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Microbial Water Quality and Contamination Drivers in Ecotourism Freshwaters of Mt. Hamiguitan Range Wildlife Sanctuary

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ABSTRACT

Freshwater ecosystems in protected ecotourism areas are increasingly threatened by microbial contamination linked to human activities, with implications for ecosystem integrity and public health. This study assessed microbial water quality along the San Isidro and Governor Generoso ecotourism trails of Mt. Hamiguitan Range Wildlife Sanctuary, Philippines. It determined total coliform and *Escherichia coli* concentrations, characterized physicochemical and hydrological conditions, examined relationships with environmental parameters, and established baseline data using a field-based cross-sectional design. Water samples from eight stations collected in April–May 2026 were analyzed using Petrifilm™ *E. coli*/Coliform Count Plates, alongside in situ measurements and spatial, temporal, and correlation analyses. Total coliform concentrations ranged from 1.33–44.78 CFU/100 mL in April and 0.22–27.00 CFU/100 mL in May, remaining within DENR Class C standards. *E. coli* ranged from 0–1.33 CFU/100 mL in April and 0–4.89 CFU/100 mL in May, with the highest level recorded at Governor Generoso Jump-Off; Camp 3 was consistently elevated, while Camp 2 showed no detectable *E. coli*. Significant spatial variation was observed for total coliform (F = 16.92, p < 0.001) and *E. coli* (F = 8.47, p < .001). Microbial indicators were associated with physicochemical variables, particularly pH (r_s up to −0.606), and site conditions reflecting human activity. Findings confirm uneven microbial contamination driven by anthropogenic and environmental factors. The baseline data highlight the need for routine monitoring, improved sanitation, and site-specific visitor management to reduce contamination risks and support adaptive, evidence-based ecotourism governance in Mt. Hamiguitan under increasing visitor pressure.

Keywords: *Escherichia coli, Microbial water quality, Mt. Hamiguitan Range Wildlife Sanctuary, Petrifilm™, Total coliforms*

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INTRODUCTION

Freshwater ecosystems provide essential ecological, social, and economic services, including the provision of drinking water, support for biodiversity, recreation, agriculture, and ecotourism. However, microbial contamination remains one of the most significant threats to freshwater quality worldwide because of its direct implications for environmental and public health (McKee and Cruz, 2021; Cortez and Siringan, 2025). Fecal contamination of surface waters may introduce pathogenic microorganisms capable of causing waterborne diseases, including diarrhea, cholera, and other gastrointestinal infections (Onichandran et al., 2014; Gomes et al., 2024). Recent reports of diarrhea outbreaks linked to contaminated water sources in Davao Oriental further highlight the importance of monitoring microbial water quality in the region (Philippine News Agency, 2025; Esteban, 2025).

Escherichia coli and total coliforms are widely recognized as reliable indicators of fecal contamination and sanitary water quality (Momba et al., 2019; Tambi et al., 2023). Their presence in freshwater systems is commonly used to evaluate potential public health risks and compliance with environmental water quality standards (DENR-EMB, 2016; DENR-EMB, 2021). Studies in the Philippines have documented microbial contamination in various aquatic environments, including Laguna Lake (Abello et al., 2021; Malajacan et al., 2023), recreational waters in Palawan (Cayabo et al., 2021), urban rivers (Gorme et al., 2010), irrigation water sources (Garcia et al., 2015), and protected landscapes such as Mt. Matutum (Martin et al., 2018). Similarly, microbial assessments in ecotourism destinations have demonstrated that visitor activities and land-use changes can influence water quality and increase contamination risks (Abris et al., 2020; Butler et al., 2021; Bureros et al., 2022). Because recreational freshwater environments are directly utilized by visitors, microbial monitoring has become an important component of recreational water quality assessment and public health protection, particularly in ecotourism destinations where human exposure to untreated water may occur (McKee and Cruz, 2021; Gomes et al., 2024).

Microbial water quality is strongly influenced by environmental conditions. Physicochemical factors such as temperature, pH, conductivity, total dissolved solids, and oxidation-reduction potential affect bacterial survival, persistence, and transport within aquatic systems (Santana et al., 2022; Dewangan et al., 2023; Loucif and Chenchouni, 2024). Hydrological conditions, including streamflow and rainfall-driven runoff, further regulate microbial loading and distribution by transporting contaminants from surrounding landscapes into freshwater bodies (Weller et al., 2022; Safari et al., 2024). Consequently, integrated assessments that combine microbial indicators with physicochemical and hydrological parameters are essential for understanding contamination dynamics and supporting effective environmental management. Studies conducted in freshwater ecosystems have further demonstrated that interactions among water quality characteristics, land use, and human activities can influence microbial occurrence and contamination patterns, emphasizing the importance of site-specific monitoring in ecologically sensitive environments (Hoven et al., 2017; Santana et al., 2022; Weller et al., 2022).

The Mt. Hamiguitan Range Wildlife Sanctuary (MHRWS) in Davao Oriental is a UNESCO World Heritage Site recognized for its exceptional biodiversity, endemism, and ecological significance (UNESCO, 2014; UNESCO, 2023). Previous studies in MHRWS have focused on floral diversity (Amoroso et al., 2018a), avifaunal assemblages (Amoroso et al., 2018b), fish diversity and physicochemical characteristics of river systems (Dapar et al., 2021), and watershed conservation mechanisms (Cereno and Roxas, 2025). Moreover, ecotourism activities within the sanctuary have increased following the reopening of selected trails (DENR–PAMO, 2023; DENR–PAMO, 2025). Despite its ecological and tourism importance, no published study has established baseline microbial water quality data for the sanctuary's rivers, streams, waterfalls, and tributaries. To date, research within MHRWS has largely focused on biodiversity conservation, ecosystem assessment, and watershed management, while microbial water quality monitoring has remained unexplored. This absence of microbial information limits the ability of protected area managers to evaluate potential public health risks, detect early signs of environmental degradation, and assess the impacts of increasing ecotourism activities on freshwater resources. This represents a significant knowledge gap because microbial contamination may affect visitor safety, freshwater ecosystem health, and long-term conservation management.

