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# Fecal Coliform Contamination in X City River Water Assessed Using the MPN Method

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**Abstract**: Fecal contamination in river water poses a significant risk to public health and aquatic ecosystems, while also indicating inadequate microbiological water quality. This study aimed to determine the level of *fecal coliform* contamination in river water and compare the results against applicable water quality criteria. Water samples were collected from four sampling points in City X. *Fecal coliform* concentrations were estimated using the *Most Probable Number* (MPN) multiple-tube fermentation method, which includes a presumptive test and a confirmatory test, utilizing *Lauryl Tryptose Broth* (LTB), *Brilliant Green Lactose Bile* (BGLB) *broth*, and EC *Broth*. Incubation was conducted at 35 ± 0.5°C for the detection of total *coliforms* and at 44.5 ± 0.2°C for the detection of *fecal coliforms*. The data were analyzed descriptively by interpreting the combinations of positive tubes using MPN statistical tables, and the resulting concentrations were then compared against a reference criterion of 1,000 MPN/100 mL. The results indicate fecal coliform counts of 35,000, 14,000, 54,000, and 92,000 MPN/100 mL for sampling points A, B, C, and D, respectively; sample D recorded the highest concentration, while sample B recorded the lowest. These findings demonstrate spatial variation in *fecal coliform* concentrations across the sampled locations.

**Keywords:** Environmental microbiology; Fecal coliform; River water; MPN method; Water quality.

## Introduction

River water is an essential resource for human sustenance and ecosystem stability. However, anthropogenic activities, particularly domestic, agricultural, and livestock practices, can introduce microbiological contaminants into river systems and degrade water quality. Rivers contain diverse microbial communities, including bacteria, fungi, and yeasts, that are transported along the hydrological gradient from upstream to downstream areas (Yoantrista & Yuniarti, 2025). Water contaminated with human and animal excreta is unsuitable for domestic consumption, particularly drinking, food preparation, and cooking, because it may contain pathogenic microorganisms that cause diarrhea and other gastrointestinal infections. Therefore, routine microbiological water quality assessments and effective pollution control strategies are essential to minimize waterborne disease risks (Riyanti et al., 2022; Mutmainnah et al., 2025).

Fecal coliforms are widely used as indicators of fecal pollution because their presence is associated with the potential occurrence of pathogenic microorganisms in aquatic environments (Puspitasari et al., 2016; Widyaningsih, 2016). Coliform bacteria can be categorized into fecal coliforms, which originate primarily from human and animal excreta, and non-fecal coliforms, which may originate from decaying organic matter and vegetation. Although total coliforms provide a general indication of microbiological contamination, Escherichia coli is a more specific indicator of fecal pollution. Elevated concentrations of these bacteria indicate an increased potential for

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pathogen occurrence and associated enteric diseases (Rophi, 2022; Febriana & Putri, 2024). Furthermore, changes in physicochemical and biological parameters can disrupt river ecosystem balance and contribute to declining water quality (Fatimah, 2020). Consequently, integrated microbiological and ecological assessments are necessary to evaluate aquatic pollution and identify potential health risks (Zakaria & Trintyani, 2025).

In X City, domestic activities, agriculture, and livestock practices around riverbanks may contribute to fecal contamination and increase microbiological pollution in river water. This condition requires attention because river water may be used for various purposes, including domestic activities, fisheries, livestock production, and agriculture. Government Regulation of the Republic of Indonesia No. 22 of 2021, Appendix VI, establishes a maximum fecal coliform concentration of 1,000 MPN/100 mL for river water, providing a regulatory reference for evaluating microbiological water quality (Pemerintah Republik Indonesia, 2021). Therefore, determining fecal coliform concentrations in rivers in X City is important for identifying contamination levels and assessing compliance with the applicable water quality standard.

The Most Probable Number (MPN) method is commonly used to estimate coliform concentrations in water through serial dilution, incubation in liquid culture media, and interpretation of positive reactions using standard statistical tables (Jiwintarum & Agrijanti, 2017; Sianipar et al., 2020). The method offers sensitivity for detecting relatively low bacterial concentrations, allows the use of different selective media, and can improve estimation reliability through appropriate replication (Sabila & Setyaningrum, 2023). Coliform analysis using this approach generally includes a presumptive test, a confirmed test using Brilliant Green Lactose Bile (BGLB) broth, and a completed test to further establish the presence of coliform bacteria (Misrofah & Purwantisari, 2021). Although the MPN method is established for microbiological water analysis, location-specific information is needed to characterize fecal coliform contamination in river water across X City and determine its compliance with the applicable standard.

