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RESEARCH ARTICLE

IN SITU WATER QUALITY ASSESSMENT AND COLIFORM CLASSIFICATION IN BATANG LAYAR, SARAWAK

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ABSTRACT

The Batang Layar river in Sarawak, Malaysia, is a vital water resource for local communities, supporting aquaculture, agriculture, transportation, recreation, and drinking water supply. Despite its significance, the river's water quality remains understudied, raising public health concerns due to potential contamination from anthropogenic activities. This research evaluates water quality of the river at four (4) sites (Nanga Tiga, Spak, Lubau, and New Layar Bridge) through in-situ physicochemical analysis and microbial profiling, focusing on coliform bacteria as pollution indicators. Results show that faecal coliform counts (FCC) and total coliform count (TCC) at these sites are classified under Class III of Malaysia's National Water Quality Standard (NWQS), indicating significant contamination and requiring substantial treatment. Notably, The New Layar Bridge site showed highest contamination levels (FCC: 817 CFU/100 mL; TCC: 12,517 CFU/100 mL), suggesting anthropogenic impacts from domestic waste or urban runoff. Molecular techniques via (GTG)5 PCR fingerprinting and 16S rRNA sequencing identified ten bacterial genera, with Acinetobacter (28.57%), Chromobacterium (14.29%), and Escherichia (9.52%) being the most prevalent genera, commonly associated with faecal contamination. This study highlights the urgent need for effective sustainable management strategies, including proper waste disposal practices and regular monitoring, to preserve the rivers in the study area.

KEYWORDS

river conservation, bacterial isolation, PCR fingerprinting, public health, anthropogenic pollution

1. INTRODUCTION

Access to clean water is a fundamental human right and crucial for sustainable living. As stated that the global water crisis continue to intensify with approximately 40% of the world's population experiencing water scarcity, creating water-stressed environments (Guppy and Anderson, 2017). This crisis is further aggravated by inadequate sanitation and contaminated water sources, particularly in developing regions, where microbial contamination often remains unmonitored. These contaminated water sources serve as vectors for waterborne diseases such as cholera and typhoid, posing significant public health risks and contributing to preventable illnesses and fatalities (WHO, 2023).

Malaysia faces similar challenges related to waterborne diseases due to deficiencies in environmental sanitation, improper wastewater disposal, inadequate water supply, and poor personal hygiene (Ho, et al., 2022; Manetu, and Karanja, 2021; Ostadtaghizadeh, et al., 2022). In the Betong region of Sarawak, communities along the Batang Layar river depend heavily on the river for drinking, fishing, irrigation and other uses. However, anthropogenic activities, including domestic sewage discharge, agricultural runoff, and industrial effluents, threaten the sustainability of this vital water resource. Consequently, these pollutants degrade water quality and pose health risks to local populations that lack access to alternative water sources or adequate treatment facilities.

Current water quality assessment in Malaysia relies on the Water Quality Index (WQI), which evaluates six physicochemical parameters: pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), ammoniacal nitrogen (NH_3-N), total suspended solids (TSS), and dissolved oxygen (DO), as outlined by the National Water Quality Standards (NWQS). Notably, the WQI does not incorporate critical biological indicators such as faecal coliform count (FCC) and total coliform count (TCC), which are crucial for assessing waterborne disease risks, despite the NWQS having established safety thresholds for these indicators by Department of Environment (DOE, 2025). This exclusion underscores a significant gap in evaluating microbial risks, particularly in socio-economically vulnerable areas like Sarawak, where river water is extensively used.

While previous researches has examined either from water samples or physicochemical parameters separately, few have integrated comprehensive water quality assessment with molecular identification of coliform bacteria (Abu-Sini et al., 2023; Leong et al., 2018; Rahman et al., 2022; Some et al., 2021). Hence, this study bridges this gap by combining *in-situ* water quality measurements with advanced molecular techniques, including $(GTG)_5$ PCR fingerprinting technique and 16S rRNA sequencing to provide a more comprehensive assessment of contamination risks

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(Johnson, et al., 2019; Sait, et al., 2023).

Our research provides multiple benefits by detecting potential pathogens and establishing connections between land-use activities and contamination profiles. The study offers critical baseline data for the Betong river system while expanding biological criteria in the water quality assessment, aligning with the 2030 Agenda for Sustainable Development's Goals of ensuring the safeguarding of water resources through monitoring and management (United Nations, 2023). These findings mark a significant step toward safeguarding both public health and aquatic ecosystems in tropical watersheds.