Recent advances in portable microbial monitoring technologies have improved the feasibility of field-based water quality assessments in remote environments. Petrifilm™ *E. coli*/Coliform Count Plates provide a practical and validated alternative to conventional laboratory methods for enumerating *E. coli* and coliform bacteria in water samples (Matner et al., 1990; Schraft and Watterworth, 2004; Tarroza et al., 2019). Their portability and ease of use make them particularly suitable for protected areas where laboratory infrastructure is limited (Brown et al., 2020; Wight et al., 2020). Despite their proven effectiveness, the application of portable Petrifilm™ technology in freshwater monitoring within Philippine protected areas remains limited. The use of Petrifilm™ plates in this study demonstrates a practical and cost-effective approach for generating reliable microbial water quality data in remote conservation landscapes where access to conventional laboratory facilities may be constrained. Furthermore, microbial indicators such as total coliforms and *E. coli* provide valuable tools for ecotourism management because they can detect early signs of contamination associated with visitor activities, sanitation deficiencies, and environmental disturbance. This study is novel in three important respects. First, it provides the first baseline assessment of microbial water quality in the freshwater ecosystems of Mt. Hamiguitan Range Wildlife Sanctuary. Second, it demonstrates the application of portable Petrifilm™ technology as a field-based monitoring tool for protected and ecotourism areas. Third, it highlights the value of microbial indicators as practical measures of ecosystem condition, visitor-use impacts, and potential public health risks in a UNESCO World Heritage Site.

This study aimed to assess the microbial water quality status of selected freshwater bodies within the San Isidro and Governor Generoso ecotourism trails of Mt. Hamiguitan Range Wildlife Sanctuary. Specifically, it sought to: (1) determine the concentrations of total coliforms and *E. coli* in selected freshwater sites; (2) characterize physicochemical and hydrological conditions associated with sampling locations; (3) examine relationships between microbial indicators and environmental parameters; and (4) establish baseline microbial water quality information to support conservation planning, ecotourism management, and public health protection within the sanctuary. Given the increasing visitor use of MHRWS, the findings are expected to contribute to evidence-based ecotourism management strategies that balance recreation, conservation, and public health protection. The study was guided by the hypothesis that microbial contamination levels vary among freshwater sites and are significantly associated with physicochemical characteristics, hydrological conditions, and the degree of human activity within the sanctuary.

MATERIALS AND METHODS

Descrioption fo the study area

The study was conducted in selected freshwater bodies within the Mt. Hamiguitan Range Wildlife Sanctuary (MHRWS), Davao Oriental, Philippines. MHRWS is a UNESCO World Heritage Site recognized for its exceptional biodiversity and ecological significance (UNESCO, 2014; UNESCO, 2023). Sampling sites were located along the two established ecotourism routes of the sanctuary: the San Isidro Trail and the Governor Generoso Trail. These areas contain interconnected freshwater ecosystems including rivers, streams, tributaries, waterfalls, and spring-fed water sources that support both biodiversity and ecotourism activities. Eight sampling stations were established based on accessibility, hydrological characteristics, and relevance to ecotourism activities: Governor Generoso Jump-off Station, Camp I, Camp II, Single Falls, Twin Falls, Camp III, Camp IV, and Puting Bato. Each monitoring site consisted of three stream positions: upstream, midstream, and downstream, resulting in a total of 24 sampling stations (Please see Figure 1). The inclusion of multiple stream positions within each monitoring site was intended to improve the representativeness of sampling by capturing potential spatial variation in microbial contamination along the freshwater continuum. All established stations were geo-tagged to ensure consistency in future monitoring and to follow established freshwater field protocols used in Philippine studies. Geographic coordinates of all stations were recorded using a handheld Global Positioning System (GPS) device .

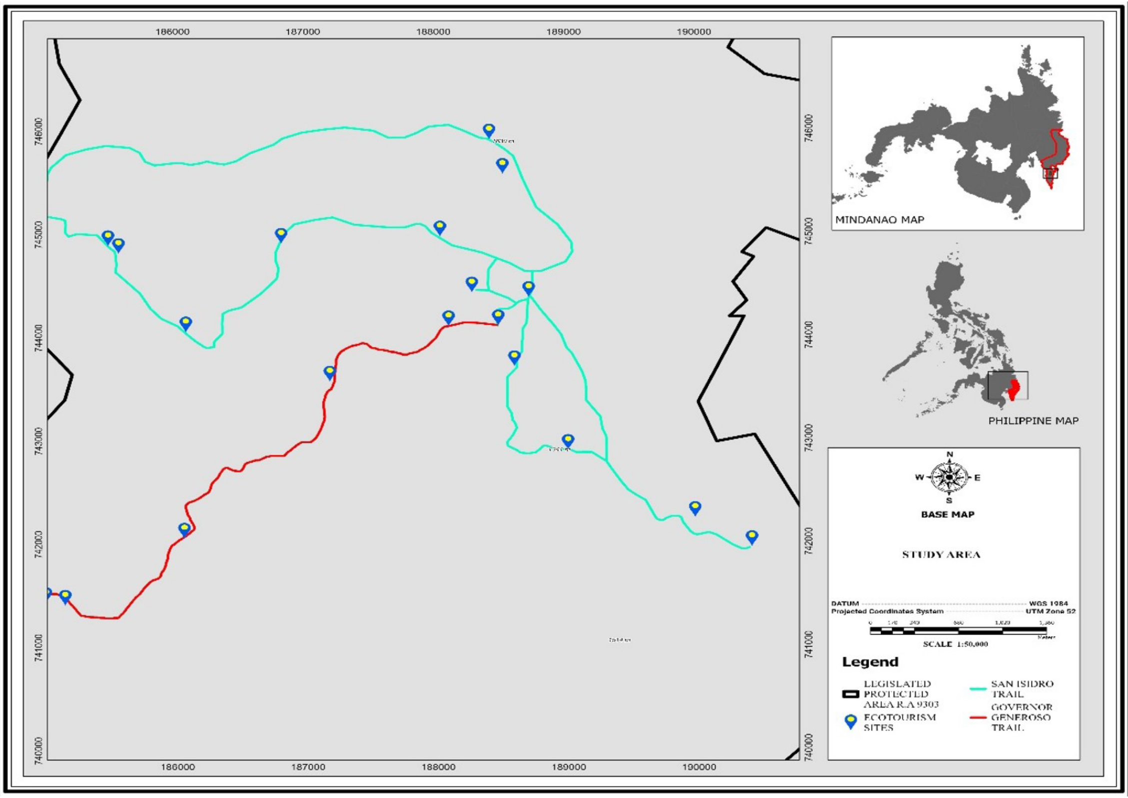

Figure 1. Map showing the study site and area.

Study design and sampling period

A field-based cross-sectional assessment was conducted during two sampling rounds in April and May 2026, representing the dry-season period. Two sampling events were undertaken to capture short-term spatial and temporal variability in microbial water quality among freshwater systems within the sanctuary. Sampling was conducted at all 24 monitoring points (eight sites × three stream positions) during each sampling round, resulting in a total of 48 water samples collected throughout the study period.