Accordingly, this study aims to analyze fecal coliform contamination in river water in X City using the MPN method and evaluate the results against the water quality standard stipulated in Government Regulation No. 22 of 2021. The novelty of this study lies in providing location-specific data on fecal coliform concentrations in the investigated river water samples and comparing the findings with the applicable regulatory threshold. These results are expected to contribute to microbiological water quality monitoring, identify potential fecal pollution concerns, and support evidence-based river water management and pollution control in X City.

## Materials and Methods

The materials utilized comprised Lauryl Tryptose Broth (LTB), Brilliant Green Lactose Bile (BGLB) broth, EC Broth, and river water samples. The instrumentation employed included an analytical balance, an autoclave, incubators, a water bath, and a Laminar Air Flow Cabinet (LAFC), sterile glass sampling bottles with a capacity of 250 mL, a cool box, thermometer, and laboratory glassware.

River water samples were collected in X City, West Sumatra, Indonesia, on February 5, 2026. Sampling locations were selected using purposive sampling based on the study objectives and the potential influence of anthropogenic activities on river water quality. Four river water samples were collected from upstream and downstream sampling points. Samples A and D represented downstream locations, whereas samples B and C represented upstream locations. The sampling design was intended to assess differences in fecal coliform concentrations between upstream and downstream river sections.

Sampling was conducted using the grab sampling technique at a depth of approximately 20 cm below the water surface, following the applicable procedures in SNI 9063:2022. Each sample was collected in a sterile glass bottle with a capacity of 250 mL. The bottles were labeled individually and placed in a cool box immediately after collection.

The samples were transported to the laboratory in a cool box maintained at a temperature below 10°C without freezing.

Sampling time, laboratory receipt time, transportation temperature, and analysis commencement time to determine the sample holding time.

Growth media preparation was performed strictly in accordance with the manufacturer's instructions. Lauryl Tryptose Broth (LTB; 35.6 g/L, pH 6.8 ± 0.2), Brilliant Green Lactose Bile (BGLB) broth (40 g/L, pH 7.2 ± 0.2), and EC Broth (37 g/L, pH 6.9 ± 0.2) were dissolved in distilled water. The prepared solutions were then dispensed into fermentation tubes equipped with inverted Durham tubes. Subsequently, all media underwent thermal sterilization using an autoclave at 121°C for 15 minutes.

River water sampling was carried out in accordance with the Indonesian National Standard (SNI) 9063:2022. Fecal coliform concentrations in river water samples were determined using the Most Probable Number (MPN) multiple-tube fermentation method. Four samples, designated A, B, C, and D, were analyzed. Samples A and D were collected from downstream locations, whereas samples B and C were collected from upstream locations. Serial dilutions of ($10^{-1}$) ($10^{-2}$) and ($10^{-3}$) were prepared for each sample. A maximum analytical volume of 1 mL was inoculated into the respective culture tubes according to the laboratory procedure.

*Fecal coliform* quantification was performed using the Most Probable Number (MPN) method, which comprises a presumptive phase and a confirmed phase. In the presumptive phase, the water samples were inoculated into *Lauryl Tryptose Broth* (LTB) using a multiple-tube fermentation series of 10 mL, 1 mL, and 0.1 mL, followed by incubation at 35 ± 0.5°C for 24 to 48 hours. The occurrence of gas production within the inverted Durham tubes indicated a presumptive positive result. Subsequently, aliquots from the positive tubes were transferred into Brilliant Green Lactose Bile (BGLB) broth for total coliform detection (incubated at 35 ± 0.5°C) and into EC Broth for fecal coliform confirmation (incubated at 44.5 ± 0.2°C) for a duration of 24 hours.

The observed positive tube combinations for samples A, B, C, and D were 5-5-1, 5-3-2, 5-5-2, and 5-5-3, respectively. Based on the recorded observations, the *fecal coliform* concentrations were 35,000, 14,000, 54,000, and 92,000 MPN/100 mL, respectively. The MPN values were interpreted using the appropriate statistical table corresponding to the analytical configuration and dilution scheme. The results were compared with the maximum fecal coliform standard of 1,000 MPN/100 mL stipulated in Government Regulation of the Republic of Indonesia No. 22 of 2021, Appendix VI. The MPN index was determined based on the specific combination of positive tubes referenced against standard MPN statistical tables, with the final values expressed in units of MPN/100 mL.

