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Fecal Enterococci Levels in Selected Tributaries of the Pampanga River Basin, Philippines, and Their Relation to Land Use

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Abstract

This study aims to generate data which can be used as a potential starting point for the updating of the Philippine Water Quality Criteria and the determination of the true impact of land use to the fecal contamination of the Pampanga River Basin (PRB), the largest subwatershed of Manila Bay. Levels of fecal indicator bacteria (FIB) were determined in the selected tributaries of the PRB, representing three land use categories, namely, the forest/woodland (control), agricultural and residential lands. FIB were quantified in order to investigate the potential contribution of the selected areas in the fecal contamination of the PRB. The study was conducted in 2021 covering March, May, June, July, and September to represent the dry (March and May) and wet (June, July, and September) seasons. Counts of FIB, namely thermotolerant coliform, *E. coli*, and enterococci were qualitatively correlated with the results of the ocular survey and key informant interview based on known fecal contributors and their relevant rainfall data. FIB counts of water bodies in the selected agricultural and residential land use categories had Geometric Mean (GM) counts that are statistically greater than those of bodies of water near the representative forest/woodland (control), and exceeded the acceptable GM limits for all FIB, regardless of the season. Notably, the GM values recorded for the waters near the selected forest/woodland (control) passed the water quality criteria for all measured FIB parameters for both seasons. Furthermore, enterococci levels in the control site were statistically lower during the wet season. These initial findings suggest that agricultural and residential land use categories could be major contributors to the unacceptable water quality of tributaries of the Pampanga River Basin. The prevalence of thermotolerant coliforms and *E. coli* was noted regardless of rainfall and land use, indicating these FIB may not be adequate as water quality indicators. With their ability to survive and persist in fecally contaminated sediments in water bodies and in nutrient-poor environments, enterococci could be more definitive indicators of fecal contamination and microbiological quality of environmental waters.

Keywords

Enterococci, Thermotolerant Coliforms, *E. coli*, Geometric Mean, Land Use

1. Introduction

Through the implementation of the mandamus rulings by the Supreme Court of the Philippine Government (GR Nos. 171947-48), three key government offices, namely: the Bureau of Soils and Water Management (BSWM), an attached agency of the Department of Agriculture (DA), the Manila Bay Coordinating Office (MBCO) and the National Mapping and Resource Information Authority (NAMRIA), both of which under the banner of the Department of Environment and Natural Resources (DENR) have determined in their respective independent reports done in 2012 [1] and 2015 ([2]; NAMRIA) that fecal contamination is present not only in the sampled tributaries of the four main subwatersheds of Manila Bay [1], but also in the Bay itself [2], which still occurs even in recent time [3]. One of the said subwatersheds that drain into the Bay is the Pampanga River Basin (PRB or Basin), one of the 18 major river basins in the country according to the River Basin Control Office (RBCO) of the DENR [4]. The PRB is composed of the majority (63%) of the entire watershed of the Manila Bay with an area of 1,237,688 hectares (ha) [1]. It was found to have tributaries that exceeded the 1990 DENR water quality criteria for fecal coliforms, confirming fecal contamination of the Basin from 2012 to 2014 [5]. These studies recommended that a link between the fecal contamination of each subwatershed and specific land use categories be established to determine the primary source of the contamination. This present study was conducted in order to address this gap through the quantification of fecal enterococci in the tributaries of the PRB, largest subwatershed of Manila Bay, while accounting for differing major land use categories present in the area. Enterococci, which are known to be in high levels in feces of humans and warm-blooded animals, have shown remarkable persistence once released into the environment [6], suggesting these microorganisms are ideal indicators of fecal contamination, and subsequently, of poor sanitation.

This present study hypothesized that the residential and agricultural land use categories are the main contributors to the fecal contamination of the subwatershed. The main objective was to preliminarily assess the potential contributions of land uses in the levels of key fecal indicator bacteria (FIB), namely, the thermotolerant coliforms, *E. coli*, and the enterococci in the PRB. The specific objectives of this study were to determine the thermotolerant coliform, *E. coli*, and enterococci counts of selected tributaries representing the different general land use categories in the PRB, to preliminarily estimate the contribution of land use to the fecal contamination within the PRB, and to appraise the suitability of the enterococci group as a microbial indicator of fecal contamination.

This study has generated baseline data on the nature and proposed source of the fecal contamination in the subwatershed that could be used as a potential starting point or reference for policymaking on water quality assessment and the impact of land use in relation to fecal contamination. The said data can also be used in the review and updating of the national Water Quality Criteria (WQC), thereby facilitating more targeted solutions that will improve the state of sanitation in the country. While this study was focused on the assessment of fecal contamination of the selected tributaries of the Pampanga River Basin only, the findings will provide valuable input on the impact of anthropogenic activities on the ecological dynamics of the Bay.

2. Materials and Methods

2.1. Selection of Sampling Site, Ocular Survey, and Key Informant Interview

Specific tributaries of the PRB were plotted as potential sampling points and grouped according to the different land use categories they represent. **Figure 1** indicates the overlay map of PRB according to DENR-RBCO [4], with the plotted sampling points designated with the following categories: A for Agricultural, R for Residential and C for Forest/Woodland (Control). The sampling sites were selected based on the following criteria: the sampling point must be in the land use category's general area as categorized by the BSWM [1] Land Use Map, the tributary must be accessible by regular means (by vehicle, boat, or on foot); and (except for the Control Sites) it must be a tributary draining to a major river of the basin. Additionally, the sites were chosen based only on their position in the BSWM [1] Land Use Map and not due to any prior knowledge of the area's sanitation laws, infrastructure, or cultures to ensure relatively unbiased selection. To maximize the representation and minimize sampling bias in lieu of limited logistics, three (3) sites per Land Use Category were selected, for a total sampling size of nine (9) sites. Furthermore, the agricultural and residential areas were labeled based on the main rivers they drain into, namely the Pampanga River (designated with the number 1), the Angat River (designated with the number 2), and the Santa Maria River (designated with the number 3). The sites that serve as Control are headwater sites located at the forest/woodland areas of Carranglan, Nueva Ecija, which is in the upper borders of the Basin, away from any potential interaction from anthropogenic factors.