Water sample collection

Water samples were collected following standard procedures for freshwater microbiological assessment (APHA, 2023). At each sampling station, approximately 100 mL of surface water was aseptically collected in sterile sampling bottles. Samples were obtained from flowing sections of the water body while avoiding disturbance of bottom sediments. Immediately after collection, samples were stored in insulated coolers maintained at approximately 4°C and processed within the recommended holding period. Field observations, including evidence of human activity, wildlife presence, surrounding vegetation, and potential contamination sources, were documented during each sampling event. To improve data reliability and permit statistical comparison among sites, the same sampling procedures were consistently applied across all monitoring stations and sampling periods.

Measurement of physicochemical and hydrological parameters

In situ measurements of physicochemical parameters were conducted at each sampling station using a calibrated portable multiparameter water quality meter. Parameters measured included temperature (°C), pH, electrical conductivity (μS/cm), total dissolved solids (mg/L), and oxidation-reduction potential (mV). Measurements were performed directly in the water column following instrument manufacturer guidelines. Instrument calibration was conducted before each field deployment to ensure accuracy and reliability of measurements. Hydrological characteristics were recorded to evaluate environmental factors potentially influencing microbial contamination. Stream width, water depth, flow condition, and general site characteristics were documented following standard freshwater hydrology procedures (Gordon et al., 2004). Qualitative observations regarding flow intensity, substrate composition, and evidence of runoff or sediment transport were also recorded.

Enumeration of *Escherichia coli* and total coliforms

Microbial analyses focused on the enumeration of *E. coli* and total coliform bacteria as indicators of fecal contamination and sanitary water quality. Enumeration was performed using Petrifilm™ *E. coli*/Coliform Count Plates (3M Food Safety, n.d.; Neogen, n.d.). For each sample, 1 mL of water was aseptically dispensed onto the center of a Petrifilm™ plate and evenly distributed using a sterile spreader according to manufacturer instructions. All Petrifilm™ analyses were performed in duplicate, and the mean colony count was used for subsequent analyses. The use of duplicate analyses was intended to minimize analytical variability and improve the reliability of microbial enumeration results. Plates were incubated in a portable field incubator at 35 ± 1°C for 24–48 h. Following incubation, colonies were counted and recorded as colony-forming units (CFU) per milliliter. Identification and interpretation of colonies followed manufacturer guidelines, where blue colonies associated with gas production were considered *E. coli*, while red colonies associated with gas production were recorded as total coliforms (3M Food Safety, n.d.). The Petrifilm™ method has been validated as an effective alternative to conventional microbiological enumeration procedures for water quality monitoring (Schraft and Watterworth, 2004; Tarroza et al., 2019; Mkhwanazi et al., 2024). Considering the 48 water samples collected and duplicate Petrifilm™ analyses performed for each sample, a total of 96 microbial observations were generated for laboratory quality assurance purposes.

Water quality evaluation

Microbial water quality results were evaluated against the microbiological criteria established under the Philippine Water Quality Guidelines and General Effluent Standards (DENR Administrative Order No. 2016-08) and subsequent updates under DENR Administrative Order No. 2021-19. These guidelines provide benchmark values for assessing the suitability of freshwater systems for recreation, fisheries, irrigation, and other beneficial uses (DENR-EMB, 2016; DENR-EMB, 2021).

Statistical analysis

Descriptive statistics were used to summarize microbial counts, physicochemical measurements, and hydrological characteristics. Mean, standard deviation, minimum, and maximum values were computed for all quantitative variables. Differences in microbial counts among sampling stations and sampling periods were evaluated using appropriate parametric or non-parametric statistical tests depending on data distribution. Relationships between microbial indicators (*E. coli* and total coliforms) and physicochemical parameters were examined using Spearman's rank correlation analysis for non-normally distributed data. Statistical significance was assessed at $\alpha = 0.05$. The statistical analyses were based on the complete dataset obtained from the 24 monitoring points surveyed during two sampling periods. Mean values from duplicate Petrifilm™ analyses were used as the final observations included in the statistical tests. Data management and statistical analyses were performed using IBM SPSS Statistics Version 29 (IBM Corp., Armonk, NY, USA). Spatial visualization and map preparation were conducted using Geographic Information System (GIS) software. Graphical presentations were generated using Microsoft Excel 365.

RESULTS

Concentrations of total coliforms and *Escherichia coli* in selected freshwater sites

Table 1 presents the mean concentrations of *E. coli* and total coliform across eight freshwater sampling stations located along the San Isidro and Governor Generoso ecotourism trails. Camp 3 recorded the highest total coliform concentrations during both sampling periods, with mean values of 44.78 CFU/100 mL in April and 27.00 CFU/100 mL in May. Camp 4 exhibited the second highest total coliform concentration during April (34.33 CFU/100 mL), followed by Twin Falls (22.44 CFU/100 mL) and Governor Generoso Jump-Off (21.56 CFU/100 mL). For *E. coli*, Governor Generoso Jump-Off recorded the highest concentration during May (4.89 CFU/100 mL), while Camp 3 consistently showed detectable concentrations during both months (1.22–1.33 CFU/100 mL). Camp 2 recorded no detectable *E. coli* during both sampling periods. A general decrease in total coliform concentrations was observed from April to May in most stations. In contrast, *E. coli* concentrations remained relatively low throughout the study area, except for the marked increase observed at Governor Generoso Jump-Off during May.

Table 1. Mean *Escherichia coli* and total coliform concentrations across ecotourism sampling sites during April and May 2026.

Site Name	Microbial Parameters Mean (CFU/mL) *Escherichia coli* April	*Escherichia coli* May	Total Coliform April	Total Coliform May
Governor Generoso Jump-Off	0.11	4.89	21.56	16.44
Camp 1	0.22	0	8	0.22
Camp 2	0	0	1.33	1.78
Single Falls	0.22	0	3.78	6.22
Twin Falls	0.33	0.11	22.44	12
Camp 3	1.22	1.33	44.78	27
Camp 4	0.33	0.33	34.33	5
Puting Bato	0.78	0	17.67	4.11

To complement the microbial concentration data, *E. coli* counts were further interpreted using the World Health Organization (WHO) microbial risk classification framework for drinking-water quality. This classification provides a practical assessment of potential public health risks associated with fecal contamination and identifies whether water samples are acceptable for direct consumption without treatment.

