Seasonal Changes and Coliform Load of Jalaur River, Province of Iloilo, Panay Island, Philippines

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Determination of the presence/absence of coliforms as the pollution indicator bacteria, total coliform count (TCC) and fecal coliform counts specifically *Echerichia coli* were carried out on specific sampling points in Jalaur River namely: Banban Pequeño (upstream), Calinog, Moroboro, Dingle, Passi near Sugar Central Mill and National Power Corporation (NPC) (midstream), and Nabitasan, Leganes (downstream) during the wet and dry months. Samples were analyzed using a defined technology, the Colilert® method, and its accuracy was verified with the conventional method (APHA Standard Methods).

Results showed that coliforms were present in all sampling sites. In Calinog, total coliform count (TCC) was high (900 $\times 10^1$ MPN/100 ml) in July. Increased coliform abundance was associated with high rainfall due to animal wastes that were carried by runoffs. In September, Passi, near NPC, TCC and *E. coli* counts were 640 $\times 10^1$ and 630 $\times 10^1$ MPN/100 ml, respectively. Reduction in counts in January, February and March was observed. Sediments in Passi near Sugar Central Mill during milling time in February showed a high TCC of 116 $\times 10^4$ MPN/100 ml which was attributed to reduced current and disturbance, and silty loam sediments texture that favored bacterial adsorption to sediments. Dingle site exhibited a domination of other coliforms over *E. coli* in January and March. In Leganes, highest TCC of 551 $\times 10^1$ MPN/100 ml was obtained in September. Coliform loads varied by season which was influenced by the availability of the nutrients and tolerance range to physical and chemical factors in the environment. Variability of the resultant interaction can also be attributed to climate changes such as extreme weather events—*El niño* phenomenon and increased nutrient loadings during heavy rains hence, increased coliform concentration in the river. The presence of coliforms in Jalaur River is indicative of contamination that can be aggravated by climate changes and implies that a potential health risk associated with pathogens causing water-borne diseases is present.

KEYWORDS: Jalaur River, biodiversity, vertebrate fauna, aquatic macro-invertebrate fauna

INTRODUCTION

Rivers are being polluted by indiscriminate disposal of sewerage, industrial wastes, and a plethora of human activities that affect not only its physico-chemical characteristics but also the microbiological quality. Waters contaminated with human feces are generally regarded as posing a greater risk to human health, as they are more likely to contain human-specific enteric pathogens. The bacterial parameters, such as colony count, total coliform, and thermotolerant coliform are widely used in the assessment of water quality because they indicate the pollution with organic matter or the fecal pollution (Ishii et al., 2005). Microorganisms are ideal sensors because they respond instantaneously to the fluctuations of environment factors by specific changes, detectable at physiology and metabolic level (Geoecomar, 2003).

Edberg et al. (2000) reported that among the coliforms, *Echerichia coli* is the best biological drinking water indicator and categorized as the far superior overall compared with other practical indicators of pollution. Its presence even in recreational waters is a strong indicator of recent sewage or animal waste contamination and the potential for the water to have other disease-causing organisms. Understanding river contamination is paramount in assessing associated health risks as well as the actions necessary to remedy the problem while it still exists (Scott et al., 2002).

Deterioration of water quality has been identified as having a potential vulnerability to climate. Two seasons, the dry and wet/rainy seasons were taken into account for the bacteriological assessment. Factors that might have affected the concentrations of these indicator bacteria were also noted. Although at present, it is hard to absolutely put a demarcation line between the rainy and dry seasons in the Philippines, this study attempted to [a] examine the bacteriological indicators of pollution in Jalaur River to determine variability under this premise; [b] correlate the bacterial pollution indicator density with the physico-chemical factors; and [c] determine the bacteriological status in terms of load of the river as affected by climate change.

