Study Of Microorganisms Present In The Yamuna River At Dussehra Ghat, Hathi Ghat And Poiya Ghat Of Agra City: An Assessment Of Ecological Consequences

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Abstract: Did you know that there are hundreds of microorganisms present in the Yamuna river out of which Proteobacteria is the most abundant? The Yamuna river water of Agra city is highly contaminated with various microorganisms. This study aims to investigate the diversity of microorganisms present in the Yamuna river within the vicinity of Agra city and explore the ecological consequences of their presence. In the present study, Yamuna river water was collected from three different sites in Agra city. The study will employ some microbial analysis techniques to identify and characterise the different microorganisms inhabiting the river waters. Analysis revealed a thriving bacterial diversity in Yamuna river water samples obtained from various locations.

Index Terms - Microorganisms, Yamuna river, Agra city, Dussehra Ghat, Hathi Ghat, Poiya Ghat, E.coli, Citrobacter, Ecological consequences.

INTRODUCTION
The Yamuna river is one of the main tributaries of the Ganga river that passes through Agra, the city of Taj Mahal. It plays a vital role in farming and agriculture. As population growth and social and economic development increase, the quality and quantity of water are becoming increasingly important. Water, also known as "Blue Gold," is essential to human civilization and is influenced by various activities such as urbanisation, industrialization, and heavy metal accumulation. Microorganisms play a crucial role in maintaining ecological balance within aquatic ecosystems, influencing water quality, contributing to biological diversity, and providing essential ecosystem services. Studying aquatic microorganisms helps identify potential contamination, assess pollution levels, and determine water suitability for various uses. They also provide vital ecosystem services, such as water purification and carbon cycling, and can mitigate risks associated with waterborne diseases. The study of aquatic microorganisms also has biotechnological implications, offering sustainable solutions to environmental and societal challenges. Thus, the study of microorganisms in aquatic systems is a multidimensional and interdisciplinary endeavour with significant implications for sustainable management of aquatic ecosystems and human health.

SAMPLING SITES
Yamuna water samples from different locations of the Agra Region (3 Ghats) District UP were collected for the isolation and identification of bacteria. Random sampling was adopted for the study. Microbiological analysis of water was conducted in the lab of St. John's College, Agra. The study was carried out in the city of Agra at three different ghats for three months from October to December 2023.
The sampling locations are as follows:

1. Dussehra Ghat (Sample 1)
2. Hathi Ghat (Sample 2)
3. Poiya Ghat (Sample 3)

The Yamuna river water samples were collected during monsoon and winter season (October to December 2023) from the Dussehra Ghat, Hathi Ghat and Poiya Ghat in polythene bottles and glass bottles of 1 litre & 500ml capacity respectively. The sampling containers were cleansed and rinsed carefully with distilled water and sterilised. The closed bottles were submerged inside the Yamuna river. Then, the bottle cap was opened to let the water in and was closed again as soon as the bottles were fully filled.

MATERIALS AND METHODS

Serial Dilution Agar Plate Method

The number of microorganisms in Yamuna water is high, exact quantification is challenging without discrete colony growth in the medium. This method involves suspending a known water sample amount (9ml) into a microbial suspension (1ml), which is further diluted serially as $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$. A 1ml aliquot from each dilution is plated on agar medium for microorganism isolation. With the help of inoculation loop streaking was done on the media plate and incubated at 37°C for 24 hours and the colonies were observed.

Most Probable Number (MPN) Method

The most probable number method is an indirect method for detection of bacteria. It is a statistical method to estimate bacterial population in a liquid when samples contain very few organisms to give genuine measure by the plate count method. Multiple tube fermentation methods which is a 3 step method is used for testing coliforms in drinking water. The 3 stages of testing includes; the presumptive test, the confirmed test and the complete test. The presumptive test involves a series of three subsets of fermentation tubes, 5 tubes in each series, each containing different amounts of lactose or lauryl tryptose broth to give an estimate of the most probable number of coliforms in the water.

Media Used

Nutrient agar, a solid microbiological growth medium for cultivating non-fastidious bacteria, remains solid at high temperatures. Prepared by dissolving 45 grams of it in distilled water, sterilised through autoclaving at 121°C for 15 minutes and poured into petri plates and dried, for sterility checking plates were incubated for 24 hours at 37°C.

Gram Staining

Gram staining also called Gram’s Method, classified bacteria into two groups i.e. Gram-positive and Gram-negative based on the properties of their cell wall. Gram-positive bacteria retain violet stain while the Gram-negative bacteria stains pink with Safranin as a counterstain.

Morphological characterization

The identification of isolated bacteria was done through analysis of their morphological features. A pure culture was obtained, and its bacterial shape, colony morphology, and gram-staining were meticulously observed and recorded. This detailed examination allowed for a precise and accurate identification of the
bacterial isolates. For identification of microorganisms Bergey's manuals of determinative and systematic bacteriology was followed and then results were made.