After initial assessment based on the identified criteria, a preliminary visit to the sites was done, wherein coordination with the relevant Local Government

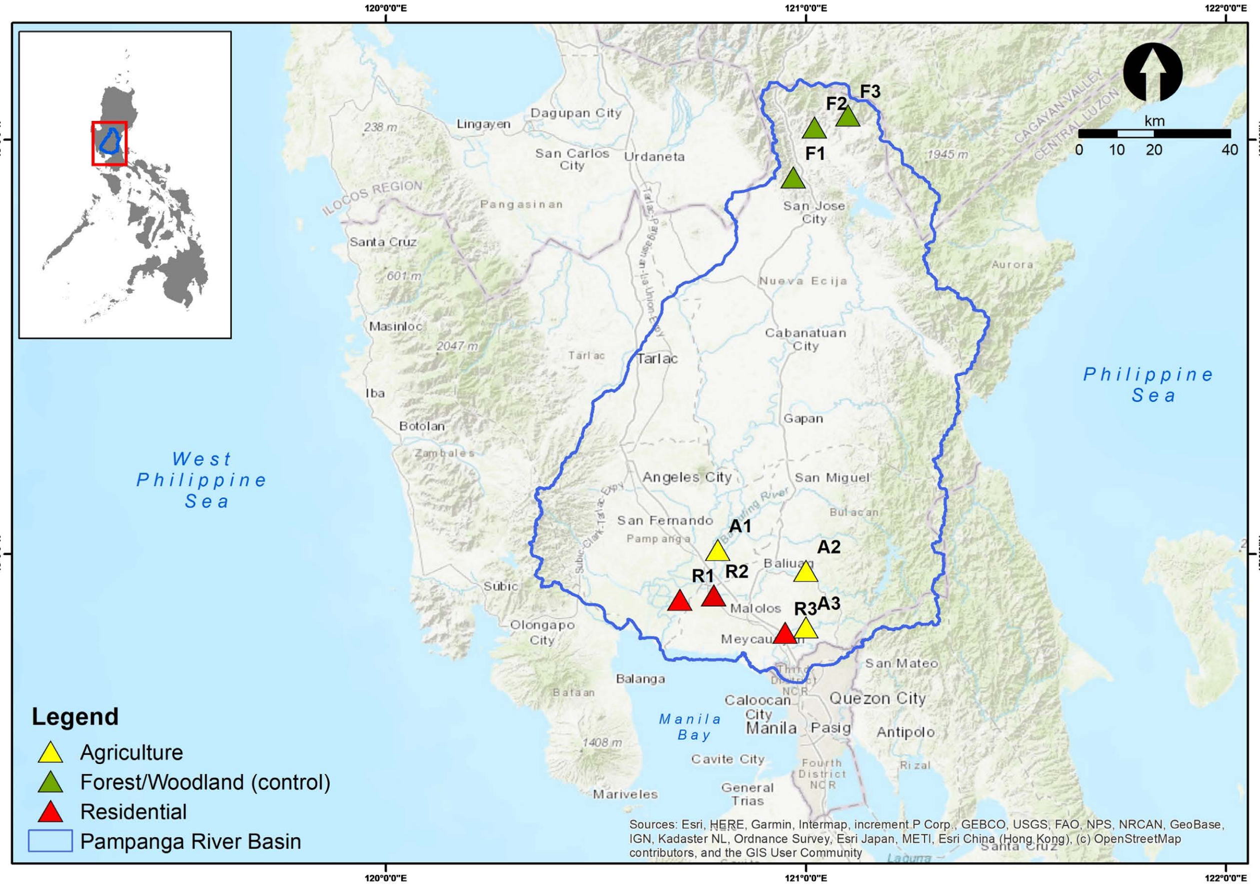

Figure 1. Location of Study Sampling Points [Agricultural 1 (A1)—Santa Cruz, San Simon, Pampanga; Agricultural 2 (A2)—Marungko, Angat, Bulacan; Agricultural 3 (A3)—Tumana, Santa Maria, Bulacan; Residential 1 (R1)—Santa Lucia Anac, Masantol, Pampanga; Residential 2 (R2)—Santo Niño, Calumpit, Bulacan; Residential 3 (R3)—Turo, Bocaue, Bulacan, Forest/Woodland (Control) 1 (C1)—Puncan, Carranglan, Nueva Ecija; Forest/Woodland (Control) 2 (C2)—Bunga, Carranglan, Nueva Ecija, Forest/Woodland (Control) 3 (C3)—Burgos, Carranglan, Nueva Ecija)].

Units (LGUs) and government officials in charge of the area where the sites are located was conducted. Furthermore, notable environmental features (e.g., wildlife, vegetation, population density) were documented. An ocular survey and key informant interview relevant to fecal contamination contributory activities at each designated sampling point were performed using a checklist. The checklist includes: sampling point code; coordinates; location; land use type; population category of the barangay; general sanitation facilities (*i.e.*, improved sanitation in residences, toilet with septic tank, unimproved open sanitation at sampling site); and presence of contributory activities based on Boehm & Sassoubre [6] such as livestock production, poultry production, wildlife, informal settlements, and open dumping of fecal wastes into water bodies. Key informants are defined as residents who are currently living, or are working for, at least 5 years in the sampling area, and who were also tapped as collectors of samples for the water body for their occupation.

