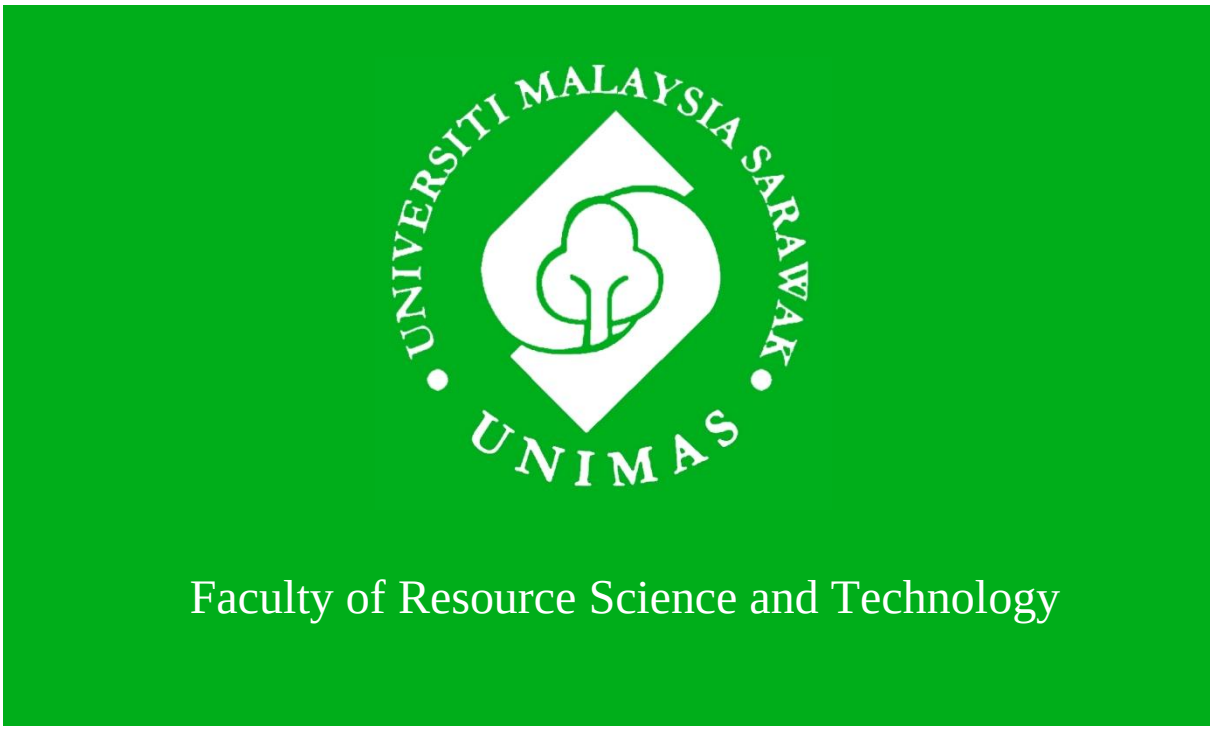


# DISTRIBUTION AND PERSISTENCE OF FECAL COLIFORM *(Escherichia coli)* in WATER AND SEDIMENT OF SEMARIANG BATU RIVER, KUCHING, SARAWAK

**MAGARATE RITA AK ELVIS SULANG (18928)**

Bachelor of Science with Honours

(Resource Biotechnology)

2010

# Distribution and Persistence of Fecal Coliform (*Escherichia coli*) in Water and Sediment of Semariang Batu River, Kuching, Sarawak

Magarate Rita Ak Elvis Sulang

This thesis was submitted in partial fulfillment of the requirements for the degree of Bachelor of Science with Honours in Resource Biotechnology

Supervisor: Prof. Prof. Madya Dr. Ling Teck Yee
Co-supervisor: Dr. Lesley Maurice Bilung

Resource Biotechnology
Department of Molecular Biology

Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2010

## DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree qualification of this any other university or institution of higher learning.