Table 2. Monthly mean *Escherichia coli* concentrations, WHO risk classification, and drinking-water acceptability across stream positions in Mt. Hamiguitan Range Wildlife Sanctuary.

Sampling Station	Highest recorded *E. coli* (CFU/100 mL)	WHO risk category*	Interpretation
Governor Generoso Jump-Off	4.89	Low Risk	Detectable fecal contamination; treatment recommended before consumption
Camp 1	0.22	Low Risk	Detectable fecal contamination
Camp 2	0.00	Very Low Risk	No detectable *E. coli*
Single Falls	0.22	Low Risk	Detectable fecal contamination
Twin Falls	0.33	Low Risk	Detectable fecal contamination
Camp 3	1.33	Low Risk	Detectable fecal contamination
Camp 4	0.33	Low Risk	Detectable fecal contamination
Puting Bato	0.78	Low Risk	Detectable fecal contamination

Note: Adapted from WHO Guidelines for Drinking-water Quality. Presence of detectable *E. coli* indicates fecal contamination and water should not be considered suitable for direct consumption without treatment. Risk categories: 0 CFU/100 mL = Conforms to drinking-water criterion; 1–10 CFU/100 mL = Low Risk; 11–100 CFU/100 mL = Intermediate Risk; 101–1000 CFU/100 mL = High Risk; >1000 CFU/100 mL = Very High Risk.

Based on WHO microbiological risk classification criteria, Camp 2 was the only station that consistently conformed to the drinking-water criterion, with no detectable *E. coli* during either sampling period. All remaining stations were classified as Low Risk because detectable *E. coli* concentrations were recorded at least once during monitoring. Although the observed concentrations remained relatively low, the presence of fecal indicator bacteria suggests that untreated water from these sites should not be assumed safe for direct consumption.

To complement the tabulated microbiological results, a Geographic Information System (GIS)-based hotspot analysis was conducted to visualize the spatial distribution of *E. coli* contamination across sampling stations within the San Isidro Protected Landscape and Seascape ecotourism trail system (Figure 2). The hotspot map identifies areas exhibiting elevated contamination risk based on WHO risk classifications and provides a spatial perspective on potential contamination sources and vulnerable recreational sites.

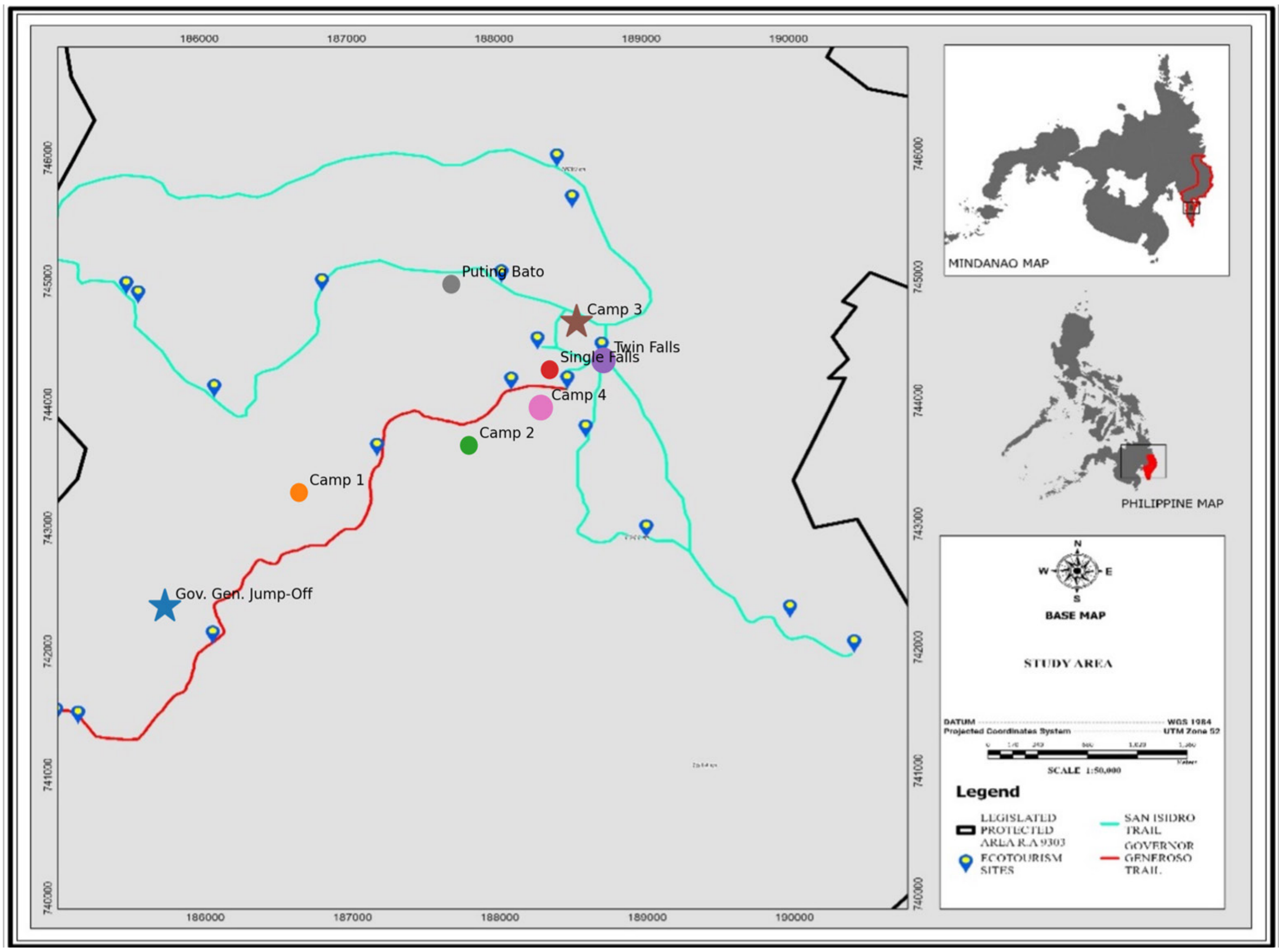

Figure 2. GIS hotspot map showing the spatial distribution of *Escherichia coli* contamination risk across ecotourism water bodies in the San Isidro Protected Landscape and Seascape, Davao Oriental. Sampling stations classified as low-risk contamination sites are highlighted according to WHO recreational water quality guidelines.