2. Materials And Methods

2.1 Study Area

Water quality assessment was conducted at four sites within Batang Layar: Nanga Tiga, Spak, Lubau, and New Layar Bridge (Figure 1). Sampling sites were chosen based on their locations in the main river's upstream, midstream, and downstream sections. These sites have different land uses along the river and within their respective catchment areas (Table 1). Each site's Global Positioning System (GPS) was recorded for accurate spatial reference (Table 1).

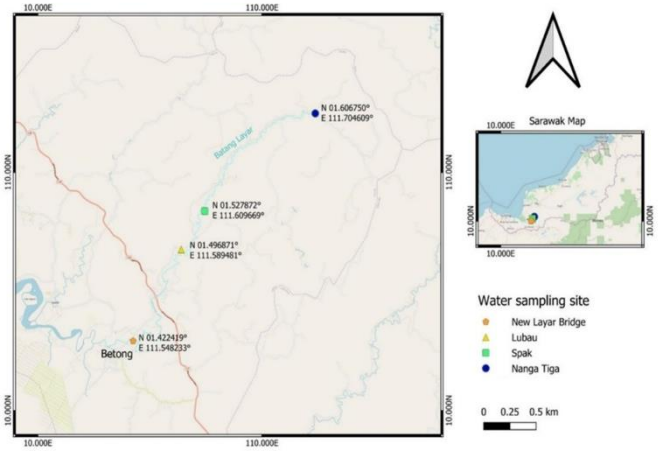

Figure 1: The localities of sampling sites (QGIS, 2023).

Table 1: GPS Coordinates and Land Use Descriptions of Four Sites in Batang Layar.

Site	Name	GPS	Description
Site 1	Nanga Tiga	N 01.606750° E 111.704609°	Upstream, Recreational, Fish rearing
Site 2	Spak	N 01.527872° E 111.609669°	Midstream, Recreational, Fish rearing, School
Site 3	Lubau	N 01.496871° E 111.589481°	Midstream, Water Treatment Plant, agricultural farm
Site 4	New Layar Bridge	N 01.422419° E 111.548233°	Downstream, flows toward the South China Sea, Industrial area, nearby construction site and factory

2.2 *In-situ* Parameters

At each sampling site, six *in-situ* physicochemical parameters, namely temperature, dissolved oxygen (DO), conductivity, total dissolved solids (TDS), pH, and turbidity, were evaluated using a YSI Multiparameter Digital Water Quality Meter. To ensure the reliability of the data, ten duplicate readings for each parameter were taken at every location. The mean values (n=10) were calculated using the Statistical Package for Social Sciences (SPSS) version 24. Table 2 outlines Malaysia's National Water Quality Standards (NWQS) for a comprehensive classification of water quality (DOE, 2025).

Table 2: NWQS for Malaysia [6]

Parameter	Unit	Class					
		I	IIA	IIB	III	IV	V
Ammoniacal Nitrogen	mg/l	0.1	0.3	0.3	0.9	2.7	>2.7
Biochemical Oxygen Demand	mg/l	1	3	3	6	12	>12
Chemical Oxygen Demand	mg/l	10	25	25	50	100	>100
Dissolved Oxygen	mg/l	7	5-7	5-7	3-5	<3	<1
pH	-	6.5-8.5	6-9	6-9	5-9	5-9	-
Colour	TCU	15	150	150	-	-	-
Electrical Conductivity*	µS/cm	1000	1000	-	-	6000	-
Floatables	-	N	N	N	-	-	-
Odour	-	N	N	N	-	-	-
Salinity	%	0.5	1	-	-	2	-
Taste	-	N	N	N	-	-	-
Total Dissolved Solid	mg/l	500	1000	-	-	4000	-
Total Suspended Solid	mg/l	25	50	50	150	300	300
Temperature	◦ C	-	Normal + 2 ◦ C	-	Normal + 2 ◦ C	-	-
Turbidity	NTU	5	50	50	-	-	-
Faecal Coliform**	count/100 mL	10	100	400	5000 (20000)[a]	5000 (20000)[a]	-
Total Coliform	count/100 mL	100	5000	5000	50000	50000	>5000

Notes:

N: No visible floatable materials or debris, no objectional odour or taste

*: Related parameters, only one recommended for use

**: Geometric mean

[a]: Maximum not to be exceeded

2.3 Coliform analysis and bacterial isolation

Although the faecal coliform count (FCC) and total coliform count (TCC) are not part of Malaysia's Water Quality Index (WQI), they are essential biological indicators of waterborne pathogens with NWQS safety

threshold set at a maximum of 5000 CFU/100 mL for FCC and 50000 CFU/100 mL for TCC (DOE, 2025). This study bridges this gap by integrating *in-situ* assessment with microbial analysis as a preliminary investigation into the river's safety.