MATERIALS AND METHODS

Water sample and sediment collection points

Water samples were taken from three identified sites of Jalaur River from July 2009 to February 2010. Upstream samples were taken from Banban Pequeno, Calinog, midstream in Moroboro, Dingle, and downstream in Nabitasan, Leganes, Iloilo. Water flowing near the National Power Corporation (NPC) and the sugar mill in Passi were also sampled to capture the real river situation where two industries influence the microbial loading of Jalaur River. One liter of water samples was collected beneath the water surface within the current at the middle portion of the river avoiding stagnation using sterilized stainless steel containers, immediately placed in ice packs in a styrofoam box to minimize temperature changes, and were brought to the laboratory for bacterial analysis. The wet weather sampling at the river was done just after a storm/heavy rain. For the sediment, grab sampling method was used. This was done only to check coliforms that might have settled, upon observation of the nature of the sediment which became distinctively dark and smelled of fermenting sugar.

Water column and sediment bacterial load analysis and identification

Detection and confirmation of *E. coli* and other coliforms, including presence-absence (P/A) and most-probable-number (MPN per 100 ml water sample) were done using Colilert test [IDEXX Lab Inc.; approved by Environment Protection Agency (EPA)], a rapid method giving sensitive results using defined substrate technology that is specific for coliforms and *E. coli*, making use of substrates, a medium formulation. The method involved simultaneous enumeration in 24 h of both total coliforms and *E. coli*. One hundred ml (100 ml) water samples and 100 g sediment contained in the prescribed sterilized sample bottles were subjected to serial dilutions, added with specific reagent, Colilert ® and incubated at 35 ±0.5 °C for 24 h. Those samples that turned yellow were considered positive for coliform, those that remained colorless were negative for coliform based on the standard Colilert comparator. Further testing was done to avoid false positive by incubating further the samples at 48 h. Total coliform analysis was based on the substrate-enzyme production β-galactosidase which hydrolyses o-nitrophenyl-β-D galactopyranoside (ONPG) and releases o-nitrophenol to produce yellow color. *E. coli* produces the enzyme β-glucuronidase which hydrolyses 4-methyl umbellyferryl-β-D glucoronide (MUG) to form 4-methyl umbelliferone and this fluoresces under the long wave UV light (365 nm) (Figure 1). *E. coli* and other coliforms present in the water sample metabolize the nutrient indicator. Serial dilutions were made to assure Most-Probable-Number (MPN) counting.

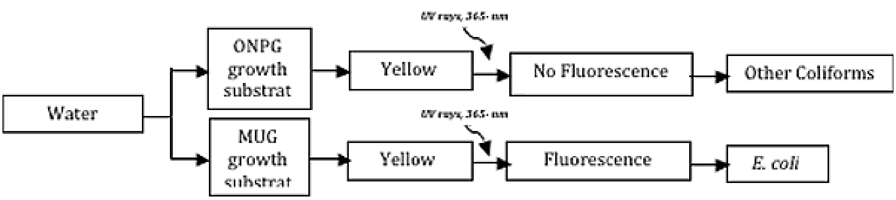

Figure 1. Coliform bacteria analysis following Colilert Quantitray system.

Total coliform (TCC) and *E. coli* counts were calculated using IDEXX Software and verified using standard Most Probable Number Table based on the assumption of a Poisson distribution (random dispersal). Verification/parallel testing, the multiple tube tests (coliform test, presumptive tests, confirmed test and completed phase) following the Standard Methods for the Examination of Water and Wastewater (APHA, 1998) were done. Results were correlated with the physico-chemical data.

Frequency counts were done on the most probable number (MPN) and total coliform count (TCC). Water samples positive for coliforms but not *E. coli* were assessed for bacterial genera identification. Results were also linked to the health status, environmental, and cultural practices of the dependent population in the area.

RESULTS

Presence and total counts of coliforms and *E. coli* in the water column and sediments

Coliforms were present in all sites during the two seasons (dry and wet) which indicates that the river water was contaminated by fecal matter. Total coliform and *E. coli* counts in all sampling sites are summarized in Figures 1 to 6.

