration in water quality (Kumaraswami and Vignesh, 2009; Arora et al. 2013; Taiwo et al. 2014).

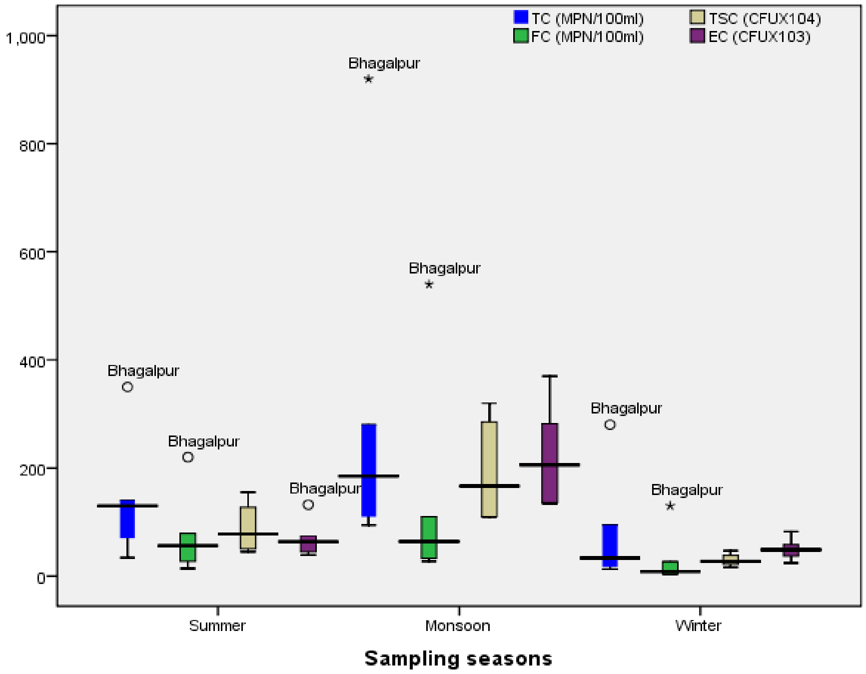

Figure 4. Temporal variation in concentration of TC, FC, TSC and EC in lower stretch of river Ganga water during 2016-17.

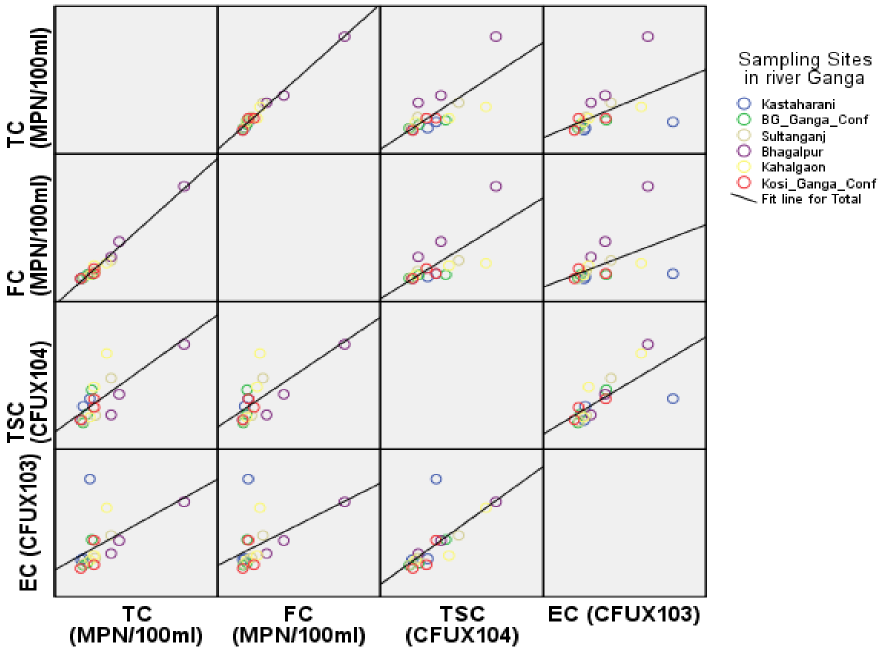

Figure 5. Correlation matrix between parameters at sites based on microbial population enumerated from lower stretch of river Ganga in different seasons.

