CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 MATERIALS

The materials for identification and culture of bacteria using nutrient agar (NA), Luria broth media (LB), nutrient broth (NB), waste cooking oil (WCO), $CaCO_3$, NH_4NO_3, $Na2HPO_4.7H_2O$, $KH_2PO_4.MNCL_2.7H_2O$, $MgSO_4.7H_2O$, yeast extract, MacConkey agar, Rhodamine B (olive oil, NaCl, agar, nutrient broth), peptone, phenol red and Gram-staining reagents were supplied by Merck, Malaysia Division. G-spin Genomic DNA extraction kit (for bacteria) and MEGA quick-spin PCR & Agarose Gel DNA Extraction System were purchased from Intron Biotechnology, Inc. Agarose power was purchased from Biosyntech Sdn. Bhd. SYBR safe DNA gel stain and 1 Kb plus DNA ladder was purchased from Invitrogen, USA. The TAE - buffer was purchased from DKSH Technology Sdn. Bhd. *Taq* DNA polymerase, $MgCl_2$ and 10 X Buffer were purchased from Invitrogen, USA.

3.2 LOCATION AND COLLECTING OF WATER SAMPLES

Water samples collected from Galing river (Kuantan), sampling station is near Kilang Jaya Gading, (M1) Street Industrial Semambu 4 (N. 03° 50.866’ E. 103° 19.976’), Setali area, (M2) Drain Lorong Seri Setali 60 (N. 03° 50.417’ and E. 103° 19.499”), (M3) Drain Street Galing 33 (N. 03° 50.022’ and E. 103° 19.863’), and (M4) Downstream Vistana Hotel (N. 03° 48.955’ and E. 103° 20.279’). Water samples were collected using sterile glass bottles with Depth Integrated Sample method. The Galing River water was preserved at low temperature before the commencement of the experiment (Table 3.1 and Figure 3.1).

The sampling locations were selected based on initial observations in the field, based on the length of the Galing River and regional division in the area of the Galing river, namely residential areas, offices and public area, industrial and agricultural areas. Galing river is divided into 4 upstream areas, middle upstream, middle downstream and downstream, is also seen by naked eye good water conditions, water color, odour, and levels of contamination by various wastes. An analysis of water quality of the Galing river was done using water quality index standard (WQIS), as a basis for determining the location of sampling points isolation of bacteria that have the ability to degrade the waste.

Table 3.1: Water Sampling Locations from Galing River

No	Locations	Landmark	Latitude (N)	Longitude (E)
1	M1	Street Industrial Semambu 4	03° 50.866’	103° 19.976’
2	M2	Drain Lorong Seri Setali 60	03° 50.417”	103° 19.499”
3	M3	Drain Street Galing 33	03° 50.022’	103° 19.863’
4	M4	Downstream Vistana Hotel	03° 48.955’	103° 20.279’

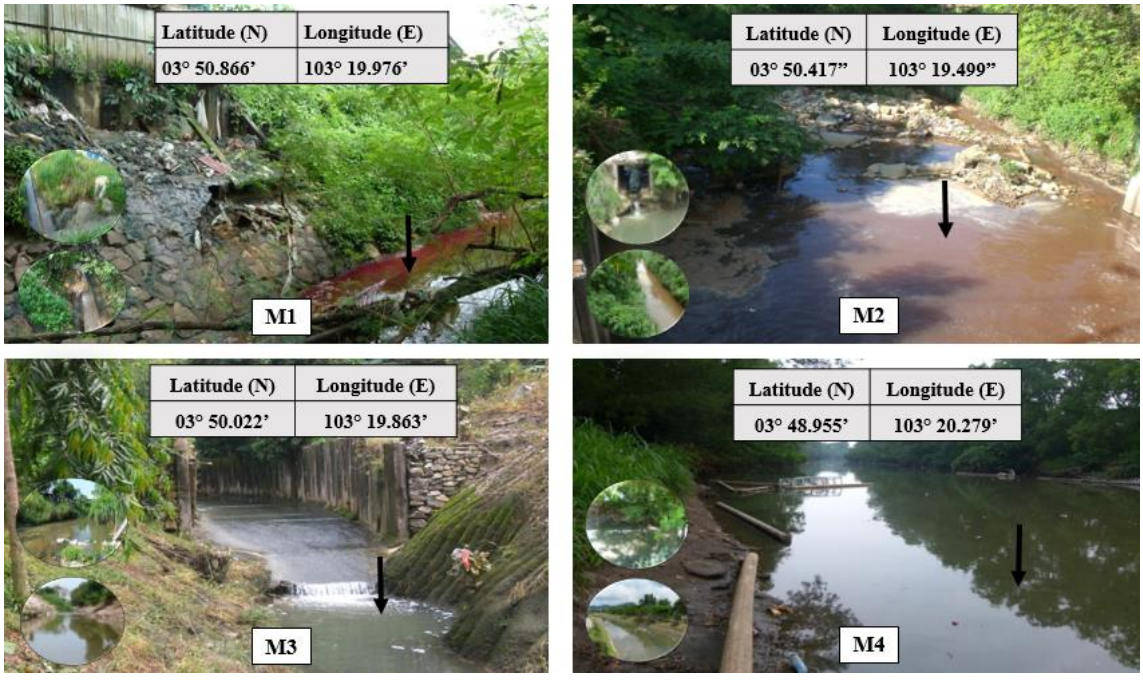

Figure 3.1: Location sampling points in Galing River (M1) Street Industrial Semambu 4, (M2) Drain Lorong Seri Setali 60, (M3) Drain Street Galing 33, (M4) Downstream Vistana Hotel.

3.3 QUALITY ANALYSIS OF GALING RIVER WATER

The location of the data collection and sampling were based on the land use in the Section 3.2. Water and sludge samples were collected using sterile glass bottles with methods *Depth Integrated Sample*. Samples were preserved in the icebox and were sent to the laboratory as soon as possible and kept refrigerated for laboratory testing. All samples were properly labelled with date, time, location and weather condition during time of sampling noted. Table 3.2 shows the parameters sampling and analysis methods which were tested *in situ* and in grab sampling technique.

Table 3.2: Sampling and Analysis Methods

No	Parameter	Sampling Method	Analysis Method
1	pH	In-situ	APHA 4500-H+B
2	Temperature (^{0}C)	In-situ	APHA 2550 B
3	Dissolved Oxygen (DO) (mg/L)	In-situ	APHA 4500 O G
4	BOD (mg/L)	Grab	APHA 5210 B
5	COD (mg/L)	Grab	HACH 8000
6	Ammonical Nitrogen (mg/L)	Grab	APHA 4500 NH3
7	Total Suspended Solid (TSS)	Grab	APHA 2540 D
8	Iron (Fe) (mg/L)	Grab	HACH 8147
9	*E.coli*	Grab	APHA 9222 G
10	Oil and Grease	Grab	USEPA 10056
11	Water Quality Index Standard (WQI)	Interim	NWQI

**American Public Health Association (APHA)*
**United States Environmental Protection Agency (USEPA)*
**HACH method is accepted by USEPA for reporting wastewater analysis*
**Environmental Protection Agency (EPA)*

Water samples were collected through *in-situ* and grab sampling technique and was preserved and analyzed in the lab. Water quality parameters that need to tested *ex-situ* are pH, temperature and dissolved oxygen (DO), while water quality parameters that were tested in the lab are Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammonia Nitrogen (NH_3-N), Total Suspended Solids (TSS), Iron (Fe) using DR-2800 spectrophotometer, *E. coli* using *E. coli* Quanty-try, and Water Quality Index Standard (WQI).