## Result and Discussion

Microbiological quality testing of river water was conducted using the Most Probable Number (MPN) method to determine the number of fecal coliform bacteria at four sampling points, namely A, B, C, and D. All samples were analyzed using dilution series of $10^{-1}$, $10^{-2}$, and $10^{-3}$ with a maximum analyte volume of 1 mL.

Based on the results of the positive tube combinations read during the confirmation stage, there was a significant variation in the number of fecal coliforms among the sampling points. The variation in positive tube combinations at each sampling point indicates differences in contamination levels among the sampling locations.

When compared with the fecal coliform criterion for river water (1,000 MPN/100 mL) specified in Appendix VI of Indonesian Government Regulation No. 22 of 2021, all four samples exceeded the reference value. The recorded concentrations were 35,000 MPN/100 mL in sample A, 14,000 MPN/100 mL in sample B, 54,000 MPN/100 mL in sample C, and 92,000 MPN/100 mL in sample D. These values were 35, 14, 54, and 92 times the Class II criterion, respectively. The highest concentration was observed in sample D, collected from a downstream location, whereas the lowest was recorded in sample B, collected from an upstream location.

Although the downstream samples had a higher mean concentration than the upstream samples, the results do not establish a consistent upstream-to-downstream gradient across all sampling locations. Furthermore, the

classification of these results as exceeding the legally applicable water quality standard requires confirmation that Class II is the designated classification or intended use of the assessed water body. Accordingly, the findings demonstrate elevated fecal coliform concentrations relative to the Class II reference criterion and indicate the need for further microbiological monitoring and assessment of potential fecal pollution sources (Government of the Republic of Indonesia, 2021).

Quantitatively, the highest fecal coliform concentration was recorded in sample D (92,000 MPN/100 mL), followed by sample C (54,000 MPN/100 mL), sample A (35,000 MPN/100 mL), and sample B (14,000 MPN/100 mL). Samples D and A were collected from downstream locations, whereas samples C and B were collected from upstream locations. Although the downstream samples exhibited higher concentrations than the upstream samples in this dataset, the limited number of sampling points and the absence of temporal replication do not support a definitive conclusion regarding an upstream-to-downstream contamination gradient.

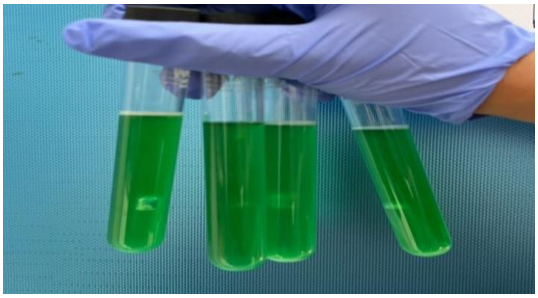


**Figure 1.** Assay results of river water samples inoculated into *Brilliant Green Lactose Bile* (BGLB) broth.

Domestic activities, livestock practices, and other anthropogenic sources may contribute to fecal contamination; however, their individual contributions cannot be established without supporting evidence, such as land-use observations, flow-direction mapping, or direct assessment of pollution sources. Therefore, the observed differences should be interpreted as spatial variations in fecal coliform concentrations among the four sampling points, highlighting the need for further investigation and repeated sampling to determine contamination patterns and potential sources.



**Figure 2.** Confirmatory assay results of river water samples in EC Broth for thermotolerant (fecal) coliform confirmation.

**Table 1.** Results of fecal coliform analysis in river water samples.

| Sample | Dilution | Maximum analytical volume (mL) | Positive tube combination | Fecal coliform (MPN/100 mL) | Environmental quality standard (MPN/100 mL) |
|---|---|---|---|---|---|
| A | $10^{-1}$<br>$10^{-2}$<br>$10^{-3}$ | 1 | 5-5-1 | 35,000 | 1,000 |
| B | $10^{-1}$<br>$10^{-2}$<br>$10^{-3}$ | 1 | 5-3-2 | 14,000 | 1,000 |
| C | $10^{-1}$<br>$10^{-2}$<br>$10^{-3}$ | 1 | 5-5-2 | 54,000 | 1,000 |
| D | $10^{-1}$<br>$10^{-2}$<br>$10^{-3}$ | 1 | 5-5-3 | 92,000 | 1,000 |

