Journal of Integrated Scientific Research (JISR)
https://jisrjournal.com/

Bacteriological Assessment of Mula-Mutha River Water, Pune, Using the Most Probable Number (MPN) Method

Triveni Sapate

Researcher, Department of Microbiology, Kamla Nehru College, Nagpur, Maharashtra, India

Email: trivenisapate02@gmail.com

Abstract

The present study aimed to determine the bacteriological quality of Mula-Mutha River in Pune, Maharashtra by enumerating total coliforms and faecal coliforms from five sampling stations, (one upstream, one in the mid city and three downstream stations) using MPN method. The multiple-tube fermentation method was used for analyzing water samples, which included presumptive testing in lactose broth, confirmation of total coliforms in Brilliant Green Lactose Bile broth, and detection of faecal coliforms in EC broth at an elevated incubation temperature. The results indicated that densities of coliforms were significantly higher at stations, downstream of the important urban drainage inflows or stations that were influenced by sewage, and then decreased to varying extents at downstream stations. However, the densities of the bacteria were still high in the city and downstream sites as compared to upstream site. Sixty-two percent of the sites were microbiologically impaired when compared to Central Pollution Control Board water-quality criteria. The spatial trend supported the idea that there was more faecal contamination down stream from urban inflow points, but would need targeted microbial source tracking analysis to be determinate. The study shows the usefulness of the MPN method as a practical approach for routine monitoring of urban river water bacteriology and the need for better sewage interception and treatment, as well as for the ongoing water quality surveillance.

Keywords: Mula-mutha River, Pune, bacteriological water quality, feacial contamination, total coliforms, faecal coliforms, most probable number (MPN)

1. Introduction

The Mula-Mutha River system created by the merging of Mula and Mutha rivers in the city of Pune is a major receiving water body to which all storm water drains of the metropolitan region flow and some stretches receive domestic sewage without treatment and some untreated. The urbanisation that occurred in Pune in last 20 years has led to an increased impervious cover, ageing water supply and water treatment works, growth of population at river banks, bringing a constant strain to the assimilative capacity of the river. Among the most immediate public health impacts of this degradation is bacterial contamination, mainly from faecal sources, posed by the use of the river for bathing, washing, religious purposes and irrigation for agricultural production downstream in parts of the basin.

Although membrane filtration (MF) testing methods are most common for the coliform enumeration of surface water bacteria, the Most Probable Number (MPN) method uses only basic incubation equipment and a selective liquid media, which can be prepared in field and laboratory environments and is the reason it is one of the more widely used coliform enumeration procedures. For this study, the standard multiple-tube MPN method is used to determine the concentrations of both total and faecal coliforms throughout an urban section of the Mula-Mutha River in order to describe the spatial nature of the contamination in relation to known sewage and storm-water inflows.

1. Objectives

- To estimate total coliform and faecal coliform bacteria at various stations of Mula-Mutha River following Most Probable Number (MPN) method.
- To assess any spatial variability of bacteriological contamination in mid city inflow points to downstream stations as resulting from contamination from the upstream residential reach.
- To compare MPN Index values observed with the water quality criteria designated by the Central Pollution Control Board (CPCB).
- To investigate the correlation between bacteriological contamination and the position of the detected urban drainage and sewage influenced inflow.

2. Materials and Methods

The sampling stations and the study area are summarized below:

The sampling stations were selected along around 12km stretch of Mula-Mutha within city limits, S1 (Upstream residential reach with minimal point inflow), S2 (Downstream reach after a major confluening stormwater drain), S3 (Urban reach, city centre, near Deccan Gymkhana), S4 (Near Mula-Mutha confluence point) and S5 (Downstream station with cumulative urban impact, just before it leaves the City limits). station selection was done using a typical point-source sampling bracketing scheme of sampling upstream and downstream of known point-source inflows.

The geographical coordinates of the sampling stations were S1 (18.483806° N, 73.825731° E), S2 (18.508886° N, 73.838403° E), S3 (18.517166° N, 73.845001° E), S4 (18.530173° N, 73.861054° E), and S5 (18.531900° N, 73.927500° E).

2.2 Sample Collection

A total of 5 sampling points were used in the Mula-Mutha river in pre monsoon sampling period and for which grab water samples were collected. The samples were taken in pre-sterilised 500 mL borosilicate glass bottles at about 30 cm below the water surface, from the mid-stream of river if accessible. To insure comparability between sampling locations, the same standardised sampling procedure was used for samples taken at each sampling station. D after collection, the samples were put in an insulated cold box and kept at about 4 °C on to the laboratory. Microbiological analysis was started within 6 hours of sample collection. To reduce potential external contamination during the collection, transport and subsequent analysis, all sampling bottles were used **and all handling and transfers were done under aseptic conditions.**

2.3 MPN Procedure

The multiple-tube fermentation technique was performed according to Standard Methods for the Examination of Water and Wastewater (dissolved oxygen) with five tube per sample dilution series.

Due to the high densities of bacteria expected to be present in river-water samples from urban areas, aseptically prepared decimal dilutions of the samples were made prior to inoculation using sterile dilution water. The dilution range in the case of each sample was chosen to produce measurable combinations of positive and negative tubes and the resulting MPN value was multiplied by the appropriate dilution factor.