2.2. Water Sampling, Transport, and Analysis

From each designated sampling point, except in the Forest/Woodland (Control) sites, a 450-m long transect was drawn, and three (3) water samples of at least 300-ml volume were collected in sterile bottles in the 0-meter, 225-meter, and 450-meter points of the transect. For the Forest/Woodland (Control) sites, the three replicate water samples were collected directly from the waterfall at 1 to 2-minute intervals per bottle, until each bottle contained at least 300 ml. The collected water samples were then stored in a cooler with ice (about ≤10˚C) and transported to the Soil Biological Resources Management Section (SBRMS) Laboratory of the Soil and Water Resources Research Division (SWRRD), or alternatively in the Soil Microbiology Section (SMS) of the Laboratory Services Division (LSD), both in BSWM, Quezon City. Samples were analyzed within the 8 to 24 hour-holding time, employing aseptic techniques. Prior to testing, the samples were serially diluted up to 10^{-4} using sterile distilled water. Aliquots of undiluted samples and their dilutions were analyzed using standard procedures described in the Standard Methods for the Examination of Water and Wastewater or SMEWW [7]. The samples collected on March 15, 16, and 17, 2021 were analyzed using the Defined Substrate Technology (DST), an IDEXX Laboratories-patented protocol. DST-based testing of samples was performed at the SMS-LSD Laboratory, BSWM. Using DST, determination of thermotolerant coliform and *E. coli* counts was performed using Colilert-18 (IDEXX, USA) according to the manufacturer's instruction based on SMEWW 9223 [8]. Enterococci counts were determined using Enterolert (IDEXX, USA) according to the manufacturer's instructions based on SMEWW 9230D [9].

For samples collected in succeeding sampling periods, namely, May 24, 25, and 26, June 28, 29, and July 1, July 26, 27, and 29, and September 13, 14, and 16, 2021, determination of thermotolerant coliform and *E. coli* counts was performed using the multiple tube technique fermentation technique (MTFT) according to SMEWW 9221B [7]. Enterococci were quantified using the multipletubetechnique (MTT) detailed in SMEWW 9230B [9]. These aforementioned methodologies were performed at the SBRMS-SWRRD Laboratory, BSWM.

2.3. Sampling Frequency and Data Analysis

Samples were collected during the months of March and May 2021 for the Dry Season, and in June, July, and September 2021 for the Wet Season to consider climate variability. The months were selected based on the historical monthly rainfall data of the specific municipalities of Bulacan, Pampanga, and Nueva Ecija, where the sampling points are located (**Figure 1**). The period from June to September 2021 was confirmed as part of the wet season with the official declaration by the Philippine Atmospheric, Geophysical, and Astronomical Services Administration's (PAGASA) on June 4, 2021, after the passage of Tropical Storm (TS) Dante [10]. As stipulated in DENR-DAO 2016-08 Section 6.3.c [11], the GM in Most Probable Number per 100 ml (MPN/100ml) of each group of fecal indicators determined for every sampling site was calculated by taking the nth root of the product of each site's averaged MPN/100ml obtained per sampling month of a climactic season [11]. The GM is used instead of the arithmetic mean because the microbial count data is expected to cover a vast range, and the GM will help in the variance homogeneity for inferential statistics purposes. The generated GM data were common log-transformed to further homogenize the variances across samples, which were then analyzed statistically via Two-Way Analysis of Variance (ANOVA) using SAS OnDemand for Academics. The thermotolerant coliform, enterococci and *E. coli* counts for each sampling point were qualitatively compared with the presence of contributory activities as indicated in the checklist and the Water Quality Criteria (WQC) for the thermotolerant coliforms set by the DENR-DAO 2016-08 [11], *E. coli* as set by the USEPA Recreational Water Quality Criteria or RWQC [12], and enterococci as set by WHO Guidelines for Recreational Water Quality or GRWQ [13]. This is to determine whether links between microbial counts and certain land use categories exist. Moreover, relevant meteorological data, like rainfall and temperature, were sourced out locally from PAGASA and internationally via the Langley Research Center of NASA, with the intention of comparing them qualitatively with the counts of the target fecal bacterial groups alongside the WQCs and the data on fecal contributory activities gathered through the survey and key informant interview.

3. Results

Statistical Analysis of FIB GM Counts

Figure 2 presents the thermotolerant coliform, *E. coli*, and enterococci counts, expressed as the common logarithm of their GM, recorded at all sampling sites at different sampling periods. Results of the study demonstrated that the thermotolerant coliform, *E. coli*, and enterococci GM levels in water bodies near the selected agricultural and residential land use categories exceeded the limits for each microbial group specified in the Water Quality Guidelines stipulated in the DENR-DAO 2016-08 (for thermotolerant coliform) [11], the USEPA RWQC of 2012for *E. coli*) [12] and the WHO GRWQ of 2021 (for enterococci) [13], regardless of season. Alternatively, the GM enterococci count of samples from the representative control sites are within the acceptable levels set by the WHO [13] in both the dry and wet seasons. No statistically significant interactions between season and land use were found in all microbial counts ($p = 0.9176$ for thermotolerant coliform, $p = 0.9602$ for *E. coli*., and $p = 0.2713$ for enterococci). Additionally, FIB counts of water bodies in both agricultural and residential land use categories had statistically significant differences over the forest/woodland (control) land use category for the thermotolerant coliform ($p = 0.0003$), *E. coli* ($p = 0.0006$), and enterococci GM count ($p = 0.0005$).