For bacterial isolation, 2 mL of each water sample was filtered through a 0.45 µm cellulose ester membrane filter (Whatman™). The filter paper was then placed on HiChrome Coliform agar and incubated at 37 °C for 24 hours (Martha, et al., 2016). FCC was determined by counting blue colonies, while TCC was determined by including all colonies (blue and non-blue). The colony-forming units (CFU/100 mL) were calculated using the formula by (Foster and Pinedo, 2015):

$$\frac{\text{CFU}}{100\ \text{mL}} = \left(\frac{\text{number of colonies}}{\text{sample volume filtered in mL}}\right) \times 100$$

Approximately three to five distinct colonies were randomly selected and cultured on Tryptic Soy Agar (TSA) using the streak-plate method, followed by incubation at 37 °C to obtain pure isolates for downstream molecular analysis (Sait, et al., 2023).

2.4 DNA Extraction

DNA from thirty-two pure isolates was extracted using the direct colony method with slight modifications from methods (Bergkessel and Guthrie, 2013). A single pure colony was mixed with 40 µL of sterile ultrapure water (UPW) in 0.2 mL microcentrifuge tubes. The mixture was heated at 95 °C for 10 minutes and then cooled at 4 °C for 5 minutes using a LabCycler (SensoQuest GmbH, Germany). The mixture was centrifuged (Wisespin, UK) at 13,500 rpm for 1 minute. The supernatant was transferred to a sterile tube and stored at -20 °C for subsequent PCR analysis (Al-Griw, et al., 2017).

2.5 $(GTG)_5$ PCR fingerprinting analysis

$(GTG)_5$ PCR fingerprinting was conducted with modifications methods from (Sait et al., 2023). The 40 µL PCR master mix included 4 µL 5× Taq Green®Flexi Buffer (Promega, USA), 4 µL 25 mM Magnesium Chloride ($MgCl_2$), 0.3 µL 25 mM Deoxyribonucleotide Phosphate (dNTPs), 2 µL 25 µM $(GTG)_5$ primer (5'- GTGGTGGTGGTGGTG-3'), 27.4 µL nuclease-free water, 0.3 µL GoTaq® Flexi DNA polymerase, and 2 µL DNA template. The amplification cycle consisted of pre-denaturation at 95 °C for 2 minutes, followed by 30 amplification cycles at 95 °C for 1 minute, 50 °C for 1 minute, 72 °C for 5 minutes, and a final elongation step at 72 °C for 5 minutes (SensoQuest GmbH, Germany). The amplified PCR products were electrophoresed on a 1% (w/v) agarose gel at 80 V for 40 minutes, with a 1 kb DNA ladder (Promega, USA) used as the DNA marker. The agarose gel was post-stained with SYBR Green dye for 30 minutes and visualised using the UV Transilluminator GelDoc Go Gel Imaging System (NuGenius, Syngene). Scoring was conducted using GelJ software (version 2.0) to construct a dendrogram, wherein isolates with a similarity index of 80% were clustered together. These clustered isolates represent similar strains, and only representatives from each cluster proceeded to 16S rRNA sequencing to minimise redundancy.

2.6 16S rRNA Sequencing

16S rRNA PCR analysis was performed following the methodologies of Sait et al. (2023) [12], using primers 27F (5'- CAGGCCTAACACATGCAAGTC -3') and 519R (5'- GWATTACCGCGGKGCTG- 3'). The PCR amplification was performed in a standard 40 µL reaction mixture, comprising 4 µL 5× Green Go Taq® Flexi Buffer, 4 µL 25 mM $MgCl_2$, 0.3 µL 10 mM dNTPs, 2 µL each of 27F and 519 primers, 23.4 µL ultrapure water (UPW), and 0.3 µL Go Taq® Flexi DNA Polymerase, with 4 µL DNA template. The amplification cycle included an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 30 seconds, and extension at 72 °C for 1.5 minutes, with a final extension at 72 °C for 10 minutes. The amplified PCR products were electrophoresed on a 1.0% (w/v) agarose gel for 40 minutes at 80 V, with a 100 bp DNA ladder (Promega, USA) serving as the DNA marker. The agarose gel was post-stained with SYBR Green dye for 30 minutes and visualised as described. The amplified DNA products were outsourced to FirstBase Sdn. Bhd. for DNA purification and Sanger sequencing. Sequencing data were analysed using the Basic Local Alignment Search Tool (BLASTn) against the nucleotide database of the National Centre for Biotechnology Information (NCBI) for bacterial classification.

