



# Bacteriological water analysis of Matutinao River in Badian, Cebu, Philippines

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## ABSTRACT

As nature tourism becomes increasingly popular, especially in tropical countries, monitoring the environment's health and making it sustainable is essential. Hundreds of daily tourists visit a popular spot between Kawasan Falls and the Matutinao River in Cebu to participate in the canyoneering activity. This study assessed the bacteriological quality of water in Matutinao River. Obtained water samples were analyzed for heterotrophic plate count (HPC), total coliform, and presence of the *Escherichia coli* (Castellani and Chalmers 1919) bacteria. It was found that the river had an HPC of 212 to  $4.57 \times 10^5$  CFU ml<sup>-1</sup> and was contaminated with coliforms (1250-1800 MPN/100 ml). The presence of *E. coli* was also detected. These values exceed the maximum permissible limit for recreation waters (i.e. 100 CFU ml<sup>-1</sup> for HPC and 1,000 MPN/100 ml for total coliforms). Ideally, *E. coli* should also be absent in recreation waters. Although not necessarily harmful, bacteria in recreational waters need to be regularly monitored to prevent potential outbreaks. It is further recommended to revisit existing local policies to help reduce sources of contamination in the river not just to protect the environment but also to promote sustainable tourism.

**Keywords:** bacterial water quality, coliforms, nature tourism

## INTRODUCTION

Rivers are an essential platform for tourism because they can be a location for recreational activities and transport services, exposure to culture and local heritage, and connect people to the environment and the natural world (Winter et al. 2019). River tourism provides a substantial income to the world's tourism ventures, brought by cruises and rafting activities. Although these activities generate money for the locals and the government, such undertakings need in-depth analysis for conservation and

sustainability (Prideaux and Cooper 2009). In the Philippines, rivers that are increasingly used for tourism include Puerto Princesa Subterranean River, Loboc River in Bohol, and Hinatuan Enchanted River in Surigao del Sur (Aquino 2017), to name a few.

Today, tourists seek thrills of adventure to experience and enjoy trekking, scrambling, climbing, jumping, and abseiling near land formations and canyoneering and swimming in bodies of water. In particular, canyoneering has become very popular for tourists who visit Cebu. Most notably, Cebu tourists try canyoneering from Kanlaob River in Alegria to



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Kawasan Falls in Badian. From Kawasan, water flows through the Matutinao River and then to the beaches of Badian.

Together, canyoneering and waterfall adventures generate millions of pesos a month for the municipality of Badian (Ambrad 2018; Erram 2020) and provide jobs for the locals who cater to the tourists' needs. But despite the upsurge in their economy, the ecological health status of these natural resources has been unattended. Studies on environmental quality, nature conservation, and sustainability are still scarce in their locality.

Song (2016) proved that the rapid growth of tourism contributes to environmental pollution resulting in a lack of long-term driving force. When there is a high influx of tourists, the river will likely become polluted by human waste without proper supervision from the local government (Savage et al. 2004). If this continues without appropriate mitigation efforts, the river may be closed off to tourists to undergo rehabilitation. Such action may result to the loss of livelihood especially for the locals in the area who only rely on tourism. As part of sustainable tourism, it is crucial to strengthen the quality of the environment and minimize pollution (Carbone and Yunis 2005). This study focused on bacterial pollution and the possible fecal contamination in Matutinao River during tourists' off-season and peak season. Thus, the objective of this study was to assess the water quality of the Matutinao River using coliform bacteria as indicators of contamination. Specifically, this study aimed to determine (1) the number of heterotrophic bacteria, (2) total coliform, and (3) detect the presence of *Escherichia coli*.

## METHODS

### Study Site

The sampling (Figure 1) was conducted in Matutinao River (9°48'36.8"N, 123°22'01.5"E), which is situated in the municipality of Badian, Cebu (9°52'9.7"N, 123°23'45.33"E). The river flows from Kawasan Falls, one of the most famous waterfalls in Cebu and popular with tourists, and empties into the Tañon Strait. The river, the waterfall, and their surrounding vegetation along Badian are considered natural reserves of Cebu (Bandel and Riedel 1998). Matutinao River was previously awarded by the Department of Environment and Natural Resources (DENR) as the cleanest inland body of water in the Philippines from 2000 to 2002 (Nuez 2018).

