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Longitudinal Assessment of Physicochemical and Microbial Water Quality along the Ganga River Continuum: Linking Anthropogenic Activities to Pollution Gradients

Prashant Singh[1], Ajad Patel[1], Vinay Singh Baghel[2], and Ranjan Singh*[1]

[1]*Department of Microbiology, Ram Manohar Lohia Avadh University, Ayodhya, India*
[2]*Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow, India*

ABSTRACT

Water is one of the most vital natural resources and is essential to a nation's socioeconomic growth. Rivers continue to be the primary source of surface water, nonetheless. About 252.8 million hectares make up the whole catchment area of India's many big, medium and minor rivers. The Indian subcontinent's largest river basin is the Ganga basin. It originated in the state of Uttarakhand and occupies more than one-fourth of the nation's land area. The Ganga basin is home to around half of the country's class 1 and class 2 towns, most of which dispose of their waste in the Gangetic River system. The increasing pollution of river systems poses a significant challenge to water quality management and public health. In this study, total 18 water samples were collected from different locations to evaluate the Ganga River's physicochemical and microbiological quality from Uttarakhand (Gaumukh) to the Bay of Bengal (Gangasagar) to determine the effects of anthropogenic activities. The study utilized crisp data summaries of key water quality parameters, including pH, total dissolved solids, total suspended solids, biochemical oxygen demand, chemical oxygen demand, and total coliform, guided by the distribution of waste discharge points. The most probable number (MPN) test is the important and observable method employed here to assess the estimation of coliform count; biochemical tests were performed for the characterisation of the bacteria isolated. An analysis of the whole Ganga River revealed that the overall middle stretch has more anthropogenic activities than the upper and lower stretch. In this study, the quantity of indicator bacteria found in the sacred Ganga water will be the main emphasis, and the water quality and health implications will be extensively examined. Since water is essential to all living things, including humans, and its quality and cleanliness are important for human health, particularly when considering microbial contamination.

Keywords: *Ganga River, Microbial Contamination, Anthropogenic Activities, Waterborne Diseases, Physicochemical Assessment, River Pollution, Faecal Indicator Bacteria.*

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Corresponding Author: *Ranjan Singh*

E-mail Address: *ranjansingh13@gmail.com*

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1. Introduction

Freshwater rivers are essential for drinking water, agriculture, ecosystem functioning, and socioeconomic development [1]. The Ganga River originates from the Gangotri Glacier (Gaumukh) in the Himalayas and flows approximately 2,700 km through northern India before discharging into the Bay of Bengal, supporting over 400 million people [2], [3]. The Ganga River originates from the Gangotri Glacier (Gaumukh) in the Himalayas and flows approximately 2,700 km through northern India before discharging into the Bay of Bengal. The river traverses diverse climatic, geological, and land-use zones, including pristine Himalayan headwaters, densely populated urban centres, agricultural landscapes, and estuarine ecosystems, making it an ideal model for investigating longitudinal changes in water quality [4], [5]. Studying microbial communities, water comes from monsoon rainfall and snowmelt in the Himalayas. The river system consists of chilly highland streams and warm water regions like deltaic habitats [6].

Studying the movement of microbial communities throughout the Ganga is appropriate since it spans more than 2,700 km, particularly between upstream, mountainous headwaters and downstream, densely inhabited places [7]. Increased human population growth, industrialisation, intensive farming practices, ritualistic traditions and climate change have all been linked to worsened human activities, urbanisation, industrial expansion and land-use change impacts on river water quality in recent decades [7], [8], [9]. To keep the Ganga River, clean, the Indian government launched the Ganga Action Plan (GAP) in 1986 intending to reduce pollution [10]. Despite the implementation of national restoration programmes such as the Ganga Action Plan and Namami Gange, water quality deterioration remains a major environmental challenge [11]. River pollution comprises a complex mixture of suspended solids, nutrients, heavy metals, organic contaminants, and pathogenic microorganisms, which collectively threaten aquatic ecosystems and human health [5], [12].

Toxic metals, plant nutrients, suspended particles, microbial illnesses, parasites, volatile, biodegradable, and resistant organic compounds are some of the main contaminants found in water [5].

Aquatic systems are particularly vulnerable because of the significance of these places for the provision of a variety of water-based activities, and anthropogenic, agricultural, or industrial activities that take place close to aquatic ecosystems can contribute to environmental pollution [13]. The Ganga basin supports one of the world's largest human populations and is subjected to intense domestic, agricultural, industrial, and religious activities, making it particularly vulnerable to water quality degradation. A systematic approach for the first basin-wide longitudinal survey of the Ganga River was recently developed and implemented [9], [14], however, reporting of the nutrient and microbial composition of water along the river continuum remains an important scientific gap which is addressed in the current study. Although numerous studies have investigated water quality in selected urban centres or river reaches, comprehensive longitudinal assessments integrating physicochemical parameters, microbial indicators, and bacterial characterization across the entire Ganga River continuum remain scarce. Therefore, the objectives of this study were to: (i) assess spatial variations in physicochemical water quality along the entire Ganga River continuum; (ii) evaluate microbial indicator bacteria and presumptive bacterial communities; (iii) investigate longitudinal pollution gradients associated with anthropogenic activities; and (iv) identify pollution hotspots requiring priority management. Unlike previous investigations limited to regional sections of the river, the present study provides a continuous longitudinal assessment from the Himalayan source (Gaumukh) to the estuarine outlet (Gangasagar). By integrating physicochemical analyses, microbial indicators, and bacterial characterisation, this study provides a comprehensive understanding of how anthropogenic pressures shape water quality along the entire Ganga River.

2. Sample Collection

To assess the physicochemical and microbiological characteristics of the entire Ganga River continuum, a total of 18 surface water samples were collected from the river's glacial source at Gaumukh (Uttarakhand) to its estuarine outlet at Gangasagar (Bay of Bengal). Sampling was conducted between November 2024 and January 2025, with the exception of the Gaumukh sample, which was collected separately because of logistical and seasonal accessibility constraints. All samples were collected during daylight hours from locations experiencing high anthropogenic activity, including major bathing ghats, pilgrimage sites, and urban riverfronts, to capture representative impacts of human activities on water quality (Table 1). Surface water samples were collected from a depth of approximately 25 cm below the water surface using sterile polypropylene sampling bottles, following standard procedures to minimise contamination from surface films and floating debris. Immediately after collection, samples were placed in insulated ice boxes (4±2°C) and transported to the laboratory under cold-chain conditions. Upon arrival, samples were processed according to standard analytical protocols, and all physicochemical and microbiological analyses were completed within 72 hours of collection to preserve sample integrity and ensure analytical reliability (Figure 1).

Table 1. Sampling Sites and Characteristics of Water Samples Collected along the Ganga River Continuum

Sample Code	Date	Location	Coordinates	River Ghat	Stretch
UG-1	20-06-2025	Gaumukh	30.92678º, 79.08079º	Gaumukh Cave	Upper Stretch
UG-2	05-11-2024	Gangotri	30.993889º,78.941393º	Gangotri Temple	
UG-3	06-11-2024	Rishikesh	30.102922º,78.300565º	Triveni Ghat	
UG-4	06-11-2024	Haridwar	29.956725º, 78.171255º	Har ki Pauri	
UG-5	07-11-2024	Bijnor	29.372842º, 78.041388º	Madhya Ganga Barrage	
MG-6	15-11-2024	Farrukhabad	27.398728º, 79.626912º	Panchal Ghat	Middle Stretch
MG-7	28-11-2024	Kanpur	26.613852º, 80.274798º	Brahmawat Ghat	
MG-8	10-12-2024	Dalmau	26.062242º, 81.028226º	Sankatmochan Ghat	
MG-9	25-12-2024	Prayagraj	25.435945º, 81.880877º	Sangam Ghat	
MG-10	28-12-2024	Varanasi	25.290474º, 83.006431º	Tulsi Ghat	
MG-11	29-12-2024	Ballia	25.732978º, 84.166192º	Mahavir Ghat	
LG-12	06-01-2025	Patna	25.600565º, 85.232026º	Kangan Ghat	Lower Stretch
LG-13	07-01-2025	Bhgagalpur	25.269224º, 87.027322º	Vikramshila Setu	
LG-14	08-01-2025	Sahibganj	25.248458º, 87.640693º	Munteshwar Ghat	
LG-15	30-01-2025	Farakka	24.809212º, 87.918348º	Farakka Barrage Township	
LG-16	14-01-2025	Howrah	22.584469º, 88.344523º	Howrah Bridge	
LG-17	14-01-2025	South 24 Parganas	21.882205º, 88.164143º	Kakdwip	
LG-18	14-01-2025	Gangasagar	21.6528º,88.0753º	Gangasagar Beach	