The GIS hotspot visualization revealed that microbiological contamination was not uniformly distributed across the study area. Stations exhibiting low-risk contamination levels were concentrated at Governor Genenoro Jump-Off, Camp 3, Camp 4, portions of Twin Falls, and downstream sections of Puting Bato. In contrast, Camp 1, Camp 2, Single Falls, and most sections of Puting Bato exhibited conformity with acceptable microbiological standards. The clustering of low-risk sites along frequently visited ecotourism routes suggests that anthropogenic activities, including trekking, camping, and recreational water contact, may contribute to localized fecal contamination. Although all recorded *E. coli* concentrations remained within the WHO low-risk category and no moderate- or high-risk hotspots were detected, the spatial clustering identified through GIS analysis indicates areas that warrant continued monitoring and management intervention. The hotspot map therefore provides a valuable decision-support tool for protected area managers by identifying priority locations for water quality surveillance, sanitation improvements, and visitor management strategies.

Consistent with the WHO risk classification presented in Table 2, stations exhibiting detectable *E. coli* concentrations generally corresponded to locations with elevated microbial contamination. Preliminary one-way analysis of variance (ANOVA) indicated significant differences in total coliform concentrations among sampling stations, $F(7,136) = 16.92$, $p < 0.001$, $\eta^2 = 0.47$, indicating a large effect of sampling station on total coliform occurrence (Table 3). Similarly, *E. coli* concentrations significantly differed among sampling stations, $F(7,136) = 8.47$, $p < 0.001$, $\eta^2 = 0.30$, also representing a large effect. Post hoc comparisons using Tukey's Honest Significant Difference (HSD) test identified Camp 3 as having relatively higher total coliform concentrations, whereas Governor Generoso Jump-Off exhibited comparatively higher *E. coli* concentrations than several other sampling stations. The Wilcoxon Signed-Rank Test showed that total coliform concentrations significantly decreased from April to May ($W = 22$, $p = 0.0003$, $r = 0.74$), indicating a large temporal effect. In contrast, *E. coli* concentrations did not significantly differ between sampling periods ($W = 28$, $p = 0.320$, $r = 0.26$), suggesting only a small-to-moderate temporal effect.

Table 3. Summary of Non-Parametric Temporal Analysis (Wilcoxon Signed-Rank Test).

Microbial parameter	Mean April (CFU/100 mL)	Mean May (CFU/100 mL)	W	n	*p*-value	Effect Size (r)	Interpretation
Total Coliform	10.33	4.83	22	24	0.0003	0.74	Large
E. coli	0.00	0.00	28	15	0.3200	0.26	Small–Moderate

Meanwhile, the Kruskal–Wallis H test demonstrated significant spatial variation in both total coliform (H = 70.6, $p < 0.0001$, $\varepsilon^2 = 0.47$) and *E. coli* (H = 38.4, $p < 0.0001$, $\varepsilon^2 = 0.23$) concentrations among the eight sampling stations (Table 4). The large effect sizes indicate that station-specific environmental conditions and anthropogenic influences substantially contributed to the observed spatial variability in microbial water quality.

Table 4. Summary of Non-Parametric Spatial Analysis (Kruskal–Wallis H Test).

Microbial Parameter	H Statistic	df	*p*-value	Effect size (ε^2)	Interpretation
Total Coliform	70.6	7	<0.0001	0.47	Large
E. coli	38.4	7	<0.0001	0.23	Large

Physicochemical and hydrological conditions associated with sampling locations

As shown in Table 5, the pH values ranged from 7.92 to 8.69, indicating slightly alkaline conditions across all sampling stations. Water temperatures ranged from 21.57°C to 28.10°C. Governor Generoso Jump-Off and Camp 1 recorded the highest temperatures, whereas Single Falls and Camp 3 recorded the lowest. Electrical conductivity ranged from 46.00 to 234.33 µS/cm, while total dissolved solids ranged from 22.67 to 117.33 ppm. Governor Generoso Jump-Off consistently exhibited the highest EC and TDS values. Oxidation-reduction potential ranged from 46.70 to 161.33 mV, with generally higher values observed during April.

Table 5. Monthly mean physicochemical characteristics across sampling stations during April and May.

Station name	pH		Temperature (°C)		EC (µS/cm)		TDS (ppm)		ORP (mV)	
	April	May	April	May	April	May	April	May	April	May
Governor Generoso Jump-Off	8.30	8.59	27.53	28.10	227.33	234.33	114.67	117.33	157.33	53.00
Camp 1	8.52	8.59	28.03	26.60	202.67	211.00	102.67	106.33	161.33	62.67
Camp 2	8.50	8.55	25.50	25.80	159.67	162.67	80.67	81.33	126.33	64.67
Single Falls	8.62	8.69	21.57	22.63	135.33	142.00	67.67	70.00	129.67	51.33
Twin Falls	8.33	8.32	23.63	23.47	46.00	54.00	22.67	26.33	133.33	86.33
Camp 3	8.18	7.92	23.50	21.60	74.33	82.33	36.67	40.67	139.33	111.00
Camp 4	8.10	8.27	23.77	23.03	157.00	180.00	77.67	90.67	106.00	72.00
Puting Bato	8.55	8.66	23.30	24.13	185.00	211.00	91.67	106.00	91.67	46.70

Note: EC = electrical conductivity; TDS = total dissolved solids; ORP = oxidation-reduction potential.

As shown in Table 6, the hydrological measurements varied among stations. Governor Generoso Jump-Off exhibited the widest stream channels, while Camp 4 recorded the greatest mean stream depth. Camp 1 showed the highest stream flow rate during April, whereas Single Falls consistently exhibited the lowest flow conditions.

Table 6. Monthly mean hydrological characteristics across sampling stations during April and May.

Station Name	Mean stream depth (m)		Stream width (m)		Mean stream flow (m/s)	
	April	May	April	May	April	May
Governor Generoso Jump-Off	0.10	0.08	16.23	14.67	0.11	0.10
Camp 1	0.13	0.12	6.97	6.40	0.17	0.10
Camp 2	0.31	0.36	6.83	6.13	0.08	0.05
Single Falls	0.09	0.07	5.28	4.93	0.01	0.01
Twin Falls	0.06	0.03	8.13	7.77	0.02	0.02
Camp 3	0.12	0.10	1.57	1.33	0.04	0.03
Camp 4	0.99	0.68	1.46	1.00	0.07	0.01
Puting Bato	0.22	0.13	3.09	2.70	0.07	0.03

Relationships between microbial indicators and environmental parameters

Spearman rank correlation analysis revealed a statistically significant moderate positive relationship between total coliform and *E. coli* concentrations ($r_s = 0.558$, $p < 0.001$), indicating that sites with elevated total coliform levels generally exhibited higher *E. coli* counts (Table 7). *E. coli* concentrations showed a significant moderate negative correlation with pH ($r_s = -0.417$, $p = 0.003$), while no significant relationships were observed with temperature, electrical conductivity, total dissolved solids, oxidation-reduction potential, stream depth, stream width, or stream flow ($p > 0.05$). Total coliform concentrations exhibited a significant moderate negative correlation with pH ($r_s = -0.606$, $p < 0.001$), a weak negative correlation with electrical conductivity ($r_s = -0.287$, $p = 0.048$), and a weak negative correlation with total dissolved solids ($r_s = -0.301$, $p = 0.038$). In contrast, total coliform concentrations were positively correlated with oxidation-reduction potential ($r_s = 0.358$, $p = 0.012$). No significant relationships were observed between total coliform concentrations and temperature, stream depth, stream width, or stream flow ($p > 0.05$).

