CHAPTER 2
EXPERIMENTAL

2.1 Sampling

2.1.1 Samples and Sampling Site

Sampling for screening survey of phenol contents in water at various sites in and around Chiang Mai city and nearby the Northern Industrial Estate(NIE), Lampoon, was conducted by consideration the water direction and the anthropogenic activities around the sites. The screening was done twice in May and June, 1994, and consequently the selected sites were adjusted in order to find the representative sites for assessing the phenols pollution. As the result, 15 out of 19 sites were chosen as shown in Figure 2.1 as a map and in Figures 2.2-2.16 for selected sites as the photographs.

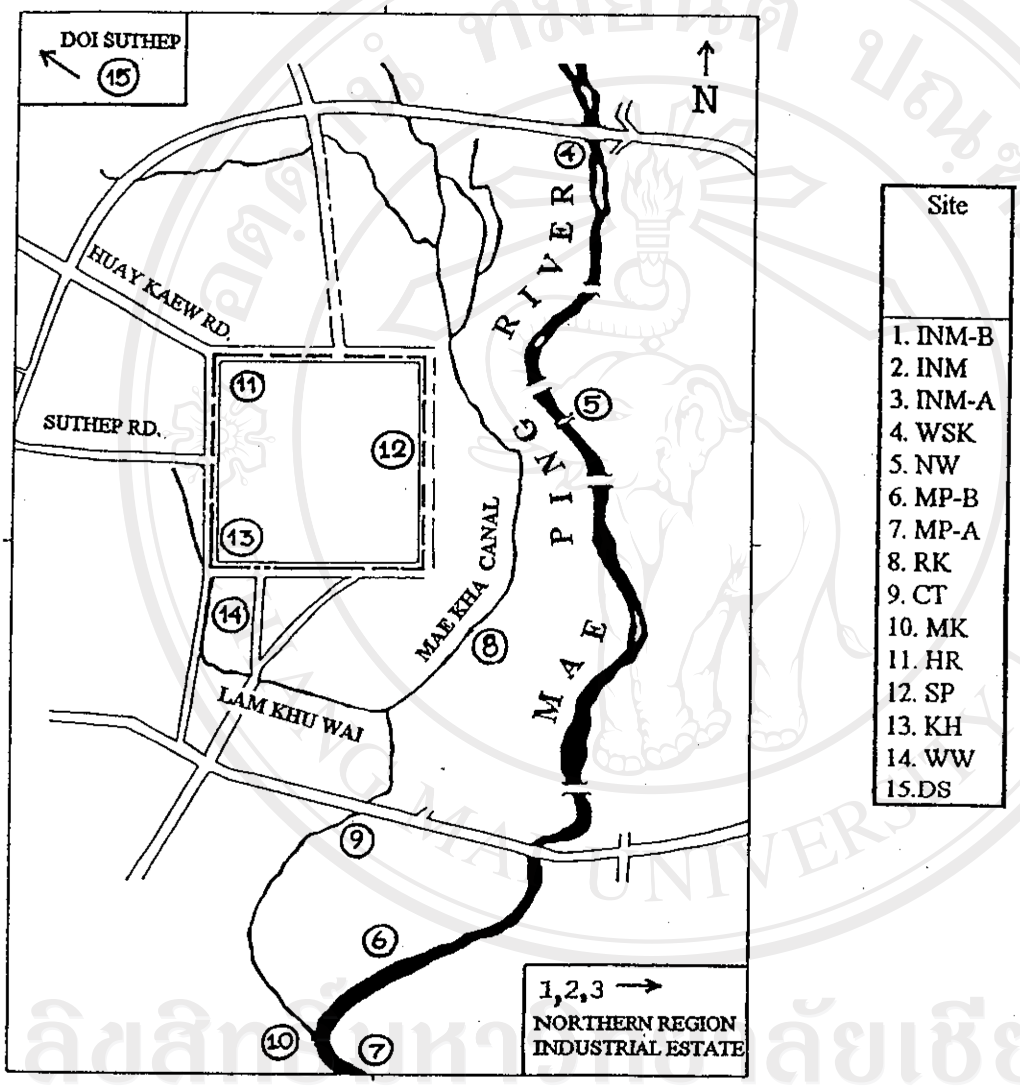
Site descriptions are shown as follows:

Site1: Before Industrial Mixing Point (INM-B)

This site is located at the Mae Kuang river in Lampoon province, nearby the NIE area. It is around 1 km before the discharging point of the NIE. There were few households around the river bank. Some waterhyacinths and grasses were found on the surface water. Fishing were observed in this area.

