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Microbiological Water Quality Assessment of Freshwater Resources in Tikamgarh, India

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ABSTRACT

Access to safe water is critical, yet microbial contamination remains a pervasive public health threat, particularly in regions under anthropogenic pressure. This study assessed microbiological water quality across four freshwater sites in Tikamgarh, India, using Membrane Filtration and Multiple Tube Fermentation methods for Total Plate Count, Total Coliform, and Fecal Coliform analysis. Water quality analysis revealed significant spatial variation in microbial load, with Pond 2 and Dam 2 showing the highest contamination among the sampled sites. A strong positive correlation and no significant difference were observed between the two analytical methods, supporting their methodological concordance for routine monitoring. The study provides baseline scientific evidence for water quality management and risk assessment in freshwater resources of Tikamgarh.

Introduction

Access to safe and clean drinking water remains a major global concern because public health, environmental sustainability, and socioeconomic development depend strongly on microbiological water quality (1, 6). Pathogenic microorganism contamination of surface water sources contributes substantially to waterborne diseases such as cholera, typhoid, and gastroenteritis, especially in developing regions where sanitation infrastructure is often inadequate (2, 7). Freshwater bodies such as ponds and dams therefore require

continuous microbiological surveillance to protect community health and support evidence-based water management (3, 8).

Traditional microbiological water assessment commonly relies on indicator organisms such as total coliforms and fecal coliforms, with Membrane Filtration (MF) and Multiple Tube Fermentation (MTD) among the most widely applied methods (4, 9). MF enables direct enumeration of colony-forming units, whereas MTD is especially useful for turbid samples and low-density bacterial populations (5, 10). A comparative analysis of

these methods, supported by statistical validation, is important for reliable interpretation of contamination status in field samples (11).

This study was designed to evaluate the microbiological quality of water from four freshwater sources in Tikamgarh, Madhya Pradesh, and to compare analytical performance between MF and MTD methods (12). The work also applies statistical tools to identify contamination trends across sites and to support interpretation of microbiological risk in the studied water bodies.

Materials and Methods

Research Site and Water Sample Collection

Water samples were collected from four freshwater sources in the Tikamgarh region of Madhya Pradesh, India, during May--June 2023: Vrindavan Pond, Mahendra Sagar Pond, Sujara Dam, and Barighat Dam (12). These sites were selected based on accessibility, local importance as water resources, and variation in surrounding human activity (13). Samples were aseptically collected in sterile 500 mL polypropylene bottles in triplicate and transported to the laboratory under cooled conditions for analysis within six hours (3).

Microbiological Analysis

All microbiological analyses were performed in triplicate using standard procedures (4, 14). Total Plate Count was determined by the MF method using sterile 0.45 μm membrane filters cultured on Plate Count Agar at 37°C for 24--48 hours, and results were expressed as CFU/100 mL (15). Total and fecal coliforms were assessed by both MF and MTD methods using selective media and confirmatory procedures, and MPN values were calculated using standard reference tables (5, 16).

Statistical Analysis

Data were analyzed using OriginPro 2023b for descriptive statistics, independent t-tests, one-way ANOVA, Pearson correlation, linear regression, principal component analysis, and hierarchical clustering (17). These analyses were used to compare methodologies, evaluate site-wise differences, and identify broader contamination patterns in the dataset (18).

Results and Discussion

Microbiological Water Quality Assessment

The microbiological analysis revealed substantial variation in bacterial contamination among the studied freshwater resources. Among all sites, Mahendra Sagar Pond (Pond 2) exhibited the highest Total Plate Count, followed by Barighat Dam (Dam 2). In contrast, Sujara Dam (Dam 1) showed comparatively lower microbial contamination levels.

The elevated microbial counts observed in Pond 2 may be attributed to increased anthropogenic activities, domestic wastewater discharge, open bathing practices, livestock access, and surface runoff carrying organic matter and fecal contaminants. Similar findings have been reported in freshwater bodies subjected to intense human activity and inadequate sanitation management.

