Bacteriological assessment of Calumpang River in Batangas City, Philippines

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Abstract - Rivers are important to society, providing water for consumption, agriculture and carrying away human wastes. The bacteriological assessment of water is important in pollution studies, as it is a direct measurement of detrimental effect of toxic waste on human health. The bacteriological water quality of Calumpang River was assessed using the multiple-tube fermentation technique to count total coliform (TC), fecal coliform (FC) and fecal streptococci (FS). Sampling was conducted to use bacterial densities to evaluate the microbial quality and to assess the sanitary condition of Calumpang River which was subdivided into three stations namely Brgy. Malitam (1), Calumpang Bridge to Bridge of Promise (2) and Tinga, Labac (3).This body of water exhibited high concentrations of TC, FC and FS which indicates a significant fecal contaminations originating from humans or other warm-blooded animals. The results obtained from all of the parameters exceeded the reference limit of water quality guidelines and general effluent standards based on DENR Administrative Order of 2008. FC/FS ratio was used to identify source of fecal contamination. FC/FS ratio results in Stations 1 and 2 were 0.74 and 3.67 respectively, indicating mixed animal and human fecal contamination while in Station 3 was 4.76, designating a human fecal contamination. Such results revealed that fecal and coliform pollution were widely distributed in the selected areas of Calumpang River making it unsuitable, unsafe and not acceptable for human use or primary contact and there may be a potential risk of infection for users of waters from Calumpang River.

Keywords - Calumpang River, total coliform, fecal coliform, fecal streptococ

INTRODUCTION

Rivers are important to society, providing water for consumption, agriculture and carrying away human wastes (Araya et al., 2003). Water is increasingly being threatened as human population grow and demand for more water of high quality for domestic purposes and economic activities (Kolawole et al., 2011). Adequate quality, amount, time and place of water are vital for the environment and people’s health (Ward and Velazquez, 2008).

Surface-associated bacterial populations and inhabitance of coliform organisms particularly in rivers are essential in the biodegradation of allochthonous substances such as contaminants derived from human activities (Araya et al., 2003). The most frequent microbial pathogens are bacteria that originate in wastewater, thus, it is necessary to assess the efficiency of the wastewater treatment and to perform microbiological analyses of the final effluent (Salem et al., 2011). The bacteriological assessment of water is important in pollution studies, as it is a direct measurement of detrimental effect of toxic waste on human health (Kolawole et al., 2011). The poor microbiological quality might be due to contamination caused by human activities and livestock. It is a common practice for people living along the river catchment to discharge their domestic and agricultural wastes as well as human excreta into rivers (Karikari and Ansa-Asare, 2004). Based on the study of Hyland (2003), it has been estimated that waterborne diseases kill more than 5 million people annually and the microbial pathogens responsible for most of these deaths originate from human and animal feces.

Water contaminated with human feces generally indicates a greater risk to human health, as they are more likely to contain human-specific enteric pathogens (Scott et al., 2002). Contamination of surface waters by fecal pollution constitutes a serious environmental and public health threat (McLellan, 2004) and it is an environmental problem of increasing importance as demographic densities increase (Lasalde et al., 2005). Fecal pollution of aquatic environments causes their degradation and may affect human industries and activities related to water, such as bathing in recreational water, shell fisheries, and the supply of drinking water. Pathogens associated with fecal pollution may cause disease in humans (Nebra et al., 2003). Animal fecal contamination, including from poultry, brought some consequences of infection due to the pathogens that are commonly found in the feces (Weidhaas et al., 2011; Nafarnda et al., 2012). River has been impaired by the presence of high levels of fecal coliform bacteria. Such contamination brings the threat of infection for people who use the water (Hubbard et al., 2004). Microbial contamination of water poses a significant risk to public health. According to the study of Gibson (2010), pathogens associated with waterborne illnesses are excreted in the feces of humans and animals.

The most frequently used microbial indicators of water quality are total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS), all are considered indicators of recent fecal contamination (Hyland et al., 2003: Djuikom et al., 2005). Coliform bacteria are a group of intestinal bacteria used as indicators to determine if treated water is acceptable for human consumption and they indicate the presence of disease-causing organisms (Uzoigwe and Agwa, 2011; Radojević et al., 2012). The use of indicator bacteria for the assessment of fecal pollution and possible water quality deterioration in various freshwater sources is widely used (Anderson et al., 2005; Byamukama et al., 2005). Enterococci and other fecal indicator bacteria contribute to gastrointestinal illnesses, as well as eye, ear, nose, skin, respiratory and other infections (Shanks et al., 2006).