Site 2: Industrial Mixing Point (INM)

This site is located at the Mae Kuang river, closely to the discharging point of the NIE. Waterhyacinth was commonly found. Oil-film was ever noted on the surface of the water. The industrial plants are mostly for electroceramics.



Site

1. INM-B
2. INM
3. INM-A
4. WSK
5. NW
6. MP-B
7. MP-A
8. RK
9. CT
10. MK
11. HR
12. SP
13. KH
14. WW
15. DS

Figure 2.1: Map Showing Locations of the Study Sites

Site 3: After Industrial Mixing Point (INM-A)

This site is a part of the Mae Kuang river which flows passing the discharging point of the NIE. It is around 1 km far from the mixing point, located after Mae Kuang Dam. Dense of aquatic plants especially waterhyacinths was observed. There were some local restaurants around 500 meters before this point but no household was found nearby the river bank.

Site 4: Wang Sing Kham (WSK)

It is a part of the Mae Ping river before running passed Chiang Mai city and situated by the side of the superhighway road. Building construction and fishing were always found at this site during the samplings.

Site 5: Nawarat Bridge (NW)

It is located in the Mae Ping river at the central part of Chiang Mai city. One side of the bank is near the market and the other have many restaurants around. No aquatic plant was found in this site.

Site 6: Mae Ping Before Mix (MP-B)

This site is in the Mae Ping river, around 1 km before the domestic wastewater discharging point (Mae Kha canal). Fishing and swimming were observed.

Site 7: Mae Ping After Mix (MP-A)

This site is situated in the Mae Ping river, around 1 km far from the domestic wastewater discharging point, Mae Kha canal. Households were widely distributed near the banks where no treatment system was available. Bathing and fishing were observed to be the activities.

Site 8: Ra Kang Bridge (RK)

This site is in the middle of the Mae Kha water route after passing the city and located on Ra Kang road. The water always had bad smell and garbages were commonly found together with the scum and gas bubbles.

Site 9: Chao Tung Restaurant (CT)

This site is situated nearby the super highway road, Chiang Mai-Lampoon. It is a part of the Mae Kha canal after passing the city. Dense of aquatic plants, waterhyacinth, morning glory and grass was found in this area, mixed up with garbages, scum, gas bubbles produced including the bad smell of water.

Site 10: Mae Kha Canal (MK)

The site is located at the edge of the Mae Kha canal before draining to the Mae Ping river. The Mae Kha canal is treated as the representative of the Chiang Mai city's main drainage route which had bad smell and always caused the huge of foam floating.

Site 11: Jang Hua Rin (HR)

This site is located in the Chiang Mai moat on Maneenoparat road. It was the water-inlet of the moat. Aquatic plants, grass and waterhyacinth were commonly found, including the garbage such as cans, plastic bags and foam boxes.

Site 12: Sompetch Market (SP)

This is located closing to the market named Sompetch at the middle of north-east side of Chiang Mai moat, on Khotchasan road. It was a standing water. The garbages usually exposed on the pathways without any suitable sanitary system.

Site 13: Jang Khu Huang (KH)

This is one of the outlet of Chiang Mai moat. It is located near Buak Had market, the flower market of Chiang Mai. Garbages were slightly found near the outlet of the moat.

Site 14: Wong Wan (WW)

This is a small drainage canal consequently followed the drainage way from Jang Khu Huang which had joined with Lum Khu Wai canal before. It is located on Wong Wan road.