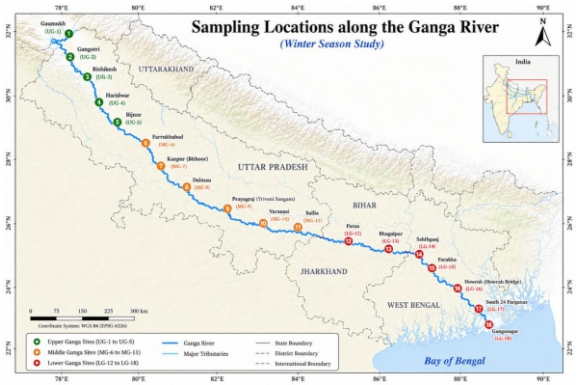

Figure 1: *Longitudinal Distribution of Water Sampling Sites across the Upper, Middle, and Lower Ganga River Continuum*

3. Materials and Methods

3.1. Water Sample Testing

Upon arrival at the laboratory, each water sample was homogenized by gentle inversion and aseptically aliquoted into sterile, autoclaved 500 mL polypropylene reagent bottles inside a Class II Biosafety Cabinet (BSC) to minimise the risk of external contamination during sample handling. Aliquots designated for physicochemical and microbiological analyses were processed immediately according to the respective analytical protocols. The remaining sample volume was stored at −80 °C for subsequent experimental analyses. All sample handling and storage procedures were performed following standard laboratory biosafety and quality assurance practices to preserve sample integrity and ensure the reliability of analytical results.

a. pH Measurement

The water sample was thawed and brought to room temperature (25 °C) and measured with a thermometer (Easy-Read® thermometer). 5ml of water sample was used in analytical replicates to measure the pH through a pH meter (Avi Scientific), and then the average was taken as shown in Table 2.

b. Turbidity

Water samples were gently mixed to ensure uniform distribution of suspended particles while avoiding air bubble formation. An aliquot of 10ml water sample was transferred into a clean, dry glass cuvette, ensuring that no bubbles adhered to the inner surface. The exterior of the cuvette was wiped with a lint-free tissue to remove fingerprints or moisture. The cuvette was then placed in the turbidity meter (Microprocessor Nephol/Turbidity meter) and was measured at a 90° light-scattering angle. Readings were recorded in nephelometric turbidity units (NTU), and measurements were performed in analytical replicates to ensure accuracy and reproducibility. The average is shown in Table 2.

c. TSS

TSS in water samples were determined using the gravimetric method. A known volume of well-mixed water sample was filtered through a pre-weighed glass fibre filter with a nominal pore size of approximately 1.2 µm. Before filtration, the filter was washed with distilled water, dried at 103–105 °C for 1 hour, cooled in a desiccator, and weighed to obtain the initial weight. After filtration, the filter containing suspended solids was dried again at 103–105 °C to a constant weight, cooled in a desiccator to prevent moisture absorption, and reweighed. The increase in filter weight represented the mass of suspended solids. TSS was calculated by dividing the mass difference by the volume of the sample filtered and expressed as milligrams per litre (mg/L). The experiment was performed in analytical replicates, and the average is shown in Table 2.

d. BOD

BOD was determined using the 5-day incubation method. Appropriate dilutions of the water sample were prepared using BOD dilution water saturated with dissolved oxygen. The initial dissolved oxygen (DO) concentration was measured immediately using a calibrated DO meter (Orion Pro Star DO213 Dissolved Oxygen Bench Meter). The bottles were then incubated at 20°C in the dark for 5 days to prevent photosynthetic oxygen production. After incubation, the final DO was measured, and BOD was calculated as the difference between the initial and final DO. Results were expressed as milligrams of oxygen consumed per litre (mg/L). The experiment was performed in analytical replicates, and the average is shown in Table 2.

e. COD

COD was determined using the closed reflux titrimetric method. A measured volume of water sample was digested with a known excess of potassium dichromate ($K_2Cr_2O_7$) solution in the presence of concentrated sulfuric acid containing silver sulfate as a catalyst. Mercuric sulfate was added where necessary to eliminate chloride interference. The mixture was refluxed at 150 °C for 2 hours in a COD digestion apparatus. After cooling, the remaining unreduced dichromate was titrated with standardised ferrous ammonium sulfate (FAS) using ferroin indicator. COD was calculated from the amount of dichromate consumed during oxidation and expressed as milligrams of oxygen equivalent per litre (mg/L). The experiment was performed in analytical replicates, and the average is shown in Table 2.

3.2. Most Probable Test

The Most Probable Number (MPN) test of coliform bacteria was determined using the multiple-tube fermentation technique. Serial dilutions of the water samples were prepared and inoculated into lactose broth tubes containing inverted Durham tubes for gas detection. A set of three or five tubes per dilution (10 mL, 1 mL, and 0.1 mL) was incubated at 37 °C for 24-48 hours for total coliform analysis. Tubes showing acid production and gas formation were recorded as positive in the presumptive test. Positive tubes were further confirmed using Brilliant Green Lactose Bile (HiMedia BGLB) broth for total coliforms and EC broth (HiMedia) incubated at 44.5 °C for faecal coliforms. The number of positive tubes at each dilution was compared with standard MPN probability tables to estimate bacterial density. Results were expressed as MPN per 100 mL of water sample. For MPN statistical analysis APHA edition were followed [15]. All analyses were performed in analytical replicates and shown as MPN index in Table 3.

3.3. Bacterial Identification and Characterization of the Ganga River

Gram staining was performed using the kit (HiMedia SKU: K001) standard protocol to differentiate bacterial isolates based on cell wall characteristics shown in Table 4. A thin smear of each isolate was prepared on a clean glass slide, air-dried, and heat-fixed. The smear was first stained with crystal violet for 1 minute, followed by the addition of Gram's iodine for another minute to form a crystal violet iodine complex. The slide was then decolourised using 95% ethanol for 10 to 20 seconds and immediately rinsed with distilled water. Counterstaining was carried out with safranin for 30 to 60 seconds. After washing and air-drying, the slides were observed under oil immersion (100×) using a light microscope. Bacteria retaining the crystal violet stain were recorded as Gram-positive (purple), whereas those taking up the safranin counterstain were identified as Gram-negative (pink/red).

3.4. Statistical Data Analysis

Standard Deviation (SD) shown in the graph bars, and the heatmap has been created by using R-programming version 4.6.1 and visualization has been done by the latest version of Rstudio.

4. Result and Data Interpretation

2.1. Physicochemical Characteristics of Ganga River

The physicochemical characteristics of the Ganga River exhibited pronounced spatial variation along the longitudinal gradient from the Gaumukh to Gangasagar (Table 2; Figure 2 a-e). Although, water quality progressively deteriorated from the pristine Himalayan headwaters towards the densely populated middle and lower reaches, reflecting the cumulative influence of anthropogenic activities, including urbanisation, industrial discharge, agricultural runoff, and religious practices. Although the river retained generally acceptable pH values, marked increases in turbidity, total suspended solids (TSS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) indicated increasing pollutant loads downstream.

The pH remained near neutral in the upper Himalayan stretch, reflecting the dominance of glacier-fed water and minimal anthropogenic disturbance. A gradual increase in alkalinity was observed downstream, particularly from Haridwar onwards, likely due to carbonate weathering, domestic wastewater inputs, agricultural runoff, and enhanced photosynthetic removal of dissolved CO_2. Despite this increasing trend, pH values largely remained within the permissible limits recommended by Bureau of Indian Standards (BIS) and World Health Organisation (WHO) (6.5-8.5), with only the Gangasagar site exhibiting slightly higher alkalinity (pH 8.9), which is consistent with estuarine conditions. Turbidity increased markedly along the river continuum, ranging from exceptionally low values at the Gaumukh to the highest levels at the Patna and Gangasagar. The low turbidity of the upper stretch reflects the clarity of glacier-fed waters, whereas progressively higher turbidity downstream is attributable to increased sediment transport, tributary inflows, municipal wastewater, industrial effluents, riverbank erosion, navigation, and religious activities. Elevated turbidity at Patna likely reflects sediment contributions from major tributaries including Gandak, Son, and Kosi rivers, while the high values recorded at the Gangasagar are further influenced by tidal mixing and estuarine sediment resuspension. Turbidity at the most downstream locations exceeded the desirable drinking water limit of 5 NTU, indicating substantial particulate contamination and reduced water clarity.