Lastly, the enterococci counts for the dry season are statistically greater than those ones recorded during the wet season ($p = 0.0002$) across land use categories, while no such differences were observed for the thermotolerant coliforms ($p = 0.8143$). All the land use categories registered a rather wide 95% Confidence Interval (CI) range in all microbial counts, particularly, for the agricultural and

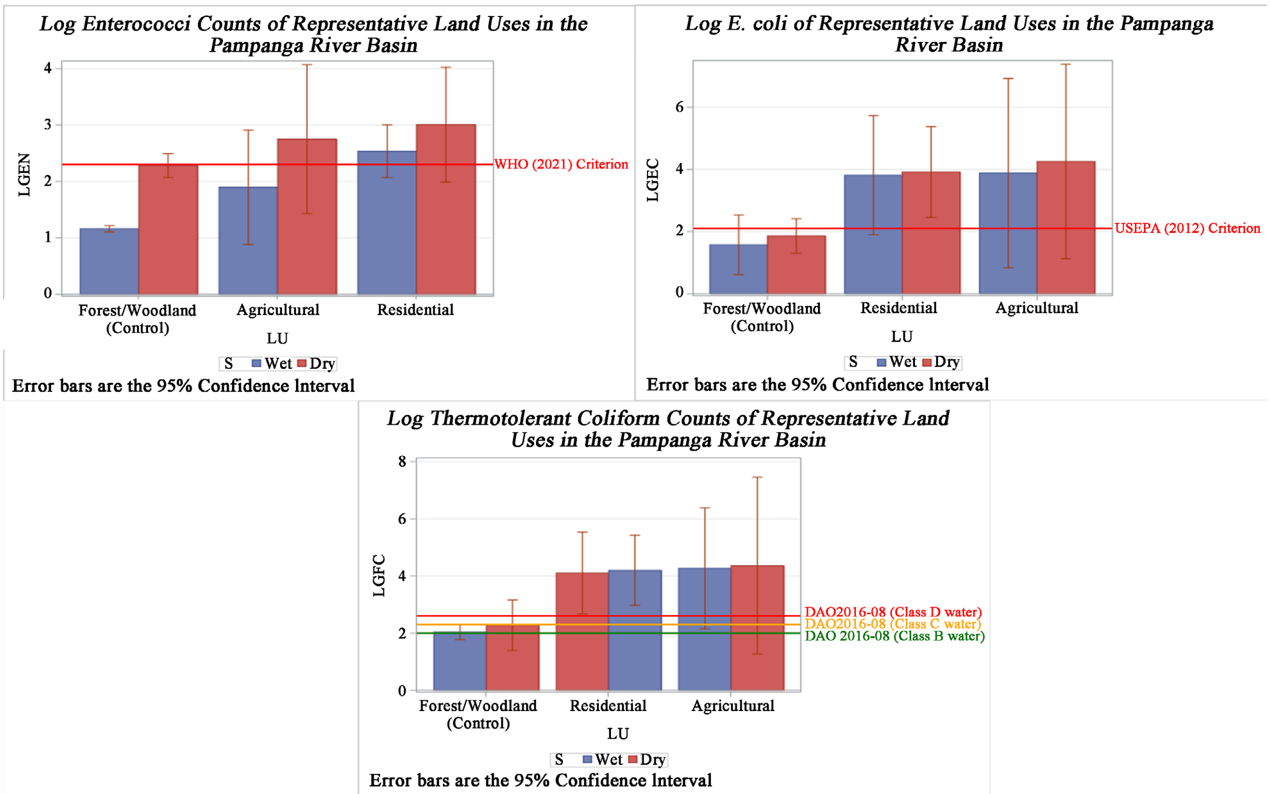

Figure 2. Log microbial counts of all representative land uses during the dry and wet seasons of 2021. The graphs are arranged in an ascending order based on mean.

residential land use categories. To illustrate, the dry season samples from the forest/woodland (control), agricultural, and residential categories yielded thermotolerant coliform counts that ranged from 25 to 1400 MPN/100ml, 18 to 28,000,000 MPN/100ml, and 470 to 340,000 MPN/100ml, respectively. It should be noted that the acceptable microbial limit for environmental waters ranges from 100 to 400 MPN/100ml [11] depending on the class of water body. This trend is also observed in the thermotolerant coliform counts recorded for the water bodies adjacent to the selected control (58 - 200 MPN/100ml), agricultural (140 to 2,400,000 MPN/100ml) and residential areas (930 to 270,000 MPN/100ml) during the wet season. The wide 95% CI range continued even in the *E. coli* counts recorded for the water bodies near the selected land use categories, during the dry and wet seasons, respectively: 20 to 260 MPN/100ml and 4 to 340 MPN/100ml for the forest/woodland (control) site; 13 to 24,000,000 MPN/100ml and 7 to 8,400,000 MPN/100ml for the agricultural site; and 300 to 240,000 MPN/100ml and 79 to 540,000 MPN/100ml for the residential area. Most of the values exceeded the acceptable microbial limit for *E. coli*, which is 126 MPN/100ml [12]. In terms of enterococci count, regardless of the dry or wet season, the wide 95% CI was also observed in water bodies near the selected agricultural (27 to 12,000 MPN/100ml and 8 to 810 MPN/100ml) and residential (98 to 11,000 MPN/100ml and 120 to 1000 MPN/100ml) sites. Meanwhile, the enterococci counts recorded for the forest/woodland (control) category had a noticeably narrower range, that is, 120 to

310 MPN/100ml for the dry season and 13 to 16 MPN/100ml for the wet season.

4. DISCUSSION

4.1. Relationship between Land Use and FIB Levels

Byappanahalli and co-authors [14] have reviewed the different ways by which FIB like thermotolerant coliforms, *E. coli* and enterococci can enter or exit water bodies. They surmised that the presence of different sources of FIB near a water body, especially those that are caused by human activities, generally lead to a higher concentration of FIB. The conducted ocular survey and key informant interview confirmed that, alongside the presence of natural fauna like birds, all the representative residential sites have sewage as a main source directly being deposited in many residences and establishments in the area. Moreover, overall agricultural soil runoff is considered as the main FIB contributor in all representative agricultural sites, alongside one or more of the following pollution sources: domesticated animals, piggeries, poultry and bird farms, and related livestock areas. The representative control (forest/woodland) sites have virtually none of those readily identifiable anthropogenic sources, owing to their inaccessibility to humans. Likely, only natural sources, such as soil, and the local wildlife (e.g., birds and boars) could have contributed to the FIB numbers in water bodies near the selected forest/woodland site. These observations concurred with the assertion of Byappanahalli *et al.* [14], that FIB numbers are significantly higher in water sources near areas with recurring anthropogenic activities due to the presence of well-defined fecal sources. This is further reinforced by the fact that in the agricultural and residential land use categories, riparian cover is greatly reduced, and that a marked decrease in riparian vegetation density increases fecal bacteria surface runoff in the water sources near it, as compared to forested areas [15].

