CHAPTER 3 : MATERIALS AND METHODS

3.1 Introduction

In this study, eight sampling points were chosen along Michu and Langat rivers. Water samples were collected from these points and the pollution parameters were studied. Analysis which were done includes physical, chemical and biological aspects of water. The eight sampling sites S1, S2, S3, S4, C2 , PN, pipe water and leachate are shown on the map (Figure 3.1).

The leachate samples were collected from the leachate pond prior to any treatment. Station S1 is the first sampling station which is located about 2 km from the leachate pond, before the settlements. This site was chosen because it represents the direct effect of leachate on the river. Station S2 is located after 5 houses and this site is expected to show changes in the river water quality due to the presence of residents. The next station is S3, which is located at the end of the settlements and this site will provide a good indication on the degradation of the water quality due to the presence of a community.

C2 is stationed at the Sungai Langat, before it meets the Sungai Michu and water samples from this station will show the impact of the operation of illegal factories, dumping untreated effluents into the river. S4 is located at the meeting point of the Michu and Sungai Langat and this station is chosen to show the mixing effect of pollutants from S3 (organic pollutants) and C2 (industrial pollutants).

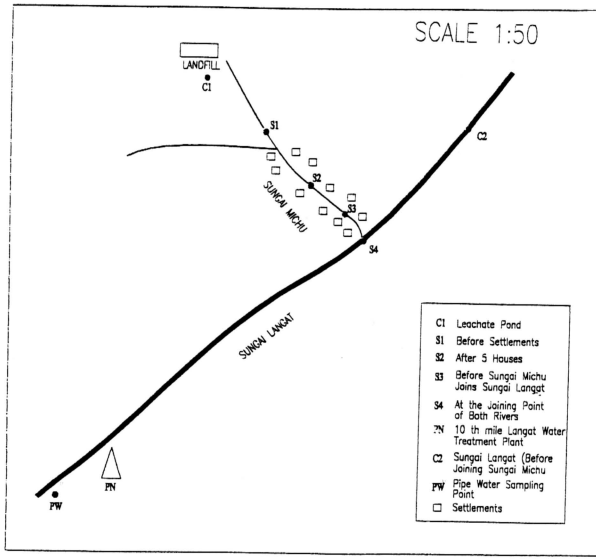

Figure 3.1 : Sampling stations along Sg Michu and Sg Langat

The second last station is PN which is the water intake point at 10th mile Langat Water Treatment Plant and the treated pipe water samples were collected from the residential area, after the 10th Mile Water Treatment Plant.

3.2 Water Sampling

Sampling was done five times, every other week and were collected in 5 replicates at each station. Samples were collected in clean plastic bottles of 500ml. For microbial analyses, samples were collected in sterile culture bottles, whereas for ICP analyses samples were taken in duplicates in High-Density Polyethylene 1-liter bottles. These bottles were washed with concentrated nitric acid to prevent the sorption of metal ions onto the sides of the container prior to metal analyses. All the samples were stored temporarily at 4°C in the laboratory.

3.3 Characterization of water samples and leachate.

3.3.1 pH

pH was determined using a pH meter (model no 8033). This analyses was done immediately after sampling.

3.3.2 Total Suspended solids

Total suspended solids are that portion of the solids retained by a filter (sometimes referred to as filterable solids, since they can be filtered out). Suspended solids and dissolved solid gives the total solids. The procedure for measuring total suspended solids is a simple gravimetric analysis involving the

difference in weights before and after a sample of water is passed through a glass fibre filter paper. Dissolved solids are considered to be the residue left after the evaporation of a known volume of the filtrate at 103°C.

The filter papers were prewashed with distilled water and oven dried for one hour at 103°C, then cooled in a dessicator before weighing. This cycle was repeated till a constant weight is obtained. Each sample was mixed by shaking and 100ml of sample was measured in a graduated cylinder. The sample was then decanted through the filter and the filter paper was oven dried at 103°-105°C for at least an hour. After the filter paper has dried, it was cooled in the dessicator before weighing. The total suspended solid was obtained by subtracting the weight of the filter before and after the filtration process.