Magarate Rita Ak Elvis Sulang

Resource Biotechnology Programme

Department of Molecular Biology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

## ACKNOWLEDGEMENTS

I am very thankful to my Heavenly Father for granting me the strength to write this report and complete the degree program in Resource Biotechnology. I would like to thank my parents for their love, support, and words of encouragement throughout my journey as an undergraduate student.

I was fortunate to have a supportive supervisor, Prof. Madya Dr. Ling Teck Yee, who always guide me in this study and let me learn more about report writing. Besides, I also thank my Co-supervisor, Dr. Lesley Maurice Bilung, who helps me a lot in Microbiology part during my study. Thanks again to both my supervisor and co-supervisor for their time, patience, and cooperation that have enabled me to become a stronger individual and at the same enabled me to reach all time heights as a student.

I graciously appreciate Dunging Buda and his colleagues for their assistance during sampling trips and sharing of new information for my writing. I would also like to thank Kai Ling and Yik Ming at Microbiology Lab for their guidance and help in conducting the experiments in the Microbiology Lab.

Finally, I am especially grateful to all my colleagues, Hanis, Gee, Nabila, Azi and Swee Wee for being there for me when I needed them the most.

## Table of Content

## List of Table



## List of Figure

## List of Abbreviation

| | |
|---|---|
| $^{\circ}C$ | Degree Celsius |
| % | Percent |
| CFU | Colony forming units |
| MPN | Most Probable Number |
| PSA | Particle Size Analysis |
| spp | Species |
| ml | Milliliter |
| µm | Micrometer |
| LOI | Loss-On-Ignition |
| cm | Centimeter |
| pH | a measurement of the acidity or alkalinity of solution [p stands for “potenz” (this means the potential to be) and H stands for Hydrogen] |
| LTB | Lauryl Tryptose Broth |
| EMB | Eosin Methylene Blue |

# Distribution and Persistence of Fecal Coliform (*Escherichia coli)* in Water and Sediment of Semariang Batu River, Kuching, Sarawak.

**Magarate Rita Ak Elvis Sulang**

Resource Biotechnology Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

## ABSTRACT

*Escherichia coli (E. coli)* is an indicator organisms used worldwide to assess microbiological safety in drinking water, recreational waters and fresh water. In this study, *E. coli* was isolated from water and sediment samples of four locations of Semariang Batu River, Kuching, which were Lemidin River (S1), Semariang Batu River (S2), Loba Kara River (S3) and Batang Mangkuang River (S4). All of the sampling stations had different activities which were construction area (S1), residential area (S2), shrimp farm (S3) and area of no obvious human activities (S4). In order to perform the survival study, *E. coli* was isolated, grown and inoculated in the sediment samples. *E. coli* was incubated under 25°C and 30°C and the population was observed until undetechable. Besides, pipette method was conducted to find the particle size of the sediment and loss-on-ignition method was performed for organic matter content. Results showed that S3 has the highest counts of fecal coliform *(E. coli)* in water sample (>1100 MPN/100ml), while S2 has the highest counts in sediment sample (12659.22 MPN/100g dry weight). It was found that there was significant difference between mean population and sampling stations in both water and sediment samples. At 25°C and 30°C there was significant difference of mean population in day two. *E. coli* in sediment from S4 survived longer due to high clay content. Further studies need to be conducted to determine the *E. coli* strain and other pathogenic organism's presence in the Semariang Batu River.

