OPEN ACCESS PEER REVIEW

DOI: https://doi.org/10.52756/boesd.2023.e02.025

Chapter- 25

Qualitative and Quantitative Assay of Coliform Bacteria in Different Water Samples & Their Role in Sustainable Development

Shrijeet Kayal, Sagar Verma, Sreenu Appikonda, Gargi Dutta, Chiradeep Basu

Keywords: Coliform Bacteria, Ganga, micro-organism, Sustainable Development, Water sample.

Abstract:

The research aims to provide valuable insights into the variation of coliform bacteria concentrations across different environmental water samples shedding light on potential sources of contamination and facilitating informed decision-making for water quality management and environmental health in the field of sustainable development. Our samples were collected from the Ganga, a pond and rainwater. Coliform bacteria, commonly used as indicators of faecal contamination, were analysed using standardized testing methods, such as the most probable number (MPN) technique, standard plate count (SPC) method and also biochemically by the IMViC test. The findings of this study contribute to our understanding of microbial ecology and support the development of targeted strategies for the prevention and mitigation of coliform bacteria-related risks in diverse settings. By MPN Test it is proved that all the 3 samples contain Coliform Bacteria, in the colony counting method 190 colonies are found in the Ganga sample while Pond and Rainwater samples contain 153 and 30 colonies respectively and the IMViC Test also concluded the presence of different strains of bacteria in the Ganga samples i.e., gave positive results.

Introduction:

Shrijeet Kayal
Member of Biotechnology Laboratory, Swami Vivekananda Institute of Modern Science, (Affiliated to MAKAUT), West Bengal, India
E-mail: shrijeetkayal999@gmail.com; Orcid iD: https://orcid.org/0009-0007-3685-5923
Sagar Verma
Member of Biotechnology Laboratory, Swami Vivekananda Institute of Modern Science, (Affiliated to MAKAUT), West Bengal, India
E-mail: sagarverma20022410@gmail.com; Orcid iD: https://orcid.org/0009-0003-5266-5952
Sreenu Appikonda
Curator & Lab in-charge of Biotechnology Laboratory, BITM
E-mail: sreenu.ncsm@gmail.com
Gargi Dutta
Mentor, Biotechnology Laboratory, BITM
E-mail: gargidutta9@gmail.com; Orcid iD: https://orcid.org/0009-0004-0362-4609
Chiradeep Basu
Mentor, Biotechnology Laboratory, BITM
E-mail: chirodipbasu@gmail.com
***Corresponding Author**: gargidutta9@gmail.com

Shubhadeep Roychoudhury, Tanmay Sanyal, Koushik Sen & Sudipa Mukherjee Sanyal (eds.), A Basic Overview of Environment and Sustainable Development [Volume: 2]. ISBN: 978-81-962683-8-1; pp. 368-376; Published online: 17th December, 2023

Coliform bacteria are a different group of microorganisms that inhabit water, soil, and the gastrointestinal tracts of mammals. They're extensively used as index organisms to assess the microbiological quality of water and food. Coliform bacteria are frequently associated with faecal impurity and can indicate the presence of pathogens that pose a threat to human health. Understanding the distribution, and characteristics of coliform bacteria is pivotal for assessing water quality, food safety and enforcing applicable public health measures.

Definition and Classification of Coliform Bacteria:

Coliform bacteria belong to the family Enterobacteriaceae and are characterized by their capability to ferment lactose with observable production of gas. They're generally facultative anaerobes, gram-negative rods, and include rubrics similar as *Escherichia*, *Enterobacter* and *Klebsiella*.

Role of Coliform Bacteria in Sustainable Development:

Coliform bacteria play a multifaceted role in sustainable development, contributing to water quality management, wastewater treatment, agricultural sustainability, environmental monitoring, and public health promotion. Properly managing coliform levels is integral to achieving a balance between human activities and environmental conservation for long-term well-being.

Sources and Transmission Routes of Coliform Bacteria:

Coliform bacteria can appear from a variety of sources, including sewage, domestic effluents, animal and human by-products. They can enter water systems through surface runoff, wastewater discharges, or defective septic systems. Spoilt food products, like raw vegetables and undercooked meat, can also be a source of coliform bacteria.

