"ANALYSIS OF GANGA WATER OF PRAYAGRAJ MAHAKUMBH MELA 2025"

A RESEARCH PROJECT

Submitted by

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UNDER THE GUIDANCE OF

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DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

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Dr. Savita D. Mali

Research Project Guide

Examiner

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Place: Kolhapur
Date: 5/05/2025

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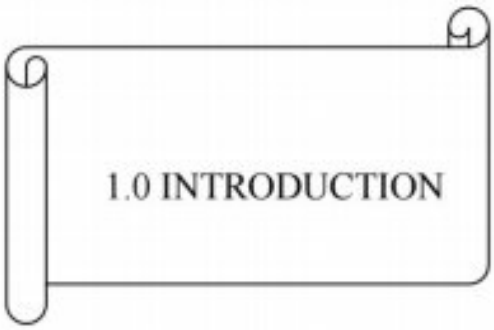
INDEX



1.0 INTRODUCTION

Kumbha mela, Hindu religious festival and the world's largest public gathering. The 2019 event at Prayagraj attracted more than 200 million people, including 50 million on the festivals most auspicious day. The Kumbh Mela, which translates to "Festival of sacred pitcher" is one of the most sacred pilgrimages for Hindus and is recognized by UNESCO on its representatives list of the Intangible cultural heritage of humanity. The main festival is celebrated among 4 sacred sites in India, each located along the banks of Holy River, in a 12-year cycle. These sites are Haridwar on the Ganga River in Uttarakhand, Ujjain on the Shipra in Madhya Pradesh, Nashik on the Godavari in Maharashtra, and Prayagraj in Uttar Pradesh where the Ganga the Yamuna, and the mythical Saraswati rivers coverage. Each sites celebration is based on a distinct set of astronomical positions of the sun the moon, and the Jupiter, the holiest time occurring at the exact moment when these positions are fully occupied. [Dubey, Sanjay, ed. (26 February 2025)]

The Ganga have numerous tributaries that join it along its course, including major rivers like Yamuna, Ramganga, Ghaghara, kasi and Sone. The 2023 Prayag Maha Kumbh Mela was the most recent iteration of the Kumbh mela, a Hindu pilgrimage festival that marked a full orbital revolution of Jupiter around the Sun. It was scheduled from 13 January to 26 February 2025, at the Triveni Sangam in Prayagraj, Uttar Pradesh, India. It was the world's largest gathering, and according to data released on 26 February, more than 660 million people and taken a dip in the river. This event marked the completion of 12-year Kumbh mela cycle and was officially termed a Maha Kumbh Mela, Spanning 45 days. The 2025 Maha Kumbh Mela made preparations for the attendance of up to 400 million visitors. The fair is over with the surpassing the 400 million attendances expected. As of 12 February 2025, the Maha Kumbh mela in Prayagraj has seen an unprecedented number of devotees participating in the sacred bathing rituals. By 6 AM on 12 February, over 7.3 million devotees had taken the ritual dip at the Triveni Sangam and other ghats during the Maghi Purnima Snan. (Barua, Kriti -21 January 2025)

Many tourists and pilgrims came from various countries such as Australia, Bhutan, Brazil, Bulgaria, Canada, China, Fiji, Finland, France, Germany, Greece, Guyana, Indonesia, Israel, Italy, Japan, Malasia, Mauritius, Mexico, Mongolia, Nepal, Netherlands, Pakistan,

Russia, Singapore, South Africa, Spain, Sri Lanka, Taiwan, Thailand, Trinidad and Tobago, Ukraine, United Arb Emirates, United Kingdom and United States.

Kumbh Mela is one of the largest and most revered Hindu pilgrimages in the world. It is a vibrant celebration of spirituality, culture, and community, attracting millions of devotees from India and abroad. The festival is held every 12 years in four different locations: Haridwar, Allahabad (Prayagraj), Nashik, and Ujjain.

History and Mythology: -

The Kumbh Mela has its roots in Hindu mythology. According to legend, during the Samudra Manthan (churning of the ocean), the gods and demons fought over the pot (kumbha) of nectar (amrit) that grants immortality. The gods, led by Lord Vishnu, successfully protected the kumbha and carried it to four different locations, where the nectar was spilled, creating sacred sites.

Significance: -

The Kumbh Mela is significant for several reasons:

1. **Spiritual Significance**: The festival is believed to offer spiritual growth, self-realization, and liberation (moksha) to devotees.

2. **Cultural Significance**: Kumbh Mela showcases India's rich cultural heritage, traditions, and values.

3. **Community Significance**: The festival brings people together, fostering a sense of community and social bonding.

Rituals and Practices: -

During the Kumbh Mela, devotees perform various rituals and practices, including:

1. **Holy Dip**: Taking a dip in the sacred rivers, such as the Ganges, Yamuna, and Godavari, is considered a sacred ritual.

2. **Pujas and Offerings**: Devotees offer prayers, flowers, and other offerings to the gods and goddesses.

3. **Processions**: Colourful processions, often accompanied by music and chanting, are an integral part of the festival.

Locations: -

The Kumbh Mela is held in four different locations:

1. **Haridwar**: Located on the banks of the Ganges River, Haridwar is one of the most sacred sites for Hindus.

2. **Allahabad (Prayagraj)**: The confluence of the Ganges, Yamuna, and Saraswati rivers makes Prayagraj a sacred site.

3. **Nashik**: Located on the banks of the Godavari River, Nashik is a significant pilgrimage site in Maharashtra.

4. **Ujjain**: This ancient city is situated on the banks of the Shipra River and is known for its spiritual significance.

Cultural and Social Impact: -

The Kumbh Mela has a significant cultural and social impact:

1. **Economic Impact**: The festival generates significant revenue for local businesses and economies.

2. **Social Impact**: Kumbh Mela brings people together, promoting social bonding and community building.

3. **Cultural Exchange**: The festival showcases India's rich cultural heritage and provides a platform for cultural exchange.

Challenges and Preparations: -

Organizing the Kumbh Mela poses several challenges:

1. **Crowd Management**: Managing millions of devotees requires careful planning and infrastructure.

2. **Infrastructure**: Providing adequate infrastructure, including sanitation, water, and medical facilities, is crucial.