The scatter plot matrix was used for the evaluation of spatial and temporal relation between the sampling sites in relation to the microbial pattern in the river to draw the conclusion regarding anthropogenic load and pollution activities in different seasons in this stretch. Results revealed that the TC was positively correlated with TSC and FC at p<0.001 while with EC it showed poor correlation. EC varied with TC, FC and TSC at Kastaharani site (Figure - 4). In the old age EC was considered as non-pathogenic bacteria but it was identified that some strains are pathogenic and harmful to the human health (Bilgrami and Kumar, 1998). In USA, TC was marked as water quality indicator for prevention of health risk under the Beaches Environmental Assessment and Coastal Health Act (2000). While it was reinvestigated and reported that the all the coliforms (environmental strains) are not dangerous to health that may reflect the false indication of faecal contamination in water bodies (Vadde et al. 2018). But still they are good indicators for the drinking water quality standards world over.

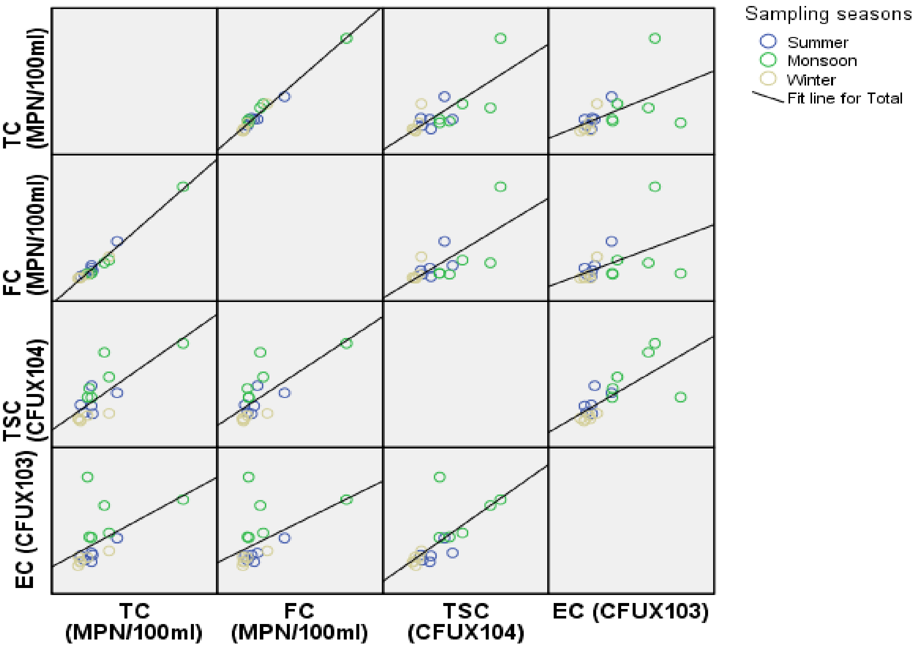

Figure 6. Correlation matrix between parameters in seasonal variation in microbial population enumerated from lower stretch of river Ganga in different seasons.

In temporal studies the FC has been not significantly correlated with EC and TSC in monsoon season while in other seasons FC was significant. It showed positive correlation with other parameters in this stretch at p<0.001 significant level (Figures 5 and 6). FC counts in water is used as crucial indicator and fixed in standard guidelines for water quality assessment by the WHO, BIS and CPCB. In the present study, all the sampling sites have higher level of FC than the limits. The monsoon receives runoffs from its wide catchments and drowns to the main flow that fluctuate the quality rapidly.

Cluster analysis

Cluster analysis (CA) was applied to detect the similarity between the microbial load and portability among the sites. A dendrogram yielded (Figure 7) by grouping all 06 sampling sites in three seasons in the river stretch. The drawn dendrogram depicted the hierarchical clustering which were extracted from the data variables like concentration of TC, FC, TSC and E. coli in the water samples at sampling sites in three seasons. The cluster 1 congregated Kastaharani, Sultanganj, Kahalgaon, Budhi Gandak – Ganga confluence (BG-Ganga Conf.) and Kosi-Ganga confluence sites. The cluster represents less pollution and it gives the clear indication of spatial diversity of microbial population in winter season and influence of confluences which diluted the river water. Cluster 2 includes four sites Kastaharani, Sultanganj, Bhagalpur and Kahalgaon in summer and monsoon months. The combination of most upstream sites, Kastaharani with highly dense populated site Bhagalpur suggests the self purification and assimilative capacity of the river Ganga.