A similar longitudinal trend was observed for TSS, with concentrations increasing from 50 mg/L at Gaumukh to a maximum of 850 mg/L at Varanasi. The highest TSS values occurred within the Ganga, particularly around Kanpur, Prayagraj, and Varanasi, where intense urbanisation, industrial activities, untreated municipal wastewater, and surface runoff substantially increased suspended particulate loads. Downstream of Patna, TSS declined gradually, likely due to sediment settling, channel widening, reduced flow velocity, and dilution by tributary inflows. Lower TSS concentrations near Gangasagar suggest enhanced sediment deposition before the river enters the estuary. Elevated suspended solids may impair light penetration, reduce primary productivity, transport absorbed contaminants, and adversely affect aquatic organisms through gill obstruction and habitat degradation.

BOD values increased consistently from the upstream to downstream reaches, ranging from 0.2 mg/L at the Gaumukh to 4.8 mg/L at Howrah. The upper stretch exhibited excellent water quality, with BOD values below 1 mg/L, indicating negligible biodegradable organic pollution. In contrast, progressively higher BOD values recorded in the lower reaches reflect increasing organic loading from municipal sewage, domestic wastewater, agricultural runoff, industrial discharge, and religious practices. Although BOD remained below the common accepted threshold of 5 mg/L, the downstream increase indicates greater microbial oxygen demand and a gradual deterioration in ecological water quality, potentially imposing oxygen stress on aquatic biota. COD showed a similar spatial pattern, increasing from 4 mg/L at the glacier-fed headwaters to maximum of 23 mg/L in Kanpur before gradually declining downstream. Elevated COD concentrations in the middle stretch coincide with major industrial centres, particularly Kanpur, where tannery operations, textile industries, chemical manufacturing, and municipal wastewater contribute substantial quantities of oxidizable organic and inorganic pollutants.

The subsequent downstream decline is likely associated with dilution, sedimentation, microbial degradation, and the river's natural self-purification capacity. Nevertheless, COD values remained consistently higher than those observed in the upper stretch, indicating persistent anthropogenic contamination. The disparity between BOD and COD further suggests the presence of considerable quantities of non-biodegradable industrial pollutants, particularly within the middle stretch. Collectively, the winter-season dataset demonstrates a distinct longitudinal deterioration in physicochemical water quality along the Ganga River continuum. The upper Himalayan stretch maintained excellent water quality, reflecting limited anthropogenic disturbance and glacier-fed hydrology. Conversely, the middle stretch emerged as the principal pollution hotspot, characterized by the highest turbidity, TSS, and Cod, particularly at Kanpur and Varanasi, highlighting the influence of industrialisation and urban expansion. Although suspended solids declined in the lower stretch, BOD remained elevated, indicating continued organic pollution from cumulative municipal wastewater and sewage discharges. These findings clearly demonstrate that increasing anthropogenic pressure progressively alters the physicochemical characteristics of the Ganga River and emphasize the need for strengthened wastewater treatment infrastructure, effective industrial effluent management, and long-term basin-scale monitoring to support sustainable river restoration.

Table 2. Physicochemical Characteristics of Surface Water at Eighteen Sampling Sites along the Ganga River Continuum

Sample Code	pH	Turbidity (NTU)	TSS (mg/l)	BOD (mg/l)	COD (mg/l)
UG-1	7.2	1	50	0.2	4
UG-2	7.5	7	70	0.6	4
UG-3	7.8	50	150	0.8	8
UG-4	8.4	90	350	1	12
UG-5	8.7	120	380	1.2	16
MG-6	7.6	170	650	1.5	20
MG-7	7.8	230	800	2.5	23
MG-8	7.5	150	350	1	12
MG-9	7.9	230	750	1.8	18
MG-10	8.2	250	850	2.5	22
MG-11	7.9	146	650	1.9	20
LG-12	7.9	300	430	3.2	15
LG-13	8.3	150	400	2.8	10
LG-14	8.5	146	200	1.7	8
LG-15	7.9	170	450	3.5	12
LG-16	8.7	200	550	4.8	18
LG-17	8.6	150	350	3.3	12
LG-18	8.9	300	250	4	13

Figure 2 (a-e). *Spatial variation in physicochemical water quality parameters (pH, turbidity, total suspended solids (TSS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD)) measured at eighteen sampling sites distributed along the Upper (UG-1–UG-5), Middle (MG-6–MG-11), and Lower (LG-12–LG-18) stretches of the Ganga River continuum*

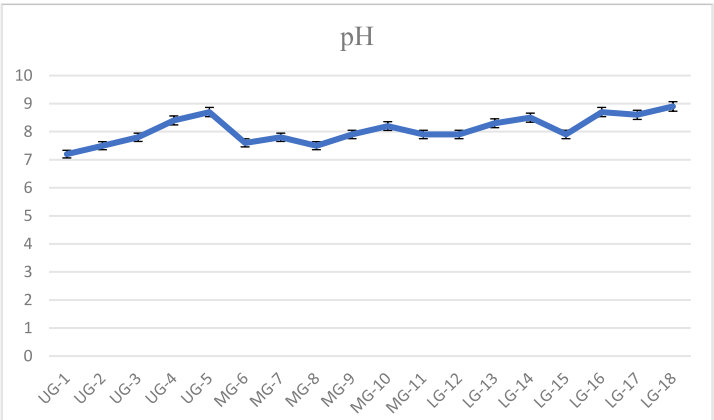

Figure 2a. *Spatial Variation in pH across Sampling Sites along the Ganga River Continuum*

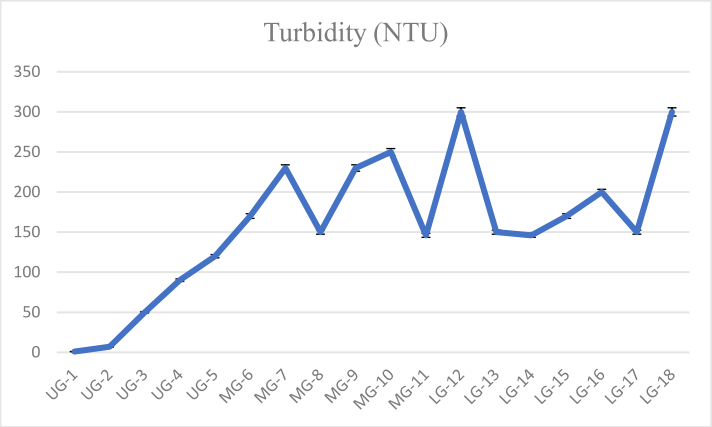

Figure 2b. *Spatial Variation in Turbidity across Sampling Sites along the Ganga River Continuum*

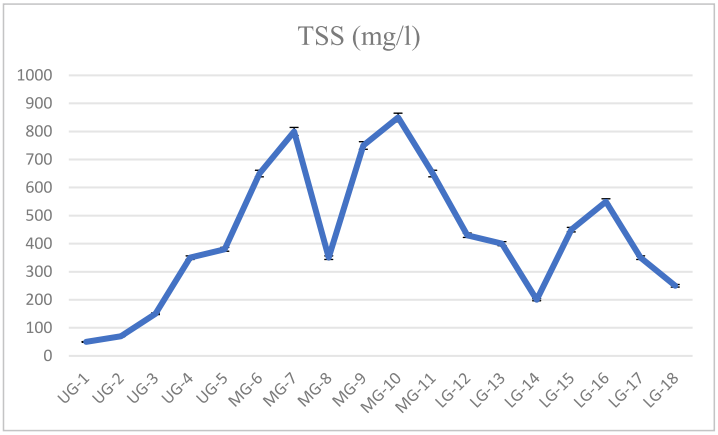

Figure 2c. *Spatial Variation in Total Suspended Solids (TSS) across Sampling Sites along the Ganga River Continuum*