Table 7. Spearman rank correlation analysis between microbial indicators and environmental variables.

Variable	*E. coli* (r_s)	*p*-value	Total Coliform (r_s)	*p*-value
Total coliform	0.558	<0.001	—	—
pH	-0.417	0.003	-0.606	<0.001
Temperature	-0.037	0.803	-0.183	0.213
Electrical Conductivity (EC)	-0.102	0.489	-0.287	0.048
Total Dissolved Solids (TDS)	-0.099	0.503	-0.301	0.038
Oxidation Reduction Potential (ORP)	0.119	0.421	0.358	0.012
Stream depth	-0.117	0.429	-0.222	0.129
Stream width	-0.165	0.262	-0.248	0.089
Stream flow	0.036	0.807	0.008	0.957

Although several physicochemical variables demonstrated statistically significant associations with microbial concentrations, site observations suggest that anthropogenic factors may also contribute to contamination patterns (Table 8 and Appendices Section). Camp 3, Camp 4, and Governor Generoso Jump-Off, which exhibited higher visitor activity, campsite proximity, and recreational infrastructure, generally recorded elevated microbial concentrations. In contrast, Twin Falls, Single Falls, and Puting Bato exhibited relatively undisturbed environmental conditions and comparatively lower microbial contamination levels.

Table 8. Observed environmental conditions, anthropogenic activities, and existing facilities across sampling stations.

Station name	Signages present	Waste presence	Visitor activity	Campsite proximity	Facilities / Utilities	Observed site condition
Governor Generoso Jump-Off	Boundary markers	None observed	Entry and jump-off activities	Present	None observed	Active human passage area
Camp 1	Locational signage	None observed	Trekking activities	Near campsite	None observed	River edge ..
Camp 2	Locational, directional, informational, regulatory, safety, educational, and landmark signages	None observed	Trekking, resting, and camping activities	Present	Porters' cabin, cooking area, water supply, restrooms	Presence of campsite facilities
Single Falls	Directional signage	None observed	Waterfall visitation and trekking activities	Absent	None observed	Natural flowing water condition
Twin Falls	Locational signage	None observed	Waterfall visitation and trekking activities	Absent	None observed	Minimal human activity observed
Camp 3	Locational, directional, informational, regulatory, safety, educational, accessibility, campground map, and landmark signages	Food scraps and waste dump near the water source	Camping, dining, and trekking activities	Present	Cabin, cooking area, dining area, activity area, steel chairs, water supply, restrooms, handwashing station, shower, potable water	Presence of recreational facilities
Camp 4	Locational, directional, informational, regulatory, safety, educational, accessibility, campground map, and landmark signages	None observed	Camping and trekking activities	Present	Cooking area, dining tables, activity area, campsite, potable water, restrooms, steel chairs	Active campsite area
Puting Bato	Locational signage	None observed	Trekking and sightseeing activities	Absent	None observed	Relatively undisturbed condition

Baseline water quality information for conservation, ecotourism, and public health

The present study established the first comprehensive microbial water quality baseline for freshwater ecosystems along the San Isidro and Governor Generoso ecotourism trails of the Mt. Hamiguitan Range Wildlife Sanctuary. Across the eight monitoring stations and 24 stream-position sampling points surveyed during April and May 2026, microbial contamination levels were generally low but exhibited significant spatial heterogeneity. Camp 3 consistently recorded the highest total coliform concentrations (44.78 CFU/100 mL in April and 27.00 CFU/100 mL in May), whereas Governor Generoso Jump-Off exhibited the highest recorded *E. coli* concentration (4.89 CFU/100 mL) during May. In contrast, Camp 2 consistently showed no detectable *E. coli* and represented the least contaminated freshwater site throughout the monitoring period.

Interpretation of microbial data using the World Health Organization (WHO) risk classification framework revealed that Camp 2 was the only station conforming to the drinking-water criterion (0 CFU/100 mL *E. coli*), while all remaining stations were classified under the Low Risk category because detectable *E. coli* concentrations were observed at least once during monitoring. Although all recorded *E. coli* values remained within the WHO low-risk threshold and all total coliform concentrations were substantially below the DENR Class C freshwater guideline of 200 MPN/100 mL, the presence of fecal indicator bacteria in seven of the eight stations indicates localized contamination inputs and highlights the need for continued microbial surveillance.

The GIS hotspot analysis further demonstrated that microbial contamination was spatially clustered rather than uniformly distributed across the sanctuary. Areas surrounding Governor Generoso Jump-Off, Camp 3, Camp 4, Twin Falls, and portions of Puting Bato emerged as localized contamination hotspots, whereas Camp 1, Camp 2, and Single Falls generally exhibited lower contamination levels. These spatial patterns corresponded closely with areas characterized by greater visitor activity, campsite proximity, recreational infrastructure, and evidence of anthropogenic disturbance, suggesting that ecotourism-related activities may contribute to localized fecal contamination.

Statistical analyses provided strong support for the observed spatial patterns. Significant differences among sampling stations were detected for both total coliform (F(7,136) = 16.92, p < .001, η^2 = .47; H = 70.6, p < .0001, ε^2 = .47) and *E. coli* (F(7,136) = 8.47, p < .001, η^2 = .30; H = 38.4, p < .0001, ε^2 = .23), indicating large spatial effects and substantial influence of site-specific environmental and anthropogenic factors. A significant temporal reduction in total coliform concentrations was also observed between April and May (W = 22, p = .0003, r = .74), whereas E. coli concentrations remained relatively stable over time (W = 28, p = .320, r = .26).