The presence of high Total Coliform and Fecal Coliform counts indicates possible fecal contamination and suggests potential health risks associated with the direct use of untreated water from these sources. Coliform bacteria are widely recognized as indicator organisms because their occurrence often reflects contamination from sewage or animal waste.

The comparatively lower contamination levels observed in Dam 1 may be associated with reduced human interference, better water circulation, and comparatively lower nutrient loading. Environmental factors such as temperature, organic matter concentration, sediment characteristics, and seasonal runoff also influence bacterial survival and proliferation in freshwater systems.

Water samples from four locations were analyzed using MF and MTD techniques, and the results indicated clear spatial differences in microbial load (12). Pond 2 exhibited the highest mean TPC, followed by Dam 2, Pond 1, and Dam 1, indicating greater microbial contamination at selected sites (13). These findings suggest location-specific influences such as anthropogenic activity, runoff, or local environmental conditions (1, 8).

The comparative dataset between Membrane Filtration (MF) and Multiple Tube Fermentation (MTD) revealed a consistent positive relationship across all contamination levels. MF values (log MPN/100mL) ranged from -3.5 to

20.0, while corresponding MTD counts (CFU/100mL) ranged from 1.2 to 15.3. As MF log values increased incrementally, MTD counts showed proportional increases, indicating strong linear agreement between the two methods. This trend corroborates the statistical analysis, confirming that both MF and MTD yield comparable results and can be used interchangeably for routine water quality assessment in the Tikamgarh freshwater resources.

Methodological Comparison: MF vs. MTD

The comparative analysis between Membrane Filtration and Multiple Tube Fermentation methods demonstrated strong methodological agreement. Statistical analysis revealed no significant difference between the mean microbial counts obtained through the two techniques ($p > 0.05$), indicating that both methods provide reliable estimates of microbial contamination in freshwater samples.

The Pearson correlation analysis demonstrated a strong positive linear relationship between MF and MTD results, confirming the consistency and reproducibility of both analytical approaches. The high correlation coefficient suggests that increases in microbial counts measured by one method were proportionally reflected in the other.

The MF technique offers several practical advantages including rapid analysis, direct colony enumeration, and improved precision for relatively clear water samples. However, highly turbid samples may interfere with membrane filtration due to clogging effects. Conversely, the MTD method is advantageous for turbid waters and low bacterial densities, although it requires longer incubation time and indirect estimation through MPN tables.

The absence of significant methodological variation indicates that both MF and MTD techniques can be effectively used for routine microbiological monitoring of freshwater systems in the study region. Similar methodological concordance has been documented in previous studies evaluating microbial water quality in environmental samples.

No statistically significant difference was observed between the MF and MTD methods for total microbial counts, demonstrating strong agreement between the two analytical approaches (5, 11). A high positive linear

correlation between MF and MTD values further supports the reliability and interchangeability of both methods for monitoring freshwater microbial contamination (9, 10).

Multivariate Water Quality Analysis

Principal Component Analysis (PCA) was performed to identify major patterns in microbiological contamination across sampling sites.

The first principal component (PC1) accounted for approximately 87.5% of the total variance, while the second principal component (PC2) explained nearly 10.0% of the variance. Together, these components represented the majority of variability within the microbiological dataset.

The PCA biplot clearly separated high-contamination and low-contamination sites. Pond 2 and Dam 2 were positioned in association with higher microbial loading vectors, indicating stronger contamination influence. In contrast, Pond 1 and Dam 1 were located comparatively farther from the major contamination vectors, reflecting relatively lower microbial pollution.

The clustering patterns obtained through hierarchical cluster analysis further supported the PCA findings. Sampling locations with similar contamination characteristics were grouped together, demonstrating consistent site-specific microbial behavior. High-contamination sites formed distinct clusters separate from low-contamination water bodies.

The multivariate statistical analyses therefore confirmed that microbial contamination patterns in the studied freshwater systems are influenced by localized environmental and anthropogenic factors.

These approaches provide valuable tools for environmental monitoring, pollution source identification, and water quality management planning.

Principal Component Analysis showed that the first two components explained most of the total variability in the microbiological dataset, separating low-contamination and high-contamination sites clearly (18).