These results indicate that the river water body at the study site has experienced significant fecal contamination. The results of microbiological water quality testing showed that all samples from the two tested locations were microbiologically contaminated based on the fecal coliform parameter According to the results of the analysis at showed that the highest concentrations were found at locations D and C, with fecal coliform counts of 92,000 MPN/100 mL and 54,000 MPN/100 mL, respectively. In contrast, at locations A and B, the values were 35,000 MPN/100 mL and 14,000 MPN/100 mL, respectively, with concentrations decreasing downstream along the river's flow.

The presence of these indicator bacteria reflects fecal contamination originating from human or animal activity in the vicinity of the watershed. Fecal coliforms are widely used as an indicator of sanitation because this group of bacteria originates from the digestive tracts of humans and warm-blooded animals; therefore, their presence in water bodies indicates fecal contamination that may carry pathogenic microorganisms (Puspitasari et al., 2016).

According to Santoso (2023), an increase in coliform concentrations in water bodies generally correlates with high levels of domestic activity and low effectiveness of wastewater management systems. Methodologically, the use of the Most Probable Number (MPN) method in this study provides a quantitative estimate of indicator bacteria density through a probability-based statistical approach. This method is widely used in water quality analysis because it can detect gas producing fermentative bacteria on selective media and is effective for identifying thermotolerant groups of fecal coliforms through high-temperature incubation (Sianipar et al., 2020).

The MPN method used in this study is a classical statistical method that is still widely used in various environmental laboratories. This method provides a quantitative estimate of the number of coliforms based on the results of lactose fermentation by bacteria, as indicated by gas formation in Durham tubes (Anggraeni, S., & Irdawati, 2025).

The varying distribution of contamination levels among sampling points indicates the presence of spatial effects in lotic water systems. Rivers, as flowing bodies of water, have dynamic characteristics, so bacterial concentrations can be influenced by dilution processes (dilution). sedimentation of organic particles, exposure to solar radiation, and interactions with indigenous microorganisms. However, these natural processes are often insufficient to reduce bacterial concentrations below the threshold when the inflow of pollutants continues uninterrupted (Sianipar et al., 2020).

High levels of fecal coliforms in river water have significant implications for public health. Fecal-contaminated water has the potential to serve as a transmission medium for waterborne diseases, such as diarrhea, typhoid, and gastrointestinal infections, other digestive systems. Puspitasari et al. (2016) state that an increase in the number of indicator bacteria statistically positively correlated with the likelihood of the presence of enteric pathogenic bacteria. Therefore, the presence of high levels of fecal coliforms should be viewed as an early warning sign of potential epidemiological risks.

Santoso (2023) reported that rivers subject to high levels of human activity tend to exhibit significant coliform levels throughout the year. particularly in areas with inadequate wastewater management systems. Water quality testing was conducted using the MPN (Most Probable Number) method for coliforms, including a preliminary test using LTB medium and a confirmatory test using BGLB medium. In addition to testing for total coliforms, fecal coliforms were also tested using EC.Broth medium.

River water quality status indicates the level of pollution in a water source at a given time, compared to established water quality standards. A river is considered polluted if it cannot be used for its intended purpose under normal conditions. The determination of water quality status is based on Ministry of Environment and Forestry Regulation No. 27 of 2021 on the Environmental Quality Index; water quality status is determined using the Pollution Index. The Pollution Index is a numerical value used to determine the level of pollution relative to permitted water quality parameters. The function of the index is to identify the impact of pollutant concentrations on surface water quality conditions. This water quality status is taken into consideration in the utilization of surface water

resources and management efforts (Novita et al., 2020).

Rivers, as lotic water bodies, are characterized by dynamic flow, so water quality is greatly influenced by conditions upstream, activities along the watershed, and the physical and chemical properties of the water. Water pollution is the excessive contamination of water with substances that can cause harm to humans and/or ecosystems. The level of water pollution depends on the abundance of pollutants, the ecology of the pollutants, and water usage. Pollutants originate from chemical, biological, or physical sources resulting from human activities (Pooja et al., 2020).