3.3.3 Total Solid (TS)

For total solid determination, a clean porcelain dish was first heated at 103°-105°C for an hour. Then the dish was cooled in a dessicator before weighing. About 100 mL of well mixed water or leachate sample was measured using a cylinder and transferred into the dish. The sample was evaporated in an oven at 103°-105°C for an hour. The dish was then cooled in dessicator before weighing.

$$\text{mg total solid/L} = \frac{(A-B) \times 1000}{\text{Sample volume, mL}}$$

Where, A= weight of dried residue and dish, g

B= weight of empty porcelain dish, g.

3.3.4 Biological Oxygen Demand (BOD)

The procedure is based on the consumption of oxygen by microorganisms that are present in the sample. Winkler method was used to determine the concentration of dissolved oxygen in the samples. This method requires the addition of three chemical reagents to the sample very soon after it is obtained. Sample was first filled in BOD bottles without trapping any air bubbles under the stopper. One ml of Mn SO_4 was added followed by 1 ml of alkaline iodide azide solution. The glass stopper was replaced and the contents were mixed by inverting the bottle several times. A brown floc was formed before and during the mixing. After the floc has settled to about two third of the bottle, 1 ml of concentrated sulphuric acid was added and this causes the floc to disappear, forming a yellow brownish solution. Exactly 200ml of this solution was titrated with 0.025 M sodium thiosulphate.

1 ml $Na_2S_2O_3$ represents 1mg/l dissolved oxygen

3.3.5 Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) is the amount of oxygen consumed by organic matter when oxidized with boiling acidified potassium dichromate solution. It provides a measure of the oxygen equivalent of that portion of the organic matter in a water sample that is susceptible to oxidation under the conditions of the test. Dichromate reflux method oxidized the organic matter chemically instead of biologically.

COD was determined by refluxing 50 ml of sample for two hours with 25.0 ml of 0.417 M potassium dichromate solution and a mixture of 1g of silver sulfate and 75.0 ml of sulfuric acid using TECATOR COD digestion unit.

After refluxing, the mixture was diluted to about twice its volume with distilled water and allowed to cool to room temperature. The excess dichromate was measured by adding a reducing agent, 0.1M ferrous ammonium sulfate, using 2-3 drops of ferroin indicator. The difference between the chromate originally added and the chromate remaining is the chromate used for oxidizing the organics. The more chromate used, the more organics were in the sample, and hence higher COD. COD was calculated using the equation below:

$$\text{Concentration of COD} = \frac{(a-b) \times c \times 8{,}000}{v} \quad \text{mg/l}$$

where: a = volume of ferrous ammonium sulfate (ml) used for blank

b =volume of ferrous ammonium sulfate (ml) used for sample

c = molarity (mol/l) of ferrous ammonium sulfate, and

v = volume of water or leachate sample (ml).

3.3.6 Ammoniacal Nitrogen

Ammoniacal nitrogen in the sample was detected by Devarda alloy method. Analysis was done by preparing a set of ammonium sulfate standard solution. Exactly 0.4715 g of dry $(NH_4)SO_4$ was dissolved in deionised

water and made volume to 1000ml (100ppm). This stock was diluted with distilled water to give 2.5 to 15 ppm of total nitrogen concentration.

10 ml of sample or standard solutions was transferred into test tubes and about 0.3-1g of $K_2 S_2 O_8$ was added to each test tube. This was autoclaved for an hour. After adding 0.2 g Devarda allov, the test tubes were left to stand over night.

About 2 ml of sample from each test tube was mixed with 8 ml of distilled water, 4ml of **Reagent 1** and 6ml of **Reagent 2.** The bluish color that developed was measured using spectrophometer at wavelength of 635nm. A standard curve was plotted for the standards and the concentration of nitrogen in the samples was obtained from this graph.

Reagent 1 :

Part 1: 15g Phenol + 0.05g Sodium Nitroprusside

Part 2 : 27.5g $Na_3 PO_4 . 12 H_2 O$ + 7.5 g $Na_3 C_6 H_5 O_7 . H_2 O$ + 0.75 g EDTA

Part 1 was dissolved in part 2 and the volume was made to 250 ml

Reagent 2:

30ml of commercial bleach was added to 400 ml of 1M Sodium hydroxide.

3.3.7 Heavy metal determination

100 ml of each sample was filtered using a preconditioned plastic filtering device with vacuum containing a filter support made of plastic. The ungrided cellulose acetate membrane filter paper with 0.45 μm pore diameter was pre-washed with 2N HNO_3 before use. After filtration, the filtrate was

acidified to pH 2 with concentrated nitric acid and analyzed directly using the Inductively coupled Plasma Atomic Emission Spectrometry (ICP-AES), Model Optima 3000.