Hierarchical clustering also grouped the sampling sites according to their contamination profiles, confirming consistent site-specific patterns in water quality (19).

Figure.1 Microbiological positives vs expected (MF and MTD)

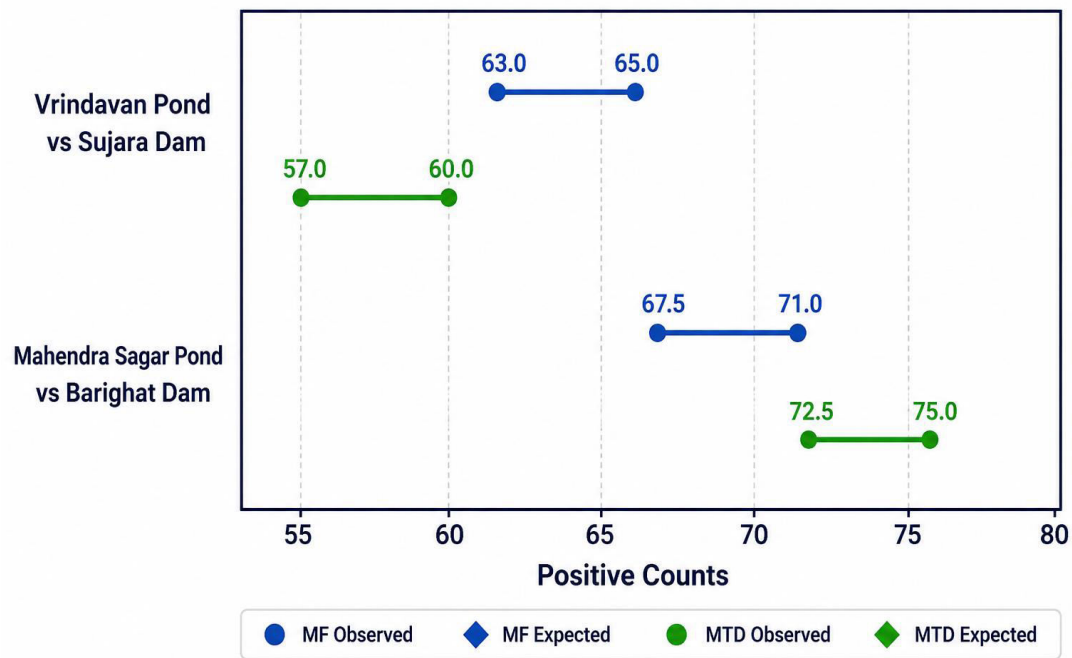


Figure.2 Displays the linear regression analysis of MF versus MTD Total Counts, with all individual data points, fitted regression line, and corresponding statistical parameters displayed on the plot.