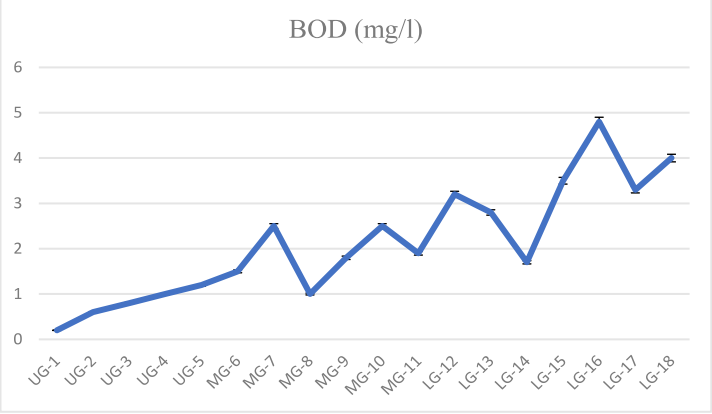

Figure 2d. *Spatial Variation in Biochemical Oxygen Demand (BOD) across Sampling Sites along the Ganga River Continuum*

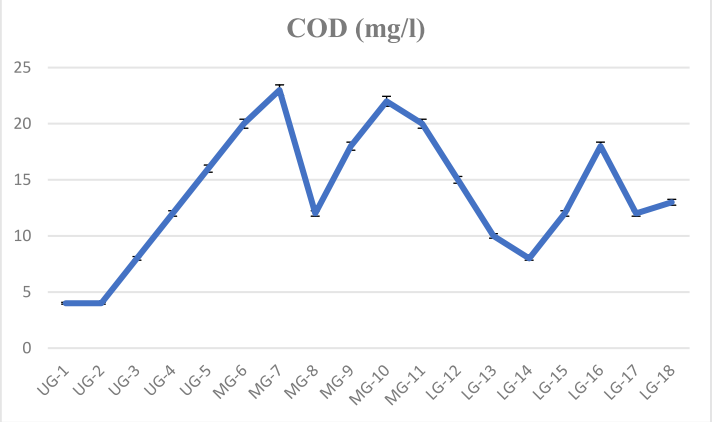

Figure 2e. *Spatial Variation in Chemical Oxygen Demand (COD) across Sampling Sites along the Ganga River Continuum*

4.2. Indicator Bacterial Characteristics of Ganga River (MPN Test)

The microbiological quality of the Ganga River was assessed during the winter season using three internationally recognised faecal indicator bacteria: Total Coliform (TC), Faecal Coliform (FC), and Faecal *Streptococci* (FS). These microbial indicators provide a reliable assessment of sanitary water quality, sewage contamination, and the potential occurrence of enteric pathogens. Their spatial distribution across eighteen sampling sites spanning the Upper, Middle, and Lower stretches of the Ganga River is presented in the Table 3 and Figure 3. A pronounced longitudinal increase in microbial contamination was observed from the glacier-fed headwaters to the downstream estuarine region. No detectable TC, FC, or FS were recorded at Gaumukh and Gangotri, confirming the excellent microbiological quality of the upper Himalayan source region and the absence of measurable faecal contamination. However, bacterial counts increased progressively from Rishikesh onwards, indicating the onset of anthropogenic influence associated with pilgrimage activities, tourism, domestic wastewater discharge, and expanding urban settlements. Total Coliform concentrations exhibited the greatest spatial variability, increasing from non-detectable levels in the upper stretch to the highest concentrations at Gangasagar. Elevated TC counts throughout the middle Ganga, particularly at Prayagraj, Varanasi, and adjacent urban centres, reflects substantial inputs of untreated municipal sewage, domestic wastewater, and urban runoff. The consistently high TC concentrations observed in the lower stretch further demonstrate the cumulative effects of upstream pollution together with additional sewage inputs from densely populated downstream cities and estuarine influences. A similar spatial pattern was observed for Faecal Coliform, which represents contamination originating primarily from humans and other warm-blooded animals. FC concentrations increased progressively downstream, with the highest values recorded at major urban centres, including Prayagraj, Varanasi, Howrah, and Gangasagar. These elevated counts indicate continuous faecal loading associated with inadequate sewage treatment, intensive religious activities, and increasing population density. Compared with Total Coliform, the FC distribution provided stronger evidence of recent faecal contamination from untreated municipal wastewater. Faecal *Streptococci* also exhibited a clear downstream increase, with minimal or undetectable concentrations in the upper stretch and substantially higher abundances throughout the middle and lower reaches. As these organisms generally survive longer than coliform bacteria under environmental conditions, their widespread occurrence indicates persistent faecal contamination and prolonged sewage influence within the river ecosystem. The concurrent increase in TC, FC and FS along the river continuum provides consistent evidence of cumulative microbiological deterioration associated with increasing anthropogenic pressure.

According to the WHO and BIS (BIS IS 10500:2012), drinking water should contain no detectable faecal coliforms or thermotolerant *Escherichia coli* (0 MPN/100mL). Except for Gaumukh and Gangotri, all sampling locations exceeded these microbiological standards, indicating that untreated river water is unsuitable for direct human consumption without appropriate treatment. The exceptionally high bacterial loads recorded in the middle and lower stretches therefore represent a significant public health concern.

The widespread occurrence of faecal indicator bacteria also suggests an increased probability of pathogenic microorganisms, including enteric bacteria, viruses, and protozoa capable of causing waterborne diseases such as cholera, typhoid, dysentery, gastroenteritis, and viral hepatitis. Furthermore, continuous sewage discharge may facilitate the persistence and dissemination of antibiotic-resistant bacteria and antimicrobial resistance genes, highlighting an additional environmental and One Health concern associated with microbial pollution in the Ganga River. Although the microbiological data demonstrate a distinct longitudinal deterioration in sanitary water quality along the Ganga River continuum. The glacier-fed upper stretch remained microbiologically pristine, whereas the middle stretch emerged as the principal hotspot of faecal contamination due to intensive urbanisation, inadequate wastewater treatment, and religious activities. Although some localised reductions in bacterial abundance were observed downstream, microbial contamination remained consistently high throughout the lower stretch, with Gangasagar exhibiting the anthropogenic activities on microbial water quality and emphasize the urgent need for improved sewage treatment infrastructure, effective wastewater management, and continuous basin-scale microbiological monitoring to protect both ecosystem integrity and public health.

Table 3. Spatial Variation in Total Coliform, Faecal Coliform, and Faecal Streptococci along the Ganga River Continuum

Sample Code	TC (MPN/100ml)	FC (MPN/100ml)	FS (MPN/100ml)
UG-1	0	0	0
UG-2	0	0	0
UG-3	150	70	30
UG-4	1500	700	350
UG-5	500	200	150
MG-6	1200	800	500
MG-7	1500	1200	800
MG-8	8000	4000	2000
MG-9	12000	8000	3500
MG-10	18000	10000	5000
MG-11	8000	4000	2000
LG-12	12000	9000	1000
LG-13	7000	5000	1200
LG-14	15000	8000	5000
LG-15	3000	1000	500
LG-16	18000	12000	7000
LG-17	8000	3000	1000
LG-18	35000	20000	10000

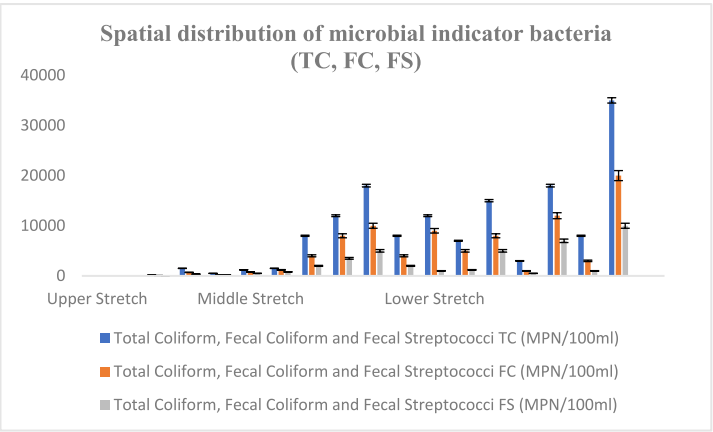

Figure 3. *Spatial distribution of microbial indicator bacteria, including Total Coliform (TC), Faecal Coliform (FC), and Faecal Streptococci (FS), measured in surface water collected from eighteen sampling sites along the Ganga River continuum. Bacterial densities are expressed as the Most Probable Number (MPN) per 100 mL of water, illustrating the progressive increase in microbial contamination from the Upper (UG-1–UG-5) to the Middle (MG-6–MG-11) and Lower (LG-12–LG-18) stretches of the river*

4.3. Bacterial Identification and Characterization of the Ganga River

The bacterial isolates recovered from water samples collected along the Upper (UG 1 to UG 5), middle (MG 6 to MG 11), and Lower (LG 12 to LG 18) stretches of the Ganga River were phenotypically characterized based on Gram-staining characteristics, cellular morphology, and presumptive bacterial genera. The isolates comprised both Gram-positive and Gram-negative bacteria with coccal and bacilli morphotypes, demonstrating distinct spatial variations in bacterial community composition along the river continuum. Gram-positive bacteria predominated in the relatively pristine upper stretch, whereas Gram-negative bacteria became increasingly abundant in the middle and lower reaches, coinciding with greater anthropogenic influence. Similarly, shifts in the relative abundance of bacilli and cocci, together with changes in the distribution of presumptive bacterial groups, reflected progressive alterations in microbial community structure associated with urbanisation, industrial activities, and sewage inputs. A detailed summary of the phenotypic characteristics is presented in Table 4, while the spatial distribution of Gram-staining characteristics, bacterial morphology, and presumptive bacterial groups is illustrated in Figures 4-7. Figure 8 represents the heatmap distribution of presumptive bacterial characteristics across the upper, middle, and lower stretch of the river Ganga.