Figure 2 summarizes the total coliform count (MPN/100 ml) in Banban Pequeño, Calinog (upstream) in both wet (July-September) and dry (January-March) seasons. Total coliform counts were 674x10^1 MPN/100 ml in August, and 520 x10^1MPN/100 ml in September. The values decreased at the onset of the dry season registering total coliform value of 213 x10^1 MPN/100 ml in January which suddenly rose in February to 792 x10^1MPN/100 ml and decreased again to 273 x10^1 MPN/100 ml in March.

In terms of *E. coli*, the month of July showed the highest mean count of 990 x 10^1 MPN/100 ml sd=0) which was noticeable. During the month of September, *E. coli* was 505 x 10^1 MPN/100 ml (sd=98), when there were domesticated animals (cattle and goats) grazing at the river bank, children swimming, and several women doing their laundry.

Other coliforms aside from *E. coli* registered zero in July and 9 x10^1 MPN/100 ml (sd=4) in August while in September it was 15

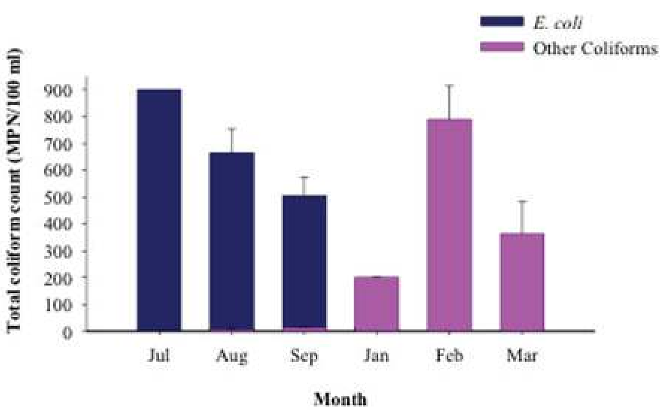

Figure 2. Mean coliform and *E. coli* counts at 10^1 dilution in Banban Pequeño, Calinog from June 2009 to March 2010.

x10^1MPN/100 ml (sd=4). Interestingly, at the onset of the dry season, there was a reverse in the relative counts between *E. coli* and other coliforms. Mean *E. coli* count was only 13 x10^1 MPN/100 ml (sd=0) against other coliforms of 200 x10^1 MPN/100 ml (sd=1) which was highlighted in February. An almost 50 % decrease in other coliform was observed in March while *E. coli* load did not change much. Water during the wet season was dominated by *E. coli*, other coliforms dominated during the dry season.

The coliform count of river water in Barangay Tabogon, Passi site where the National Power Corporation (NPC) is located in all sampling months has a mean TCC range of 137-346 x 10^1 MPN/100 ml which was below the prescribed standard, and hence, within the acceptable level. The trend was going down from the month of September to the last sampling in March (Figure 3). Effluent was being discharged during this period. The same trend as in Calinog site was observed whereby *E. coli* count of 321 x 10^1 MPN/100 ml (sd=32) was higher in September (wet season) than other coliforms, which dramatically dropped in January with a value of only 21 x 10^1 MPN/100 ml (sd=25) then rose again in February of 215 x 10^1 MPN/100 ml sd=15). In March, *E. coli* count was only 58 x 10^1 MPN/100 ml (sd=28). Other coliform counts in this area were highest in January (251 x 10^1 MPN/100 ml (sd=47) and decreased in February to March with values 21 and 79

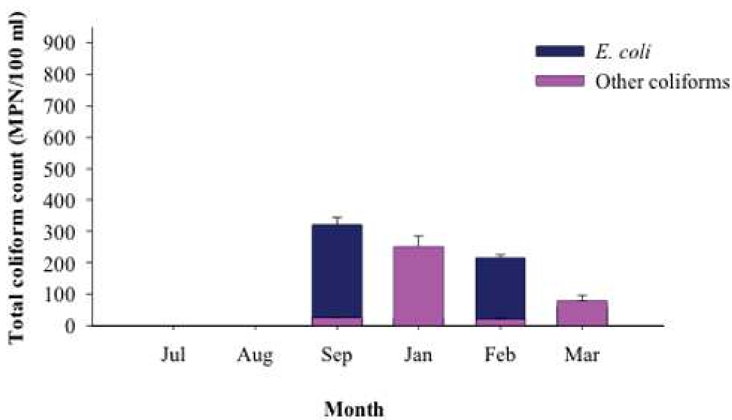

Figure 3. Mean coliform and *E. coli* counts at 10^1 dilution in Tabogon, Passi site (NPC) from June 2009 to March 2010.