Presumptive test: Relative sizes of the samples were inoculated into lactose broth with the help of inverted Durham tubes. If larger volumes of inoculum were used, double-strength media was employed. Tubes were incubated at 35 ± 0.5°C for 24-48 hours, and gas production considered as a presumptive positive reactions.

Confirmed total coliform test: The samples in presumptive-positive tubes were transferred onto Brilliant Green Lactose Bile (BGLB) broth and then cultured at 35 ± 0.5°C for up to 48 h. The presence of gas in wells was used to confirm total coliforms.

Faecal coliform test: Samples from the suitable positive tubes were taken and put into ec broth and kept at 44.5 ± 0.2°C for 24 h. Production of gas, as indicated by its positive faecal-coliform reaction, was recorded.

In addition, FDA mentions that the EC broth shall be inoculated and left to grow for four hours at 44.5°C, and that for faecal-coliform MPN, the incubated complete broth should be gas positive. MPN values were determined from the positive tubes by using the appropriate set of MPN probability tables and reported as MPN/100 mL. 95% confidence intervals were obtained for each estimate.

2.4 Reference Criteria

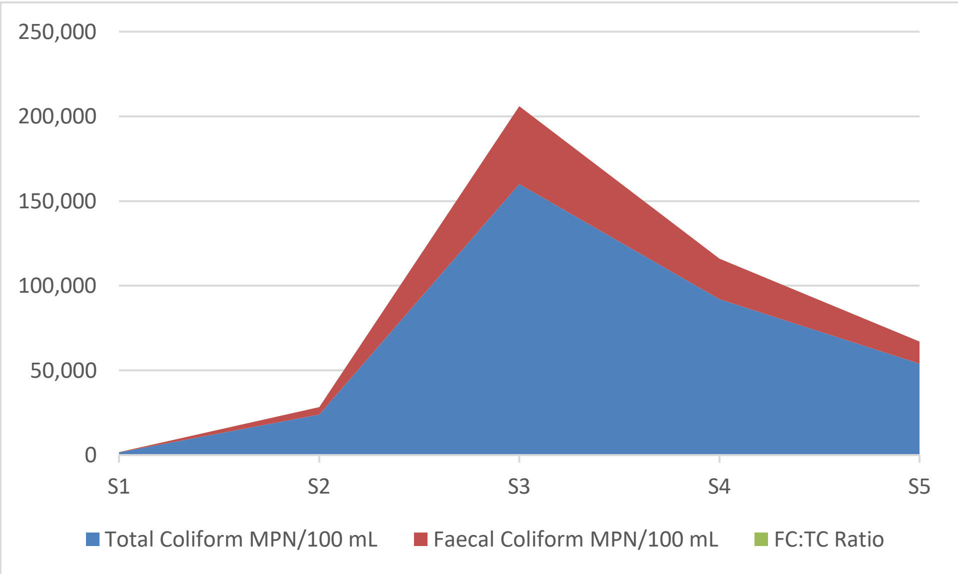
The observed values of total coliform have been compared with the water-quality Designated-Best-Use (DBU) criteria set by CPCB. Based on CPCB criteria, the total coliform organisms in Class B water for organised outdoor bathing should be 500 MPN/100ml or less and for Class C water for treatment as drinking water after conventional treatment and disinfection, total coliform organisms should not exceed 5000MPN/100ml. These criteria served as reference values to interpret the microbiological status of the river stretches sampled.

3. Results

The results of total coliform and faecal coliform MPN values of the five sampling stations in Mula-Mutha River is shown in Table 1. It was noted that at any one point in the study reach, there was a distinct spatial fluctuation in the bacteriological contamination.

Table 1. Illustrative MPN index values by station (sample dataset for methodological demonstration).

Station	Location Description	Total Coliform MPN/100 mL	Faecal Coliform MPN/100 mL	FC:TC Ratio
S1	Upstream residential reach	1,600	220	0.14
S2	Below stormwater drain confluence	24,000	4,300	0.18
S3	Below sewage outfall, city centre	160,000	46,000	0.29
S4	Near Mula–Mutha confluence	92,000	24,000	0.26
S5	Downstream, city exit point	54,000	13,000	0.24



The results indicated that there was a significant rise in bacteriological contamination from the upstream station S1 to the urban stations S2 and S3. The total coliform counts rose from 1600 MPN/100mL at sample point S1 to 24000 MPN/100mL at sample point S2; at sample point S3, the counts employed the highest recorded value of 160000MPN/100mL. The trend in counts of faecal coliforms was similar: the counts were 220 MPN/100mL at sample station 1 (S1), 4300 MPN/100mL at sample station 2 (S2), and 46000 MPN/100mL at sample station 3 (S3). The total and faecal coliform counts were reduced at both S4 and S5 from S3, although they were still much higher than the upstream station. The total coliform were found in all the five stations and were found to be higher than the CPCB Class b (total coliform reference value is 500 MPN/100 ml of water in organised outdoor bathing). Furthermore, the results of total coliform analysis at S2-S5 sites went beyond Class C reference value of 5000 MPN/100mL.