3. **Security**: Ensuring the safety and security of devotees is a top priority.

Urban Transformation of Prayagraj

1. Creation of a Temporary Megacity

To accommodate the massive influx of pilgrims, a temporary city was established over 4,000 hectares, featuring 200,000 tents, 250 miles of roads, and 30 pontoon bridges. This "tent city" was designed to host up to 660 million visitors, making it the largest human gathering in history.

2. Infrastructure Overhaul

The Uttar Pradesh government invested over ₹7,500 crore to enhance Prayagraj's infrastructure. This included the construction of new flyovers, expanded roads, upgraded railway stations, and a modern airport terminal. Additionally, ₹1,500 crore was allocated specifically for Kumbh Mela arrangements.

3. Beautification and Cultural Integration

The city underwent extensive beautification, with 38 major junctions and 75 km of urban routes enhanced through landscaping, green belts, and thematic development. Street art and murals adorned approximately 1 million square feet of walls, and four thematic gates—Saraswati Dwar, Shiv Dwar, Ganga Dwar, and Yamuna Dwar—were constructed to symbolize the confluence of spirituality and urban aesthetics.

4. **Digital Infrastructure and Crowd Management**

Advanced technologies were employed for crowd control and safety. AI-powered cameras and integrated command centers were set up to monitor and manage the vast crowds. Traffic management systems were integrated with Google Maps to provide real-time updates, and digital surveillance ensured security throughout the Mela area.

Economic Impact and Urban Legacy

1. **Economic Boost**

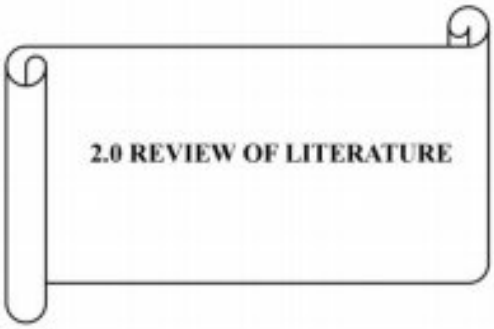
The Maha Kumbh Mela generated significant economic activity, with an estimated business turnover exceeding ₹3 lakh crore. This encompassed sectors such as hospitality, transport, food, and consumer goods, benefiting local businesses and strengthening the regional economy.

2. **Employment Opportunities**

The extensive preparations and operations created substantial employment opportunities across various sectors, including construction, hospitality, logistics, and security. This provided a livelihood to thousands of individuals, contributing to the local economy.

3. **Long-Term Urban Development**

The infrastructure improvements and urban planning initiatives undertaken for the Mela have left a lasting legacy in Prayagraj. The enhanced roads, bridges, and public amenities continue to serve the city's residents, contributing to its overall development and modernization.



2.0 REVIEW OF LITERATURE

The kumbha melas, each lasting several weeks each is observed at various times and locations according to the Hindu traditions. These gathering holds great spiritual significance and attracts millions of devotees around the world. The frequency of the melas varies, some occurring annually and the Maha kumbha Mela taking place every 144 years in Prayagraj.

Mela	Occurrence	Location
Magh Mela	Once a year	Prayag Raj
Kumbh Mela	Once every 3 years	Haridwar, Prayagraj, Nashik and Ujjain
Ardh Kumbh Mela	Once every 6 years	Haridwar and Prayagraj
Purna Kumbh Mela	Once every 12 years	Haridwar, Prayagraj, Nashik and Ujjain
Maha Kumbh Mela	Once every 144 years	Prayagraj

Table no. 1: - Occurrence and location of Kumbh

Types of Kumbh Mela: -

The water quality of Ganga River at Prayagraj during the Maha Kumbh Mela 2025 was subject of analysis of by central pollution control board (CPCB). According to their report, the water quality was found to be suitable for bathing, with key parameters such as pH, dissolved O2, biochemical oxygen demand and faecal coliforms within permissible limit.

However, it's worth noting that an earlier CPCB report had indicated that the water quality at several location in Prayagraj did not meet primary bathing water quality standards due to high faecal coliform levels, nevertheless a subsequent analysis confirmed the overall river water quality during the Maha Kumbh was suitable for bathing. The Uttar Pradesh government had taken a significant measure to ensure the water quality including installing to sewage treatment. Additionally, the government had allocated a substantial budget for river for river cleaning efforts, with Rs 7421 crore allocated to national mission for clean Ganga for the period of 2022 – 2025

Tradition ascribes the Kumbh Melas origin to the 8th century philosopher Shankar, who instituted regular gatherings of learned ascetics for discussion and debate the founding myth of Kumbh mela – attributed to purana (Collections of Myth and legend) – recounts how the gods and demons fought over the pot (Kumbh) of Amrit, the elixir of immortality produced by their

joint churning or the milky ocean. This churning of the ocean is widely known as the Samudra Manthan/ Sagar Manthan. To prevent the demons from winning the elixir. Devotees from all over the world, travel the sites of the mela to take bath in the holy rivers. The act of bathing is believed to cleanse the soul of all the impurities and sins and help attain moksha.

Yajanas: -

Yajnas are sacred fire rituals conducted by priests and spiritual leaders' offerings such as ghee, grains and herbs are poured into the consecrated fire amidst the chanting of mantras from the sacred Vedic texts, these ceremonies aim purify the environment, seek divine Favor, and maintain the natural order of nature.

Stampedes: -

Despite being illustrative of India's rich cultural and religious heritage, Kumbh mela has been moved by tragedy, notably, stampedes and occasional violence, leading to substantial loss of life raising concerns about crowd Management and safety protocols.

One of the earliest and most catastrophic One of the earliest and most catastrophic stampedes occurred during 1954. Kumbh mela considerable security measures were put into effect, event so, subsequent Kumbh melas have witnessed similar tragedies. In 2003, during the Nashik Kumbh mela, a stampede led to the deaths of at least 39 pilgrims and injured more than 100 people. The incident was reported caused by a scramble among devotees to collect coins thrown by sadhus. A stampede at 2010 Haridwar Kumbh mela claimed seven lives and injured 17 people. Two individuals drowned in separate stampede at the mela. A particularly tragic event unfolded on February 10,2013, during the Kumbh mela in Allahabad a stampede at the railway station resulted in 36 fatalities and numerous injuries. At least 15 people lost their lives, and many other were insured during the shahi snan in 2025 Kumbh mela.