Correlation analyses further indicated that microbial contamination was associated with environmental conditions. *E. coli* and total coliform concentrations were positively related (r_s = 0.558, p < 0.001), while both indicators exhibited significant negative relationships with pH. Total coliform concentrations also showed significant associations with electrical conductivity, total dissolved solids, and oxidation-reduction potential. Nevertheless, observational data suggested that anthropogenic factors, particularly visitor activity, campsite presence, food waste accumulation, and recreational infrastructure, likely exerted a stronger influence on microbial occurrence than physicochemical conditions alone.

Collectively, these findings provide a robust baseline dataset for future environmental monitoring, conservation planning, ecotourism management, and public health protection within Mt. Hamiguitan Range Wildlife Sanctuary. The baseline information establishes reference conditions against which future changes in microbial water quality can be evaluated and provides a scientific foundation for adaptive management strategies aimed at maintaining freshwater ecosystem integrity while supporting sustainable ecotourism development.

DISCUSSION

The results demonstrate clear spatial and temporal variability in microbial contamination and physicochemical conditions across freshwater sites within the ecotourism trails of Mt. Hamiguitan Range Wildlife Sanctuary. These patterns reflect the combined influence of localized anthropogenic pressures, environmental conditions, and hydrological processes that govern microbial water quality in tropical protected ecosystems.

Concentrations of total coliforms and *Escherichia coli* in selected freshwater sites

The observed spatial heterogeneity in total coliform and *E. coli* concentrations strongly suggests that contamination is driven primarily by site-specific human activities rather than basin-wide pollution. Elevated microbial loads at Camp 3 and Governor Generoso Jump-Off align with evidence that recreational use, campsite proximity, and inadequate sanitation infrastructure are key contributors to fecal contamination in freshwater systems (Butler et al., 2021; McKee and Cruz, 2021). The presence of food waste and intensive visitor activity at Camp 3 further strengthens the likelihood of direct or indirect fecal inputs into nearby water bodies. These findings are consistent with studies in other Philippine freshwater and ecotourism systems where anthropogenic pressure has been linked to elevated microbial indicators (Abris et al., 2020; Cayabo et al., 2021). Similar patterns have been reported in Laguna Lake tributaries, where fecal contamination hotspots were associated with human settlements and land-use intensity (Abello et al., 2021; Malajacan et al., 2023). Collectively, these comparisons indicate that even protected and relatively intact ecosystems are not exempt from microbial contamination when exposed to concentrated recreational activities.

From a conceptual standpoint, microbial indicators function as sensitive proxies of environmental disturbance, responding rapidly to localized inputs before broader ecological degradation becomes apparent (Momba et al., 2019). The detection of *E. coli* across most stations in this study reinforces the notion that low-intensity tourism can still generate measurable microbial impacts in freshwater ecosystems. However, the generally low concentrations compared with highly urbanized systems such as the Pasig River (Gorme et al., 2010) and Mega Manila waters (Castro and Obusan, 2023) suggest that current land protection measures in Mt. Hamiguitan still provide substantial buffering capacity. The observed temporal decline in total coliform concentrations from April to May may reflect seasonal hydrological variation, particularly dilution effects and changes in environmental conditions influencing bacterial survival. Seasonal fluctuations in microbial abundance have been widely documented in freshwater systems and are often associated with rainfall patterns, flow variability, and nutrient availability (Weller et al., 2022; Martínez-Játiva et al., 2025). This reinforces the importance of multi-season monitoring to accurately characterize microbial dynamics.

Physicochemical and hydrological conditions associated with sampling locations

The physicochemical conditions across all stations indicate generally intact freshwater environments typical of minimally disturbed tropical headwater systems. Slightly alkaline pH values and low electrical conductivity are consistent with baseline conditions reported in Mt. Hamiguitan river systems and other forested catchments in Mindanao (Dapar et al., 2021). Such conditions are generally favorable for microbial persistence, as neutral to slightly alkaline waters can enhance bacterial survival and metabolic activity (Dewangan et al., 2023). However, physicochemical variables alone do not fully explain microbial distribution patterns. The clustering of contamination at specific sites suggests that anthropogenic inputs play a more dominant role than baseline water chemistry. Elevated conductivity and total dissolved solids at Governor Generoso Jump-Off likely reflect increased surface runoff and human disturbance, consistent with findings that conductivity can serve as an indirect indicator of catchment disturbance and anthropogenic influence (Panda et al., 2018; Kasa and Reddythota, 2023).

Hydrological characteristics further explain spatial variability in microbial concentrations. Sites with lower flow velocity, reduced flushing capacity, and greater retention time tended to exhibit higher bacterial counts. This aligns with hydrological principles indicating that reduced flow enhances microbial accumulation and persistence in surface waters (Gordon et al., 2004). Similar relationships have been observed in recreational and tropical river systems, where stagnant or slow-moving waters promote microbial survival (Safari et al., 2024). Thus, microbial contamination in Mt. Hamiguitan is best understood as the product of interacting hydrological and anthropogenic drivers rather than isolated environmental factors.

Relationships between microbial indicators and environmental parameters

The positive correlation between total coliform and *E. coli* confirms their shared response to fecal contamination sources and supports their complementary roles in water quality assessment (Tambi et al., 2023; Momba et al., 2019). The significant negative relationship between microbial indicators and pH suggests that more alkaline conditions may slightly suppress bacterial persistence, although this effect appears secondary to contamination inputs. Weak to moderate correlations between microbial indicators and conductivity, total dissolved solids, and oxidation-reduction potential further indicate that physicochemical conditions influence bacterial survival but are not primary drivers of contamination. These findings are consistent with studies emphasizing that microbial water quality is governed by complex interactions among land use, hydrology, and environmental conditions rather than single-factor control (Loucif and Chenchouni, 2024; Weller et al., 2022).

Importantly, observational data highlight the role of tourism infrastructure and human activity as dominant contributors to microbial distribution. Stations with campsites, cooking areas, sanitation facilities, and high visitor traffic consistently exhibited higher bacterial loads. This pattern supports the recreational carrying capacity framework, which posits that ecological degradation increases when visitor intensity exceeds ecosystem assimilative capacity (Butler et al., 2021). Even in World Heritage Sites, such as Mt. Hamiguitan, localized ecological pressures can emerge when tourism is not matched with adequate waste and sanitation management. From a One Health perspective, the detection of fecal indicator bacteria in recreational waters underscores potential exposure pathways affecting human health, wildlife, and aquatic ecosystems. Similar concerns have been highlighted in studies linking environmental microbial contamination with public health risks in recreational water settings (Gomes et al., 2024; McKee and Cruz, 2021).