### Sampling Collection and Handling

The first water sampling was done on 22 September 2019, an off-season for tourists. The sampling was made in two stations (Figure 1): one



**Figure 1.** Sampling sites at Matutinao River, Badian, Cebu (Image lifted from Google Maps). MR2 and MR4 were the sampling sites during September, and MR1 and MR3 were the sampling sites in December.

near Kawasan Falls (MR2, 9°47'59.05"N, 123°22'40.61"E) and one near the river bank (MR4, 9°48'35.3"N, 123°22'03.6"E) approximately 50 m away from where housing and commercial establishments are situated. Samples were collected from the center of the river and 15 cm below the water's surface, with the mouth of the sterile glass bottle facing the stream's flow when opened slightly and closed underneath the water. Each bottle contained 100 ml of the water sample with two replicates per station. Sample bottles were labeled and placed in an icebox with a thermometer for temperature monitoring. Regular ice replacement was also done to maintain the temperature inside the icebox between 0-4°C to prevent the bacteria from multiplying. Samples were then processed within 24 h in the laboratory (Baird et al. 2017). The second sampling was during the peak season in December 2019 when two more sites were added: one before (MR1, 9°47'59.3"N, 123°22'40.1"E) Kawasan Falls, where canyoneering ends, and one in between Kawasan Falls and Matutinao river bank (MR3, 9°48'40.9"N, 123°22'21.9"E). The off and peak seasons were decided based on experience and consultation with the Local Government Unit of Badian and with the Cebu Provincial Tourism Office. The off-season is during the rainy season (June to November; PAGASA 2021), and the peak season is during the dry season (December to May; PAGASA 2021).

### Bacteriological Analyses

Heterotrophic plate count (HPC), multiple tube fermentation technique (MTFT), and *E. coli* detection using eosin methylene blue agar (EMBA; Sigma-Aldrich, USA) culture medium were conducted following the protocols from standard methods for the examination of water and wastewater (Baird et al. 2017) and were performed at the Microbiology Laboratory of the University of the Philippines Cebu.

**HPC.** To determine the number of heterotrophic bacteria, the HPC was performed by serially diluting water samples ( $10^0$  and  $10^{-2}$ ) and spread plated onto nutrient agar (NA; HiMedia, India) plates. The plates were incubated at  $37^\circ\text{C}$  for 24 h, and the number of colonies was counted to calculate the CFU  $\text{ml}^{-1}$ .

**MTFT.** To determine the total coliforms, multiple tube fermentation technique, presumptive, confirmatory, and completed tests were performed. In the presumptive test, 10, 1, and 0.1 ml of the water samples were inoculated to 10 ml lauryl tryptose broth (Sigma-Aldrich, USA) tubes (containing an inverted Durham tube inside) and were incubated at  $37^\circ\text{C}$  for 24 h. An acidic reaction (i.e. yellow colored-medium) and the formation of gas bubbles inside the Durham tubes denotes a positive presumptive test. The positive presumptive tubes were subjected to the confirmatory test that undergoes the same method and interpretation as the presumptive test but uses the brilliant green lactose bile broth (Sigma-Aldrich, USA).

#### *Escherichia coli* Detection

To detect the presence of *E. coli*, positive confirmatory tubes then underwent the completed test using EMBA. Positive water samples were spread plated onto EMBA plates and were incubated at  $37^\circ\text{C}$  for 24 h. Typical coliform colonies (i.e. *E. coli*) had a green metallic sheen. All of these were performed in two replicates.

#### Data Analysis

The HPC and total coliform were determined by calculating its colony forming unit (CFU) and most

probable number (MPN), respectively (Maturin and Peeler 2001; Feng et al. 2017). The differences in HPC and total coliform values were then compared across sampling events. Lastly, *E. coli* was only reported as present (+) or absent (-).

## RESULTS

### Heterotrophic Bacteria

As shown in Table 1, the heterotrophic bacterial counts in September ranged from 45 to 3,187 CFU  $\text{ml}^{-1}$ . These values had changed from 812 to 1,953 CFU  $\text{ml}^{-1}$  in the original sites in December. However, the new sampling locations recorded the highest HPC (i.e.  $1.62 \times 10^4$  to  $4.57 \times 10^5$ ). HPC was generally higher during December with an 8-43 fold increase.

### Total Coliform

The total coliform were low in the morning and in the afternoon sampling at MR2 (< 1,000 MPN/100 ml) but high (i.e. 1,700 MPN/100 ml) at MR4 in September (Table 2). However, in December, all sites had high total coliform (> 1,000 MPN/100 ml). The most probable number (MPN) was also higher in December than in September, with an increased of 1-2-fold.

### *Escherichia coli*

*Escherichia coli* was detected in all sampling locations in September but only during afternoon sampling in December (Table 3).