3. Results And Discussion

3.1 *In-situ* parameters

The mean value of the Batang Layar *in-situ* assessment at the four sampling sites are presented in Table 3.

Table 3: Average In-situ parameters of Batang Layar at each site

Parameter	Nanga Tiga	Spak	Lubau	New Layar Bridge
Temperature (°C)	25.28	25.34	25.55	27.11*
Dissolved Oxygen (mg/L)	7.83	7.85*	7.80	7.08
Conductivity (µS/cm)	17.80	17.49	17.40	21.04*
Total Dissolved Solids (mg/L)	11.26	11.29	11.18	13.15*
pH	6.54	6.48	6.67*	6.52
Turbidity (NTU)	1.28	9.63	11.66	25.11*
*: Highest value				

The findings indicate that all parameters exhibit statistically significant differences ($p<0.05$) within each site. The New Layar Bridge site recorded the highest mean water temperature (27.11 °C), likely due to its proximity to an industrial area. The elevated temperature levels at the New Layar Bridge site were possibly attributed to extensive industrial pollution, leading to thermal pollution that could impact aquatic ecosystems by facilitating the formation of harmful organic matter in rivers (Vallero, 2019). Dissolved Oxygen (DO) concentrations correlate with water temperature, with the New Layar Bridge site exhibiting the lowest DO (7.08 mg/L), which may hinder the growth of aquatic organisms (Speight, 2020). In terms of conductivity and total dissolved solids (TDS), the New Layar Bridge site showed the highest values. Notably, all sites fell within the acceptable conductivity range (0-200 µS/cm). Aquatic species generally require a specific range of water conductivity for survival. These findings align, which states that higher conductivity values may indicate elevated amounts of TDS in water (Bwire et al., 2020). Deviations in pH from the neutral range (pH 7) can affect aquatic life, potentially enhancing heavy metal solubility or resulting in elevated ammonia levels (Saalidong, et al., 2022). Although Spak recorded the lowest pH (6.48), it is still considered safe, as the observed pH values (around pH 6) suggest the suitability of the water for aquatic life conservation. At the New Layar Bridge, the turbidity level reached 25.11 NTU (Nephelometric Turbidity Units), suggesting potential industrial contamination. The rapid flow rate in industrial regions may have contributed to the discharge of suspended solid due to anthropogenic activities, aligning with the findings from (Ling et al., 2017).

3.2 Coliform count

As reported that faecal coliforms serve as critical indicators of faecal pollution in water catchments, posing an environmental concern for human health (Zhang et al., 2021). In this study, FCC ranged from 550 to 817 CFU/100 mL, surpassing the safe threshold of 400 CFU/100 mL for recreational waters (DOE, 2025). TCC recorded in this study varied from 4,800 to 12,517 CFU/100 mL, with the New Layar Bridge sites recording the highest TCC at 12,517 CFU/100 mL. These findings indicated contamination from upstream sources and within the catchment, including household wastewater runoff, latrines, animal discharge, and untreated wastewater from nearby anthropogenic activities (Digaletos, et al., 2023). Despite its distance from urban pollution hotspots, Nanga Tiga, a rural upstream site, recorded the highest FCC (817 CFU/100 mL), possibly due to frequent recreational activities and fish rearing in the area. This finding aligned, which indicated that recreational activities could increase the detection of *Escherichia coli* (Sanchez et al., 2021). The impact of fish rearing through the *tagang system* may result from leftover feed, which introduces excess nutrients into the water, eventually transforming water bodies into bacterial reservoirs (Raza, et al., 2025). Significant concentrations of faecal coliforms in the river suggest the presence of opportunistic pathogenic bacteria that could pose risks to public health, particularly for communities that closely engage with the river for bathing or irrigation.