In this study, the researchers analyzed the water quality of Calumpang River using microbial indicators since people residing near the river are still using it for fishing, bathing and laundering. Increased effluent discharges on the said river are caused by the high level of industrialization in the locale. Because it was and still is very expensive and time consuming to test for all the pathogens, it is suggested that a single group of microorganisms that come from the same source as pathogens can be used to indicate the presence of pathogens (Yongsi, 2010), so microbial indicators will only be including the total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS).

Coliform bacteria are group of bacteria including Enterobacter, Klebsiella, Eschirichia, Cirtobacter, and Aeromonas. They make up around 10% of the intestinal microflora of the human and animal intestine (Yongsi, 2010). Total coliform and fecal coliform are useful in assessing the sanitary condition of water (Scott et al., 2002). Fecal coliform are a subset of the total coliform and include group of microorganisms like Citrobacter, Hafnia, Klebsiella and Serratia. Their presence in water could indicate fecal contamination (Yongsi, 2010). Fecal streptococci comprise two genera, Enterococcus and Streptococcus (Dionisio and Borrego, 1995).

The ratio between fecal coliforms and fecal streptococci gives a fecal index, which indicates the origin of pollution (human, animal or mixed) (Scott et al., 2002; Djuikom et al., 2005; Chitanand et al., 2008). High number of indicators that will be detected would reveal that the microbiological quality of the water sources used was poor, unsafe and not acceptable for human consumption (Obi et al., 2002).

Thus, the purpose of this study is to use bacterial densities to evaluate the microbial quality of Calumpang River in the assessment of its sanitary condition.

MATERIALS AND METHODS

Sampling Area

This study focused on the Calumpang River, a perennial body of water with a catchment area of approximately 472 square kilometres and forms the Southeastern boundary of the Poblacion and it flows into the Batangas Bay at a point approximately two kilometers south of Batangas Port with the following coordinates: 13°45′43.37″N 121°04′28.12″ E. As shown in Figure 1, the river was subdivided into three stations namely Brgy. Malitam (1), Calumpang Bridge to Bridge of Promise (2) and Tinga, Labac (3).

Figure 1
Map of Calumpang River from Tinga, Labac to Barangay Malitam
showing the Water Collection Sites

Water Sample Collection

The water samples from the three stations, as depicted in Figure 1, were collected last July 2012. Three (3) sampling sites were selected for every station in the collection of water samples, which were then combined to produce a composite sample that represented each station.

Bacteriological Analysis

Three parameters were evaluated in order to assess the bacterial and coliform densities of the river. These are the analysis for total coliform (TC), fecal coliform (FC) and fecal streptococci (FS) test. The first two tests were performed at the Optimal Laboratory, while the latter was conducted at Natural Sciences Research Institute at UP Diliman.

Test for coliform group were carried out by the multiple tube fermentation technique in duplicate tubes and dilutions were reported in terms of Most Probable Number (MPN). Lauryl tryptose broth was used for the presumptive portion of the multiple - tube test and incubated for 35±0.5oC. After 24±2h, tubes were examined for growth, gas formation, and acidic reaction (shades of yellow color). No gas or acidic formation was reincubated and reexamined at the end of 48±3h. All positive presumptive tubes were submitted to the confirmatory phase, the brilliant green lactose bile broth. The inoculated brilliant green lactose bile broth tubes were incubated at 35±0.5oC. Gas formation in any amount at any time within 48±3h constitutes a positive confirmed phase and calculated for the MPN value of total coliform. For fecal coliform, positive brilliant green bile broth tubes were inoculated into Escherichia coli broth (EC) and incubated in a water bath at 44.5±0.2°C for 24±2h. Gas production in an EC broth culture in 24 h or less was considered a positive for fecal coliform bacteria and counted for MPN value.