Site 15: Doi Suthep (DS)

This site is a small waterfall located on Doi Suthep, around 300 meters from Ruae Si cave, around 200 meters from main road. There was no activity found near the site. The vegetation is evergreen forest. This is used as the controlled site.

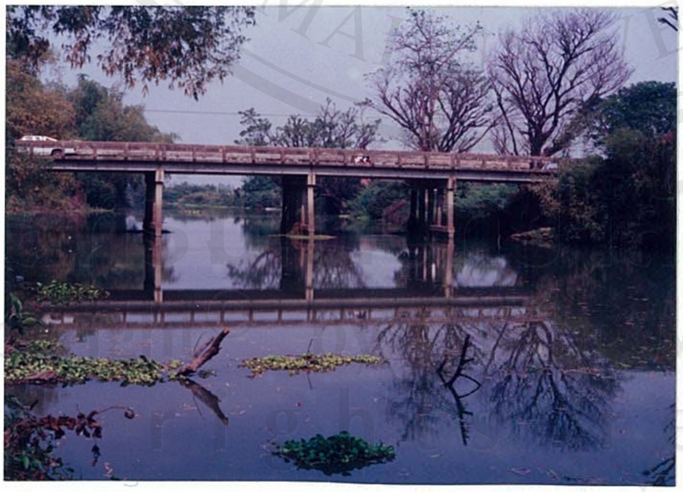


Figure 2.2: Before Industrial Mixing Point (INM-B)

Figure 2.3: Industrial Mixing Point (INM-A)

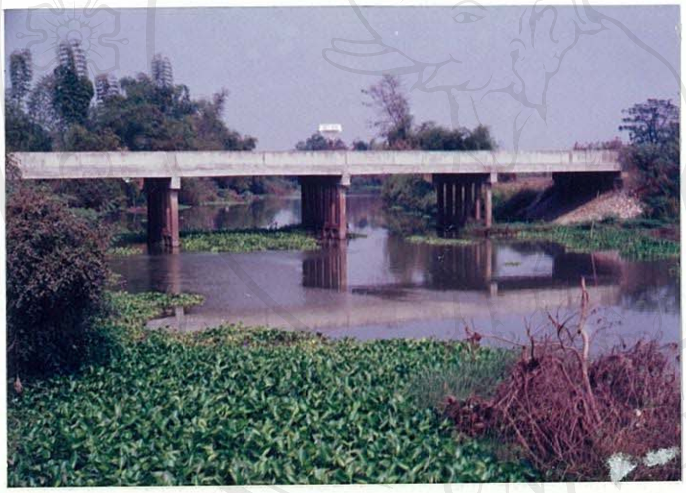


Figure 2.4: After Industrial Mixing Point (INM-A)

Figure 2.5: Wang Sing Kham (WSK)

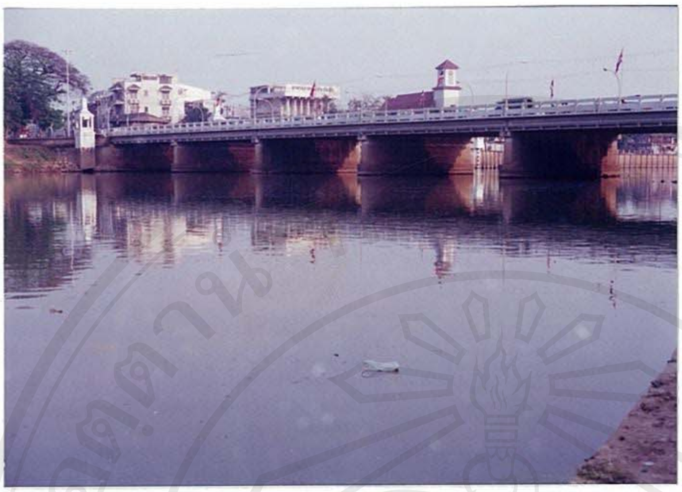


Figure 2.6: Nawarat Bridge (NW)

Figure 2.7: Mae Ping Before Mix (MP-B)

Figure 2.8: Mae Ping After Mix (MP-A)