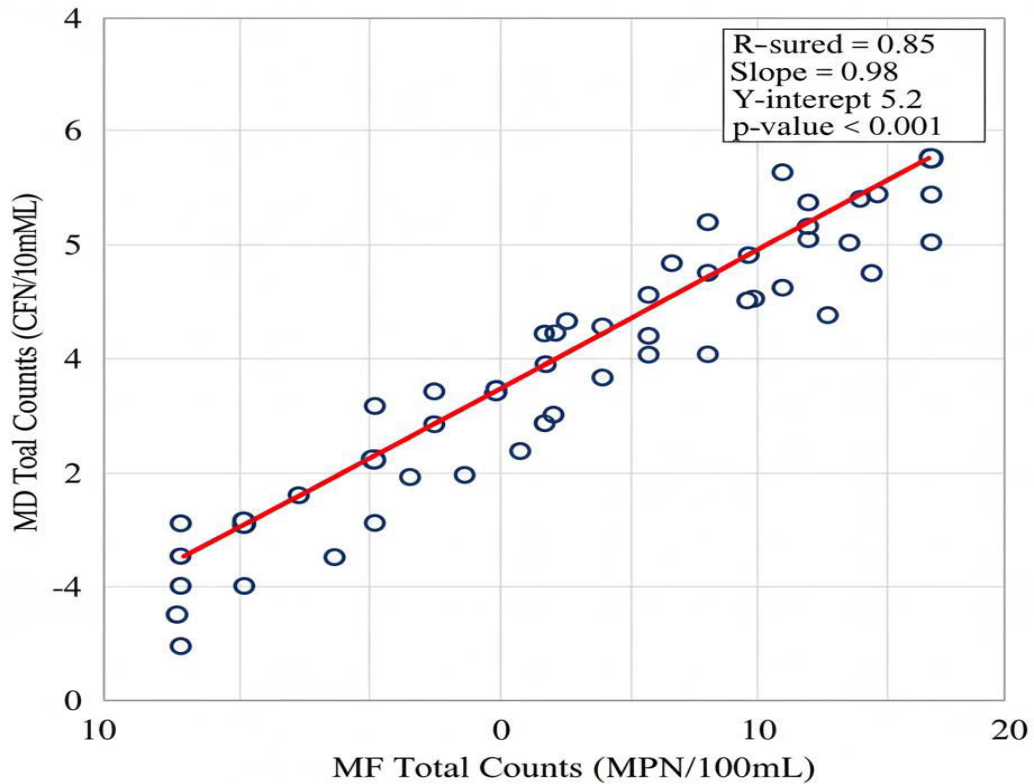


Figure.3 Grouped comparison of mean Total Counts between both methodologies, with error bars representing standard deviation and *t*-test annotations.

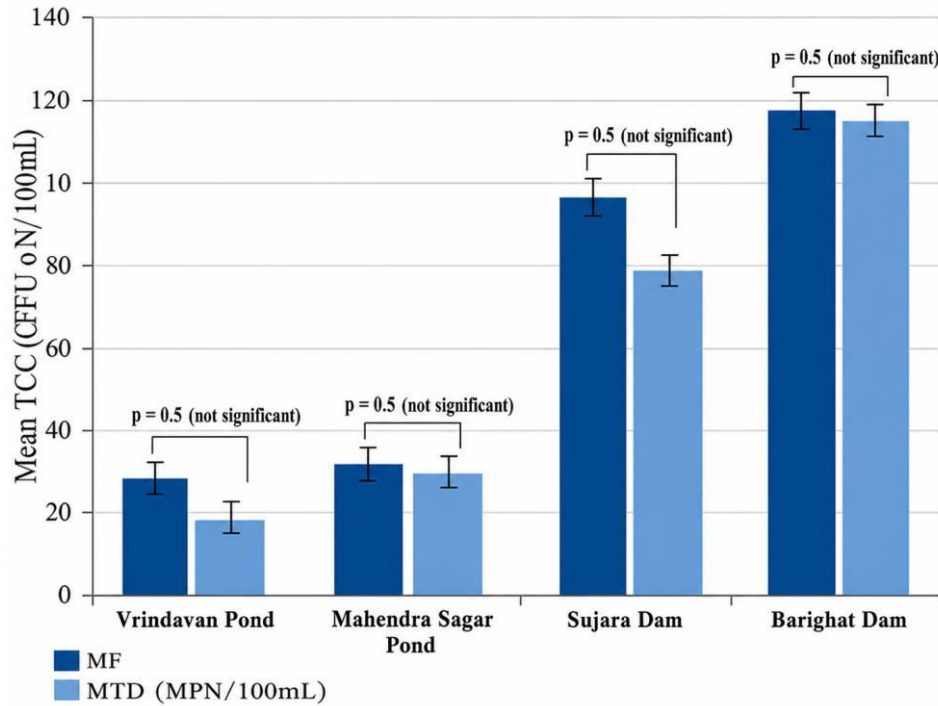
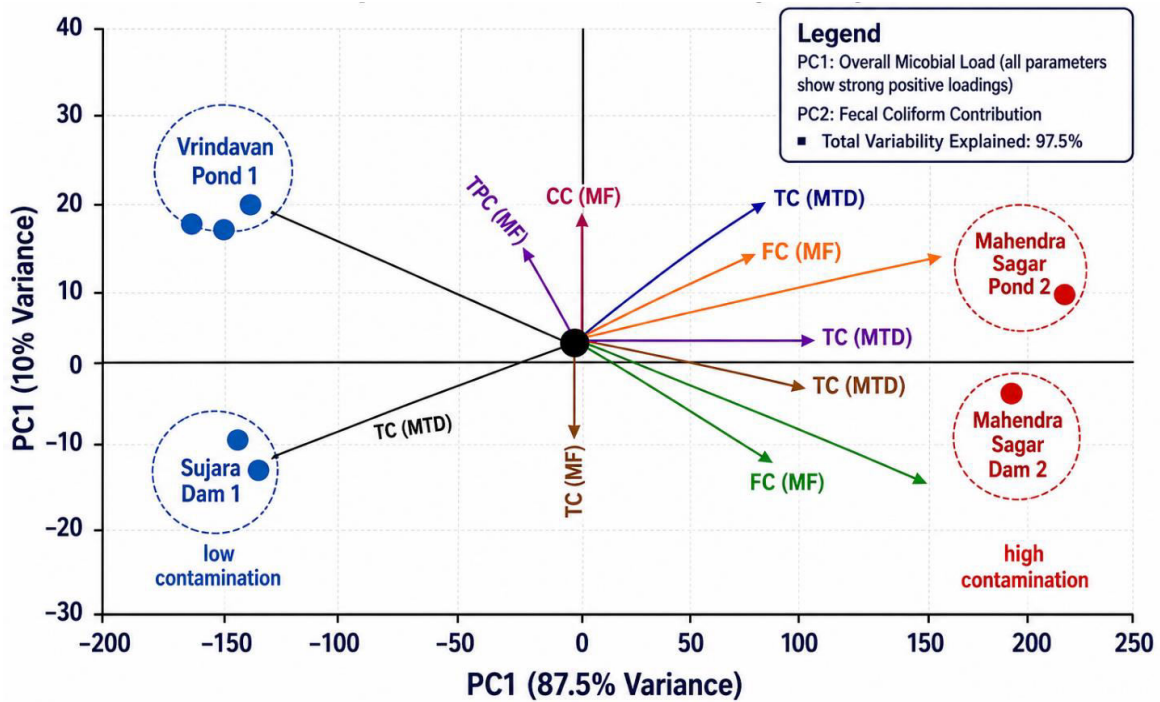