3.3 Dendrogram and genetic analysis

The dual-method approach in this study, combining the $(GTG)_5$ PCR fingerprinting with 16S rRNA sequencing, enabled efficient strain grouping and precise taxonomic identification (Sait, et al., 2023; Kathleen, et al., 2014). The fingerprint technique reduced redundant sequencing efforts by efficiently grouping representative strains through the clustering of isolates at 80% similarity, followed by 16S rRNA sequencing to provide genus/species-level identification of coliform bacteria, which is crucial for assessing potential health risks associated with waterborne pathogens in contaminated water sources (Sait, et al., 2023; Hamdi, et al., 2023; Lihan, et al., 2020). This methodology addresses the gap in sufficiently addressing the impacts of socio-economic development on waterborne pathogens, particularly in Sarawak rivers, such as Batang Layar, despite the heavy reliance of rural communities on river water.

The banding profiles of 32 coliform bacterial isolates from the Batang Layar are shown in Figure 2. $(GTG)_5$ PCR fingerprinting of 32 isolates was organised into 21 clusters with a similarity index of 80%. The dendrogram was constructed based on the Dice similarity method with a tolerance value of 5.0 through UPGMA linkage of $(GTG)_5$ PCR fingerprinting. The isolates clustered within the same genetic branch, with some isolates forming groups closely, indicating a shared ecological niche. Clusters 17 and 19 were the largest groups of isolates, comprising three isolates each. This preliminary grouping by $(GTG)_5$ PCR fingerprinting in this study provides a rough estimation of genetic similarities among bacterial isolates, streamlining subsequent 16S rRNA sequencing efforts (Sait, et al., 2023; Kathleen, et al., 2014; Lihan, et al., 2020).

Upon generating a dendrogram through $(GTG)_5$ PCR fingerprinting analysis, 21 representative isolates were selected for 16S rRNA sequencing analysis. Table 4 presents the classification of these selected isolates, each exhibiting a similarity level of at least 97%, thereby meeting the threshold for clustering of unknown strains (Sait, et al., 2023; Srinivasan, et al., 2015). *Acinetobacter* sp. was the most prevalence, accounting for 21.88%, detected in 7 out of the 32 isolates. $(GTG)_5$-PCR method effectively facilitated the screening of unknown bacterial strains, revealing a distinct bacterial community isolated from Batang Layar river.

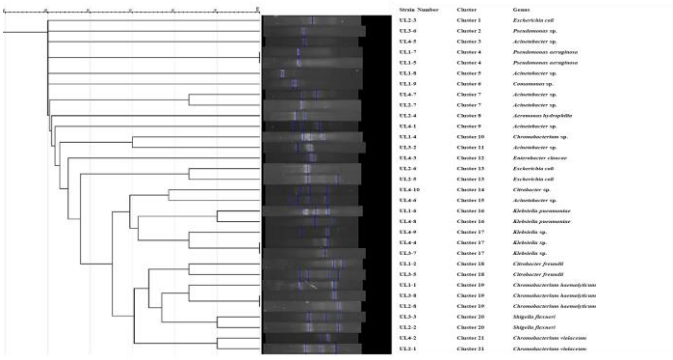

Figure 2: Dendrogram based on the Dice similarity method with a tolerance value of 5.0 using UPGMA linkage of $(GTG)_5$ PCR fingerprints.

Table 4: Bacterial isolates classification and prevalence with percentage similarity above 97%

Cluster	Isolate chosen for sequencing	Source	Genus	Accession Number (percentage similarity)	Prevalence percentage (%)
1	UL2-3	Spak	*Escherichia coli*	AY078065.1(99%)	3.13
2	UL3-6	Lubau	*Pseudomonas* sp.	MG674651.1(99%)	3.13
3	UL4-5	New Layar Bridge	*Acinetobacter* sp.	MK696218.1(99%)	3.13
4	UL1-7	Nanga Tiga	*Pseudomonas aeruginosa*	MT646431.1(100%)	6.25
5	UL1-8	Nanga Tiga	*Acinetobacter* sp.	OQ933252.1(99%)	3.13
6	UL1-9	Nanga Tiga	*Comamonas* sp.	MK571159.1(99%)	3.13
7	UL2-7	Spak	*Acinetobacter* sp.	MN437597.1(99%)	6.25
8	UL2-4	Spak	*Aeromonas hydrophilia*	MT416424.1(100%)	3.13
9	UL4-1	New Layar Bridge	*Acinetobacter* sp.	LC602846.1(99%)	3.13
10	UL1-4	Nanga Tiga	*Chromobacterium* sp.	MN122133.1(99%)	3.13
11	UL3-2	Lubau	*Acinetobacter* sp.	MN437597.1(99%)	3.13
12	UL4-3	New Layar Bridge	*Enterobacter cloacae*	KJ605845.1(99%)	3.13
13	UL2-6	Spak	*Escherichia coli*	AY078065.1(99%)	6.25
14	UL4-10	New Layar Bridge	*Citrobacter* sp.	MT415798.1(100%)	3.13
15	UL4-6	New Layar Bridge	*Acinetobacter* sp.	LC602846.1(99%)	3.13
16	UL4-8	New Layar Bridge	*Klebsiella pneumoniae*	OP136157.1(100%)	6.25
17	UL3-7	Lubau	*Klebsiella* sp.	JF322941.1(99%)	9.38
18	UL3-5	Lubau	*Citrobacter freundii*	MT470968.1(99%)	6.25
19	UL1-1	Nanga Tiga	*Chromobacterium haemolyticum*	MN691330.1(100%)	9.38
20	UL2-2	Spak	*Shigella flexneri*	MT604864.1(100%)	6.25
21	UL4-2	New Layar Bridge	*Chromobacterium violaceum*	MN880157.1(99%)	6.25