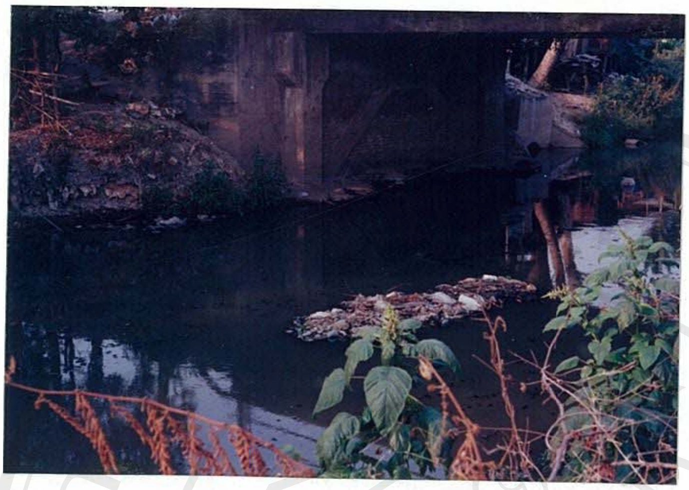


Figure 2.9: Ra Kang Bridge (RK)

Figure 2.10: Chao Tung Restaurant (CT)

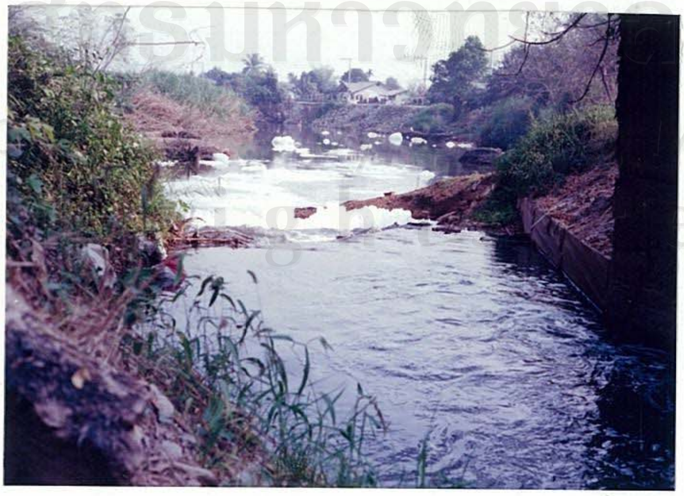


Figure 2.11: Mae Kha Canal (MK)

Figure 2.12: Jang Hua Rin (HR)

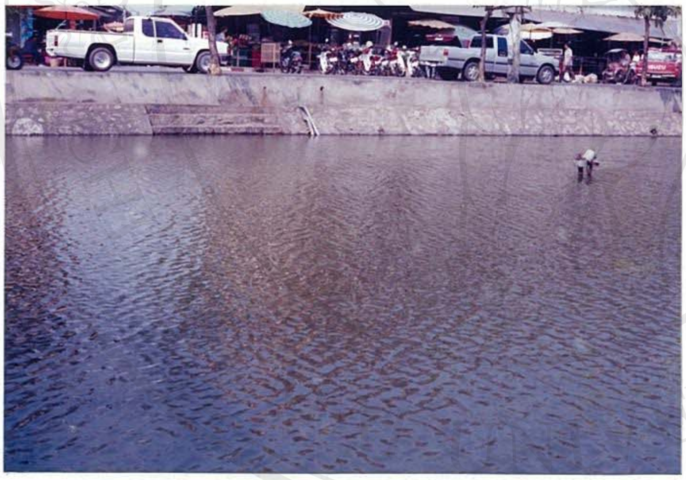


Figure 2.13: Sompetch Market (SP)

Figure 2.14: Jang Khu Huang (KH)

Figure 2.15: Wong Wan (WW)

Figure 2.16: Doi Suthep (DS)

2.1.2 Sampling Method and Sample Preservation

Water sample was collected at 30 cm depth at the center of waterbody. By using water sampler (Figure 2.17) for the site where the bridge is nearby, the others by direct hand-sampling using the boat. The water samples were kept in the brown-glass bottles with teflon screw caps (cleaned with water and acetone). The bottles were not prerinsed with sample before collection.(37)

The preservation was done by acidifying with phosphoric acid to pH 2 (2 ml phosphoric acid/ l of water sample). The samples were cooled (4°C) from the time of collection until extraction.