According to Hindu Mythology, Haridwar is one of the holiest places on account of the belief that the gods have left their foot prints in the Haridwar. The holy city of Haridwar is site to some of the most sacred Hindu rituals and one can always see pilgrims and devotees from around the globe gather at Haridwar to offer prayer an auspicious occasion, having a dip in holy river Ganga. millions of devotees and visitors, a dip in the holiest river Ganga during Kanwar, Ardh Kumbh, Maha Kumbh and festive occasions. It is not possible for any city municipality to make proper arrangements. For doing and other civic facility for millions of pilgrims during festive occasions.

Haridwar city is famous for such big festive occasions like kanwar, Ardh kumbh and Maha Kumbh. As a consequence of insufficient facilities, the available places, grounds, fields and riparian city forest areas are used as latrines and toilets. The municipal water points turn as quick wash places. The 4-5 km stretch of Ganga river and river canal banks are used a bathing places and sites of holy water collection too.

The pilgrims also offer flowers, cloths, old of Gods and Goodness, besides last remains of their loved to dispose in the river Ganga at Haridwar. At the beginning of the year 2010 from the month of January heavy influx of pilgrims pored in Haridwar city to attend the Maha Kumbh (14^{th} January 2010 to 30^{th} April 2010) for taking a holy dip in river Ganga to wash away their past sins. According to newspapers and local admiration, more than 8 million pilgrims took holy dip in river Ganga at Haridwar during Purna Kumbh. It can be well presumed that such a massive bathing would affect the quality of water of any river in ecosystem. Therefore, a chemical analysis of water was done to assess the impact of mass bathing.

Therefore, a project was undertaken to analyse water of Ganga River (chemically and microbiologically) collected during Maha Kumbh mela 2025.

3.0 MATERIAL AND METHODS

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3.1 Collection of samples:

Ganga water from Maha Kumbh mela 2025is used as sample for different physical, chemical and biological testing. Sample was collected on 24th January 2025 in the morning (On 13th day of Mela). Sample collected in sterile glass bottles and it was tightly packed, labeled with detail and bought to laboratory and stored in refrigerator till its analysis.

3.2 Physical Characterization of Ganga water

3.2.1 Taste: -

The collected sample from Maha Kumbh mela was tasted by all 4 group member and result was noted down.

3.2.2 Odour: -

The smell of collected sample was tested and results were noted down.

3.2.3 Texture: -

The texture of collected sample was observed and noted down.

3.2.4 Colour: -

Colour of Ganga water was analysed by observing the sample.

3.3 Chemical testing of Ganga water.

3.3.1 pH: -

Ganga water sample from Kumbh mela was tested for pH. First pH strip was taken and dipped into the sample.

The colour of paper pH strip was compared with standard pH paper strip and pH was noted down.

3.3.2 BOD: -

The BOD values of water sample was determined as follows.

(A) Filling Bottles

1. Distilled water was aerated for about 24 hours. Then this aerated water was used for diluting Ganga water collected from Kumbha Mela.
2. Four BOD bottles were taken and labelled as BI, BF, SF, SI
3. SI and SF bottles were added with diluted Ganga water sample (300ml) and care was taken to avoid any bubble formation
4. BI and BF bottles were filled with aerated water.
5. BF and SF bottles were kept for incubation in BOD incubator at 26°C – 28°C for 5 days. After incubation dissolved oxygen was calculated as follows.
6. BI and SI bottles were taken for analysis of dissolved oxygen on first day.

(B) Determination of dissolved oxygen:

1. 2ml MnSO4 and 2ml alkali azaide solution was added in BOD bottles

Brown ppt was observed

2. 2ml conc. H_2SO_4 was added to dissolve the PPT and BOD bottle were shaken.
3. 100ml sample was taken in conical flask and 1ml of 2% starch indicator was added in it and blue colour was appeared in solution.
4. Solution was titrated against 0.0125 N sodium thiosulphate till Blue color turns to colourless. (As shown in photograph no.1.)

BOD was Calculated by using formula: -

$$\text{BOD (mg/lit)} = \frac{\text{(A-B) N x 1000 x dilution factor}}{\text{Amount of sample for titration}}$$

Where,

A = Sample Initial - Sample Final (SI - SF)

B = Blank Initial – Blank Final (BI – BF)

N = Normality of Sodium thiosulphate

1000 = Conversion factor

3.3.3 COD:

1. 20ml diluted sample of Ganga water was taken in conical flask and to this of 0.25N $K_2Cr_2O_7$ was added,
2. Traces of $AgSO_4$ or $HgSO_4$ were added that contains high amount of Chloride
3. 30ml Conc. H_2SO_4 was added
4. Sample was refluxed for 24 hours on reflux unit
5. After refluxing that flask was removed, cooled and total volume was made 130 ml by using cold distilled water.
6. 2-3 drops of ferroin indicator were added in the solution and titrated against 0.1N FAS.
7. Burette reading was recorded when colour changes from bluish green to wine red.
8. COD of sample was then calculated with following formula.(as shown in photograph no.3)

$$\text{COD (mg/lit)} = \frac{\text{(A-B) N x 8 x 1000}}{\text{Amount of sample used}}$$

Where,

A = Blank reading

B = Sample reading

N = Normality of FAS (0.1N)

1000 = Conversion Factor.

8 = Atomic number of oxygen.

3.3.4 TDS & TSS:

Procedure:

1. **Determination of Total Dissolved Solids (TDS):**

   i. Accurately weight the clean dry silica crucible.

ii. Transfer 100ml of unfiltered Ganaga water sample into the crucible and take weight of the container.

iii. Evaporate the sample by placing in hot air oven at 150°C for one hour.

iv. Take out crucible and cool it properly by placing in desiccator.

v. Record the weight of crucible.

vi. Calculate the presence of TDS in Ganga water sample by following fomula.

$$\text{TDS (mg/lit)} = \frac{\text{WI-WF}}{\text{S}} \text{ X } 1000$$

Where,

WI= Initial weight of crucible

WF= Final weight of crucible

S = Amount of sample taken.