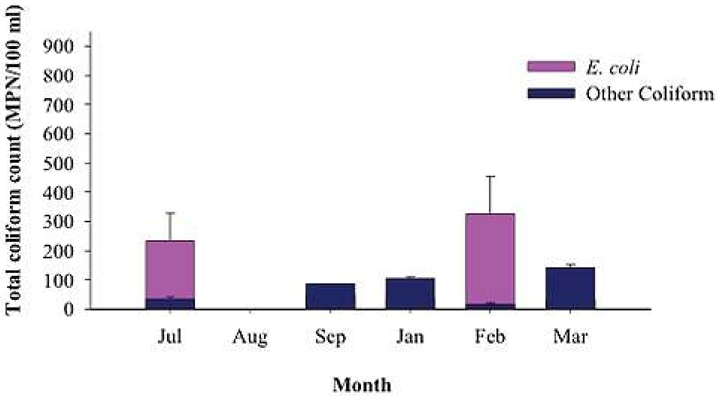

Figure 4. Mean coliform and *E. Coli* counts at 10^1 dilution in Moroboro, Dingle (dam site) from June 2009 to March 2010.

x 10^1 MPN/100 ml respectively. The water in this area was darker in color in January than in February and March due to continuous discharge.

Water level in Moroboro, Dingle sampling site was relatively higher than in other sites even during the low tide owing to the presence of a dam intended for irrigation and the coliform concentration was considered mild during the sampling months (Figure 4). Mean TCC was relatively high in July 267 x 10^1 MPN/100 ml but decreased in September (133 x 10^1 MPN/100 ml) and values were still within the DENR accepted limit. During the dry season, *E. coli* mean count was highest in February (326 x 10^1 MPN/100 ml; sd=181) and lowest in March (31 x101 MPN/100 ml; sd=0). January samples showed the domination of other coliforms over *E. coli* in this area in January and March with values 104 x 10^1 MPN/100ml (sd=7); and 143 x 101 MPN/100 ml (sd= 16) respectively. As to the other coliform count, January (104 x 10^1 MPN/100 ml) and March registered high count but not too high compared to Calinog area.

The first sampling in Passi site specifically of the river water flowing near the sugar mill was in September when there was no milling activity. A high TCC during the wet month of September (640 x 10^1 MPN/100 ml) was obtained which abruptly lowered towards the dry season. Other coliforms were dominated by *E. coli* during the time of sampling (Fig. 5).

The dry months showed an almost doubled reduction in TCC

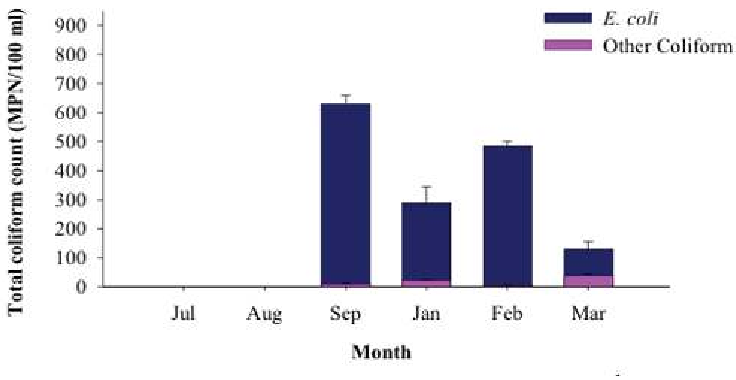

Figure 5. Mean coliform and E. coli counts at 101 dilution in Passi site (near the Sugar Central Mill) from June 2009 to March 2010.

count in February (489 x 10^1 MPN/100 ml. Came March, the TCC decreased again. *E. coli* count recorded lowest (128 x 10^1 MPN/100 ml; sd=37) and highest in September (630 x 10^1 MPN/100 ml; sd= 23). During this period, other coliform counts were at their lowest in all months.