4. Discussion

The distribution of coliform bacteria in space showed a significant reduction in the bacteriological water quality in the urban stretch of Mula-Mutha River. The lower counts obtained at S1 than at the sampling points at S2 and S3 suggest that a significant amount of bacteria had taken place between the sampling sites. This increase can be attributed to a convenience of these stations being close to large urban drainage and sewage-influenced inflows. But the current MPN method of analysis only reveals bacterial level and geographic extent of faecal contamination, and does not necessarily have the power to differentiate between human, animal or other faecal sources. The microbial source-tracking technique and/or sanitary-survey evidence would be needed to confirm sources of contamination.

Methodologically, the MPN technique employed here exhibits practical benefits for a study such as this: does not require any specific apparatus for filtration, not easily clogged by samples containing a moderate amount of turbidity and is suitable for field or resource-limited conditions. The major limitations are the two-stage presumptive-confirmatory analysis, which makes it slower than membrane filtration, and loss of analytical precision that is reflected by the confidence intervals around the value of the MPN indices. Alternatively, membrane filtration (MF) followed by colony forming units (cfu) on selective media can be used as a complimentary method or for confirmatory testing where greater throughput or greater accuracy are desired, especially for regulatory compliance monitoring. Faecal coliform concentrations at all stations from S3 to S5 are consistently hundreds of times higher than the Class B bathing water standards of CPCB, so that any direct contact activity along those areas has important bacteriological risk. This has direct connection with public health messages to oppose religious bathing (ghats) and informal recreation which is seen along the river inside the city limits.

5. Conclusion and Recommendations

With respect to limits of urban stretch of Mula-Mutha River in Pune, this study showed that there was considerable spatial differences in bacteriological water quality. The levels of total and faecal coliforms rose significantly downstream from the major urban inflow locations, and the maximum total coliform and faecal coliform concentrations were found at S3. The concentration of bacteria decreased at other downstream stations, but they are still many bacteria per 100 milliliters compared to the upstream station. The total coliform concentration for all sampled stations were found to be above the class B standard for organised outdoor bathing as laid down by CPCB owing to which microbiological impairment of all stations during sampling period was considered as severe. The results justify sewage influenced urban discharges interception, treatment and regular bacteriological monitoring of the river.

- Target investigation, interception and treatment of sewage and drainage related inflow upstream of stations S2 and S3, the stations which saw the greatest bacterial count increases.

- The routine bacteriological monitoring will take place at 3 fixed stations upstream, midcity, and downstream; with standard frequencies of sampling during periods of potential increased pollution risk.

- Publish proper public-health advice or signage at facilities where there are direct water-contact activities during periods when the bacteriological water-quality criteria are not achieved.

- Continue to sample future spring and autumn periods as well as the monsoon period to assess spring, autumn and monsoon effects and seasonal variation, to measure outgoing/outwash effects due to rain, and to measure sewage related bacterial loads.

References

1. Jadhav, S. D., & Jadhav, M. S. (2017). Analysis of Water Quality Using Physico-chemical Parameters of Mula-Mutha River, Pune Maharashtra. *International Journal of Trend in Scientific Research and Development*, 250–256. https://doi.org/10.31142/ijtsrd2509

2. Jagtap, S. S., & Manivanan, R. (2019). Water Pollution Status of Mula-Mutha Rivers in Pune City: Review. *Zenodo (CERN European Organization for Nuclear Research)*. https://doi.org/10.5281/zenodo.3609976

3. Kale, S. S., Vibhute, N. P., & Shinde, R. M. (2026). Smart city mission's impact on Mula-Mutha river pollution (2015-2025): An analysis based on MPCB data. *International Journal of Humanities and Arts*, *8*(3), 115–117. https://doi.org/10.33545/26647699.2026.v8.i3b.324

4. Sawalkar, N., & Sawalkar, N. (2024). "Assessment of Water Quality in the Mula-Mutha River: Challenges, Solutions, and Analysis. *INTERANTIONAL JOURNAL OF SCIENTIFIC RESEARCH IN ENGINEERING AND MANAGEMENT*, *8*(4), 1–5. https://doi.org/10.55041/ijsrem32020

5. Singh, P., Dar, J. G., Dhanorkar, M., & Warke, A. (2025). Multivariate Statistical Analysis of Water Quality of Major Rivers in Pune: A Case Study. *International Journal of Mathematical Engineering and Management Sciences*, *10*(4), 1013–1031. https://doi.org/10.33889/ijmems.2025.10.4.049

6. Some, S., Mondal, R., Mitra, D., Jain, D., Verma, D., & Das, S. (2021). Microbial pollution of water with special reference to coliform bacteria and their nexus with environment. *Energy Nexus*, *1*, 100008–100008. https://doi.org/10.1016/j.nexus.2021.100008

7. Vibhute, N. P. (2026). Assessment of water quality in the Mula river, Pune: A study on temperature, pH, and dissolved oxygen (DO). *International Journal of Humanities and Arts*, *8*(3), 103–107. https://doi.org/10.33545/26647699.2026.v8.i3b.321