2. Determination of Total Solids (TS):

i. Accurately weight the clean dry silica crucible.

ii. Transfer 100ml of unfiltered Ganaga water sample into the crucible and take weight of the container.

iii. Evaporate the sample by placing in hot air oven at 150°C for one hour.

iv. Take out crucible and cool it properly by placing in desiccator.

v. Record the weight of crucible.

vi. Calculate the presence of TS in Ganga water sample by following fomula.

$$\text{TS (mg/lit)} = \frac{\text{WI-WF}}{\text{S}} \text{ X } 1000$$

Where,

WI= Initial weight of crucible

WF= Final weight of crucible

S = Amount of sample taken.

3. **Determination of Total Suspended Solid (TSS): -**

   i. TSS can be determined by subtracting TDS from TS.

TSS (mg/lit) = TS – TDS

3.4 Biological testing of Ganga water:

3.4.1 Determination of load of microorganisms in Ganga water sample:

A method used to estimate viable bacteria or fungi in sample by plating a diluted sample onto a growth medium and counting the resulting colonies after incubation.

3.4.1.1 SPC for bacteria:

1. Sample preparation: Ganga water sample was diluted in sterile diluent (e.g sterile water/saline) to archive a concentration of 1:10 to 1:100
2. Plating: By using sterile pipette 0.1ml of each dilution was transferred onto a sterile solidified nutrient agar plate and spreading was done.
3. Incubation: The plates were incubated at 25°C for 24 hours/ 48 hours/ 72 hours.
4. Colony counting: Colonies from each plate were counted and CFU was calculated with following formula,

$$\text{CFU/ml} = \frac{\text{Number of colonies x dilution factor}}{\text{Amount of sample taken}}$$

3.4.1.2 SPC for Fungi:

1. Sample preparation: Ganga water sample was diluted in sterile diluent (e.g sterile water/saline) to archive a concentration of 1:10 to 1:100
2. Plating: By using sterile pipette 0.1ml of each dilution was transferred onto a sterile solidified Potato dextrose Agar (PDA) plate and spreading was done.
3. Incubation: The plates were incubated at 25°C for 24 hours/ 48 hours/ 72 hours.
4. Colony counting: Colonies from each plate were counted and CFU was calculated with following formula,

$$\text{CFU/ml} = \frac{\text{Number of colonies x dilution factor}}{\text{Amount of sample taken}}$$

3.4.1.3 Determination of MPN:

It is statistical technique used to estimate the concentration of viable Microorganisms in a sample (Mostly coliforms)

1. 15 tubes were taken. (10 large size tube and 5 jumbo tubes)
2. Large ten tubes were added with 5ml MacConkey's broth. (Single strength)
3. Jumbo tubes were added with 5ml of double strength MacConkey's broth
4. Out of 10 tubes of single strength MacConkey's broth 5 tubes were added with 1ml Ganga water sample and remaining 5 tubes of single strength MacConkey's broth were added with 0.1ml of Ganga water sample.
5. Jumbo tubes were added with 10ml of Ganga water sample.
6. All 15 tubes were added with Daharms tube in inverted position.
7. Tubes were then incubated at 37°C for 24 hours.
8. After incubation tubes were observed for colour change and gas production
9. Then no. of positive tubes was compared with the MPN table to get value of No of Microorganisms in the water sample.

3.5 Detection of pathogens:

3.5.1 Detection of *Staphylococcus aureus.*

1. **Enrichment-**

   For enrichment of *Staphylococcus aureus* 100ml of Nutrient broth with 8% NaCl was prepared.

   In that 1ml Ganga water sample was added and flask was incubated at 37°C for 24 hours

2. **Isolation-**

   A loopful of enriched sample was taken with the help of nichrome wire loop and streaked on the sterile mannitol salt agar plate. The plates were incubated at 37°C for 24 hours and plates was observed for development of yellow colonies with yellow zones which indicates the presence of *Staphylococcus aureus.*

3. **Identification-**

   Colony was checked and further tested for identification.

   Colony characters of well isolated colony were noted down.

Further gram staining and biochemical testing were performed to identify *Staphylococcus aureus.*

3.5.2 Detection of *Pseudomonas aeruginosa*:

1. **Enrichment.**

   For enrichment of *Pseudomonas aeruginosa* Nutrient broth with 1% Dettol was prepared.

   In that 1ml Ganga water sample was added (1%), flask was incubated at 37°C for 24 hours.

2. **Isolation.**

   A loopful of enriched sample was taken with the help of nichrome wire loop and streaked on the sterile cetrimide Agar. The plates were incubated at 37°C for 24 hours and plates was observed for development of Greyish colonies which indicates the presence of *Pseudomonas aeruginosa.*

3. **Identification**

   Colony was checked and further tested for identification.

   Colony characters of well isolated colony were noted down.

   Further gram staining and biochemical testing characters were performed to identify *Pseudomonas aeruginosa.*

3.5.3 Detection of *E. coli*:

1. **Enrichment-**

   For enrichment of *E. coli* MacConkey's broth was prepared.

In that 1ml Ganga water sample was added (1%), flask was incubated at 37°C for 24 hours

2. **Isolation-**

   A loopful of enriched sample was taken with the help of nichrome wire loop. Streaked on the sterile MacConkey's agar plate. The plates were incubated at 37°C for 24 hours and plates were observed for development of pink colonies which indicates the presence of *E. coli.*

3. **Identification:**

Colony was checked and further tested for identification. Colony characters of well isolated colonies were noted down and, on the basis, it was identified. Further gram staining and biochemical testing characters were performed to identify *E. coli.*

3.5.4 Detection of *Salmonella-*

1. **Enrichment:**

   For enrichment of *Salmonella* MacConkey's Broth was prepared. In that 1ml Ganga water sample was added (1%) flask was incubated at 37oC for 24 hours.