As of the February 2020 Annual Action Plan + Investment Report (APIR) of the Manila Bay Sustainable Development Master Plan (MBSDMP) [3], it was seen that the water quality in the Bay continues to deteriorate, and this includes fecal pollution. Many monitoring stations in the Bay have reported fecal pollution levels beyond the WQC set by the DENR-DAO [11] for Class SB (Fishery water class, tourism zones and recreational water Class 1 in marine waters), that is 100 MPN/100 ml, as recent as 2018. The report attributed this to several factors, the first being many Metro Manila households are still "not yet being served by sewerage and septage systems". Households outside Manila that are not properly connected to proper septage systems were also seen as contributors. Further, effluents with non-complying FIB levels from livestock farms, industries and commercial establishments and the like, were also recognized as point sources of fecal contamination. Finally, wastewater from non-point sources like agricultural or aquacultural runoff was also noted as a main contributing factor. These factors could be linked to the results of this study, as the agricultural and residential sites sampled in the PRB generally have numerous activities that contribute to fecal pollution of the water body. Moreover, some sites have poor or non-existent sanitation systems as observed in Calumpit, Bulacan (Residential 1) and Bocaue, Bulacan (Residential 3) where pipes directly discharge sewage into the nearby water bodies and numerous stilt houses with no sanitation facilities are located on top of the tributary river of the Masantol, Pampanga site (Residential 2). This led to the overall non-compliance of the sampled tributaries in the agricultural and residential land use categories to the WQCs set for thermotolerant coliforms, *E. coli*, and enterococci, regardless of season. Such contaminated tributaries drain into the even bigger rivers that eventually drain into the Manila Bay, hence their impact to the fecal pollution of the Bay is inevitable. This phenomenon further reinforced the general position of many previous studies that land use categories with continuous anthropogenic activities, are inextricably tied with high FIB levels of their nearby water bodies [14] [16].

4.2. FIB Survivability and Persistence during Hydrologic Transit

Devane *et al.* [17], citing numerous previous works, stated that the transport of microorganisms between the sediment and water column in river systems is a "dynamic process that occurs during base river flows and during high flow events". Haller *et al.* [18] had also established that FIB like thermotolerant coliforms and *E. coli* can survive and remain culturable for up to 40 days in freshwater sediment, while enterococci could remain culturable for up to 50 days. In an earlier concurrence with the study of Haller *et al.*, [18], Lleo *et al.* [19] had elucidated that for certain *Enterococcus* species that originated from human and animal feces like *E. faecium*, *E. casseliflavus* (formerly *E. flavescens*), and *E. durans* [14], their cultivability can last for about 55 to 61 days in a freshwater microcosm and about 51 to 57 days in a seawater microcosm. Since sediments like soil and animal excrement are the primary vehicles of the FIB during hydrologic transit, the continued persistence and survivability of enterococci are ensured provided the sediment particles remain intact or new sources, like sewage from pipes or soil runoff, are added into the environment. Nutrients from sewage, soil, and interstitial water column regardless of flow rate, could enhance the growth of FIB in water bodies [17]. However, there are other environmental factors that may also play a role in decreasing their survivability. The most common environmental factors that could induce stress to FIB survival include sunlight, salinity, disinfection, starvation, and predation [14]. However, this present study did not determine these environmental parameters due to lack of appropriate equipment and funding constraints. In future studies, an investigation on the effects of these factors to the survivability and persistence of the FIB may be undertaken. Previous studies reported that FIB could be inactivated through exposure to higher sunlight intensity [20] and disinfectants like chlorine [21]. Scarcity of nutrients which leads to starvation could have more inhibitory effect on *E. coli* than on enterococci [22], suggesting that the latter FIB have better survivability in the environment. In addition, the presence of predators like protozoa can lead to a net decrease of FIB numbers in water [23].

Relating this information in the context of the PRB, the sampled agricultural and residential sites were in close proximity to tributaries that drained to three main rivers, the Pampanga River, the Angat River, and the Santa Maria River. With the Manila Bay as the endpoint, the Pampanga River has a total length of around 260 km, the Angat River at around 153 km, and the Santa Maria River at around 26 km (as per Google Maps estimate). In addition, according to the 2013-2018 monthly and yearly discharge data collected by Macalalad *et al.* [24], the volume of water that drains from the rivers of the PRB to the Manila Bay, which generally increases from June to December, can exceed 500 m^3 per second when at least two (2) tropical cyclones occur. As previously stated, the survival of FIB could be extended when attached to nutrient-rich sediments that harbor feces, decaying organic matter, and other debris, which are commonly discharged into the water bodies near areas with poor or non-existent sanitation systems. These sediments continually get replenished due to the mixing of water inflows coming from the other tributaries of the PRB, which in turn have their own stockpile of nutrients like organic matter, phosphorus, nitrates, and ammonium-nitrogen, all of which mostly obtained from anthropogenic sources like sewage [14] [16] or agricultural runoff [1]. Furthermore, enterococci can enter either the starvation state or the viable but not culturable (VBNC) state if certain environmental conditions such as oligotrophy, presence of predators and lower ambient temperatures occur [17] [19]. All of these factors can enhance the survivability of FIB, especially the enterococci group, during transit, and subsequently, during their transport to Manila Bay while in a culturable state. This could possibly take place even in FIB that originated in headwater sites (e.g. Carranglan, Nueva Ecija), which could be verified through a more extensive study on FIB level determination in the central area of the PRB. This future study will facilitate the validation of the survivability potential of FIB during hydrologic transit, especially for the enterococci group.