The samples for fecal streptococci were analyzed by the multiple tube fermentation technique using 3 of 100 up to 10-3 dilutions on Azide Dextrose Broth (ADB). The tubes were incubated at 37oC for 48 hours. To confirm the presence of fecal streptococci, turbid culture tubes were streaked on Pfizer Selective Enterococcus (PSE) agar. Plates with brown colonies and haloes were considered presumptive for fecal streptococci.

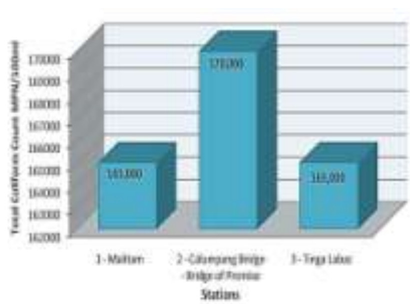
Statistical Analysis

The data were elaborated statistically using the software package SPSS 18. In cases in which bacterial levels exceeded the count where the value is unlimited, a value was assigned for statistical analysis. The mean and standard deviation were calculated, and regression analyses were performed to analyze significant relationship. Analysis of variance (ANOVA) was used to determine if there were differences in pattern of bacterial population between stations. t-test was also used to assess the difference between the two parameters (fecal coliform and fecal streptococci). Cluster analyses were done to identify similarity.

RESULTS AND DISCUSSION

The results, as presented in Figure 2, show the total coliform count in 3 selected stations. Stations 1 and 3 obtained a value of 165,000 MPN/100ml while Station 2 got 170,000 MPN/100ml. All stations gained total coliform count >160,000MPN/100ml.

Figure 2
Bacteriological Test of TotalColiform Count (MPN/100ml)



Based on DENR Administrative Order of 2008, water quality guidelines and general effluent standards, the maximum level for total coliform count is 25,000MPN/100ml, thus the results obtained exceeded the reference limit which revealed that all the stations were highly contaminated with coliform bacteria.

Presence of total coliform in water signifies fecal contamination due to dumping of human and animal feces into and/or near the river (Amadi et al., 2012) and other bacteria from the soil (Zamxaka et al., 2004).

Figure 3
Bacteriological Test of Fecal ColiformCount (MPN/100ml)

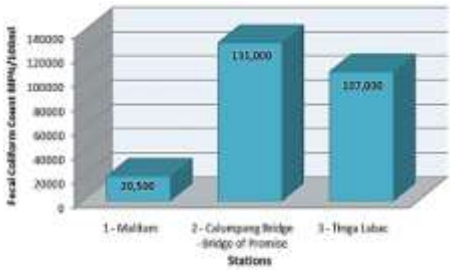
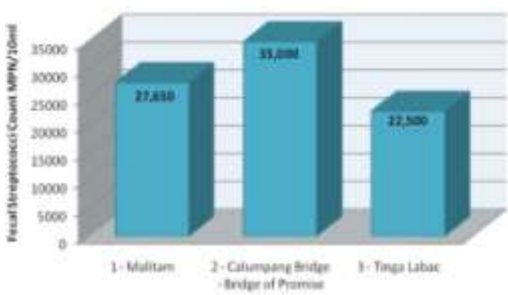


Figure 3 presents the bacteriological test of fecal coliform count in the three stations. The results for stations 1, 2 and 3 were 20,500MPN/100ml, 131,000MPN/100ml and 107,000MPN/100ml respectively. The maximum level for fecal coliform count presented by DENR Administrative Order 2008, water quality guidelines and general effluent standards, is 800MPN/100ml. Therefore, all the results were out of the reference range. This indicates that there is high concentration of fecal contamination. Thus, it indicates that the water is contaminated with fecal of human or animal waste (Zamxaka et al., 2004).

Figure 4
Bacteriological Test of FecalStreptococci (MPN/100ml)



Fecal streptococci are always present in manure, and then the presence of these microbes in a water sample is a strong evidence of fecal contamination (Valenzuela et al., 2009). The result of the bacteriological test for fecal streptococci is presented in Figure 4. The results obtained from Stations 1, 2 and 3 were 27,650MPN/100ml, 35,000MPN/100ml and 22,500MPN/100ml, respectively. The results proved that the three stations are contaminated with fecal materials. This may be due to the fact that there are several families residing in the entire stretch of the Calumpang River.