2. **Isolation:**

   A loopful of enriched sample was taken with the help of nichrome wire loop. Streaked on the sterile MacConkey's agar plates. The plates were incubated at 37°C for 24 hours and plates was observed for development of pale colonies which indicates the presence of *Salmonella.*

3. **Identification:**

   Colony was checked and further tested for identification. Colony characters of well isolated colonies were noted down and, on the basis, it was identified. Further gram staining and biochemical testing characters were performed to identify *Salmonella.*

3.5.5 Detection of *Klebsiella*.

1. **Enrichment:**

   For enrichment of *Klebsiella* MacConkey's broth was prepared. In that 1ml Ganga water sample was added (1%), flask was incubated at 37°C for 24 hours.

2. **Isolation:**

   A loopful of enriched sample was taken with the help of nichrome wire loop. Streaked on the sterile MacConkey's agar plate. The plates were incubated at 37°C for 24 hours and plates was observed for development of pink mucoid colonies which indicates the presence of *Klebsiella.*

3. **Identification:**

   Colony was checked and further tested for identification. Colony characters of well isolated colonies were noted down and, on the basis, it was identified. Further gram staining and biochemical testing characters were performed to identify *Klebsiella.*

3.6 Antimicrobial testing of Ganga water sample.

3.6.1 Agar well diffusion method-

3.6.1.1 Anti-microbial activity of ganga water against *Staphylococcus aureus*

1. Sterile nutrient agar with 8% NaCl was prepared and after sterilization freshly prepared suspension of *Staphylococcus aureus* was added in sterile molten cooled nutrient agar flask and plates were prepared.
2. Then with the help of cork borer at the centre well was prepared.
3. After that 0.1 ml Ganga water sample was added in that well.
4. Plates were kept in the fridge for 10 min for diffusion.
5. Then plates were kept in incubator at 37°C for 24 hours and observed for clear zone around the well.

3.6.1.2 Anti-microbial activity of Ganga water against *Klebsiella pneumoniae*

1. Sterile Nutrient agar medium was prepared and after sterilization freshly prepared suspension of *Klebsiella pneumoniae* was added in sterile molten cooled nutrient agar flask and plates were prepared
2. Then with the help of cork borer at the centre well was prepared.
3. After that 0.1 ml sample was added in that well.
4. Plates were kept in the fridge for 10 min for diffusion.
5. Then plates kept in incubator at 37°C for 24 hours and observed for clear zone.

3.6.2 Paper disc method

3.6.2.1 Anti-microbial activity of Ganga water against *Candida*

1. Sterile plates of potato dextrose agar were prepared
2. The 0.1ml sample of test organism were spread on the PDA plates.
3. Then with the help of punching machine small circular disc of Whatman filter paper were prepared
4. Discs were soaked into the Ganga water
5. Then disc was placed on Potato dextrose agar (PDA) plates and spread with candida species with the help of forceps.
6. Plates were kept in fridge for 10min for diffusion.

7. Then plates were kept in the incubator at 37°C for 24 hours.
8. Finally, plates were observed for clear zone around the disc.

3.6.2.2 Anti-microbial activity of Ganga water against *E. coli*

1. Sterile plates of nutrient agar were prepared.
2. The 0.1ml sample of test organism were spread on the NA plates.
3. Then with the help of punching machine small circular disc of Whatman filter paper were prepared.
4. Discs were soaked into the Ganga water.
5. Then disc was placed on NA with the help of forcep.
6. Plates were kept in fridge for 10min for diffusion.
7. Then plates were kept in the incubator at 37°C for 24 hours.
8. Finally, plates were observed for clear zone around the disc.

3.7 Isolation of coliphages

1) Debacterification

i. 10ml Ganga water sample was taken in centrifuge tube
ii. In that tube 0.5 ml chloroform was added.
iii. Then centrifuge tube at 5000 RPM for 15 minutes.
iv. Then supernatant was carefully collected in test tube.
v. Then test tube heated in water bath at 45oC for 10 min, this is called treated lysate.

2) Enrichment

i. Two tubes of phage broth were taken. (10ml per tube)
ii. Tubes were labelled as Host (H) and Lysate (L) tube.
iii. Then 0.5 fresh host (E. coli) suspension was added in both tubes.
iv. Then tubes were incubated at 37°C for 3 hours
v. After incubation 1ml treated lysate was added in L tube and continue incubation at 37°C for overnight.

3) Isolation

i. Tubes were observed for loss of turbidity in "L" tube.
ii. Then 0.5 ml chloroform was added in "L" tube.
iii. Then tube was centrifuge at 5000RPM for 15 minutes.
iv. Then supernatant was carefully transferred in another test tube.

v. Then test tube was heated in water bath at 45°C for 10-15 minutes. This is called enriched lysate.

vi. Then dilution (10-fold) of enriched lysate was prepared. (10^1 to10^5)

vii. Then sterile mineral salt solution tubes were taken.

viii. Then 0.1ml form each dilution (10^1 to 10^5) were added in each mineral salt solution tube.

ix. Then 0.5ml host (*E. coli*) from H tube was added in mineral salt tube.

x. Tubes were mixed well and incubated at 37°C for 10 minutes.

xi. Then entire content of tubes was added in soft agar tube (2.5ml)

xii. Content was mixed well and poured on bacto-tryptone agar plate.

xiii. Plates were incubated at 37°C for 24 hours

xiv. Finally, after 24 hours. Plates were observed for plaques.

4.0 RESULT

4.0 RESULT

The analysis of Ganga River water sample collected during the Maha Kumbh mela 2025 included physical, chemical and biological assessments along with antimicrobial susceptibility testing. The results ser detailed below:

4.1 Physical Characterization: -

4.1.1 Taste-

The taste of Ganga water sample was reported to be mildly metallic, which may be due to the presence of dissolved minerals or trace elements such as iron, copper or zinc.

4.1.2 Odour-

The Ganga water sample collected during the Maha Kumbh mela 2025 exhibited a faintly musty odour. This characteristics smell may be attributed to the presence of decaying organic matter, alga and microbial metabolites

4.1.3 Texture-

Upon tactile examination, the water felt generally smooth, but fine particles were noticeable. These suspended solids likely originated from soil and runoff, ash residues and flower offerings.

4.1.4 Colour-

The water appeared pale yellow and slightly turbid. The yellowish hue could result from the presence of dissolved organic compounds and pollutants introduces from mass bathing and immersion activities.