In Leganes, which was the downstream site, TCC count recorded highest in September (537 x10^1 MPN/100 ml), and the lowest value in the dry month of March. *E. coli* count of only 4 x 10^1 MPN/100 ml was

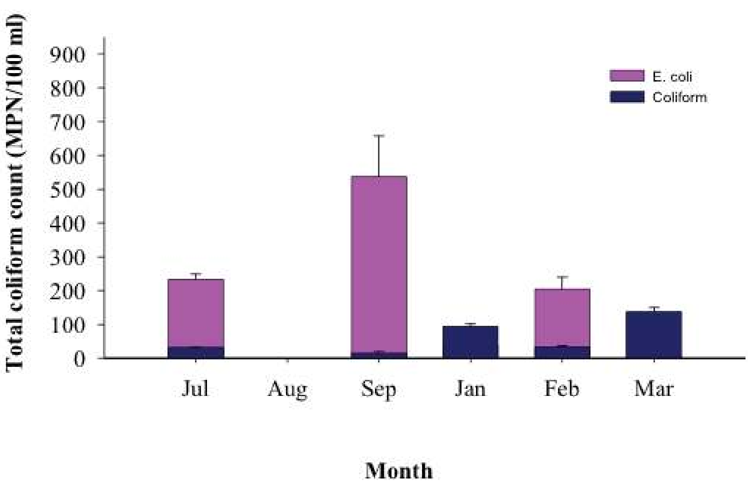

Figure 6. Mean coliform and *E. coli* counts at 10^1 dilution in Nabitasan, Leganes from June 2009 to March 2010.

recorded. It was low tide during the time of sampling. Fluctuations in the results can be due to the difference in physical and chemical factors such as water flow rate and water level brought about by diurnal tide changes and other physico-chemical factors (Figure 6), since the site was located at the mouth of the river and changes were very likely.

To check whether the decrease in the coliform loading has something to do with "sedimentation" or settling of the bacteria, sediment samples from Calinog and Passi areas were sampled for coliform loading. The water level was low during the time of sampling. Figure 7 shows a very high total coliform count of 116 x 104 MPN/100 ml in Passi (near Sugar Central Mill) in February where carabaos were bathing in the river. This was dominated by *E. coli* load of 95 x 10^4 MPN/100 ml. In March the load decreased abruptly to 21.5 x 10^4 MPN/100 ml.

Negligible TCC in the sandy sediment of Calinog site was obtained but high in Passi with silty sediments. The nature of the sediment perhaps provided favorable environment for the coliforms. There was not much resuspension of coliforms in the water during dry month of February (120 x10^4 MPN/100 ml). Varying sediment texture compared to Calinog site brought stronger association of bacteria with silty and clay particles rather than sandy, hence high counts.

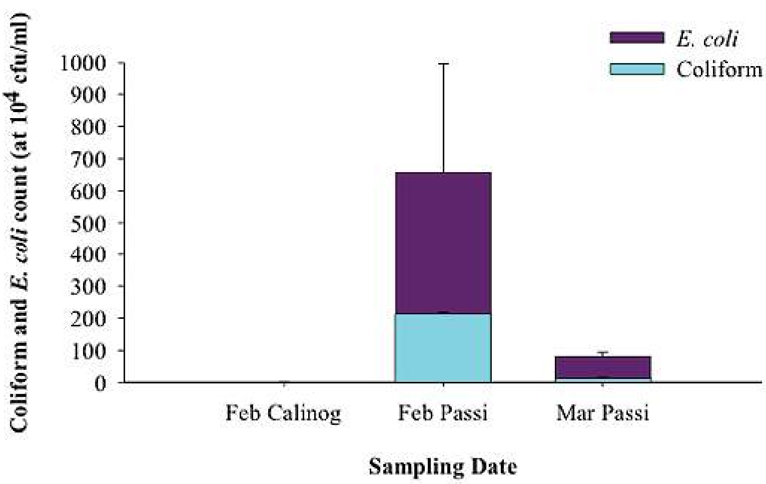

Figure 7. Mean *E. coli* and coliform counts in sediment at 10^4 dilution in Calinog and Passi sites.