**Table 1.** Matutinao River (MR) mean heterotrophic plate count in September and December.

Location and Time (AM/PM)	HPC (CFU $\text{ml}^{-1}$ ) in September 2019	HPC (CFU $\text{ml}^{-1}$ ) in December 2019
MR1 (AM)	No samples collected	16,178
MR1 (PM)	No samples collected	457,500
MR2 (AM)	212	1,691
MR2 (PM)	45	1,953
MR3 (AM)	No samples collected	479
MR3 (PM)	No samples collected	1,574
MR4 (AM)	3187	1,521
MR4 (PM)	100	812

**Table 2.** Matutinao River (MR) mean total coliform in September and December.

Location and Time (AM/PM)	Total Coliform (MPN/100 ml) in September 2019	Total Coliform (MPN/100 ml) in December 2019
MR1 (AM)	No samples collected	1,250
MR1 (PM)	No samples collected	1,800
MR2 (AM)	865	1,800
MR2 (PM)	975	1,800
MR3 (AM)	No samples collected	1,700
MR3 (PM)	No samples collected	1,800
MR4 (AM)	1,700	1,800
MR4 (PM)	1,700	1,800

**Table 3.** Presence of *Escherichia coli* in Matutinao River (MR) in September and December.

Location and Time (AM/PM)	<i>E. coli</i> in September 2019	<i>E. coli</i> in December 2019
MR1 (AM)	No samples collected	-
MR1 (PM)	No samples collected	+
MR2 (AM)	+	-
MR2 (PM)	+	+
MR3 (AM)	No samples collected	-
MR3 (PM)	No samples collected	+
MR4 (AM)	+	-
MR4 (PM)	+	+

## DISCUSSION

### Heterotrophic Plate Count

A high HPC indicates that the environment is suitable for bacterial growth. However, an increased number of HPC bacterium, such as *E. coli*, can be detrimental to human health (Allen et al. 2004). This implies that humans exposed to these bacteria are at risk of infection. MR1 is the last point of the canyoneering activity, where many tourists swim and wash their muddy footwear. Tourists were particularly many in the afternoon, attributed to the higher HPC value of MR1 (Table 1). Vignesh et al. (2014) noted a proportional relationship between water recreational activities and bacteria density. As observed in this study, bacterial density is relatively higher during the tourist peak season in December than in the off-season in September. This also coincides with what Zao et al. (2017) reported that local contamination sources (i.e. land use, population density, and economic development) could lead to an increase in total bacterial numbers (HPC). The HPC at MR4 (AM) could be attributed to its proximity to the residential area. This is supported by Pepper et al. (2004) where they reported that the major source of bacteria is from the household distribution system or the household tap and that the average number of bacteria in household tap is 3,072 CFU ml<sup>-1</sup>, which is quite close to the value obtained in the current study.

Based on available data cited in Edberg and Allen (2004), there are concerns of infection from drinking or exposure to contaminated water such as: some species of HPC bacteria are associated with disease (e.g. gastroenteritis) and physical contact with pathogenic HPC bacteria can cause illness than ingesting water. However, they did point out that HPC concentration must be high (i.e. 10<sup>4</sup>-10<sup>8</sup> CFU ml<sup>-1</sup>) to cause infection.

As seen in Table 1, most of the HPC concentrations are within 10<sup>3</sup> or below. At this concentration, the risk of disease is low, and thus, the concern for public safety is lessened. The acceptable limit set by the World Health Organization for HPC is only 100 CFU ml<sup>-1</sup> (Bartram et al. 2003). Nonetheless, the HPC concentration was observed to reach 10<sup>4</sup>-10<sup>5</sup> CFU ml<sup>-1</sup>, within the infectious dose range (Edberg

and Allen 2004). The spike in HPC concentration could be attributed to the nearby human establishment downstream and human activities upstream, where tourists engage in recreation activities like swimming, bathing, and canyoneering. This is congruent to the findings of Glińska-Lewczuk et al. (2016), who reported an increase in river HPC near households and recreational areas by locals and tourists. Tourist and domestic wastes might also contribute to the increase of bacteria count in waterways and the resuspension of bacteria from the sediment due to water activities (Mwanamoki et al. 2014).

### Total Coliform

The Matutinao River can be classified as a body of water for recreational purposes (Class B) under DAO No. 34 (EMB-DENR 1990). Under this classification, the permissible limit for total coliform is 1,000 MPN/100 ml. Like HPC, a high MPN value suggests plausible risks of infection if the coliform number exceeds the permissible limit (Leclerc et al. 2001). As recorded in September (Table 2), upstream of Matutinao River (MR2) nearly reached the maximum acceptable limit for total coliforms in recreational waters. In contrast, the downstream of the Matutinao River (MR4) exceeded the limit. In December, all sites had MPN values that exceeded the limit. As mentioned, MR1 is the terminal for canyoneering activities. Restrooms had been established in the vicinity of Kawasan Falls at MR2. At MR3, local fauna such as birds and monkeys might have contributed to an increase in total coliform if they defecated directly to the river or bacteria from their feces were transported to the river by surface runoffs (Divya and Solomon 2016). Furthermore, MR4 is near the residential areas. Similarly, some local butchers, livestock and poultry are near the river. These are all possible sources of contamination (Hoyer et al. 2006) that might have resulted in the high total coliform observed in this study. Nevertheless, the results of this study resemble the study of Zao et al. (2017), where total coliform did not differ by stream location (i.e. upstream, midstream, and downstream).