4.3.1. Gram Staining

The bacterial isolates recovered from surface water samples collected across the Upper (UG 1 to UG 5), Middle (MG 6 to MG 11), and Lower (LG 12 to LG 18) stretches of the Ganga River exhibited distinct spatial variations in Gram-staining characteristics, cellular morphology, and presumptive bacterial groups. Both Gram-positive and Gram-negative bacteria, represented by cocci and bacilli morphotypes, were detected throughout the river continuum; however, their relative abundance varied considerably among river stretches. Gram-negative isolates became increasingly abundant in the middle and lower stretches, reflecting the progressive influence of urbanisation, industrial activities, and sewage inputs. Similarly, shifts in the distribution of bacilli, cocci, and presumptive bacterial groups indicate a gradual transition in the phenotypic characteristics of bacterial populations along the longitudinal pollution gradient of the Ganga River.

Gram Staining Characteristics

The distribution of bacterial isolates exhibited a distinct longitudinal shift along the Ganga River continuum, corresponding to increasing anthropogenic pressure (Figures 4-7). The upper stretch was dominated by Gram-positive bacteria, with only occasional Gram-negative isolates detected at Rishikesh and Haridwar. Most isolates were represented by Gram-positive bacilli and cocci, consistent with the relatively pristine, glacier-fed environment and limited anthropogenic disturbance. These bacterial groups are commonly associated with natural freshwater, soil, and sediment ecosystems, indicating comparatively good microbiological quality in the upper reaches. In contrast, the middle stretch exhibited a pronounced increase in Gram-negative bacteria, particularly at Kanpur, Dalmau, Prayagraj, and Varanasi. Gram-negative bacilli and cocci were more frequently isolated from these locations, suggesting increased inputs of municipal wastewater, industrial effluents, agricultural runoff, and urban drainage.

The predominance of Gram-negative bacteria in this stretch is consistent with the elevated organic pollution and faecal contamination indicated by the physicochemical and microbiological analyses, reflecting the ability of these organisms to persist and proliferate under nutrient-rich and polluted environmental conditions. The lower stretch contained a mixed assemblage of Gram-positive and Gram-negative bacteria; however, Gram-negative isolates became increasingly abundant at Farakka, Howrah, and Gangasagr. This distribution likely reflects the cumulative effects of upstream pollutant transport together with additional sewage inputs from densely populated downstream urban centres. Although, the progressive transition from Gram-positive dominance in the upper stretch to an increased abundance of Gram-negative bacteria in the middle and lower stretches indicates a clear longitudinal shift in the phenotypic characteristics of bacterial isolates, corresponding with increasing anthropogenic influence and the deterioration of microbiological water quality of the Ganga River continuum.

4.3.2. Bacterial Morphology

Two major bacterial morphologies were observed: Cocci and Bacilli. Bacilli were the dominant morphology throughout the river. They included both Gram-positive bacilli and Gram-negative bacilli. The predominance of bacilli reflects their greater ecological adaptability, spore-forming ability (particularly Bacillus and Clostridium), and enhanced survival under varying environmental conditions. Cocci occurred across all stretches but became more frequent in urban regions. Gram-positive cocci were commonly observed at: Haridwar, Prayagraj, Patna and Howrah. Gram-negative cocci were particularly abundant in the middle and lower stretches, indicating increasing sewage-derived contamination. The coexistence of cocci and bacilli demonstrates increasing bacterial diversity with increasing anthropogenic disturbance.

4.3.3. Distribution of Presumptive Bacterial Genera

(a) Gram-positive Bacilli

Gram-positive bacilli constitute one of the predominant phenotypic groups recovered from the Ganga River, particularly in the upper stretch, and remained frequently detected at several downstream locations. Based on colony morphology and Gram-staining characteristics, these isolates were presumptively assigned to the genera *Bacillus*, *Clostridium*, *Listeria*, and *Corynebacterium*. Their widespread occurrence is consistent with the natural distribution of these bacteria in freshwater, soils, and river sediments and may also reflect contributions from agricultural runoff and terrestrial inputs. The frequent recovery of presumptive Bacillus spp. Is likely associated with their spore-forming ability, which enhances persistence under fluctuating environmental conditions. Similarly, the detection of presumptive *Clostridium* spp. may indicate the long-term persistence of spore forming anaerobes within river sediments and could be suggestive of historical faecal contamination.

(b) Gram-positive Cocci

Gram-positive cocci were throughout the river continuum and were presumptively identified as *Staphylococcus*, *Streptococcus*, and *Enterococcus*. Their relative abundance increased at densely populated urban locations, particularly Haridwar, Kanpur, Prayagraj, Varanasi, Patna, and Howrah.

The occurrence of presumptive *Enterococcus* and *Streptococcus* is consistent with the elevated faecal indicator bacteria recorded at these sites and may reflect increasing sewage contamination. In contrast, presumptive bacteria *Staphylococcus* spp. may originate from multiple environmental and anthropogenic sources, including human skin, recreational water use, and religious bathing activities. Although, the increased occurrence of Gram-positive cocci in urban stretches supports the microbiological evidence of progressive anthropogenic influence along the river continuum.

(c) Gram-negative Bacilli

Gram-negative bacilli became increasingly abundant from the middle stretch onwards, with most isolates being presumptively assigned to the family *Enterobacteriaceae*. Although species-level identification requires biochemical and molecular confirmation, the observed phenotypic characteristics are consistent with members of this family, including *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Salmonella*, and *Shigella*. The highest frequencies were observed at Kanpur, Dalmau, Prayagraj, Varanasi, Howrah, and Gangasagar, coinciding with locations exhibiting elevated Total Coliform and Faecal Coliform counts. This spatial distribution is indicative of increasing anthropogenic influences and is consistent with contamination from untreated municipal wastewater, urban runoff, and sewage discharges. The predominance of presumptive *Enterobacteriaceae* in the middle and lower stretches further supports the progressive deterioration in microbiological water quality observed along the Ganga River continuum.

(d) Gram-negative Cocci

Gram-negative cocci were predominantly from the middle and lower stretches of the Ganga River and were presumptively assigned to the genera *Neisseria*, *Moraxella*, and *Veillonella* based of Gram-staining characteristics and colony morphology. Although definitive taxonomic identification requires biochemical and molecular confirmation, the occurrence of these presumptive genera was more frequent at sites influenced by intensive urbanization and sewage discharge. Several members of these genera are commonly associated with the human oral and respiratory microbiota and may enter aquatic environmental through domestic wastewater and untreated sewage. Their increased occurrence in the downstream reaches is therefore consistent with the elevated faecal indicator bacteria (TC, FC, and FS) observed these locations and further supports the progressive influence of anthropogenic activities on the microbiological quality of the Ganga River, the phenotypic characterization of bacterial isolates revealed a distinct longitudinal shift in the distribution of presumptive bacterial groups along the Ganga River continuum. The upper stretch was dominated by Gram-positive environmental bacteria, whereas the middle and lower stretches exhibited a progressively greater abundance of Gram-negative bacteria together with presumptive faecal-associated groups. The highest diversity of phenotypically distinct bacterial isolates was observed in the middle Ganga, particularly at Kanpur, Prayagraj, and Varanasi, corresponding with areas subjected to intensive urbanization, industrial activities, and untreated sewage discharge. Although the lower stretch maintained a diverse bacterial assemblage, the increased occurrence of presumptive Gram-negative and faecal-associated bacteria likely reflects the cumulative effects of upstream pollutant transport combined with additional municipal wastewater inputs from densely populated downstream regions, including Howrah and Gangasagar.