Key words: Fecal coliform, *E. coli*, die-off rate, organic matter, particle size

## ABSTRAK

*Escherichia coli (E.coli) merupakan organisma penunjuk yang digunakan di seluruh dunia untuk menilai tahap keselamatan mikrobiologikal di dalam air minuman, air untuk rekreasi dan air tawar. Dalam kajian ini, E. coli dipencilkan daripada air sungai dan keladak sungai di empat kawasan yang berbeza di Sg. Semariang Batu, Kuching, iaitu Sg. Lemidin (S1), Sg. Semariang Batu (S2), Sg. Loba Kara dan Sg. Batang Mangkuang (S4). Kesemua stesen mempunyai aktiviti yang berbeza iaitu kawasan pembinaan (S1), Kawasan perumahan (S2), kawasan penternakan udang (S3) dan kawasan yang tidak dicemari oleh aktiviti manusia (S4). Untuk menjalankan kajian terhadap ketahan E. coli, E. coli telah dipencilkan, dibiarkan dan dicampurkan di dalam sampel keladak sungai. E. coli dieramkan pada suhu 25°C dan 30°C. Selain itu, "pipette method" dijalankan untuk menentukan saiz partikel keladak sungai, manakala "loss-on-ignition method" dilakukan untuk menentukan kandungan bahan organik. Keputusan menunjukkan S3 mencatatkan bilangan fecal coliform (E. coli) yang tertinggi bagi sampel air sungai (>1100 MPN/100ml) manakala S2 mencatatkan bilangan tertinggi bagi sampel keladak sungai (12659.22 MPN/100g dry weight). Didapati bahawa terdapat perbezaan kepentingan antara min populasi dan stesen bagi kedua-dua air sungai dan keladak sungai. Pada suhu 25°C dan 30°C juga tedapat perbezaan kepentingan dalam min populasi pada hari ke-dua. E. coli di dalam keladak sungai dari S4 hidup lebih lama disebabkan oleh kandungan tanah liat yang tinggi. Kajian lanjut perlu dijalankan untuk menentukan jenis E. coli dan organisma patogenik yang hadir dalam Sg. Semariang Batu.*

*Kata kunci: Fecal coliform, E.coli, kadar kematian, bahan organik, saiz partikel*

## 1.0 Introduction

Problem of surface water sanitation remain an issue since the water is polluted to some degree with microbial population. Microbial contamination of groundwater used for drinking purposes is world-wide concern (Kator & Rhodes, 2003). In other words, we are more concern on human health since many waterborne diseases such as gastroenteritis and traveler's diarrhea had risen (O'Connor 2002).

Microorganisms which contributed to the sanitation of natural water were mainly derived from domestic sewage and non-point runoff containing excreta of human and animals (McLellan *et al*., 2001). Furthermore, improper operating septic systems or direct injection of effluent had led to the serious contamination of groundwater and water quality problems (Ksoll *et al*., 2007).

The microorganisms were not only found in surface water but also in the sediment. Some research showed that the microorganism may be concentrated in bottom sediments as a result of sedimentation of bacteria-bound particles (Fish & Pettibone, 2008).

According to Ling *et al.* (2009), Semariang Batu River is a tidal influenced river located near Kuching city. Rapid development of the city is affecting the river where traditionally a village is located upstream and in the 1990's due to blooming of shrimp industry. Besides, other land use activities such as construction area and residential area also give an impact to water quality of Semariang Batu River. At the same time, we were interested to conduct the die-off study for the species of fecal bacteria found in river. The term "die-off" here refers to loss of culturability on standard coliform enumeration culture media, either by loss of the ability to divide and form colonies or by the death of the cell (Shiaris, 1992).

Besides being potential pathogens, fecal bacteria such as *E. coli* can indicate the presence of other waterborne pathogens. Therefore, *E. coli* was used as *an* indicator to contact with the extent of fecal contamination in the water column and sediments which may be impacted by different land use activities.

This study characterizes the distribution of Escherichia coli by enumeration using 3-tubes Most Probable Method (MPN) and determination of the persistence of *Escherichia coli* in the sediment of the Semariang Batu River.

The major objectives of this study were to:

1. determine the impact of different land use activities on the concentration and distribution of *E. coli* at Semariang Batu River
2. determine the sediment characteristics by pipette method for particle size analysis and loss-on ignition (LOI) method for organic matter content and whether they affect the die-off of the E. coli
3. conduct the die-off study or survival of *E. coli* in sediments of Semariang Batu River.