RESULTS
The result of this study has illustrated the quality of Yamuna water supplied in the selected region of Agra, where the sites were Dussehra Ghat, Hathi Ghat and Poiya Ghat. Coliform bacteria, *E. coli* and *Citobacter* are concerned with microbiological parameters.

**E.coli**

Scientific Classification
- Domain: Bacteria
- Kingdom: Eubacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: *Escherichia*
- Species: *coli*

**Cultural Examination of E.coli**

<table>
<thead>
<tr>
<th>Cultural Characteristics</th>
<th>On Nutrient Agar Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic &amp; facultative anaerobic, optimum temperature for growth is 37°C, grows well on ordinary media.</td>
<td>It produces large, smooth, opaque &amp; dice shaped colony.</td>
</tr>
</tbody>
</table>

Microscopic Examination: Gram-negative, straight rods, arranged singly or in pairs.

![Figure 2: (a) E.coli growth on Nutrient agar, (b) Microscopic picture of E.coli](image)

The rod-shaped, facultative anaerobic, Gram-negative bacteria *Escherichia coli*, also known by its abbreviation *E. coli*, is frequently detected in the lower intestine of warm-blooded (endotherm) organisms. While the majority of *E.coli* strains cause no harm, some can result in severe food poisoning due to the contamination of food. The ability of a cell to survive outside the body is for a limited time only. Therefore, they are the perfect organisms to check environmental samples for faecal contamination.
Citrobacter

Scientific classification
Domain: Bacteria
Phylum: Pseudomonadota
Class: Gammaproteobacteria
Order: Enterobacterales
Family: Enterobacteriaceae
Genus: Citrobacter

Cultural Examination of *Citrobacter*

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<tr>
<td>Aerobic &amp; optimum temperature for growth is 37℃, and grows well on ordinary media.</td>
<td>Small, circular, convex &amp; shows the white colonies.</td>
</tr>
</tbody>
</table>

Microscopic Examination: Gram-negative, bacilli, long rod-shaped bacteria.

*Figure 3: (a) Citrobacter growth on Nutrient agar (b) Microscopic picture of Citrobacter*

*Citrobacter* is a genus of rod-shaped Gram-negative coliform bacteria belonging to the Enterobacteriaceae family. The ability to utilise malonate, ferment lactose (*C. koseri* is a lactose fermenter), and convert tryptophan to indole-positive distinguishes distinct species of *Citrobacter*. These are 1.0×2.0-6.0µm in size. They are either single or in pairs, and are motile. Optimum temperature for their growth is 37℃. Opportunistic infections such as respiratory tract infections, urinary tract infections and bloodstream infections are caused by the *Citrobacter*.

Other microorganisms
Yamuna river water samples collected from different ghats were checked under the microscope for the presence of algae, fungi and the microscopic animals. The observations are as follows (Figure 4):

*Figure 4: (a) Microscopic view of algae, (b) Microscopic view of fungal hyphae, (c) Microscopic animal (Nematode)*
DISCUSSION
This study examines the impact of urbanisation on the quality and quantity of flow in the Yamuna River. Since waterborne infectious diseases can have serious consequences, controlling the microbiological quality of drinking water should be the top concern in all countries (WHO, 2004). Scientists of All India Institute of Medical Sciences (AIIMS) reported a high frequency of disease-causing microbes in drinking and recreational water. The causative microbial agents, also called pathogens, multiply in the intestinal tract of the persons suffering from the disease and are discharged in their faeces in large numbers (Sharma, 2001, CPCB 2001). Agra is a city where people from all over India keep visiting year around and waterborne infections are prevalent without checks. Moreover, in our country due to close association of animals with human beings, unhygienic conditions in proper drainage systems, poor methods of animal husbandry and the free-roaming of animals with their access to houses, latrines and sewage, the chances of water pollution with harmful microbes increase manifold.

The risk of contracting infections is a significant public health problem. It is hoped that the findings incorporated in this report will aid in understanding public health, the role of different government agencies in disease control, and the importance of vigilance and proper control measures.

In three months' work (October to December) two microorganisms were isolated and identified namely *Citrobacter* and *E.coli* in Yamuna water, Agra, U.P. From the results it was observed that the maximum contamination during three-month periods in the Agra region was in Poiya Ghat and Hathi as compared with Dussehra Ghat.

CONCLUSION
Microscopic examination of the Yamuna river uncovers a high level of microbial pollution, indicating that the river is highly polluted. The presence of these harmful microbes in water can affect nearby humans and animals drastically. As the Yamuna water is used for drinking and irrigation purposes by millions of people, it is a huge environmental concern. Therefore, immediate attention is required to control Yamuna pollution which will help in protecting public health and the environment.

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REFERENCES