The observed transition from predominantly environmental bacterial groups in the upper stretch to increasing numbers of presumptive sewage-associated bacteria downstream is consistent with the physicochemical deterioration and elevated faecal indicator bacteria (TC, FC, and FS) recorded throughout the river continuum. These findings collectively demonstrate the progressive influence of anthropogenic activities on the microbiological quality of the Ganga River and reinforce the utility of culture-based phenotypic characterization as a preliminary indicator of microbial pollution. Because bacterial identification in the present study was based on Gram-staining characteristics and colony morphology, the reported taxa should be regarded as presumptive assignments. Confirmation of bacterial identity using biochemical characterization and molecular approaches, such as 16S rRNA gene sequencing or MALDI-TOF mass spectrometry, is necessary to accurately determine species composition, evaluate pathogen prevalence, and investigate the occurrence of antimicrobial resistance and associated public health risks within the Ganga River ecosystem.

Table 4. Presumptive Identification and Phenotypic Characterization of Bacterial Isolates Recovered from Surface Water Samples along the Ganga River Continuum

Sample Code	Gram Staining	Morphology	Presumptive Bacterial genera
UG-1	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
UG-2	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
UG-3	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Negative	Bacilli	*Enterobacteriaceae*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
UG-4	Negative	Bacilli	*Enterobacteriaceae*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
UG-5	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
MG-6	Negative	Bacilli	*Enterobacteriaceae*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Negative	Bacilli	*Enterobacteriaceae*
MG-7	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
MG-8	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
MG-9	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
MG-10	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
MG-11	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
LG-12	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
LG-13	Negative	Bacilli	*Enterobacteriaceae*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
LG-14	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
LG-15	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
LG-16	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
LG-17	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Cocci	*Staphylococcus, Streptococcus, Enterococcus*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*
LG-18	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Bacilli	*Enterobacteriaceae*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Negative	Cocci	*Neisseria, Moraxella, Veillonella*
	Negative	Bacilli	*Enterobacteriaceae*
	Positive	Bacilli	*Bacillus, Clostridium,Listeria, Corynebacterium*

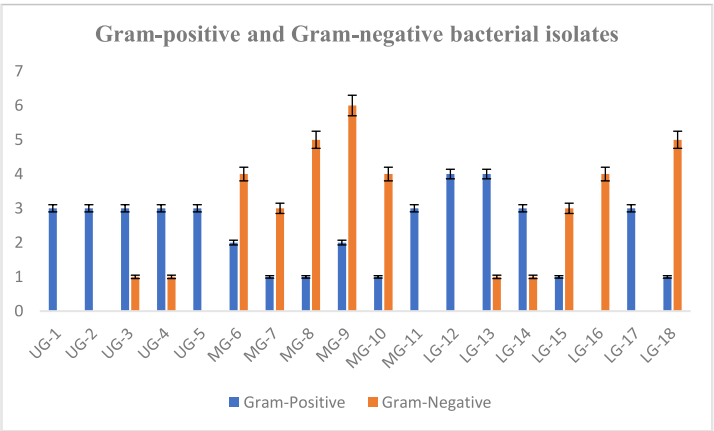

Figure 4. *Comparison of Gram-positive and Gram-negative bacterial isolates recovered from surface water collected at eighteen sampling sites along the Upper, Middle, and Lower stretches of the Ganga River*

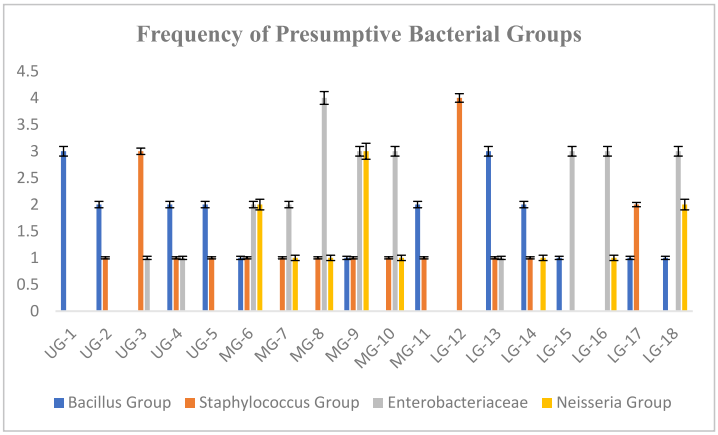

Figure 6. *Relative abundance of major presumptive bacterial groups isolated from surface water samples collected at eighteen sampling sites along the Ganga River. Values represent the number of isolates assigned to each presumptive*

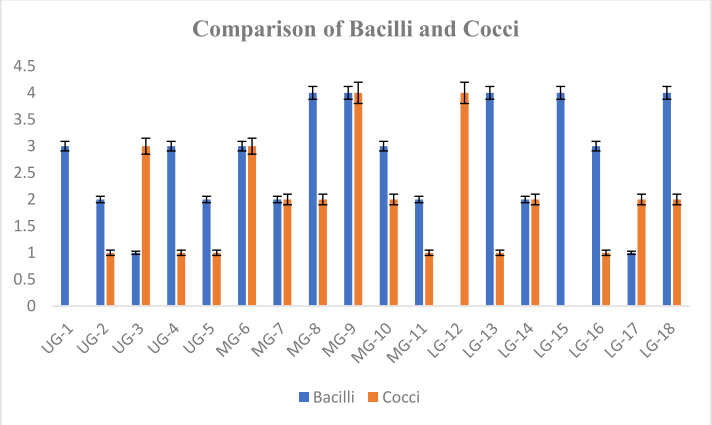

Figure *5. Comparison of bacterial morphology (bacilli and cocci) recovered from surface water samples collected at eighteen sampling sites along the Upper, Middle, and Lower stretches of the Ganga River*

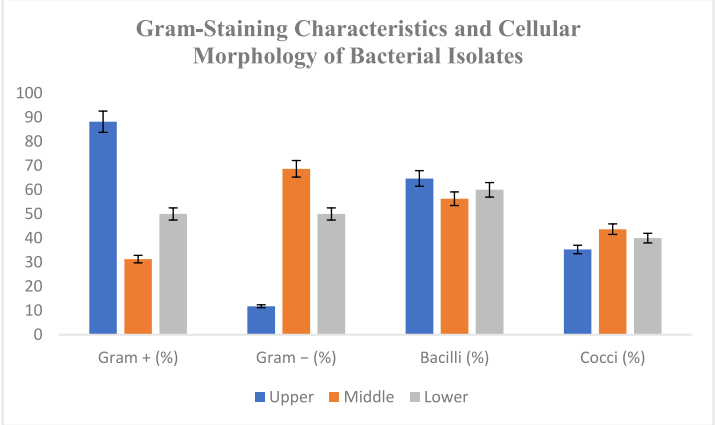

Figure 7. *Summary of Gram-Staining Characteristics and Cellular Morphology of Bacterial Isolates across the Upper, Middle, and Lower Stretches of the Ganga River*