## 2.0 Literature Review

### 2.1 Fecal Coliform

Coliform bacteria are a group of normal microflora in the intestines of warm-blooded animals (Elahe, 2009). According to Feng *et al.* (2002), fecal coliform was first defined by Eijkman where he found that fecal coliform is a subset of total coliform that grows and ferments lactose at different incubation temperature. Fecal coliform was analysed at 44.5$^{\circ}$C for water, shellfish and shellfish harvest water (Feng *et al*., 2002; APHA, 1998). Fecal coliform group may consist of *E. coli* and other enteric such as *Klebsiella, Enterobacter* and *Citrobacter* that can ferment lactose (Kenneth, 2005). An excessive amount of fecal coliform concentrations in water bodies such as lakes, rivers and streams can pose a public health threat when humans come in contact with the water (Runholt *et al*., 2007). Recently, Runholt and his colleagues (2007) had used Total Maximum Daily Loads (TMDLs) to achieve state water quality standards. This TMDL process is based on the relationship between pollution sources and in-stream water quality conditions. Based on TMDL, fecal coliform water quality standard for aquatics and recreational water shall not exceed 200 CFU/100 ml.

### 2.2 Sanitary Significance of Fecal Coliforms in the Environment

The presence of some microorganisms in water is used as an indicator of possible contamination and index of water quality. Coliform group of bacteria was mainly used to determine the safety of potable water (Borrego *et al*., 2002). The presence of coliform bacteria in potable water indicates unsuitable sanitation practices. This may be the result of poor water treatment, plant design problems, improper procedures, inadequate hygienic practices or after growths in the distribution system (Graves, 2003). According to Graves

(2003), nearly all natural waters are populated by coliform bacteria. Therefore, for the sake of human who has direct contact with the water, the populations of such coliforms should be curtailed by treatment and with the disinfection.

The presence of enteric pathogens in potable and recreational waters becomes a great concern. Therefore, it is important to determine the microbiological safety of the water by analyzing the presence of the specific pathogens. However, it is impractical to look for every pathogen potentially present in the water since there are hundreds of different microorganisms associated with waterborne diseases. As a result, groups of indicator microorganisms are used to determine the biological safety of the water such as total coliforms, fecal coliforms, *Escherichia coli*, fecal streptococci, *enterococci* and bacteriophages (Borrego *et al*., 2002).

The quality of recreational waters especially for activities such as swimming, surfing, boating or fishing is of great value to our society. Thus, an appropriate indicator should be used to determine the risk of enteric or other types of disease and the indicators sometimes are not appropriate for all the conditions. The statistical correlation had proved that total coliforms can be used as indicators in analysis of recreational waters. However, *E. coli* is much more reliable indicator in the presence of fecal pollution (Graves, 2003).

### 2.3 *E. coli* as an Indicator Organism for Water Sample

In 1892, Shardinger first proposed the use of *Escherichia coli* as an indicator of fecal contamination (Feng *et al*., 2002). *E. coli* which reside in the gastrointestinal tracts of humans and animals are used in the United States and throughout the world in assessing the microbiological safety in drinking water, recreational waters, and fresh water (Kon *et al.*, 2007). According to Ishii *et al.* (2006), *E. coli* has been used as indicator of fecal contamination due to the correlation between elevated *E. coli* counts in the water and the

occurrence of gastrointestinal symptoms or diseases. *E. coli* is considered as a good indicator because it has important criteria, including: (1) it is present in the feces of human and warm-blooded animals at numbers exceeding those of pathogen; (2) it shows minimal growth in aquatic systems and at slower rates than pathogens; (3) it is readily detectable by simple procedure that result in unambiguous identification of the fecal coliform group; (4) it is consistently present when pathogens are present; and (5) it shows increased resistance to disinfectants as opposed to pathogens (Elmund *et al.*, 1999; Redman, 2003).