4.3. The Wet Season in Relation to FIB Persistence

Alongside the aquatic environment's physico-chemical characteristics, the persistence of FIB in waters is also heavily affected by climactic variations [16]. In relation to this, increased rainfall generally leads to heightened concentrations of FIB in ambient waters due to increased runoff and resuspension from sediments like soil, animal excrement, decaying plant matter, and, in the case of more urban areas, exfiltrated sewage [6], which was already reflected in some representative PRB tributaries that generated high thermotolerant coliform counts in previous studies [1] [2] [5]. Moreover, the sampling sites in this study are all within a Type III Climate according to the Modified Corona Classification (MCC) System, wherein after a relatively dry season from November to April, a relatively wet season characterized with increased tropical cyclone incidence during the rest of the year is a common phenomenon. The increased runoff due to high precipitation is therefore considered a main contributing factor to fecal contamination in the sampled areas, especially during the last two sampling periods, namely, in July and September 2021, when three typhoons significantly increased rainfall in all sampling sites (*i.e.*, TY *Fabian* on July 16 - 23; TY *Jolina* on Sept. 6 - 9, and TY *Kiko* on Sept. 7 - 12). This is evidenced not only by observably higher depth and flow of the water sources during the conduct of the sampling proper during the wet season regimes, but also according to the daily adjusted precipitation data obtained from the National Aeronautics and Space Administration Prediction of World Energy Resources (NASA POWER) Data Viewer (**Figures 3-5**).

Isobe *et al.* [25] stated in their work that, in general, FIB levels in tropical waters apparently tend to be lower during the dry season, and it is attributed to the lower flow of water. However, this was not reflected in this present study since the measured FIB, for the mean GM thermotolerant coliform and *E. coli* counts and 95% CI range remained statistically similar regardless of season and land use. Moreover, the mean GM values for the enterococci count and their corresponding 95% CI range observed during the wet season are significantly lower than those observed uring the dry season. These results suggest that enterococci are not resuspended in non-fecal sediments to the river basins as effectively as the thermotolerant coliforms and *E. coli*. Likely, with the lower inflow due to lack of rainfall, sediments that harbor fecal matter from wildlife and human could get concentrated in the area. Moreover, this finding concurs with the report on the natural presence and prevalence of thermotolerant coliforms and *E. coli* in water bodies [7] [16], as their numbers did not statistically differ even with the increased precipitation caused by the passing of three typhoon events, regardless of the nearby land use. Earlier studies had demonstrated the survival

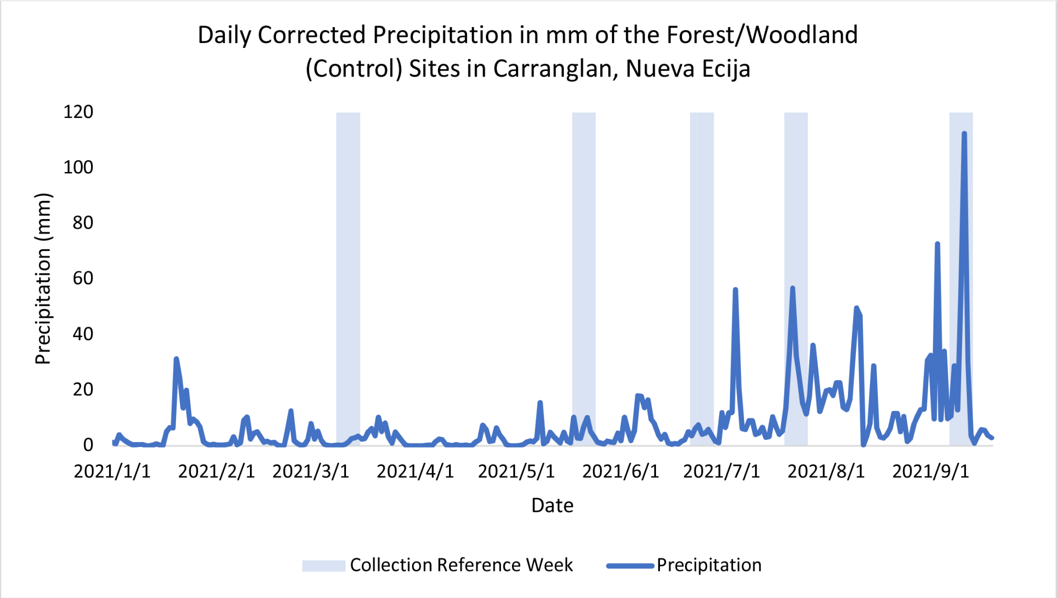

Figure 3. Daily adjusted precipitation records of all Forest/Woodland (control) from January 1 - September 19, 2021 (Source of basic data: https://power.larc.nasa.gov/data-access-viewer/). Note: The Collection Reference Week is the sampling day proper plus the six days before it.