Figure.4 Illustrates the PCA biplot of water quality parameters, showing principal components PC1 (87.5%) and PC2 (10.0%), with four site positions and six parameter loading vectors.



In conclusion, the present study provides a comprehensive microbiological assessment of selected freshwater resources in Tikamgarh district, Madhya Pradesh, using Membrane Filtration and Multiple Tube Fermentation techniques. The results revealed significant spatial variation in microbial contamination among the investigated water bodies. Mahendra Sagar Pond and Barighat Dam exhibited comparatively higher microbial loads, indicating substantial anthropogenic influence and possible fecal contamination.

The comparative methodological evaluation demonstrated strong agreement between MF and MTD techniques, with no statistically significant difference observed between the two methods. The high positive correlation between analytical results confirms the reliability of both approaches for freshwater microbiological monitoring. Multivariate statistical analyses including PCA and hierarchical clustering effectively differentiated the sampling locations according to contamination characteristics and highlighted site-specific pollution patterns. These findings emphasize the importance of continuous microbiological surveillance of freshwater resources, particularly in regions experiencing increasing human and environmental pressure.

The study provides valuable baseline data for water quality management in Tikamgarh and suggests the necessity of implementing improved sanitation practices, regular monitoring programs, and public awareness initiatives to minimize microbial contamination and associated health risks. Future studies should incorporate seasonal monitoring, physicochemical characterization, and molecular identification of pathogenic microorganisms for a more comprehensive understanding of freshwater ecosystem health.

Author Contributions

Rajni Roy: Investigation, formal analysis, writing—original draft. Jitendra Malviya: Validation, methodology, writing—reviewing. Mukesh Kumar Ahirwar:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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