According to Kospa et al. (2019), approximately 60 to 70% of river pollution is caused by domestic wastewater, while only 6.1% of wastewater is treated. Although river pollution from industrial wastewater has decreased by 40%, the high contribution of domestic wastewater means that rivers remain polluted. Broadly speaking, domestic waste is divided into two categories: organic waste and inorganic waste.

Sewage systems are one indicator of urban river pollution; poorly regulated sewage systems result in many sewage outlets discharging wastewater into rivers (Xu et al., 2019).

According to a study by Syafri et al. (2020), the behavior of communities living near watersheds (DAS) contributes to a decline in environmental quality and water pollution. Organic waste comes from feces, vegetable scraps, and food waste, while inorganic waste can include plastic, paper, and chemicals resulting from the use of detergents, shampoo, soap, and other chemical products. In areas without domestic waste management systems, waste is generally disposed of directly into the environment, particularly water bodies (rivers and lakes) where it is then carried downstream and deposited along the watercourse (Kholif, 2020).

Positive LTB media samples were not only sent for confirmatory testing but also for Fecal Coli testing, by taking one loopful of the positive coliform culture and inoculating it onto EC.Broth medium. After inoculation, the media are incubated in an incubator at 45°C. According to Mukhlis (2019), for non-fecal coliforms, incubation is performed at 35°C, as the bacteria row optimally at that temperature, while fecal coliforms can still survive at 44°C.

According to Amprin (2020), microbial water contamination can occur in agricultural areas. Sources of water contamination include fecal bacteria, stormwater runoff, and waste. In densely populated residential areas, where houses are very close together and landfills and septic tanks tend to be located near water sources, the habit of residents living along rivers to dispose of trash into the river can lead to contamination by coliform bacteria. From a public health perspective, water with high levels of fecal coliforms is unsuitable for domestic use without prior treatment. Using contaminated water for bathing, washing, or even indirect consumption can increase the risk of exposure to pathogens. Therefore, it is necessary to interventions include improving sanitation systems and constructing communal wastewater treatment plants (WWTPs), and educating the public on the importance of maintaining environmental cleanliness.

Irdawati et al. (2012) noted that heavy rainfall can affect the presence of coliform bacteria in a body of water. This is because rainwater seeps into the soil, which affects the movement of coliform bacteria. Flooding exacerbates inadequate sanitation systems, making the community vulnerable to waterborne infectious diseases, such as diarrhea and others.

An increase in fecal coliform concentration may be caused by seasonal changes; specifically, during the dry season, river temperatures rise, which can lead to an increase in the concentration of total coliforms in the river. Having a fecal coliform concentration the low contamination levels are influenced by the fact that the area is still sparsely populated. and the distance between homes and the river is considerable, resulting in less contamination from domestic wastewater on land. If a river water sample tests positive, it means it contains coliform bacteria. This is indicate by all Durham tubes showing gas bubbles and the water sample becoming cloudy; the formation of gas bubbles in the Durham tubes and the color change in the medium indicate that lactose fermentation has occurred in the liquid medium as a result of microbial activity in the sample, thereby producing gas (Hidayat et al.,2020).

In the coliform confirmation test, LTB media that test positive are then subjected to an E coli (fecal coliform) test using *Escherichia coli Broth* (ECB) media. ECB media, which contains lactose, enables the detection of fecal coliforms because fecal bacteria can ferment lactose into acid and gas, which appear as bubbles in a Durham tube. Incubation is carried out at 45°C ± 0.5 for 24 hours to optirize the growth of fecal coliform bacteria; this temperature is suitable for fecal coliform bacteria, which can survive at this temperature, whereas non-fecal coliform bacteria grow better at 35°C (Hadiansyah et al., 2021).

## Conclusion

The analysis of river water samples collected in X City on February 5, 2026, revealed fecal coliform concentrations of 35,000 MPN/100 mL in sample A, 14,000 MPN/100 mL in sample B, 54,000 MPN/100 mL in sample C, and 92,000 MPN/100 mL in sample D. The highest concentration was recorded in sample D, whereas the lowest was observed in sample B. These results demonstrate variations in fecal coliform concentrations among the four sampling points on the sampling date. The findings provide location-specific information on the microbiological characteristics of the analyzed river water samples. However, because the study involved a single sampling occasion and four samples, the results do not establish temporal trends or identify definitive contamination sources. Further studies involving repeated sampling and additional environmental observations are recommended to provide a more comprehensive understanding of fecal coliform distribution in river water in X City.

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