Health Risks Associated with Coliform Bacteria:

While not all coliform bacteria are pathogenic, their presence in water and food can indicate the possibility of harmful diseases. Some coliform species, similar to certain strains of *Escherichia coli*, can beget severe gastrointestinal infections, leading to diarrhoea, abdominal pain and might be fatal in elevated situations.

Microbial-mediated water pollution is seen as one of the great challenges to the aquatic environment worldwide. The influx of faecal matter, hospitals, industrial effluence and cattle farms increase the bacterial load in a given water body (Mondal et al., 2022). Coliform bacteria group is conventionally viewed as an indicator organism of microbial contamination. From the coliform, *Escherichia coli* is the indicator of faecal contamination. De Man-diphasic tube fermentation technique is one of the casually employed methods for the detection of coliforms through sugar lactose fermentation with the production of either acid or gases. Potability of water has been assessed by bacteria of faecal origin either absent or present within the tolerance level as specified by MPN/100 ml (Some et al., 2021).

It is believed that bacteria in patients' faeces cause and spread many human diseases such as cholera, typhoid fever, dysentery and gastroenteritis which contaminate food and water thereby rendering them unsafe for consumption. Coliform bacteria are the traditional microbiological indicators most commonly used to assess water quality. Two of the most commonly used pollution indicator bacteria that have been employed as hygiene metrics for the assessment of drinking water cleanliness are the total and faecal coliform counts (Aram et al., 2021).

The source of drinking water is surface waters, i.e., lakes and reservoirs, mostly in rural areas where water availability is a problem (Biswas & Saha, 2021; Bandyopadhyay et al., 2023). This fresh water is full of coliform bacteria nowadays. During the course of two years between 2018 and 2019, this study examined two drinking water reservoirs: Klingenberg and Small Kinzig Reservoir. In summer four orders of magnitude coliform bacteria were detected per 100ml than in wintertime and concentrations up to 2.4 × 104 bacteria per 100ml (Reitter et al., 2021).

River constituents can change their genetically linked physical, physiochemical, and biological characters due to the introduction of artificial constructions in them (Bhattacharya et al., 2016; Roy et al., 2022). Coliform bacteria are important quality indicators of water linked to human health. An investigation was done on eight weir stations constructed in Nakdong River, an important river in South Korea, to consider the relationship between coliform bacteria and water quality parameters. From 2012 to 2016 these sites' fifteen water quality parameters were studied by multiple regression and correlation analysis. The results for each station proved the analytical validity; the average adjusted R2 values for total and faecal coliforms were high, more than 0.6 and 0.8, respectively (Seo et al., 2019).

Comparison of antibiotic-resistant coliform bacteria occurrence between Czech and Slovak Republic hospital wastewater effluents was done. As a resistance mechanism and persistent virulence factor, it has also reviewed a few isolates that are resistant to antibiotics. The biggest amount of drug-resistant germs was found in samples taken from the hospital in the Czech Republic, Valašské Meziříčí. More than half of the resistant isolates were found to produce biofilm and they were also phenotypically multidrug resistant. TetA and TetE genes were found to be co-overexpressed together with efflux pump overproduction in 42% of isolates (Lépesová et al., 2020).

Aims and objectives:

The primary objectives are as follows-

- To quantify and compare the microbial load of coliform bacteria in different water samples including Ganga water, Pond water and Rainwater.

- To identify different strains of Coliform Bacteria.
- To identify different transmission routes and sources of coliform bacteria, aiming to focus on factors contributing to their presence continuity.

Materials and methods:

Sample Collection and Processing:

Samples were collected from Ganga, Pond and Rainwater samples were collected using standard sampling techniques.

Study design:

Selection of Study Sites and Sample:

The study was conducted in the metropolitan area of Kolkata and the water samples were collected from the river Ganga (Lat. 22.566^0, Lon. 88.339^0), a local pond (Lat. 22.535^0, Lon. 88.363^0) and from direct rainwater.

In the area from where the Ganga water is collected various human activities go around daily including washing clothes, bathing and also idol immersion, which might suggest that the Ganga water is highly polluted. On the other hand, the pond water was collected from a pond residing inside the Birla Industrial and Technological Museum (BITM), which is regularly cleaned and maintained so that it might be less polluted.