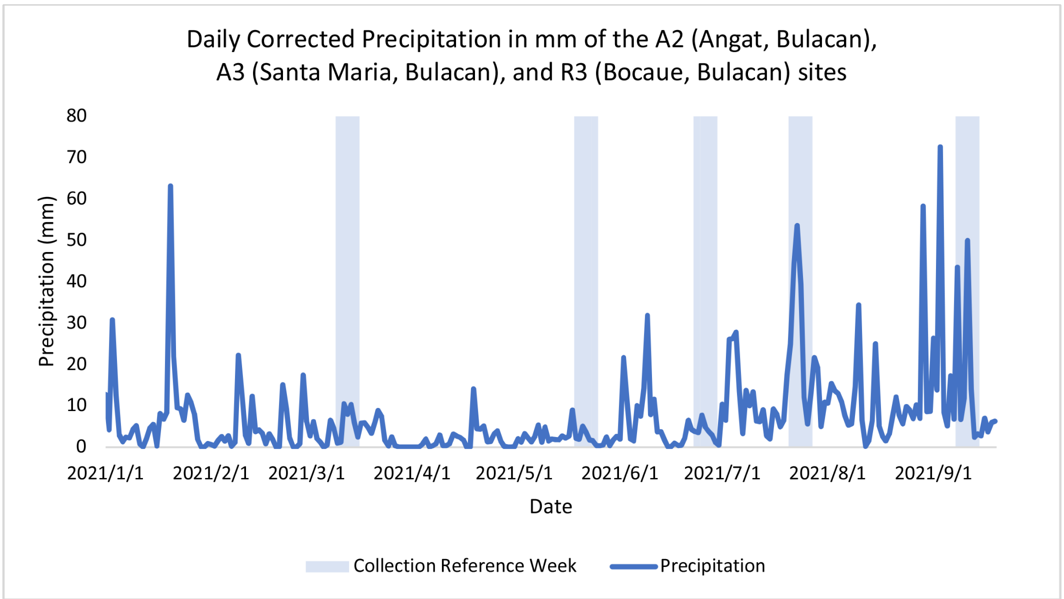

Figure 4. Daily adjusted precipitation records of Agricultural 2, Agricultural 3, and Residential 3 sites from January 1 - September 19, 2021 (Source of basic data: https://power.larc.nasa.gov/data-access-viewer/). Note: The Collection Reference Week is the sampling day proper plus the six days before it.

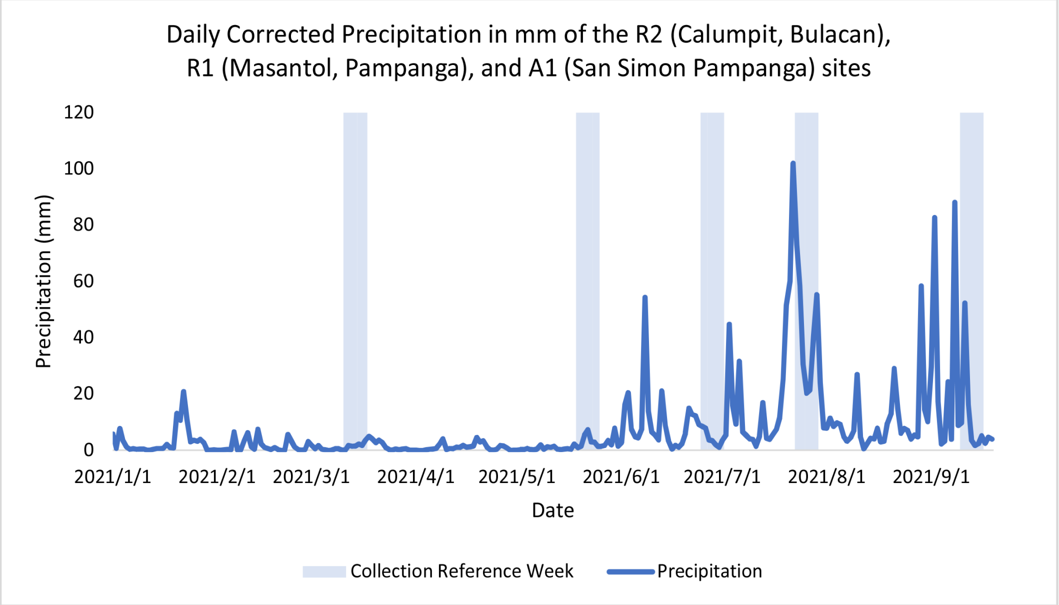

Figure 5. Daily adjusted precipitation records of Residential 2, Residential 1, and Agricultural 1 sites from January 1-September 19, 2021 (Source of basic data: https://power.larc.nasa.gov/data-access-viewer/). Note: The Collection Reference Week is the sampling day proper plus the six days before it.

of thermotolerant coliforms like *E. coli* in river water and under laboratory conditions for up to 260 days [26] and their persistence in tropical environments, where *E. coli* could be found naturally in pristine areas of tropical rainforests [27]. Coliforms and *E. coli* survive indefinitely after introduction from natural reservoirs like soil until they eventually become part of the natural microflora [28]. Results of this present study concurred with their observations.

4.4. Evaluation of Enterococci as a Fecal Microbial Indicator

The data generated from this present study indicate that the presence of enterococci in the sampled waters was a consequence of fecal matter concentration and not by environmental runoff like soil especially in the forest/woodland (control) land use category, unlike the thermotolerant coliforms and *E. coli*, whose numbers remained relatively similar regardless of rainfall amount and land use. This is because the sampled water bodies from the forest/woodland (control) land use category had no consistent source of fecal matter outside of occasional wildlife. No anthropogenic sources like toilets, sewers, or latrines were observed in the control sites. These observations were corroborated by the data derived from the results of the ocular survey and key informant interview related to fecal contributory activity in the selected sites. According to the informants, human settlements in areas near the forest/woodland site are generally located at least one to three kilometers from the site of collection. On the other hand, there are more persistent sources of fecal contamination in the tributaries near the selected agricultural and residential sites. For example, all residential sites examined in this study had sewage as a consistent source, which directly drains from the residences or establishments beside and atop the water body (such as in the Masantol, Pampanga site), or above the water body via dikes (like in the Bocaue and Calumpit sites in Bulacan). This is also coupled with other sources like plant litter, leftover food, animal carcass, and manure from domesticated animals that may have flowed from the upstream areas. Meanwhile, the sampled water bodies in the selected agricultural sites have received runoff from the irrigation of the farms, the cleaning of the livestock shelters harboring feces from chicken, ducks, cattle and goat and the roaming domesticated animals. There is likely a continuous introduction of enterococci from the agricultural and residential areas to the adjacent bodies of water and these FIB may have been more concentrated in these areas during the dry season.