Total coliform count (TCC) by area

In a summary of the total coliform count by area at different months covering wet and dry seasons (Figure 8), it is noticeable that the dry season counts for total coliforms were lower than during the wet season in all areas.

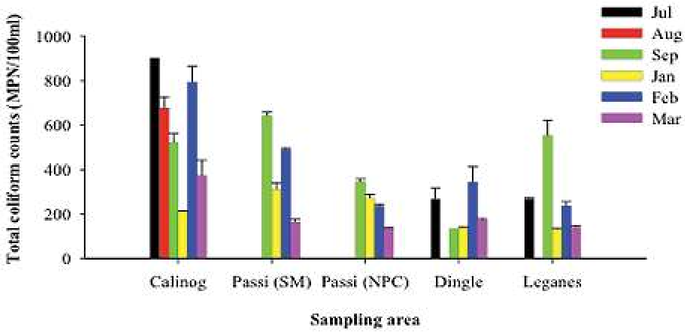

Figure 8. Mean total coliform and *E. coli* counts in the water column at 10^1 dilution in Calinog, Passi, Dingle, and Leganes sites.

Coliforms identified aside from *E. coli* was *Klebsiella* sp. in Banban Pequeño, Calinog, Passi City, and Moroboro Dingle stations. Other coliforms included *Enterococci* sp. but confirmed tests on *Salmonella* or *Proteus* sp. were not established. The presence of *Enterococci* sp. also indicated that Jalaur River was indeed contaminated by feces.

In Banban Pequeño, Calinog, the surface water temperature of the river during the wet season ranged from 23.2-26.9 °C and a significant positive relationship between the temperature and the *E. coli* load and other coliforms was obtained ($r= 0.96; p<0.01$). The general trend was that the TCC and *E. coli* counts were lower during the dry/summer season than during the rainy/wet season.

The level of ammonia during the sampling season significantly influenced the total coliform load of the river. The pH of Jalaur River water (7.8–8.0) during the wet season did not differ much during the dry season (7.2–8.0) but influenced the growth of *E. coli* and other coliforms ($r=0.90$). It is very likely that coliforms tolerated well the alkaline environment which was well within the permissible range of 8.2–8.7 for their growth. Nitrite, as a naturally occurring ion in the environment, positively influenced the coliform growth during the wet season ($r=0.60$) and negatively during the dry season ($r=-0.77$). Bacteria in water quickly convert nitrites to nitrates. The same result was obtained in phosphate as in ammonia-N which significantly influenced the total coliform load in Calinog, Dingle, and Leganes during the wet season *(r=0.98)*.

Total suspended solids (TSS) in the river during the wet season was most directly influenced by runoffs from the upstream site *(r=0.92)*. However, this could not be a direct prediction of high *E. coli* counts and high TSS, while dissolved oxygen (DO) highly, and positively influenced the growth of total coliforms in the upper and middle stream during the data gathering period such that as the D.O decreases, the growth of the coliforms increases *(r=0. 77)*.

DISCUSSION

Water quality depends in part on land use and how water resources are managed and protected. Freshwater bodies are directly or indirectly affected by point and non-point contamination such as industrial, urban, and agricultural operations. Direct deposit by domesticated animals, wildlife, livestock farms, dumping of septic systems, sewage leaks, swimmers bodies, dirty diapers, and agricultural runoff that occurs when rain comes in contact with the soil containing the bacteria, were the sources of contamination. In Calinog area, 86.7% of the residents use Jalaur River for bathing their livestock. In Dingle, hog raising is one of the income generating activities of the people. It is also worth noting that only few locals were using compost pits and depend on the river for draining animal wastes. The water then works its way to the river resulting into increased bacteria levels in the rivers due to the large amount of fecal material washed away as runoff during a rainfall.