3.4 Spatial Variation and Potential Health Risks

Nanga Tiga, situated upstream of Batang Layar in the rural area, exhibited the highest bacterial diversity with six different genera, similar to Lubau, which had five genera, surpassing Spak and New Layar Bridge. This diversity may be influenced by land-use activities near the catchment, such as fish rearing, school areas, and agriculture. Effluents from schools and organic waste from agriculture or aquaculture contributed to the bacterial diversity in Nanga Tiga. Similarly, the Lubau catchment was in proximity to settlements that might have introduced domestic household waste into its catchment. Although no illness caused by *Pseudomonas* sp. has been reported in this area, the presence of *Pseudomonas aeruginosa* in

Nanga Tiga suggests a potential risk as a pathogenic bacterium that causes pneumonia (Elfadadny, et al., 2024).

Spak, also known as Spak Clearwater, is a popular recreational river among tourists and local communities. This study identified the presence of *E. coli* and *Shigella flexneri* in Spak, both are known to induce diarrhea (Afum, et al., 2022). Previous reports on the presence of pathogenic Enterobacteriaceae in recreational rivers emphasise the potential health threats associated with recreational activities (Lihan et al., 2017). Consequently, vulnerable populations, including children, the elderly, and immunocompromised individuals, are at heightened risk of waterborne diseases (Sanborn, and Takaro, 2013).

The New Layar Bridge, located in an industrial urban area, is subject to pollution from factory effluent discharge, agricultural runoff, and waste dumping, resulting in water quality that surpasses river standards (Habibu, et al., 2025). Waterborne pathogens such as *Enterobacter cloacae* and *Klebsiella pneumoniae* were detected at the New Layar Bridge site. Both bacteria can cause a rare occurrence of septic arthritis in healthy individuals, as mentioned, posing a health risk to those exposed to contaminated water (Zandi et al., 2022). These findings highlighted the necessity for water quality monitoring and public health precautions, particularly in high-risk areas.

4. Conclusion

This study determines the water quality condition of Batang Layar, Sarawak through a combined *in-situ* physicochemical analysis and molecular identification of coliform bacteria. Elevated FCC and TCC levels, particularly at industrial and recreational areas, indicate contamination from industrial, domestic, and agricultural sources. Nanga Tiga and Lubau exhibited 6 out of the 10 identified bacterial genera, underscoring the microbial genus variation within river ecosystems. The detection of opportunistic pathogenic bacteria, including *E. coli*, *S. flexneri*, *P. aeruginosa*, and *K. pneumoniae*, reflects significant public health risks, especially for the communities that live within the watershed that rely on the river for drinking, recreation and irrigation. This study provides a more comprehensive assessment framework by integrating biological indicators with traditional water quality metrics that addresses a critical gap in Malaysia's WQI. Future research should explore additional parameters (e.g, Biochemical Oxygen Demand (BOD), ammoniacal nitrogen), seasonal variations, and antimicrobial resistance (AMR) patterns for comprehensive risk assessment. The findings are vital for policymakers, environmental agencies, and local communities by offering insights to improve sanitation infrastructure, enforce pollution controls and enhance public awareness. Proactive implementing sustainable water management strategies based on these findings can protect environmental health, benefiting the well-being of Batang Layar local communities and ecosystems.

Conflict Of Interest Statement

The authors declare that they have no conflict of interest.

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