An indicator organism typically provides evidence of presence or absence of pathogenic organism in the river water (USEPA, 2008). Whitman *et al*. (2006) said that contributions of point source in indicator organism were generally more straightforward than non-point sources. Therefore, *E. coli* is still considered as a good indicator organism. Other than *E. coli*, *Enterococcus* was used as indicator organism for the past decades (Teplitski & Butlers, 2008). Based on Teplitski and Butlers (2008), fecal enterococci mainly appear to persist in seawater for longer periods of time and they are not sensitive to solar radiation. Therefore, *E. coli* is still considered as a good indicator organism.

## 2.4 Standard Values for Fecal Coliforms in Environment

In order to monitor the quality of water, the guideline value for safety and clean water are important. According to Tennessee Department of Environment and Conservation (2009), a safe, clear, potable, aesthetically pleasing, and acceptable public water supplies can be obtained from raw surface water containing up to 10,000 coliform/100 ml or 2000 fecal coliforms/100 ml. However, the desired levels are less than 100 coliforms or 20 fecal coliforms/100 ml. In many other cases, a limit value for swimming is <235 CFU/100ml (Whitman *et al*., 2006). The standard value for fecal

coliform counts for total body contact (swimming) is 200 fecal coliform / 100 ml while for partial body contact (boating) is 1000 fecal coliform / 100 ml (USEPA, 1992). In shellfish harvesting areas, the geometric mean of fecal coliform should not exceed 14 bacteria / 100 ml (WHO, 1977). According to DOE Malaysia (2008), geometric mean of fecal coliform for CLASS IIB which is recreational use with body contact is 400 fecal coliform / 100 ml.

### 2.5 MPN method

The Most Probable Number (MPN) method is applicable to the enumeration of coliforms, fecal coliforms and aerogenic *Escherichia coli* in water in sealed containers including mineral and spring water. The MPN procedure involves a multiple tube fermentation technique where three or more decimal dilutions of the sample are inoculated into tubes of broth medium and incubated at specific temperature and time (GoC, 2003).

The method is progressive where first step is to determine the presence of coliforms in the tubes, then determining if these tubes also contain fecal coliforms and finally confirming whether *E. coli* is present. The gas produced in the tube is used as an indication of ability to ferment lactose from Lauryl Trptose Broth (presumptive coliform test) while gas production from Brilliant Green Lactose Bile broth is considered confirmation of coliform presence (Food Microbiology Lab, 2006). The number of tubes indicating the presence or absence of the three groups of organisms can be estimated from a standard statistical MPN table.

### 2.6 Die-off Rate of Fecal Coliform

There are many factors affecting the die-off rate of fecal coliform. In this study, *E. coli* will be the main focus of the fecal coliform group. In the studies of Ling *et al*. (2005), both temperature and pH gave an impact toward the survival of *E. coli*. The result of the studies showed that die-off rate of *E. coli* increased in acidic environment and in the high

temperature. Sampson *et al*. (2006) also reported that cooler water temperatures can increase the survival rate of *E. coli* while Bogosian *et al*. (1996) found that *E. coli* can survived in nonsterile river water up to six days at 37°C, eight days at 20°C and twelve days at 4°C.

In addition to the effect of temperature on survival, findings indicate that *E. coli* can survive in sediments and soils over extended periods of time (Anderson *et al*., 2005). Sediment are essentially sand with fines that are poorly differentiated and structured material and mostly lying within the stream or along the bank (American Heritage Dictionary of the English Language, 2000). *E. coli* concentration in the water can be directly affected by naturally occurring *E. coli* population in the sediment (Byappanahalli *et al*., 2006; Whitman & Nervers, 2003). This is because once *E. coli* adsorbed onto the particulate matter such as sediment, they are protected from environmental stresses, such as desiccation and solar radiation (Whitman *et al*., 2004) and therefore can be deposited into river water. Research suggests that *E. coli* probably survive longer in sediments as compared to their shorter survival rate in the overlying water due to the increased levels of organic matter in the sediments or the particle size of the sediment (Ling *et al.*, 2003).