Additionally, fecal contamination of water bodies near the selected residential areas is apparent and alarming since there is a continuous deposition of sewage and occurrence of contributory anthropogenic activities throughout the year. Furthermore, while all the key informants interviewed within the sites under the residential land use category reported that they have improved sanitation facilities, it was observed that the communities near the sites, such as those in Bocaue and Calumpit, Bulacan, were directly disposing their wastes into the river. In some sites, open defecation still occurs in certain areas, like in a community of stilt houses atop the sampled Pampanga River tributary in Masantol, Pampanga (Residential 1). On the other hand, the FIB levels in the agricultural and forest/woodland (control) land use categories, were observed to be more fluctuating. Agricultural sites have regular periods of planting, fertilization, irrigation, and cleaning. In these sites, septic tanks are the main improved sanitation facility which are regularly emptied and disposed. Meanwhile, generally random rainfall events caused by climactic factors influence the flow of fecal matter the most for the water bodies within the forest/woodland (control) land use category, as there are no nearby human settlements, and no sanitation facilities which could consistently contribute to their FIB levels. All these events and factors are reflected through the 95% CI of the FIB counts of the water bodies near the selected agricultural and forest/woodland sites (control), in which they have periods when the counts are within acceptable limits (**Figure 2**). This is more pronounced with the enterococci counts observed in forest/woodland (control) land use category, where counts were found to be fluctuating between conforming and non-conforming to the WHO WQC [13] during the dry season, while strictly conforming to the said WQC during the wet season. With the prevalence of thermotolerant coliforms and *E. coli* in water bodies within PRB and in similar tropical waters where these FIB became part of the natural microflora over time [28], these FIB may not be sufficient microbial parameters in determining the microbiological quality of environmental water. The suitability of enterococci as FIB is also exemplified due to their higher survival and persistence in fecal sediments as compared to thermotolerant coliforms and *E. coli* [17], attributable to its morphology and ability to respond to unfavorable conditions. With their coccoidal morphology, the cells have less surface area which enables them to require less nutrients for survival, avoid predators, and attach to smaller sediments [29]. Notably, enterococci have the ability to enter either the starvation state or the VBNC state within 14 - 61 days (depending on species group) during periods of unfavorable growth conditions such as poor nutrient levels in the environment and be resuscitated to culturable state when nutrients, such as those coming from anthropogenic sources in the PRB (*i.e.*, sewage from pipes located downstream), become available within that time frame [19].

The enterococci group is not only suitable as an alternate microbial indicator for fecal contamination of water bodies, but it is also a better microbial quality parameter when compared with thermotolerant coliforms and *E. coli* in the context of this study, due to the occurrence of host/reservoir-specificity. Unlike thermotolerant coliforms and *E. coli*, species of enterococci have specific hosts, which includes humans and warm-blooded animals such as wildlife and domesticated animals [9]. *Enterococcus cecorum*, *E. sacharrolyticus*, *E. dispar*, *E. cecorum* and *E. asini* are harbored by specific animal reservoirs, namely, chickens, cattles, humans, pigeons and donkeys, respectively [14]. Detection of these enterococci will lead to more definitive tracking of direct fecal sources of contamination. The data gathered through the ocular survey and key informant interview can serve as an initial guide for this endeavor.

5. Conclusions

Both the agricultural and residential land use categories may have contributed more to the fecal contamination of their nearby water bodies compared to the forest/woodland (control) category for all FIB determined. This is most likely caused by the presence of numerous sources of fecal contamination in both the agricultural and residential land use categories that contribute to the high FIB values in the tributaries that exceeded the established microbial limits, during the dry and wet seasons, as compared to the control. Moreover, the findings also reinforce the fact that anthropogenic activities in the selected sampling sites are the main sources of fecal contamination of the adjacent water bodies, which were also observed and reported in previous studies [14] [16] [30] [31] [32].

This study showed that the enterococci group is a better indicator of fecal contamination compared to thermotolerant coliforms, the current microbial quality indicators adapted by the Philippines for environmental waters [11], and to *E. coli*, the microbial indicator recommended by the USEPA [12] to be used alongside the enterococci group. Results of this study also concurred with the well-established literature that the thermotolerant coliforms and *E. coli* are part of the natural microbial fauna of tropical waters.

However, the FIB counts recorded for the water bodies near the selected sites do not fully reflect the actual contribution of each land use to the fecal contamination and the FIB levels, as there are other factors to be considered and investigated. There is a need for a long-term investigation that will assess the inter-annual variation of environmental factors that may affect the thermotolerant coliform, *E. coli*, and enterococci counts in the sampled river bodies, like rainfall, temperature, and the water body's chemical composition, with special focus on the contribution of land uses and their sanitation levels. Moreover, it is recommended to have more extensive sampling selection to encompass the under-represented areas of the PRB, such as the industrial, commercial and other built-up areas. Also, to further establish the suitability of the enterococci as an alternative fecal indicator in differing tropical water compositions and conditions, it is recommended that studies on enterococci levels in other freshwater and marine bodies in the country be undertaken. In these proposed future studies, physico-chemical parameters of the water bodies such as pH, temperature, nitrates, phosphorus, dissolved oxygen, and chlorides will be measured to determine their effects on the persistence and survival of these FIB in local aquatic environmental conditions. Finally, the enumeration of enterococci in water bodies in the country through conventional or molecular methods should be conducted as part of a Microbial Source Tracking (MST) initiative, allowing for an even more specific recognition of fecal contributors which can lead to more targeted solutions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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