Enumeration and Identification of Coliform Bacteria:

MPN Test:

Soil surveys carried out on several sites in India showed that coliforms are present in potable water as a result of the presence of CO_2-releasing bacteria while they ferment lactose. An easy, fast, and cheap field test for screening of drinking water for the presence of faecal contamination which is based on the recognition of CO_2 gas by the new test is comparable to the standard MPN. It proved highly successful in the field when it was used to detect faecal pollution and monitor water quality during an outbreak of water-borne hepatitis A infection in the city of Gwalior.

Colony Counting Method:

After checking the presence of Coliform bacteria in the water samples to check the load of the present bacteria Colony Counting method is done.

Sterile Skimmed Milk agar plates are taken and 100µl of inoculum from each sample are added. Then using a sterile glass spreader, the inoculum is evenly spread on the agar plates. After that the inoculated petri plates are inverted and incubated for 48hrs at 37°C and results are observed.

IMViC Test:

The IMViC set is a group of four distinct tests that are widely employed to identify bacterial species, namely coliforms.

The letter in “IMViC” means one of the tests. “I” means indole; “M” is for methyl red; “V” is for Voges-Proskauer, and “C” is for citrate. The small letter “i” is an addition to improve pronunciation. IMViC is an acronym consisting of four different tests.

Indole Test:

It is grown on the sulphide-indole-motility (SIM), tryptophan broth, or the motility urease indole (MIU) medium. After the addition of Kovac’s reagent, the result is read. The appearance of red layer on the top of the tube after Kovács reagent has been added indicates a positive result. A negative result implies the absence of colour change at the top of the tube after adding Kovács reagent.

Methyl Red (MR) Test:

Methyl red and Voges–Proskauer tests are carried out in methyl red-Voges-Proskauer (MR-VP) broth where the added reagents vary depending on the tests. Presence of positive methyl red test is confirmed by turning red colour post methyl red reagent addition. No colour change after methyl red reagent has been added is indicative of negative methyl red test.

Voges Proskauer (VP) Test:

Negative VP reaction is indicated by no colour change following the addition of the first and second Barritt's reagents. The presence of red-brown colour after mixing Barritt’s A and Barritt’s B reagents is the indicator of a positive Voges-Proskauer’s test.

Citrate utilization Method:

The test is performed on Simmons citrate agar: Better the previous thing and greater the next. Absence of both growth and colour change in the tube denotes a negative citrate utilization test A positively reacted citrate is identified by growth and a blue colour change.

Results

MPN Test:

In this method air bubble confirming the presence of coliform bacteria is found in all the samples. But with varying sizes of the bubble. The increasing order of the bubble is Ganga>Pond>Rain. Therefore, the presence of bubbles in all the samples gives a positive test for MPN.

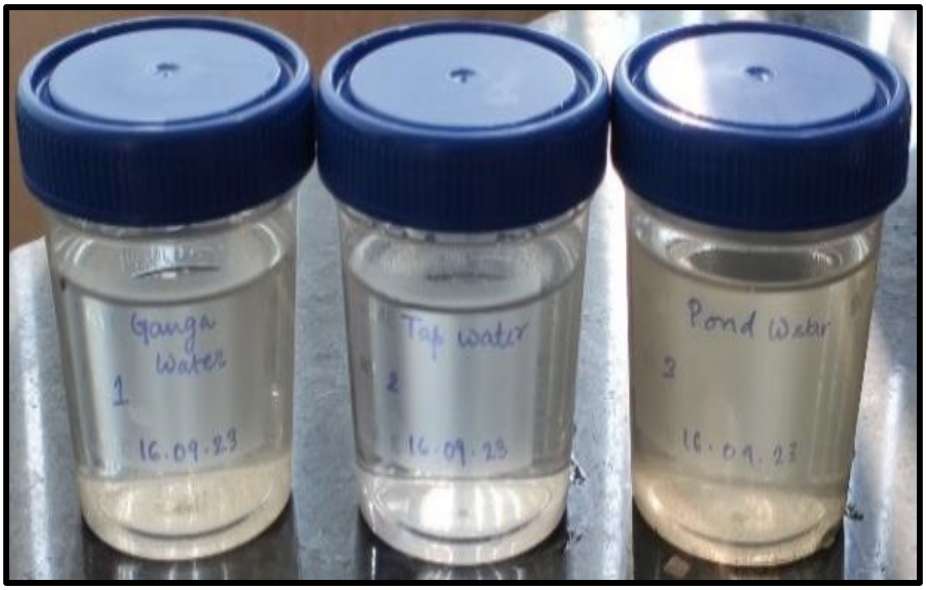


Figure 1. Collected water samples.