3.3.8 Other determinations

Chloride, water hardnesss, sulphite, and total alkalinity were determined using Hanna Instruments HI 4817 test kit.

Chloride

The chloride level was determined by a mercuric nitrate titration. The pH of the sample was lowered to 3 by addition of nitric acid. Mercuric ion react with chloride ions in the sample to form mercuric chloride. When excess mercuric ions are present, it complexes with diphenylcarbazone to form purple solution. The color change from yellow to purple determined the end point of this titration.

Water hardness

The hardness level as mg/l calcium carbonate was determined by an EDTA titration. The sample was first adjusted to a pH of 10 using a buffer solution. Eriochrome black T indicator which was used chelates with metal ions such as Mg and Ca to form a red colored complex. As EDTA was added, metal ions complex with it. After all the free metal ions have been complexed, and excess EDTA removes the metal ions complexed with the eriochrome black T to form a blue colored solution. This color change from red to blue is the end point of titration.

Sulphite

An idiometric mehod was used in this determination. Iodide ions was reacted with iodate ions to form iodine in the presence of sulphuric acid. The sulphite ions present in the water sample then reduces the iodine back to iodide. An excess of iodate ions will generate additional iodine, which will from a blue complex with starch. This color change determines the end point of this titration.

Total Alkalinity

Total alkalinity was determined by nuetralization method. The sample was first nuetralized to a pH of 8.3 using dilute HCl acid and phenolphtalein indicator. This process converts hydroxide ions to water and carbonate ions to carbonic acid and the end point is indicated by a color change to yellow.

3.4 Microbiological analysis

3.4.1 Detection of coliforms in water (MPN)

A primary concern in the bacteriological analysis of water is whether there is fecal pollution. This because feces can carry disease causing microorganisms such as *Salmonella* sp, *Shigella* sp, *Campylobacter* sp, *Escherichia* sp, *Vibrio* sp, *Leptospira* sp and *Yersinia* sp. The test is based on the ability of *E.Coli* to ferment lactose with the production of acid and gas.

The presumptive coliform test

Selective media are used to assay for coliforms. They contain ingredients such as bile salts which can be tolerated by enterobacteria, but inhibits the growth of most non-enterics. The media also contain lactose and a color indicator to show acid production, and this can be determined with the use of Durham tubes. Mc Conkey broth is used as selective medium in the initial testing for coliforms. Three set of tests was prepeared:

10 ml of sample was added to each 3 bottles of 10ml McConkey broth

1ml of sample was added to each 3 bottles of 5ml Mc Conkey broth

0.1ml of sample was added to each 3 bottles of 5 ml Mc Conkey broth

All these bottles were incubated at 37°C for 24-48 hours

Bottles that shows gas trapped in Durham tubes were recorded as positive.

The confirmed presumptive coliform test.

The presumptive coliform test indicates the presence of bacteria that could digest lactose with the formation of acid and gas. Differential media, EMB (Eosin Methylene Blue agar) is used. Bacteria colonies that grow on the EMB plates and which have a green metallic sheen are probably *E. coli*.

From the test bottles of Mc Conkey broth, those that shows positive results and has the lowest inoculum was inoculated on the EMB plates. These cultures were incubated at 37°C for 24 hours. The appearance of green metallic sheen bacterial colonies on the EMB plates confirms that enteric coliforms are present in the water sample.

From each of the positive bottles of the presumptive coliform test, a loopful of culture was inoculated into 5 ml of fresh lauryl tryptose broth which have been incubated in a 37°C water bath. Immediately the inoculated bottles were placed in a 44°C water bath for 24 hours. Tubes showing gas production are considered to contain *E. coli*.

3.4.2 Salmonella-Shigella isolation

Salmonella-Shigella Agar (SS) was used as the selective media in this isolation. About 100µL of sample was pipetted out and spread on the SS agar plates. These plates were incubated at 30°C for 24-48 hours. Lactose fermenting bacteria such as *E. coli* or *Klebsiella pnuemonia* will appear as small pink or red colonies whereas lactose nonfermenting bacteria, such as *Salmonella* species, *Shigella* species and *Proteus* species will appear as colorless colonies. Certain *Salmonella* species are capable of producing hydrogen sulphide which turns the center of the colonies black. Number of colorless and black centered colonies were enumerated and recorded.