4.2 Chemical testing:

4.2.3 pH-

The pH of the eater sample ranged from 7-7.2 indicating that the eater was nearly neutral pH reflects balanced chemical conditions, suitable for most aquatic organisms.

4.2.4 BOD: -

The biological oxygen demand of the analysed water sample was found to be 0.35 mg/l. This value indicates excellent water quality with very low organic pollution. According to standard water quality classifications, BOD values below 1mg/l are typical of unpolluted water

sources. The low BOD indicates minimal microbial activity due to organic matter, implying sample contains negligible biodegradable organic contaminants.(shown in photograph no 2)

Photograph no.1: SI and BI mixture before titration

Photograph no. 2: SI and BI mixture after titration

4.2.5 COD: -

The chemical oxygen demand of Ganga River water sample was measured to 8.8mg/l. This value falls within the range typically observed for moderately clean surface water and indicates the presence of low to moderate level of oxidizable organic and inorganic substances. According to environmental quality standards, COD values below 10mg/l are generally acceptable and this water is fit for outdoor bathing, recreation or propagation of aquatic life.

Photograph no. 3 Sample and Blank before titration

Photograph no. 4 sample and blank after titration

(As shown in photograph no 4)

4.2.6 TDS and TSS: -

The total dissolved solids (TDS) concentration in the Ganga River water sample to be 668 mg/l, which remains well within the permissible limit prescribed by the bureau of Indian standards (BIS). This indicates a moderate level of dissolved mineral content.

The total suspended solid level was recorded at 636mg/l which confirms that the dissolved phase of water quality remains within acceptable ecological and usage thresholds.

Table no. 2 Physio-chemical parameters of ganga river water at Prayagraj and comparison with WHO and BIS standards.

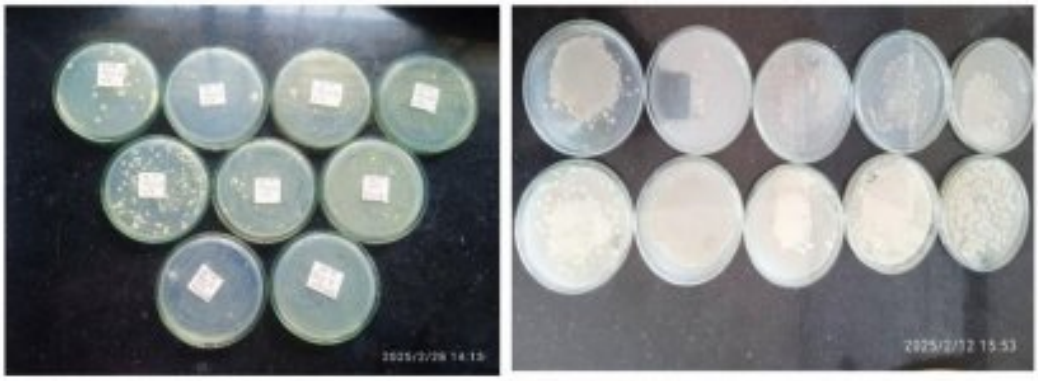
Parameter	Range	Mean ± SD	WHO/BIS limit
pH	7.0 – 7.2	7.6 ± 0.2	6.6 - 8.5
BOD (mg/lit)	0.28 – 0.41	0.35 ± 0.05	≤ 3.0 (CPCB)
COD (mg/lit)	8.2 - 9.4	8.8 ± 0.4	≤ 10 (CPCB)
TSS (mg/lit)	645 - 690	668 ± 18	50 (Desirable)
TDS (mg/lit)	615-660	636 ± 15	No fixed BIS; < 100

4.3 Biological testing of ganga water

4.3.1 Determination of load of microorganism in ganga water: -

4.3.1.1 SPC for bacteria.

The standard plate count (SPC) method is widely used microbiological technique to estimate the number of viable bacteria in a sample. The following table shows the number of bacterial colonies counted at various dilution after 24,48 and 72 hours of incubation. As expected, lower dilutions (i.e. higher concentrations) resulted in more colonies, often to may to count that marked as 'uncountable'. As dilution increases the number of colonies become manageable and countable overtime, colony counts generally increase due to bacterial growth, we pick growth often observable at 72 hours.



Photograph no.5 SPC of Bacteria

Table no 3 Colony counts at different dilution and incubation time

Dilution of Ganga water sample	Number of colonies		
	After 24 Hours	After 48 Hours	After 72 Hours
10^{-1}	8	8	10
10^{-2}	12	13	18
10^{-3}	17	20	32
10^{-4}	20	23	34
10^{-5}	29	34	42
10^{-6}	41	48	52
10^{-7}	55	58	66
10^{-8}	72	Uncountable	Uncountable
10^{-9}	Uncountable	Uncountable	Uncountable
10^{-10}	Uncountable	Uncountable	Uncountable

To determine the viable bacterial count, colonies with the statistically acceptable range (30 – 300 colonies) were selected from the plates and incubated for 72 hours. Dilutions of 10^{-5}, 10^{-6} and 10^{-7} were considered for calculation, as they yielded the colony counts of 42,52 and 67 respectively. The colony forming units per ml (CFU/ml) were calculated by using the formula.

$$\text{CFU/ml} = \frac{\text{Number of colonies x dilution factor}}{\text{Amount of sample taken}}$$