As the river travels downstream, the pollution load increases by receiving sewage and wastewater from townships located nearby. Cluster 3 represented by single sampling site i.e. Bhagalpur in monsoon season correspond to highly polluted sites. Along the selected stretch the Bhagalpur site is the class I city without any sewerage treatment facilities and it receives pollution load not only from sewage but also from cottage industries as point source and at the same time it has catchment of big Diyara land (Flood plain) which supports agriculture practices that adds non-point sources of pollution mostly from agriculture.

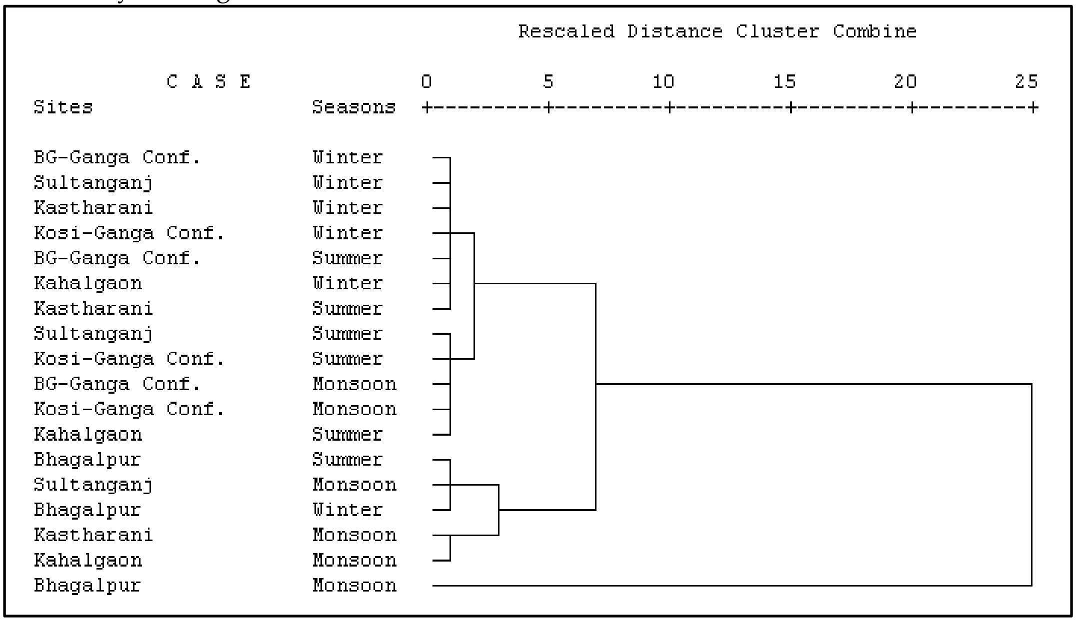

Figure 7. Dendrogram showing clustering of sampling sites based on microbial population enumerated from lower stretch of river Ganga in different seasons.

CONCLUSION

The microbiological analyses were carried out at the six selected sites in lower stretch of river Ganga between Munger and Katihar districts of Bihar. All the water samples examined during the study period exceed limits of WHO, BIS and CPCB for drinking and bathing water standards. The microbiologically evaluated results indicate that the river water cannot be considered potable without primary treatment. The quality deteriorates further in the lower stretches and in the area with higher population densities. Its direct use either for consumption or bathing may be at risk to the health of the residents and pilgrims. The TC and FC analysis showed that the water is contaminated by direct sewage discharge to the river that increase the organic load and proliferate dangerous forms of bacteria. The river stretch may be categorized based on microbial enumeration, so that in future cost effective monitoring system can be developed. The results are alarming for the stretch; under study the microorganisms load identified in this study may cause serious damage to the population, especially to the child population. Coordination between the water restoration, cleanup activities and awareness programme among basin stakeholders should be established so that they may better understand about health care and river conservation.

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