Moreover, concentration of *E. coli* in the open water system is not only affected by biotic and abiotic factors but soon settles or become diluted, such that concentration decrease exponentially with depth of the water (Whitman *et al*, 2006). Besides, natural input such as rain event and tidal condition also become the factors that affect *E. coli* concentrations. This was proved after as little as 1 to 2 cm of rainfall, the levels of microorganisms in the water increased by several orders of magnitude (Schwab, 2007). Solo-Gabriele *et al.* (2000) reported that multiplication of *E. coli* in riverbank soil located in Fort Lauderdale, Florida, during drying and wetting cycles in laboratory experiment simulating tidal activity.

## 2.7 Previous Studies of Fecal Bacteria Counts in River water and Sediment

According to Salmore *et al*. (2006), the mean of *E. coli* level in the Menomonee River, Wisconsin, USA, was 1700- 10,000 CFU/100ml. This level was increased by one to three orders of magnitude after the storm events. In another research (Riebschlleager & Karthikeyan, 2008), *E. coli* is the leading cause of water impairments in the United States. Through the Total Maximum Daily Load (TMDL) program, mandated by the Clean Water Act Section 303, is a process to develop pollutant specific management plans integrating water quality assessment for protection of impaired watershed. A stream segment considered as impaired due to pathogens if 25% of the samples exceed 394 CFU/ 100mL or if the geometric mean of the samples exceed 126 CFU/100mL for indicator organism such as *E. coli* (Riebschlleager & Karthikeyan, 2008). Meanwhile Whitman *et al.* (2006) said that *E. coli* densities in main branch ranged from $10^2$ to $10^3$ CFU/100ml. This indicates the trend of *E. coli* increasing from headwaters to outfall. *E. coli* were recovered from organic soil or sediment with ranging from 5 to 1150 CFU/g (Ishii *et al*., 2006). Furthermore, Byappanahalli *et al*. (2006) found that the unpaired t test showed that the mean *E. coli* count downstream (921 CFU/ 100 ml) was significantly higher than that upstream with 486 CFU/ 100 ml ($P \leq 0.001$, $df = 660$). Meanwhile, the median concentration of *E. coli* in water was 1089 MPN/ 100 ml.

## 2.8 Sediment Analysis

### 2.8.1 Pipette Method

Particle size analysis of sediment is needed for the characterization, classification and is used to evaluate texture. Soil particles smaller than 2000 µm are generally classified as sands, silts and clays (Gee & Bauder, 1986). From this particle size analysis, percentage of sand, silt and clay can be determined (Gasparotto *et al.,* 2003). There two types of procedure for particle size analysis: (a) Pipette method (b) Hydrometer method. The pipette method is more convenient and it is recognized as more precise method (Chadwick & Quick, 2007). First step in the chemical pretreatment of any soil is removal of organic matter. Recently used reagent to remove the organic matter is hydrogen peroxide (Malo & Doolittle, 2000).

The pipette method is a direct sampling method. The principle is based on the variation of density at a point as a function of time. In practice, the principle used is that in a settling suspension at time, t and depth, z. Moreover, a sample must taken from a thin layer gives the original concentration of all particle (James, 2007).

### 2.8.2 Loss-On-Ignition Method

Total soil organic matter is estimated by loss-on-ignition method (LOI). Soil organic matter can be defined as series of fraction that comprise a continuum based on decomposition rate. Davies initially described the procedure of LOI in 1974 (Janice, 2008).