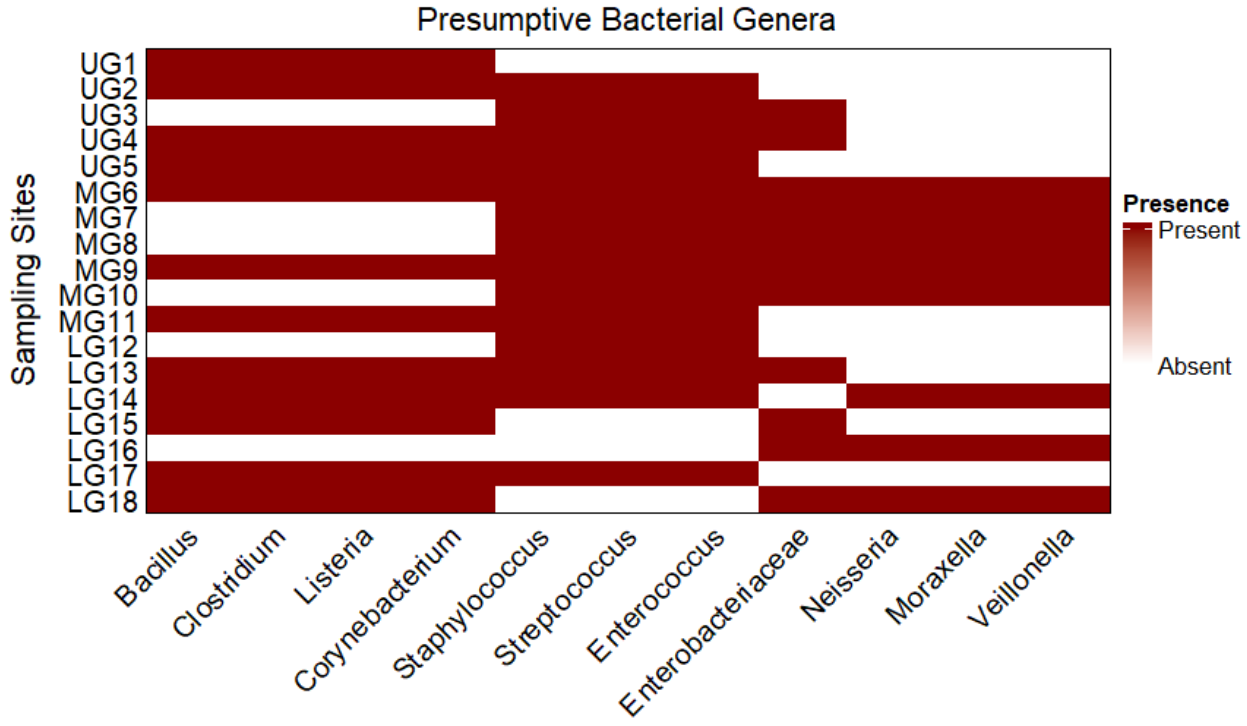

Figure 8. *Heatmap showing the distribution of presumptive bacterial genera isolated from water samples collected along the Upper, Middle, and Lower stretches of the Ganga River during the winter season. Presence (red) and absence (white) of bacterial genera are based on Gram staining, morphology, and presumptive identification. The side annotation indicates the sampling stretch, highlighting the longitudinal variation in bacterial community composition along the river continuum*

Table 5. Comparative water quality standards prescribed by ICMR, WHO, CPCB, BIS (IS 10500:2012), ISI, and USEPA, [16]

Parameter	ICMR	WHO	CPCB Surface Water Criteria	BIS IS 10500:2012 (Drinking Water)	ISI	USEPA
pH (units)	7.0–8.5	7.0–8.5	Class A: 6.5–8.5; Class B: 6.5–8.5; Class C: 6.5–9.0	Acceptable: 6.5–8.5; No relaxation	–	6.5–8.5*
Total Dissolved Solids (TDS, mg L^{-1})	500	500	–	Acceptable: 500; Permissible: 2000	–	500 (Secondary Standard)
Temperature (°C)	–	–	No significant deviation from natural conditions	–	–	–
Turbidity (NTU)	5	2.5	–	Acceptable: 1–5; Permissible: 10	–	≤ 1–5
Biochemical Oxygen Demand (BOD, mg L^{-1})	–	–	Class A: ≤2; Class B: ≤3; Class C: ≤3	Not specified	–	–
Dissolved Oxygen (DO, mg L^{-1})	–	–	Class A: ≥6; Class B: ≥5; Class C: ≥4	Not specified	–	–
Free CO_2 (mg L^{-1})	–	10	–	10	–	–
Electrical Conductivity (µS cm^{-1})	–	–	–	750	–	–
Faecal Coliform (MPN 100 mL^{-1})	–	–	Class B (Bathing): ≤500 desirable; ≤2500 permissible	–	–	–
Faecal *Streptococci* (MPN 100 mL^{-1})	–	–	≤100 desirable; ≤500 permissible for bathing waters	–	–	–
E. coli / Thermotolerant Coliforms	–	Not detectable in 100 mL	–	Not detectable in 100 mL	–	Zero detectable organisms in drinking water
Total Coliforms	–	–	–	Not detectable in 100 mL	–	Total coliform rule applies

Discussion

Longitudinal investigations encompassing the entire Ganga River continuum remain remarkably limited, despite the river's immense ecological, cultural, and socioeconomic significance. The present study provides one of the most comprehensive basin-scale assessments of physicochemical and microbiological water quality conducted from the glacial source at Gaumukh to the estuarine outlet at Gangasagar. By integrating physicochemical indicators, faecal indicator bacteria, and phenotypic characterization of bacterial communities, this investigation offers a holistic understanding of the progressive transformation of water quality along the river continuum and demonstrates how increasing anthropogenic pressures influence the ecological health of one of the world's most important freshwater ecosystems. The longitudinal approach adopted in this study enables the identification of spatial pollution gradients and provides valuable baseline information for future river management and restoration strategies. The physicochemical characteristics observed in the present investigation revealed a gradual deterioration in water quality from the pristine Himalayan headwaters to the densely populated downstream reaches. The measured Ganga River parameters like pH, Turbidity, BOD, COD, TC, FC and FS specified the permissible contamination limits prescribed by the agencies like WHO, BIS, [16] Table 5. The progressive increase in organic pollution and microbial contamination clearly corresponds with expanding urbanization, industrial development, agricultural runoff, religious activities, and untreated municipal wastewater discharge along the river basin [16], [17]. Comparable observations have been reported by Gani et al., (2025) [18], during water quality assessment at Haridwar, where pH ranged between 6.06 to 8.47, turbidity varied from 0.3 to 11.8 NTU, and BOD values ranged from 0 to 1.6 mg L^{-1}, indicating relatively good water quality in the upper Himalayan stretch. These findings closely correspond with the present study, in which the upper stretch exhibited low turbidity, minimal organic pollution, and near-pristine physicochemical conditions, thereby confirming the comparatively undisturbed nature of the glacier-fed headwaters.

The influence of large religious gatherings on river water quality has been extensively documented. Mishra et al., (2025) [19], reported substantial deterioration in water quality during the Maha Kumbh at Prayagraj, where TSS, BOD, and COD increased to 210 mg L^{-1}, 8.7 mg L^{-1}, and 70 mg L^{-1}, respectively. These values were considerably higher than those recorded during the pre-Kumbh period (45 mg L^{-1}, 2.8 mg L^{-1}, and 18 mg L^{-1}) and remained elevated even after the event (70 mg L^{-1}, 3.7 mg L^{-1}, and 26 mg L^{-1}). Although the present investigation was conducted outside major festival periods, elevated physicochemical parameters observed in the middle stretch similarly indicate the cumulative effects of continuous anthropogenic inputs, including domestic sewage, industrial effluents, and intensive human activities. Spatial variations observed in the present study are also supported by the longitudinal assessment conducted by, Balkrishna et al., (2024) [20]. Their investigation reported turbidity values ranging from 2.02 to 37.53 NTU and pH values between 7.50 and 8.16 in the upper stretch from Gaumukh to Gangotri, while the middle stretch exhibited turbidity between 4.66 and 61.31 NTU with relatively stable pH values ranging from 7.60 to 7.86. Exceptionally high turbidity (694.3 NTU) was recorded at Diamond Harbour near Kolkata, reflecting intensive sediment transport and downstream anthropogenic disturbances. Similarly, the present study documented a progressive increase in turbidity from the upper to lower reaches, supporting the hypothesis that sediment resuspension, urban runoff, and tidal influences collectively contribute to declining water quality downstream. Further evidence of downstream alkalization has been reported by [21], who observed pH values ranging from 8.0 to 9.5 across lower Ganga locations, including Barrackpore, Dakshineshwar, Kadamtala, Kakdwip, Kachuberia, and Gangasagar. These observations are consistent with the slightly alkaline conditions recorded in the present investigation and may be attributed to increased bicarbonate concentrations, enhanced primary productivity, wastewater discharge, and estuarine mixing processes. Seasonal fluctuations in water quality have also been highlighted by Sharma et al., (2025) [22], at Haridwar.

Their study demonstrated significant temporal variability, with pH reaching 10.27 during winter, turbidity decreasing to 0.009 NTU in winter compared with 1.73 NTU during the monsoon, and BOD attaining a maximum value of 4.48 mg L^{-1} during summer while declining to 0.6 mg L^{-1} during the monsoon. Although the present study represents a winter-season assessment, the observed physicochemical trends align with the reported seasonal dynamics, emphasizing that river water quality is strongly influenced by hydrological conditions, climatic variability, and anthropogenic activities. Kanpur COD was highest because of sewage discharge directly in the river Ganga from the industries like leather processing industries, textile manufacturing industries, heavy engineering and chemical industries. TSS decreased in the lower stretch because of tidal sea water, and Gangasagar location has highest microbial population may be because of Makar Sankranti religious bathing.