Figure 2. Gas bubble formation in the 3 Samples.

Colony Counting Method-After plating:

After 48 hours of incubation the following results are obtained.

Table 1: CFU count in agar plates.

Number of CFU found in the Skimmed Milk Agar Medium	
Ganga	190
Pond	153
Rain	30

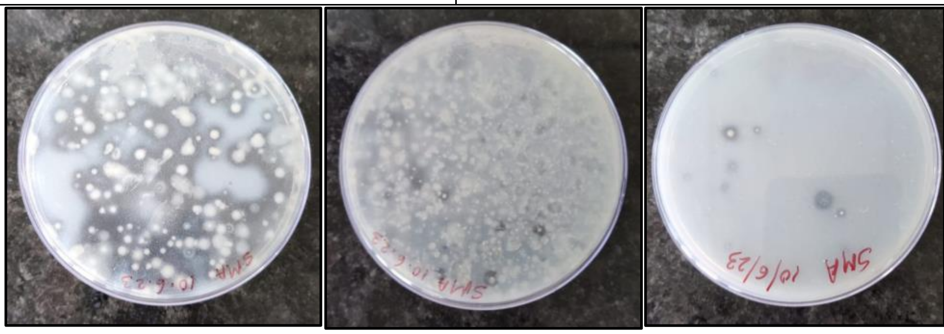


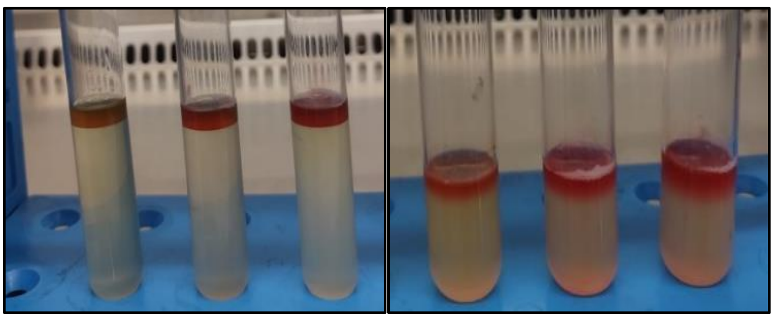
Figure 3. The colony was formed by plating the 3 Samples in petri dishes.

IMPVIC:

In the four different tests i.e. Indole Test, Voges Proskauer (VP), Methyl Red (MR) and Citrate Agar all the samples were tested and they gave different results according to different strains of bacteria present in the samples.

Table 2: Result of IMViC Test of the samples.

Samples	Indole	VP	MR	Citrate Agar
Sample 1	+	+	+	+
Sample 2	+	-	-	+
Sample 3	-	-	-	+



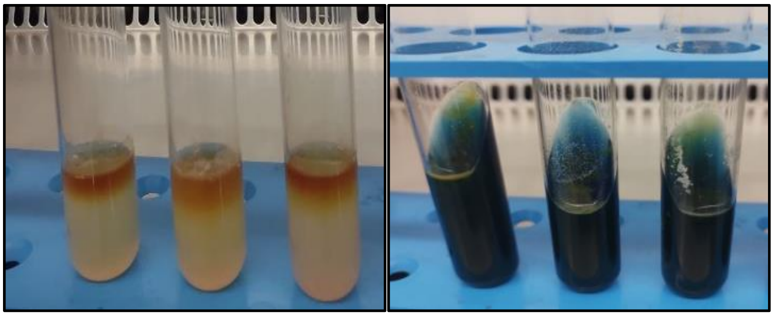


Figure 4. Results of IMViC.

Discussion:

Implications of the Findings:

In the first experiment i.e., the MPN Test which is a qualitative test, a bigger gas bubble is observed in the Ganga water compared to pond and rainwater when all three samples are inoculated in the same media and incubated at similar conditions for a particular period of time which implies that the concentration of Coliform bacteria in the Ganga water is much more than the other two samples. In the second experiment i.e., the Colony Counting method, 190 colonies of coliform bacteria are observed while pond water and rainwater contain 153 and 30 colonies respectively. By this quantitative method, it is seen that Ganga water contains more Coliform Bacteria compared to pond and rainwater.