Increase concentrations of TC, FC and FS in all of the selected areas of Calumpang River indicate significant fecal contaminations originating from humans or other warm-blooded animals (Djuikom et al., 2005). As stated by Bohra et al. (2012), presence of coliform simply indicates that pathogens are expected to be present. The alarming high number of total coliforms indicates high level of fecal pollution of the river which potentially poses a high health risk. According to Kolawole et al. (2011), gastrointestinal illness was experienced by swimmers who swam in water with a median coliform density of 2,300 coliforms per 100 ml. High total and fecal coliform counts in water are usually manifested in the form of diarrhea, fever and other secondary complications (Zamxaka et al., 2004). Enterococci are opportunistic pathogen that can cause infections of urogenital tract, endocarditis and wound infections in humans (Abbas et al., 2007). Since there are still fishing in Calumpang river, fishes which live in these polluted sites can easily intake these bacteria while feeding along with contaminated aquatic foods. Migration of the bacteria from water to fish may cause spoilage of fish and outbreak of disease (Rahman et al., 2010).

Table 1 shows the comparison of the results value in three stations which can also be seen in the three previous figures. In terms of total coliform, the three stations obtained >160,000MPN/100ml. The fecal coliform results garnered 20, 500 MPN/100m at station 1, 131,000 MPN/100ml at station 2 and 107,000 MPN/100ml with station 3, which makes the second station with the highest fecal contamination and station 1 with the least. Both total and fecal coliform count exceeded the reference range set by DENR Administrative Order of 2008. Higher bacterial population was obviously due to addition of more sewage and fecal matters through greater human activities (Bohra et al., 2012)

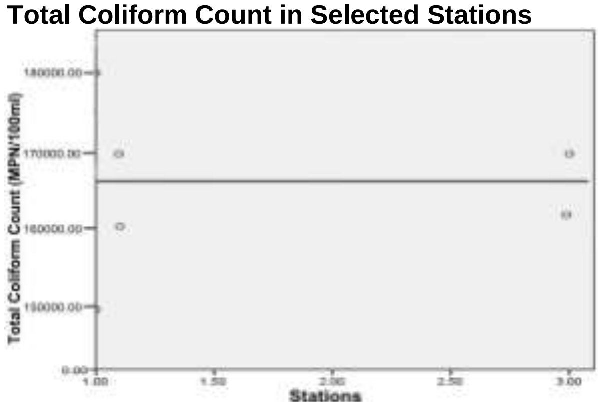
Table 1
Bacteriological Quality of Water Samples

Stations	TC (MPN/ 100mL)	FC (MPN/ 100 mL)	FS (MPN/ 100 mL)	FC/FS
1	>160, 000	20, 500	27, 650	0.74
2	>160, 000	131,000	35, 000	3.67
3	>160, 000	107, 000	22, 500	4.76

**Station 1 – Malitam; *Station 2 – Calumpang Bridge - Bridge of Promise; *Station 3 – Tinga, Labac*

Another test to assess fecal contamination is the fecal streptococci which evaluate the amount of non-human fecal sources like animals. Analyses of fecal streptococci revealed 27,650MPN/100ml at station 1, 35,000 MPN/100ml at station 2 and 22, 500 MPN/100ml at station 3. Highest amount of fecal streptococci is seen in station 2 and lowest amount in station 3. The FC/FS ratio is used for determination of fecal pollution whether it is from human or non-human sources. For human fecal contamination, the FC/FS ratio is >4.0; FC/FS ratio <0.7, the source of pollution is animal wastes and if the ratio is between the two, there is a mixed animal and human wastes pollution. FC/FS ratio results in Stations 1 and 2 were 0.74 and 3.67 respectively, indicating mixed animal and human fecal contamination while in Station 3 was 4.76, designating a human fecal contamination.