The principle of the direct estimation of organic matter requires one to separate it from inorganic materials, which approximately makes up 90% or more of the weight in soil (Nelson & Sommers, 1996). The most commonly used methods in destruction of organic matter were either by oxidation of the organic matter with hydrogen peroxide ($H_2O_2$) or ignition of the soil at high temperature (Nelson & Sommers, 1996). Besides, LOI is an inexpensive and reliable technique that has been used for decades by soil scientists, geologists, geographer and limnologists. In LOI method, soil organic is oxidized at moderate to high temperature with weight loss being proportional to the amount of soil organic matter in the sample (Konen *et al.*, 2002).

The relationship between LOI at 550 ºC ($LOI_{550}$) and organic carbon content; and between LOI at 950 ºC ($LOI_{950}$) and inorganic carbon content are currently accepted as a standard. However, these relationships are affected by sediment compositions which are clays, salts, and other variable content of organic carbon (Santisteban *et al*., 2004).

## 3.0 Materials and Methods

### 3.1 Sampling Site Description

In this study, both water and sediment sample were taken from four stations at tributaries of the Semariang Batu River with different land use activities. Station S1 was located at Lemidin River where construction work to develop a new river front was observed; Station S2 was located at Semariang Batu River itself with almost 250 houses along the river. Most of the villagers were fisherman and still depend on the river for the daily incomes. Station S3 was located at Loba Kara River which was shrimp farm effluent discharge point; and station S4 was located at Batang Mengkuang River which no obvious human activities was being observed.

Table 1: Study area and land use activity

| Station | Name of River | Land Use Activity |
|---|---|---|
| 1 | Sg. Lemidin | Construction Area |
| 2 | Sg. Semariang Batu (Village) | Residential Area |
| 3 | Sg. Loba Kara | Shrimp Farm |
| 4 | Batang Mengkuang | No Obvious Activity/Control |

.


Fig. 1: Map of study area with construction area (S1), residential area (S2), shrimp farm (S3) and area of no obvious human activity (S4).

### 3.2 Sample Collection

The bottles and plastics bag for sediment collection were sterilized by autoclave. During sampling, the *in situ* parameter such as temperature and pH were taken for both water and sediment samples. Both water and sediment were collected during low tide in three sampling trips (Table 2).

Table2: Sampling Dates

| No. Trip | Date of Trip |
|---|---|
| 1 | 10/9/2009 |
| 2 | 2/2/2010 |
| 3 | 2/3/2010 |

#### 3.2.1 Water Sample Collection

Triplicates of water samples were taken from each station. The water sample was taken by dipping the sterile bottle onto the water surface. After that, the bottle was sealed with the parafilm and covered the whole bottle with aluminium foil to avoid sunlight. Then, the water sample directly placed inside the ice box and was processed within six hours of collection (U.S. Environment Protection Agency, 2000).

#### 3.2.2 Sediment Sample Collection

For triplicate sediment collection, only half spade of the sediment was taken and put an inside sterile plastic bag. The spade was rinsed with 70% ethanol prior taking the sediment for each station. This was to ensure that no contamination occurred. Next, the plastic bag was placed into an ice box for immediate transport to the laboratory (Shiaris, 1992).

### 3.3 Enumeration of Fecal Coliform

Fecal coliform especially *E. coli* was enumerated by three series tubes of Most Probable Number (MPN) Method.

#### 3.3.1 Enumeration of Fecal Coliform from Water Sample

Most Probable Number (MPN) method was used to enumerate the fecal coliform (Shanks *et al*., 2006). Three tubes MPN series (9 tubes) which contains Lauryl Tryptose Broth (LTB) was prepared. Then three tubes of Double Strength LTB was inoculated with 10 ml of water sample and another three tubes of Single Strength was inoculated with 1.0 ml of the water sample and the last three tubes of Single Strength was inoculated with 0.1 ml of water sample. All of the tubes were incubated for 48 hours at $37^{\circ}$ C. After incubation, the presence of gas in the Durham tubes was observed. The positive tube was transferred to another tube which contains Brilliant Green Lactose Bile Broth. The tube was incubated for 24 hours in $37^{0}$ C. The positive tube was observed for the presence of the gas. Next, the colonies from positive tubes were streaked onto Eosin Methylene Blue (EMB) Agar and incubated for another 24 hours in $35^{\circ}$C. The streaking on the EMB Agar step was done to confirm the presence of the *E. coli.* The pattern of positive and negative tubes was recorded and the most number of organisms per unit volume of the original sample was determined by using a standardized MPN table (Refer to Appendix Table 3).