At 10^{-5} dilution: 4.2×10^{6} CFU/ml

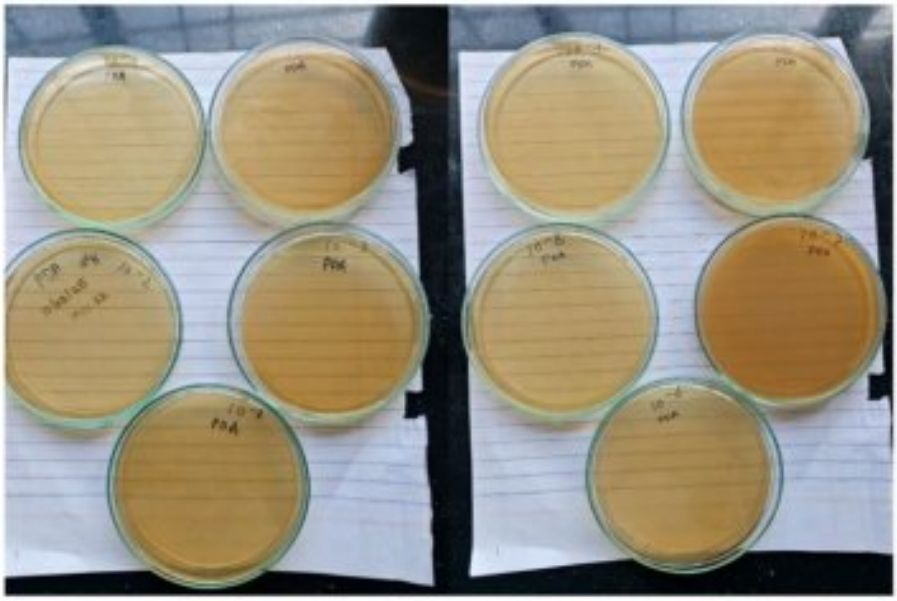
At 10^{-6} dilution: 5.2×10^{7} CFU/ml

At 10^{-7} dilution: 6.7×10^{8} CFU/ml

Thus, the bacterial load was estimated to be approximately 2.81×10^{7} CFU/ml.

4.3.1.2 SPC for fungi

SPC was also performed to determine the presence of viable fungal colonies using the serial dilution and plating method as for bacteria. The inoculated plates were incubated under appropriate fungal growth conditions. However no fungal colonies were observed on any of the dilution plates even after the complete incubation period. The absence of fungal colonies suggests that either fungal species were not present in the original sample or their concentration was below the detectable limit of SPC method. This result highlights a potential low fungal or absence of viable fungal spores in the sample under the tested condition. (as shown in photograph no.6)



Photograph no. 6 SPC of Fungi

4.3.2 MPN: -

The Most Probable Number (MPN) test was conducted using a three-tier dilution series to estimate the coliform count in the Ganga River water sample. The test involved inoculating five tubes each with 10 mL (double-strength), 1 mL (single-strength), and 0.1 mL (single-strength) of the water sample into MacConkey broth. After incubation, positive reactions were observed in one tube of the 10 mL dilution and one tube of the 0.1 mL dilution, resulting in a positive tube combination of 1-0-1. According to standard MPN tables for a 5-tube, three-dilution series, this combination corresponds to an MPN value of 4 coliforms per 100 mL of water. This low coliform count suggests that the water sample has minimal faecal contamination and is within acceptable limits for human use, provided other water quality

parameters are water sample. The test involved inoculating five tubes each with 10 mL (double-strength), 1 mL (single-strength), and 0.1 mL (single-strength) of the water sample into MacConkey's broth. After incubation, positive reactions were observed in one tube of the 10 mL dilution and one tube of the 0.1 mL dilution, resulting in a positive tube combination of 1-0-1. According to standard MPN tables for a 5-tube, three-dilution series, this combination corresponds to an MPN value of 4 coliforms per 100 mL of water. This low coliform count suggests that the water sample has minimal faecal contamination and is within acceptable limits for human use, provided other water quality parameters are also satisfactory.

Photograph. no. 7MPN tubes

Table no. 4 MPN Index

Number of tubes giving positive reaction out of				Number of tubes giving positive reaction out of			
5 undiluted samples (dilution factor-1)	5 dilutions of 10 (dilution factor-10)	5 dilutions of 100 (dilution factor-100)	MPN Index per 100 mL	5 undiluted samples (dilution factor-1)	5 dilutions of 10 (dilution factor-10)	5 dilutions of 100 (dilution factor-100)	MPN Index per 100 mL
0	0	0	< 2	4	2	1	26
0	0	1	2	4	3	0	27
0	1	0	2	4	3	1	33
0	2	0	4	4	4	0	34
1	0	0	2	5	0	0	23
1	0	1	4	5	0	1	30
1	1	0	4	5	0	2	40
1	1	1	6	5	1	0	30
1	2	0	6	5	1	1	50
2	0	0	4	5	1	2	60
2	0	1	7	5	2	0	50
2	1	0	7	5	2	1	70
2	1	1	9	5	2	2	90
2	2	0	9	5	3	0	80
2	3	0	12	5	3	1	110
3	0	0	8	5	3	2	140
3	0	1	11	5	3	3	170
3	1	0	11	5	4	0	130
3	1	1	14	5	4	1	170
3	2	0	14	5	4	2	220
3	2	1	17	5	4	3	280
4	0	0	13	5	4	4	350
4	0	1	17	5	5	0	240
4	1	0	17	5	5	1	300
4	1	1	21	5	5	2	500
4	1	1	26	5	5	3	900
4	2	0	22	5	5	4	1600
				5	5	5	> 1600

* Multiply the MPN Index by the smallest dilution factor from the series used when dilutions other than 1, 10, and 100 are used. *(Standard Methods for the Examination of Water and Wastewater, 18th ed.)*

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4.3.3 Detection of pathogen

Photograph no. 8 Enrichment of sample of E.coli, Pseudomonas aeroginosa, Staphylococcus aureus, Candida

4.3.3.1 Detection of *Staphylococcus aureus.*

Plates of MSA stricked with Ganaga river water sample showed no viable growth of *Staphylococcus aureus*. Specifically, there were no yellow colonies surrounded by yellow zones which are characteristic indicator of *Staphylococcus aureus* due to mannitol fermentation and subsequent acid production the absence of *Staphylococcus aureus* suggest that the ganga water sample does not exhibit contamination from this particular pathogen at the tested site and time.

4.3.3.2 Detection of *Pseudomonas aeruginosa.*

Cetrimide agar plates inoculated with the Ganga River water sample showed no growth of *Pseudomonas aeruginosa.* Specifically, no greyish colonies with surrounding pigmentation were observed, which are typical of *Pseudomonas aeruginosa* due to production of pyoverdine & pyocyanin pigments. The absence of *Pseudomonas aeruginosa* suggests that the sampled Ganga River waters does not exhibit contamination by this opportunistic pathogen

4.3.3.3 Detection of *E. coli.*

MacConkey's agar plates inoculated with the Ganga River waters sample showed no bacterial growth Notably, no pink to red lactose fermenting colonies were observed which indicates presence of *E. coli*. The absence of *E. coli* suggests that the tested water sample does not show evidence of recent fecal contamination at the time and site of collection.