At last, in the IMViC Test which is a biochemical test, Ganga water was detected positive for all four tests while pond water was positive for 2 tests and rainwater was for only one. This implies that Ganga water contains a various number of bacterial strains compared to pond and rainwater.

Conclusion:

This experiment concludes that Ganga water contains a high concentration of Coliform Bacteria mainly due to the day-to-day activities performed there. While the load of Coliform Bacteria in pond water is less since it is maintained periodically. The rainwater contains a slight or negligible number of coliform bacteria since there is no direct contamination pathway involved.

From this, we can say that Coliform Bacteria help to detect water pollution or otherwise serve as indicators of overall environmental health. Monitoring of these coliform levels helps to assess the safety of water for consumption and recreational activities.

References:

Aram, S.A., Saalidong, B.M., & Lartey, P.O. (2021). Comparative assessment of the relationship between coliform bacteria and water geochemistry in surface and ground water systems. *PLoS One*, 16(9), e0257715.

Bandyopadhyay, A., Sinha, A., Thakur, P., Thakur, S., & Ahmed, M. (2023). A review of soil pollution from LDPE mulching films and the consequences of the substitute biodegradable plastic on soil health. *Int. J. Exp. Res. Rev.*, *32*, 15-39. https://doi.org/10.52756/ijerr.2023.v32.002

Bhattacharya, P., Samal, A., & Bhattacharya, T. (2016). Sequential extraction for the speciation of trace heavy metals in Hoogly river sediments, India. *Int. J. Exp. Res. Rev.*, *6*, 39-49.

Biswas, S., & Saha, S. (2021). A report groundwater arsenic contamination assay in the delta area of West Bengal. *Int. J. Exp. Res. Rev.*, *25*, 84-88. https://doi.org/10.52756/ijerr.2021.v25.008

Lépesová, K., Olejníková, P., Mackuľak, T., Cverenkárová, K., Krahulcová, M., & Bírošová, L. (2020). Hospital wastewater—Important source of multidrug resistant coliform bacteria with ESBL-production. *Int. J. Environ. Res. Public Health*, *17*(21), 7827.

Mondal, P., Adhikary, P., Sadhu, S., Choudhary, D., Thakur, D., Shadab, M., Mukherjee, D., Parvez, S., Pradhan, S., Kuntia, M., Manna, U., & Das, A. (2022). Assessment of the impact of the different point sources of pollutants on the river water quality and the evaluation of bioaccumulation of heavy metals into the fish ecosystem thereof. *Int. J. Exp. Res. Rev.*, *27*, 32-38. https://doi.org/10.52756/ijerr.2022.v27.003

Reitter, C., Heike Petzoldt, H., Korth, A., Schwab, F., Stange, C., Hambsch, B., Tiehm, A., Lagkouvardos, I., Gescher, J., & Hügler, M. (2021). Seasonal dynamics in the number and composition of coliform bacteria in drinking water reservoirs. *Science of The Total Environment*, *787*, 147539.

Roy, S., Das, N., Saha, S., & Ghosh, D. (2022). Idol immersion in Ichhamati river and its impact on water quality parameters. *Int. J. Exp. Res. Rev.*, *29*, 40-47. https://doi.org/10.52756/ijerr.2022.v29.004

Seo, M., Lee, H., & Kim, Y. (2019). Relationship between coliform bacteria and water quality factors at weir stations in the Nakdong River, South Korea. *Water* (Basel), 11(6), 1171.

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.

HOW TO CITE

Shrijeet Kayal, Sagar Verma, Sreenu Appikonda, Gargi Dutta, Chiradeep Basu (2023). Qualitative and Quantitative Assay of Coliform Bacteria in Different Water Samples & Their Role in Sustainable Development. © International Academic Publishing House (IAPH), Shubhadeep Roychoudhury, Tanmay Sanyal, Koushik Sen & Sudipa Mukherjee Sanyal (eds.), *A Basic Overview of Environment and Sustainable Development [Volume: 2]*, pp. 368-376. ISBN: 978-81-962683-8-1. DOI: https://doi.org/10.52756/boesd.2023.e02.025