#### 3.3.2 Enumeration of Fecal Coliform from Sediment Sample

The preparation of sediment sample for fecal coliform enumeration was based on the analysis of 25 g analytical unit at 1:9 sample dilution ratio. First, 25 g sediment was suspended in 225 ml of 0.1 % peptone water in 250 ml beaker (Patel & Payne, 2004). Then, 10 ml, 1 ml and 0.1 ml respectively were pipetted from the beaker into three tubes of

MPN series. The methodology was the same as bacterial enumeration from water sample (Ontario Ministry of the Environment, 2004).

### 3.4 Inoculum Concentration

In order to begin the die-off study, concentration of starter inoculums needed to be determined. Type of organism used was pure culture of *E. coli* isolated from Serin River and grown in Eosin Methylene Blue agar. A single colony was inoculated into nutrient broth and incubated for five hours to reach the exponential phase. After five hours, 10% pure culture v/v nutrient broth was added into another nutrient broth. The culture was incubated for 210 min ($3^{1/2}$ hours) until the cells grew up to $10^4 – 10^5$ per ml. For every 30 minutes, the culture was spreading on EMB agar to determine the counts and at the same time the optical density was determine. The concentration of the starter inoculums was determined by optical density measurements at 650 nm (UV-Visible Spectrophotometer, Libra S11 & S12, Biochrom) and by using the standard curve developed (Ling *et al*., 2003). The optical density used was 0.014 and the concentration of bacteria was approximately in $10^4 – 10^5$ CFU per ml. The standard curve developed is shown in Appendix (Fig. 11).

### 3.5 Die-off Experiment

Fifty grams of sediment was placed in a 250 mL sterile beaker and 25.5 mL of sterile distilled water was added to saturate the sediment. The beaker was wrapped with aluminium foils to exclude light and the sediment was inoculated with 5 mL of inoculum. The inoculum and the sediment were mixed well with sterile glass rod. Experiment was conducted in triplicate and incubated at $25^0$ C and $30^0$ C. Sampling was done alternate day until *E. coli* was undetectable. One gram of the sediment was taken into saline water in

tube from the beakers. The counts of bacteria were determined using spread plate method. The plates were incubated $37^0$ C for 24 hours.

Sediment sample from the station S1, S2, and S4 were chosen in the experiment of *E. coli* die-off. The stations were chosen based on the different land use activities. The pH of each station was determined by adding one gram of sediment into 10 ml of deionised water. The pH was determined by pH detector (Eutech Instrusment, pH/Ion 510).

### 3.6 Particle Size Analysis (PSA) Method

Pipet method was used in this studied because it was a standard method from which other PSA methods were compared. The procedure has been adapted from Day (1965) and Green (1981). The sediment samples which contain high amounts of organic matter (> 5%) were transferred to 1000 ml beaker. 5 ml of hydrogen peroxide ($H_2O_2$) was added to the sediment suspension. $H_2O_2$ was added to completely destroy the organic matter. To determine the sand fraction, sodium hexametaphosphate was added to the sediment sample and 100 ml distilled water was added. Sodium hexametaphosphate is a chemical dispersant use in removing the cement or others flocculating agents. The sample must be dispersed and maintained in a dispersed state until sedimentation measurements are complete. Then, the mixture was poured into nest of sieves arranged from top to bottom with decreasing size in the following order: 1 mm, 500 µm, 250 µm, 125 µm and 63 µm. The sieves were shaken on a sieve shaker for 3 minute. The sand, silt and clay fraction were determined according to Day (1965) and Green (1981).