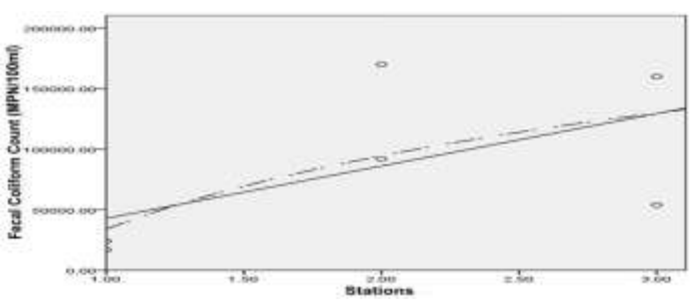
Figure 5
Total Coliform Count in Selected Stations



**Station 1 – Malitam; *Station 2 – Calumpang Bridge – Bridge of Promise; *Station 3 – Tinga, Labac*

Figure 5 is the linear presentation of total coliform count in selected stations. From station 1 to station 3, total coliform count is constant. Increase concentrations of TC in all of the selected areas of Calumpang River indicate significant fecal contaminations originating from humans or other warm-blooded animals (Amadi et al., 2012; Djuikom et al., 2005) and other bacteria from the soil (Zamxaka et al., 2004).

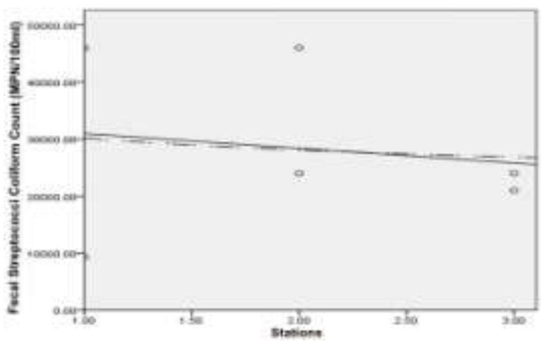
Figure 6
Fecal Coliform Count in Selected Stations



**Station 1 – Malitam; *Station 2 – Calumpang Bridge - Bridge of Promise; *Station 3 – Tinga, Labac*

Figure 6 is the linear presentation of fecal coliform count in selected stations. From station 1 to station 3, fecal coliform count is increasing. Thus, it means that fecal contamination in station 3 is greater than station 1. On the study of Jamieson (2003), it has recently been demonstrated that fecal bacteria can survive for weeks, and sometime months, within surface water systems. Extension of survival was due to their attachment to sediment particles present in the river and settled to the bottom. It appeared that in station 3, there was substantial reservoir of fecal bacteria which act as a major source of bacteria during both low and high flow conditions of the river. Any turbulence could cause the bacteria to remove from the sediment, where it could continue to migrate to succeeding stations.

Figure 7
Fecal Streptococci Coliform Count in Selected Stations



**Station 1 – Malitam; *Station 2 – Calumpang Bridge - Bridge of Promise; *Station 3 – Tinga, Labac*

The fecal streptococci count in selected stations is shown in Figure 7. Based on the linear graph, the fecal streptococci count is slightly decreasing from station 1 to station 3. This means that there are more fecal streptococci contaminants in station 1 than station 3.

Fecal streptococci are not as ubiquitous as coliforms. There was lower value of FS at station 3 because FS were unable to multiply in sewage contaminated water (Dionisio and Borrego, 1995) and they die off rapidly in the environment than other bacterial indicators (Valenzuela, 2009). Since station 1 is directly connected to Batangas Bay, a seawater, they live longer in coliforms (Dionisio and Borrego, 1995) and were able to survive for over 140 days (Valenzuela, 2009).

Based on the study of Karikari and Ansa-Asare (2004), poor microbiological quality of the river might be due to contamination caused by human activities and livestock. There is a usual practice for people living along the river catchment to discharge their domestic and agricultural wastes as well as human excreta/wastes into rivers. Wild and domestic animals that are drinking into the water can also contribute to the contamination of water through direct defecation and urination.

There were no significant differences observed in terms of total coliform, fecal coliform and fecal streptococci count. Results of the river microbial quality reveals that all of the parameters in the water samples from selected areas were not within the acceptable limits. Thus, all of the selected areas of Calumpang River indicate significant fecal contaminations originating from humans or other warm-blooded animals (Djuikom et al., 2005).

CONCLUSION

Fecal and coliform pollution were widely distributed in the selected areas of Calumpang River. The study illustrates a significant fecal contaminations originating from humans or other warm-blooded animals making the microbial quality and sanitary condition of the river poor, unsafe and not acceptable for human use or primary contact. Thus, Calumpang River is unsuitable for domestic uses because it implicates a potential risk of infection for the users of the said river due to increase coliform and fecal streptococci contamination.

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