Total coliform levels of more than 1,000 MPN/100 ml indicate a considerable and growing risk of infectious disease transmission (Kapwata et al.

2018). Moreover, the high total coliform count obtained in the samples might indicate that the water sources were contaminated with fecal matter (Suthar et al. 2009; Shittu et al. 2010; Mabvouna et al. 2020). Miao et al. (2018) reported that total coliforms could also indicate the presence of enteroviruses that cause waterborne diseases, such as gastroenteritis and hepatitis. Although a high total coliform may not necessarily result in health problems, the presence of coliforms denotes that pathogens might be present in the water (Leclerc et al. 2001; Suthar et al. 2009; Mabvouna et al. 2021). Pathogenic coliforms could cause gastrointestinal, respiratory, skin, eye, ear, nose, and throat diseases (Donovan et al. 2008). Hoyer et al. (2006) reported that high total coliform is correlated with *Pseudomonas aeruginosa* Migula 1900, which causes skin rashes and otitis externa in swimmers.

### Presence of *E. coli*

The presence of *E. coli* was also confirmed in the two sampling sites. *Escherichia coli* has been an indicator organism for fecal contamination since the 1890s (Abdullah et al. 2018). Its mere presence renders water unfit for drinking. When *E. coli* counts are high in recreational waters, bathing, swimming, or even fishing will no longer be allowed (O'Flaherty et al. 2019; Rossi et al. 2020). Usually, other animals only act as a reservoir for *E. coli* but occasionally cause diarrhea (Ramos et al. 2020). This bacterium also could reproduce and survive for long periods in the environment (Jang et al. 2017). This implies a greater risk of harboring waterborne infections resulting in possible morbidities. Human exposure to high quantities of fecal bacteria such as *E. coli* in recreational waters has increased the risk of illness and disease, including gastrointestinal and respiratory disorders and skin, ear, and eye infections (Mwanamoki et al. 2014). According to Khan et al. 2018, fecal bacteria could also cause hepatitis, intestinal disorders, diarrhea, dysentery, cholera, typhoid fever, jaundice, scabies, and vomiting in children, older and younger adults, as well as people with weak immune systems/immunocompromised. *Escherichia coli* could also cause abscesses, urinary tract and wound infections (Suthar et al. 2009) and might be associated with *Vibrio cholerae* Pacini 1854, the causative agent of cholera (Mabvouna et al. 2020).

In a study by Stocker et al. (2016), *E. coli* detection is not affected by sampling time throughout the day. This corresponds to the result of the current study during the September sampling but not during December, where *E. coli* was only detected in the afternoon samples. Moreover, Blaustein et al. (2013) discussed that a decline of *E. coli* could be brought about by its prokaryotic and eukaryotic predators. They noted that *E. coli* has higher nutritional value than indigenous microorganisms, and these predators can even select their prey based on nutritional value (Balustein et al. 2013). The researchers infer that an

influx of tourists in the afternoon can disrupt this prey-predator interaction, add more *E. coli*, and allow the bacterium to multiply.

In the Philippines, the rainy season is from June to November, and the dry season is from December to May (PAGASA 2021). The absence of *E. coli* in the morning sampling in December can be attributed to sunlight, which is an essential factor in controlling the bacterial population in tropical countries with high solar radiation intensities during the dry season and the high light penetration depths in clear river waters (Conan et al. 2008; Cho et al. 2010). Troussellier et al. (2004) tested this on river-isolated *E. coli* and showed that the bacterium's survival significantly decreased. The presence of *E. coli* can also vary with increasing time scales (Muirhead and Meenken 2018).

In conclusion, the microbiological water quality of the once cleanest rivers in the Philippines is now contaminated with fecal coliforms. Both HPC and MPN displayed values that exceeded the maximum permissible limits of bacteria, 100 CFU ml<sup>-1</sup> (Bartram et al. 2003) and 1,000 MPN/100 ml (EMB-DENR 1990), respectively wherein some of which can potentially be pathogenic or opportunistic. Hence, the river needs regular monitoring to ensure safety for both locals and tourists from waterborne infections. It is our hope that the result of this study will push the local government of Badian for an immediate response to mitigate the river contamination with proper waste disposal and wastewater treatment. Furthermore, quarterly bacteriological tests must also be conducted in the river to monitor the effectiveness of the mitigation efforts toward the conservation of the tourist site.

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**ROLE OF AUTHORS:** *KJCB* – conceptualized the study, designed the experiment, analyzed the data, wrote the research paper; *JLL* and *KJD* – collected the samples, conducted the experiment, analyzed the data, contributed to the discussion.