4.3.3.4 Detection of Salmonella

MacConkey agar plates inoculated with the Ganga River water sample exhibited no bacterial growth and non-lactose fermenting pale colonies typically associated with *S.spp* were observed furthermore there was no indication of H_2S gas production, which is commonly associated with *Salmonella* metabolism when grown on appropriate differential media. The absence of *Salmonella* colonies & lack of H_2S production may indicate a lower immediate risk of salmonellosis.

4.3.3.5 Detection of *Klebsiella*

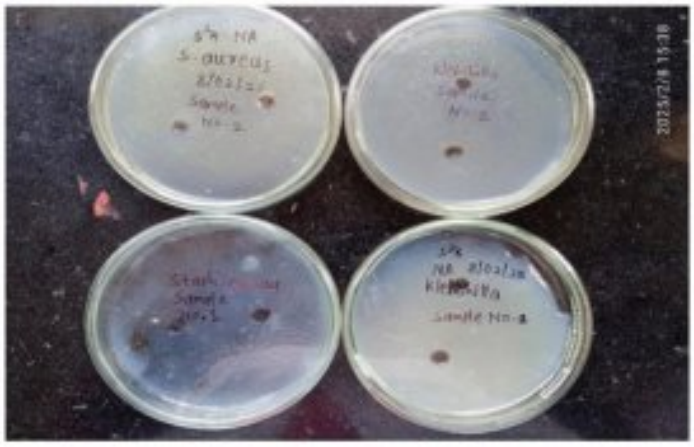
MacConkey's agar plates inoculated with the Ganga River waters sample exhibited no visible bacterial growth. Specifically, no large, mucoid, pink-to-yellow colonies surrounding with zones typically inactive of *Klebsiella* due to lactose fermentation & capsule production were observed the absence of *Klebsiella* colonies suggests that the waters sample lacks detectable levels of this pathogen at the time & site of collection. Their absence may reflect a low level of enteric pollution at this location.

4.4 Antimicrobial testing of ganga water sample.

4.4.1 Agar well diffusion method.

4.4.1.1 For *Staphylococcus aureus*

The agar well diffusion assay performed using the Ganga River water sample showed no zone of inhibition around the well. This indicates that the water sample exhibited no observable antimicrobial activity against *Staphylococcus aureus* under the tested condition. as shown in photograph no.9



Photograph no. 9 Antimicrobial activity by Staph. Aureus and Klebsiella

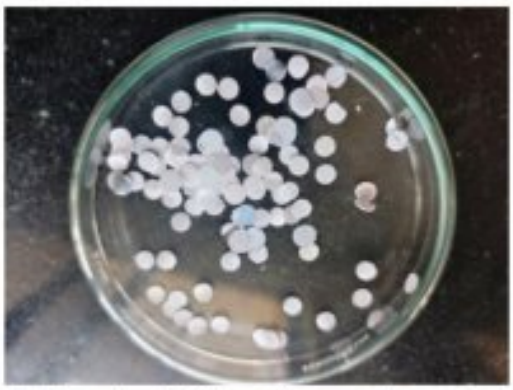
4.4.1.2 for *Klebsiella*

The agar well diffusion assay using the Ganga River waters sample showed no zone of inhibition around the well. This indicates an absence of detectable antimicrobial activity against *Klebsiella* under the tested conditions.(as shown in photograph no.9)

4.4.2 Paper disc method

4.4.2.1 For *Candida*

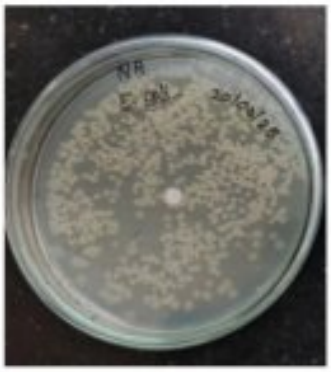
The papers disc diffusion assay conducted with the Ganga River water sample showed no visible zone of inhibition around the disc. This indicates that the sample exhibited no delectable antifungal activity against *Candida* under the experimental conditions.



Photograph no. 10 Wattman filter paper discs

4.4.2.2 For *E. coli*

The paper disc diffusion assay performed using the Ganga River waters sample exhibited no clear zone of inhibition surrounding the disc This indicates that the water sample showed no measurable antimicrobial effect against *E. coli* under the tested conditions.(as shown in photograph no.11)



Photograph no. 11 Antimicrobial activity by paper disc method

4.5 Isolation of coli phages.

The absence of plaques after incubation suggests that no coliphages were detected in your sample. This outcome indicates that either coliphages were absent in the collected water sample or present in concentrations below the detection limit of the assay.

Photograph no. 12 Isolation of coliphages

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5.0 CONCLUSION

The comprehensive analysis of Ganga river water collected during the Maha Kumbh Mela 2025 at Prayagraj indicates that the water quality remained largely within acceptable and safe limits for public use and ritual bathing. The physical parameters such as taste, which was mildly metallic, odour that was slightly musty, and appearance being pale yellow and slightly turbid, suggested the presence of minor organic matter and natural elements. Chemical analysis revealed that the pH was near neutral, ranging between 7.0 and 7.2, while the Biological Oxygen Demand (BOD) was 0.35 mg/L and the Chemical Oxygen Demand (COD) was 8.8 mg/L—both well within the permissible limits set by the Central Pollution Control Board (CPCB). Total Dissolved Solids (TDS) were measured at 636 mg/L and Total Suspended Solids (TSS) at 668 mg/L, with the relatively high suspended solids not posing any immediate concern.

The biological assessment showed a bacterial load of approximately 2.81×10^7 CFU/mL, no fungal growth, and an MPN value of only 4 coliforms per 100 mL, indicating minimal faecal contamination. Importantly, pathogenic bacteria such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, *Klebsiella*, and *Pseudomonas aeruginosa* were not detected, suggesting no significant health hazards at the site and time of sampling. Additionally, antimicrobial testing showed no inhibitory activity of the water sample against various microbial strains, indicating the absence of natural antimicrobial properties in the water.

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6.0 BIBLIOGRAPHY

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