The microbiological assessment further demonstrated a pronounced increase in faecal contamination along the river continuum. Total coliform, faecal coliform, and faecal *streptococci* counts increased progressively from the glacier-fed upper stretch to the densely populated lower reaches, reflecting cumulative inputs of untreated domestic sewage, livestock waste, agricultural runoff, and urban wastewater. The absence or negligible occurrence of faecal indicator bacteria in the upper Himalayan region confirms the relatively pristine condition of these headwaters, whereas the markedly elevated bacterial loads in the middle and lower stretches indicate severe faecal pollution associated with increasing human population density and inadequate sanitation infrastructure. The present findings are consistent with those reported by Mishra et al., (2025) [19], during the Maha Kumbh at Prayagraj, where total coliform, faecal coliform, and enterococci counts increased dramatically to 50,000, 14,000, and 900 MPN per 100 mL, respectively. In comparison, pre-Kumbh concentrations were substantially lower (3,500, 1,200, and 80 MPN per 100 mL), while post-Kumbh values remained elevated (12,000, 1,300, and 300 MPN per 100 mL). These observations clearly demonstrate the substantial influence of mass religious gatherings on microbial water quality and reinforce the importance of effective sanitation management during large-scale public events. Comparable longitudinal trends were also reported by Balkrishna et al., (2024) [20], who observed faecal coliform counts ranging from 0 to 1,100 MPN per 100 mL between Gaumukh and Bijnor, whereas considerably higher concentrations reaching 11,000 MPN per 100 mL were recorded at Bithoor, Mirzapur, and Varanasi. Similarly, the lower stretch exhibited faecal coliform counts ranging from 30 to 11,000 MPN per 100 mL, with the highest concentrations recorded at Gangasagar. The present investigation similarly demonstrated progressively increasing microbial contamination from the upper Himalayan reaches towards the estuarine zone, thereby confirming that faecal pollution intensifies downstream in response to cumulative anthropogenic activities. Likewise, Srivastava & Verma, (2025) [23], reported elevated total coliform concentrations throughout the middle Ganga basin, with approximately 1,800 MPN per 100 mL recorded at Bithoor, Prayagraj, Varanasi, and Mirzapur, whereas comparatively lower counts (592–920 MPN per 100 mL) were observed at Rishikesh and Haridwar. These findings further corroborate the spatial pollution gradient identified in the present study and emphasize the disproportionate impact of urbanization and municipal wastewater discharge on microbial water quality within the middle Ganga basin.

Overall, the present investigation demonstrates a distinct longitudinal deterioration in both physicochemical and microbiological water quality from Gaumukh to Gangasagar. The combined evidence indicates that the middle Ganga basin functions as the principal pollution hotspot, primarily due to intensive industrialization, urban expansion, untreated sewage discharge, and religious activities, while the lower stretch experiences cumulative downstream pollution loads compounded by estuarine processes. The integration of physicochemical parameters with microbiological indicators across the entire river continuum provides a comprehensive understanding of pollution dynamics at the basin scale and establishes an important scientific baseline for future environmental monitoring, public health risk assessment, and sustainable management of the Ganga River ecosystem. The elevated microbial loads observed in the lower Ganga, particularly at Gangasagar, may be further influenced by estuarine processes operating at the freshwater–marine interface. Tidal mixing continuously redistributes microorganisms throughout the estuary, while sediment resuspension releases particle-associated bacteria back into the water column. In addition, estuarine sediments function as long-term reservoirs for faecal bacteria, allowing microbial persistence even after reductions in upstream contamination. The accumulation of organic matter and the formation of biofilms further enhance bacterial survival by providing nutrient-rich microhabitats and protection from environmental stress. Collectively, these processes contribute to the prolonged persistence of microbial contaminants in the lower Ganga estuary despite dilution by seawater. Although pollutant concentrations increased markedly throughout the middle Ganga, partial reductions in TSS and COD observed in some downstream locations suggest the operation of natural self-purification mechanisms. Rivers possess an intrinsic capacity to improve water quality through dilution, sedimentation, microbial biodegradation, reaeration, photoinactivation, adsorption, and biological interactions. Suspended particles carrying microorganisms and organic pollutants gradually settle to the riverbed, while indigenous microbial communities degrade biodegradable organic matter, thereby reducing oxygen demand. Simultaneously, atmospheric reaeration replenishes dissolved oxygen, enhancing aerobic decomposition processes. Ultraviolet radiation, protozoan grazing, and naturally occurring bacteriophages further contribute to the decline of microbial populations. However, despite these natural recovery mechanisms, the magnitude of untreated sewage discharge and continuous anthropogenic inputs throughout the middle and lower Ganga exceeds the river's self-purification capacity, resulting in persistent physicochemical deterioration and elevated microbial contamination.

Limitation

A limitation of this study is the absence of direct source-tracking, metagenomic validation, and high-resolution temporal monitoring, which may further refine the understanding of microbial dynamics.

Conclusion

The findings revealed a pronounced spatial deterioration in water quality from the pristine Himalayan headwaters towards the densely populated middle and lower reaches of the river during the winter season.

The upper stretch was characterised by low turbidity, suspended solids, organic pollution, and negligible microbial contamination, reflecting the limited influence of human activities and the dominance of glacier-fed hydrology. In contrast, the middle stretch, particularly Kanpur, Prayagraj, and Varanasi, emerged as the principal pollution hotspot, exhibiting elevated turbidity, total suspended solids, chemical oxygen demand, and faecal indicator bacteria, indicative of substantial inputs from untreated municipal wastewater, industrial effluents, agricultural runoff, and intensive religious activities. Although suspended solids decreased in the locations of lower stretches because of sedimentation and hydrological processes, biochemical oxygen demand and microbial contamination remained consistently high, demonstrating the cumulative effects of upstream pollutant transport and continuous sewage discharge. Microbiological analyses further demonstrated a clear longitudinal increase in Total Coliform, Faecal Coliform, and Faecal *Streptococci*, with bacterial densities substantially exceeding recommended drinking water standards at nearly all downstream sampling locations. These observations confirm extensive faecal contamination throughout much of the river and indicate a considerable risk for the transmission of waterborne pathogens in areas where untreated river water is used for domestic or recreational purposes. The progressive increase in microbial indicators also reflects inadequate wastewater treatment infrastructure and persistent sewage inputs across the Ganga basin. Phenotypic characterisation of bacterial isolates revealed a distinct ecological transition from predominantly environmental Gram-positive bacterial communities in the upper stretch to increasingly Gram-negative bacteria in the middle and lower stretches. The predominance of presumptive *Enterobacteriaceae* together with *Enterococcus*, *Streptococcus*, *Neisseria*, *Moraxella*, and *Veillonella* in downstream locations provides additional evidence of progressive sewage contamination and increasing anthropogenic disturbance. Although bacterial identification was based on culture-dependent phenotypic characteristics, the observed community shifts strongly corroborate the physicochemical and microbiological evidence of declining ecological health along the river continuum. Collectively, these findings demonstrate that anthropogenic activities have fundamentally altered both the physicochemical characteristics and the microbial ecology of the Ganga River, producing a well-defined longitudinal pollution gradient from the Himalayan headwaters to the Bay of Bengal. The strong agreement between physicochemical deterioration, increasing faecal indicator bacteria, and shifts in bacterial community composition highlights the value of integrating conventional water quality assessment with microbiological characterisation for comprehensive river health evaluation, this study provides robust baseline information for future river restoration and environmental monitoring programmes. Protecting the ecological integrity of the Ganga River will require basin-wide implementation of advanced sewage treatment systems, strict enforcement of industrial effluent regulations, effective management of diffuse agricultural pollution, and continuous long-term monitoring using standardised physicochemical, microbiological, and molecular approaches. Future investigations should incorporate high-throughput sequencing, metagenomics, quantitative PCR, and antimicrobial resistance surveillance to resolve microbial community dynamics at species level, identify emerging pathogens, and evaluate the environmental dissemination of antibiotic resistance within the Ganga River ecosystem. Such multidisciplinary and basin-scale monitoring frameworks will be essential for achieving sustainable river restoration, protecting freshwater biodiversity, and safeguarding public health under principles of one health.

Acknowledgement

Prashant Singh conceptualized and designed the study, conducted field sampling, performed the laboratory experiments, analysed and interpreted the data, and prepared the original draft of the manuscript. Ajad Patel, Vinay Singh Baghel, and Ranjan Singh contributed to data interpretation, critically reviewed the manuscript for important intellectual content, and proofread the final version. All authors read, revised, and approved the final manuscript for publication.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

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