Seasonal variations have influenced bacterial loading in Jalaur River. The contrast in the bacterial load between wet and dry seasons may be attributed to the temporal variations of *E. coli* concentration exhibited in the rivers aside from tidal and longitudinal variations and the inputs of other coliform species (Schilling et al., 2009; Mill et al., 2006; Cabral & Marques, 2006). Basically, different sampling points in Jalaur River showed varied coliform counts which indicated the state of the river at that period of time only. During the stormy months of July to September, the upper elevation site showed about three times (from 213–674 x 10^1MPN/100 ml) more contamination than in January, the onset of warm climate. The aftermath of the typhoon “Jolina” after a long drought has added much to the runoffs.

Also, flushing out of the soil of these bacteria might have contributed to the high concentration of *E. coli*, and played a significant role in the contamination. Similar observations and results were found by Byers et al. (2005) on sediment and *E. coli* loads in unfenced streams of a beach in the U.S. According to Ackerman and Weisberg (2003), rainfall and runoff due to rainfall events has been associated with increasing microbial levels along numerous beaches. Sampson et al. (2006) examined the effects of rainfall on *E. coli* and total coliform levels in a lake using the same method as in this study. However, they found no relationship between rainfall amount and bacterial concentration. This finding is in contrast to the result obtained in this study, perhaps because Jalaur River is a flowing body of water while a lake is mostly still.

In Passi, where the sugar mill is located, the contamination indicator was highest in February (484×10^1 MPN/100 ml). This could probably be due to sedimentation of the bacteria due to less water and disturbance, i.e., water is calmer during dry than during the rainy season. There was less 'mixing up' of water and sediments during the dry season compared to the rainy season where flow of water stirs up the sediment. During the dry months of January, February and March, the level of *E. coli* and total coliform concentrations decreased. This may be due to the lower loading of fecal material matter when there is no or less rain. Also, this could be accounted for by the ecological conditions of the river whereby vigorous grazing of the bacteria by other organisms such as protozoa, having a greater chance to utilize them as food due to reduced water current, caused a pronounced reduction of the *E. coli* in warmer temperature.

The sediment in Passi site, during the time of milling showed a high coliform count (640×10^1 MPN/100 ml). Sediment at the time of sampling was gray to black in color, water was murky, and with a characteristic smell of fermenting sugar. *E. coli* and other fermenting bacteria at this point in time were perhaps utilizing the sugar effluents producing gas. Added to the dry climate resulting in a low water flow rate, evaporation and less disturbance, the coliforms proliferated. There was not much transport of bacteria and they probably stayed or remained in the sediment which was less sandy and less pebbled compared to the upstream Calinog (TCC of 0.778×10^4 MPN/100 ml). This bacterial adsorption was also demonstrated in the study on transport and deposition of sediment-associated *E. coli* in natural streams of Jamison, et al. (2005) and Pachepsky et al. (2009) on lakes. Water sediments can serve as environmental reservoirs for

E. coli. The difference in adsorption could be attributed to the stronger association of bacteria with silty and clay particles with more organic content as compared with sandy particles (Pachepsky et al., 2009). This observation is similar with the results of the study of Youn-Joo et al. (2002) wherein enumeration of bacteria in bottom sediment showed that the densities of *E. coli* and total coliforms in sediment were much higher compared to those in water. *E. coli* levels increased in depth and bottom water samples had higher densities due to their association with particles. During high flow events such as heavy rains and storms bottom sediments release bacteria.

Taking into account the effect of the Moroboro Dam in Dingle (Jalaur river midstream), it allows the river to flow downstream when open, and to empty into the Leganes area. When it is closed, the dam acts more like a lake or a reservoir ("damming effect"), creating a static environment allowing change in bacterial load (grazing by zooplankton). Thus, in addition to rainfall, the dam operation cycle does affect the level of bacteria in the Jalaur River.