Baseline water quality for conservation, ecotourism, and public health

This study establishes a critical baseline for microbial water quality in Mt. Hamiguitan Range Wildlife Sanctuary, providing a reference point for long-term ecological monitoring. Baseline datasets are essential in protected areas because they allow differentiation between natural variability and anthropogenic change over time (Cortez and Siringan, 2025). While biodiversity monitoring has traditionally dominated protected area assessments, increasing evidence highlights the importance of integrating microbial indicators as early-warning tools for ecosystem integrity (Castro and Obusan, 2023; UNESCO, 2023). The results demonstrate that although overall microbial concentrations remain low, localized contamination hotspots are already present in high-use ecotourism zones. This finding is consistent with global evidence that even low-density tourism can influence microbial water quality in sensitive ecosystems (Abris et al., 2020; Butler et al., 2021). Accordingly, integrating microbial monitoring into routine protected area management is essential for sustaining both ecological integrity and visitor safety.

Regulatory interpretation and management implications

All recorded total coliform concentrations remained within DENR Class C freshwater guidelines (DENR-EMB, 2016; 2021), indicating general compliance with national water quality standards. However, the detection of *E. coli* in most stations signals localized fecal contamination and underscores that regulatory compliance does not necessarily equate to absence of health risk. The identification of Camp 3 and Governor Generoso Jump-Off as contamination hotspots provides actionable insights for targeted management interventions. Focused strategies such as improved waste management, sanitation infrastructure upgrades, and visitor regulation are more appropriate than blanket restrictions, aligning with adaptive management approaches in protected areas (Cereno and Roxas, 2025). Recent waterborne disease outbreaks in Davao Oriental further emphasize the public health relevance of continuous microbial monitoring in recreational waters (Philippine News Agency, 2025; Esteban, 2025).

Synthesis of findings, study limitations, and future research directions

Overall, the findings demonstrate that microbial water quality in Mt. Hamiguitan is influenced by a combination of anthropogenic activities, hydrological conditions, and environmental factors, with GIS-based hotspot analysis effectively revealing spatial clustering of contamination and providing a useful decision-support tool for conservation planning; however, several limitations should be considered in interpreting these results, including the restricted sampling period (April–May 2026) that does not capture full seasonal variability across wet and dry cycles, the reliance on culturable indicator organisms (*E. coli* and total coliforms) which may not fully represent the diversity of waterborne pathogens, the absence of microbial source tracking which prevents definitive identification of contamination sources, and the relatively limited number of sampling stations which may reduce the spatial resolution of hotspot analysis, thereby underscoring the need for future research to incorporate year-round monitoring, expanded microbial and molecular indicators, microbial source tracking techniques, higher-density spatial sampling, and predictive modeling approaches to more accurately distinguish between natural and anthropogenic contamination sources and to strengthen long-term water quality assessment and ecotourism management strategies in the sanctuary.

CONCLUSION

This study successfully achieved its objectives by determining the concentrations of total coliforms and *E. coli* in selected freshwater sites, characterizing the physicochemical and hydrological conditions across sampling stations, analyzing the relationships between microbial indicators and environmental parameters, and establishing a baseline microbial water quality dataset for the San Isidro and Governor Generoso ecotourism trails of Mt. Hamiguitan Range Wildlife Sanctuary. The findings demonstrate that microbial water quality is generally acceptable in terms of total coliform levels, as all recorded values remained within the DENR Class C freshwater standards. However, the consistent detection of *E. coli* in most sampling stations indicates localized fecal contamination, highlighting potential public health risks associated with the use of untreated stream water within recreational areas. Spatial analysis revealed significant variation in microbial contamination among sites, with higher levels observed in areas of greater human activity such as Camp 3 and Governor Generoso Jump-Off, while relatively undisturbed sites like Camp 2 showed minimal or no contamination. These results confirm that microbial contamination is not uniformly distributed but is influenced by site-specific conditions. The study also found that microbial indicators are associated with physicochemical and hydrological characteristics, as well as the degree of anthropogenic disturbance, supporting the hypothesis that contamination levels vary significantly among freshwater sites and are shaped by both environmental factors and human activity. Thus, the hypothesis is supported and not rejected. The findings imply that even within a protected and ecologically intact ecosystem, increasing ecotourism activities can contribute to measurable microbial impacts when sanitation and waste management are inadequate. These results underscore the need to strengthen monitoring systems, improve sanitation infrastructure, and implement site-specific visitor management strategies to maintain water quality. The study provides essential baseline information to support evidence-based ecotourism management, conservation planning, and public health protection within Mt. Hamiguitan Range Wildlife Sanctuary.

RECOMMENDATIONS

- Strengthen sanitation facilities in high-use ecotourism sites (especially Camp 3 and Governor Generoso Jump-Off) by improving toilets, waste disposal systems, and water management to reduce fecal contamination risks.
- Implement site-specific visitor management strategies, including limiting overcrowding in sensitive areas and designating proper washing and waste disposal zones near campsites and jump-off points.
- Establish a localized water quality monitoring program led by the Protected Area Management Office (PAMO) and local stakeholders to regularly track *E. coli* and coliform levels in key recreational sites.
- Conduct continuous environmental awareness campaigns for tourists, guides, and local communities emphasizing proper waste disposal and discouraging direct consumption of untreated stream water.
- Integrate long-term microbial water quality monitoring into existing ecotourism management plans to support evidence-based decision-making for sustainable tourism development in Mt. Hamiguitan, with recommendations for future research including year-round monitoring, microbial source tracking, molecular analyses, and expanded GIS-based assessments.

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AUTHOR CONTRIBUTIONS

M.D.C: Conceptualized the study, conducted the field sampling and data analysis, and prepared the initial draft of the manuscript. P.N.C: Supervised the overall research process, contributed to the study design and data interpretation, and critically reviewed and approved the final manuscript for publication.

DECLARATION

Informed consent statement

The study involved environmental water sampling and did not involve human participants, human tissues, or vertebrate animals; therefore, formal human research ethics review was not required. Prior permission to conduct sampling activities within Mt. Hamiguitan Range Wildlife Sanctuary was obtained from the Department of Environment and Natural Resources–Protected Area Management Office (DENR–PAMO) and relevant site authorities. All field activities were conducted in accordance with protected area regulations and environmental protection guidelines to minimize disturbance to natural ecosystems.

Conflicting interests

The authors have no conflict of interest related to this study.

AI Declaration

The authors declare that no Artificial Intelligence (AI) or AI assisted technologies were used in the preparation of this manuscript.

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