Physical and chemical factors responsible for variations in the survival of fecal coliforms are extremely complex in water from different localities and in different points. In the Jalaur River, bacteria might have developed mechanisms that allow them to minimize inhibitory effects of the environmental factors which account for the differences in the bacterial load and the pathogen genera present. Varied concentrations of coliform including *E. coli* were observed to undergo spatio-physical fluctuations as shown in Figures 1 to 5. Runoff from agricultural, domestic, and industrial areas may contain iron, aluminum, ammonia, mercury, or other elements, and pH of the water will determine the toxic effects, if any, of these substances.

The study highlighted the coliform status of the Jalaur River as influenced by change in season and specifically changes in climate within two seasons in a year. I was expected that results would vary from year to year. Transport and dissemination of microbial agents via rainfall and runoff were affected by extreme precipitation (typhoon) which affected water quality of the river causing increasing human exposure to pathogens as contaminants.

The relative importance of temperature on its survival was seasonally dependent since *E. coli* and some coliforms are considered thermotolerant. During summer, animals, the major reservoir of *E. coli*, shed this organism more frequently when stressed. This region had been experiencing drought brought about by the *El Niño*, a situation that almost worsened from March to June 2009. If climate variability increases, current and future deficiencies in areas such as watershed protection, infrastructure and storm drainage systems will probably increase the risk of contamination events (Rose et al., 2001). The reduction of wind caused by change in climate affects the amount of dissolved oxygen. Aerobic to facultative bacteria are affected by the dissolved oxygen which when not available or limited, can also adapt and grow.

It is quite revealing that the coliform loading in water column and sediment involves an interplay of different factors. According to Davis et al. (2005) persistence *in situ* of changing climate remains within the species' tolerance limit, disregarding adaptation as a biotic response. Anthropogenic pollutants or substances emitted by industrial and domestic activities deposited into the river can cause an adverse impact on the microbial activity, ecology and population dynamics of the indigenous microbiota. The changes in climate could worsen the situation. Changing climate and environment will likely trigger genetic changes in bacteria that could enable pathogens to survive not only in water but also in soil.

Finally, waterborne diseases, such as a variety of diarrheal illnesses and infections such as fungal skin diseases, eye infections and respiratory illnesses are likely to occur in the areas.

CONCLUSIONS AND RECOMMENDATIONS

The Jalaur River is contaminated with feces and is unsafe for drinking but still meets the standards for recreational water at some period of the year based on the acceptable Geometric Mean Standard Value coliform load for freshwater set by the DENR in the Philippines (5,000 MPN/100 ml). The river harbors pathogenic microorganisms which can be detrimental to the health of the dependent population. The finding that *Klebsiella* sp. was present confirmed this which could lead to infectious diseases like pneumonia. Moreover, contamination varied markedly over distances of a few kilometers.

Seasonal variations adversely affected the growth and survival of coliforms in water column such that elevated counts were obtained during the rainy season compared to the dry season. Typhoon "Jolina" brought about runoffs which carried feces from the domesticated animals from the banks and nearby houses to the river causing the total coliform and *Echerichia coli* counts to rise to an alarming value.

The high total coliform count in sediments suggests adsorption of the bacteria during dry months after the long drought in the region. Moreover, the summer heat was well tolerated by the bacteria which are considered thermotolerant. Increase in pH, DO (mg/l) concentration during wet season favored coliform abundance; hence, physico-chemical factors adversely affect the growth and survival of coliforms. This was highlighted and most specific in the increase in some ions like nitrite and ammonia and phosphate in Jalaur River.

Based on the results obtained, growth and survival of *E. coli*, hence the load in Jalaur River, depend upon the interplay of, and tolerance range to different physical and chemical factors coupled with the availability of the nutrients, and changes in climate. Jalaur River setting undergoes a relative instability probably due to variability of resultant interaction network, and the observed differences in climate being one of the major factors.

A thorough monitoring of the incidence of water-borne related infections in Municipal and Rural Health Centers of towns where the river passes must be done as well as the establishment of an information dissemination campaign on the danger posed by water-borne diseases. Similar studies of the river should be conducted with more sampling points, more frequent sampling, and for a longer period of time to obtain a more reflective condition of the river as affected by climate change. Confirmation of suspected sources of fecal coliform contamination should be given attention.

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