The samples were kept for a maximum of 28 days for spectrophotometric method and flow injection analysis (FIA) technique(30, 52). For HPLC technique, the samples were extracted within 2 days of collection(5) and completely analysed within 40 days of extraction(37).

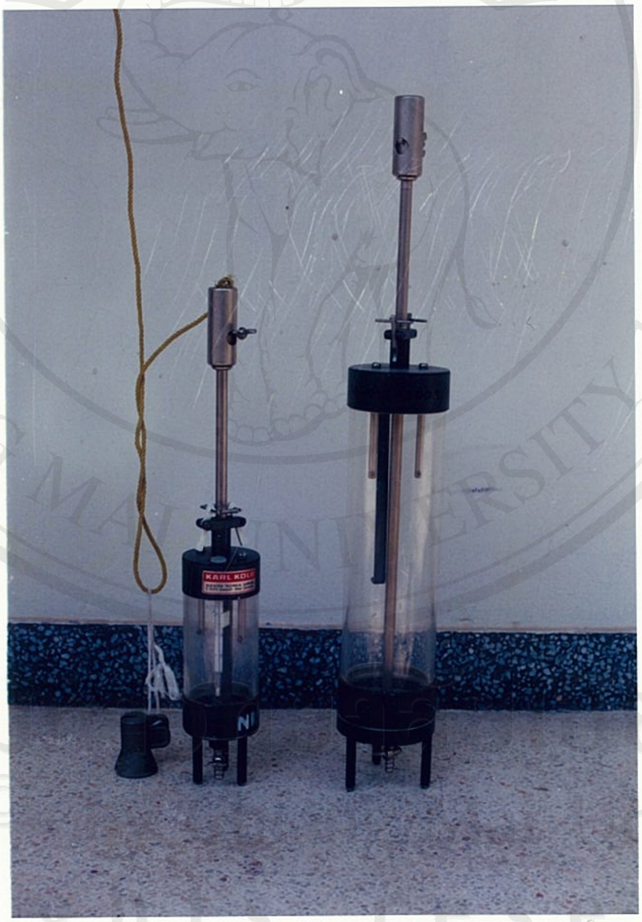


Figure 2.17: Water Samplers

2.2 Physico-Chemical Parameters Measurement

Measurement of some physico-chemical parameters; pH, temperature, conductivity, dissolved oxygen and oxygen saturation capacity were conducted in the field using the environmental test kits; microprocessor oximeter, pH meter and conductivity meter which all manufactured by WTW Wissenschaftlich-Technische Werkstaetten, Germany. Other parameters, such as secchi depth, depth of water body, odor, color and flow rate of water were also carried out.

2.3 Spectrophotometric Method

2.3.1 Apparatus and Chemicals

2.3.1.1 Apparatus

(1) UV-VIS Spectrophotometer,
Shimadzu UV-1604, Japan

2.3.1.2 Chemicals

(a) Phenol, A.R. grade, BDH Chemicals, England

(b) 4-Aminoantipyrine, >98%, A.R. grade, Fluka, Switzerland

(c) Ammonium chloride, A.R.grade, BDH Chemicals, England

(d) Potassium hexacyanoferrate(III), >98%, A.R.grade,
Fluka, Switzerland

(f) Sodium sulphate anhydrous, A.R.grade, Merck, Germany

(g) Ammonia solution 25%, A.R. grade, Carlo Erba, Italy

(h) Chloroform, A.R. grade, Merck, Germany

2.3.2 Preparation of Solutions

2.3.2.1 Reagent

(a) 4-Aminoantipyrine(4-AAP) solution

An aminoantipyrine solution was prepared daily by dissolving 2g of 4-AAP in distilled water of a final volume of 100 ml.

(b) Buffer solution

A buffer solution employed in this experiment was a mixture of 350 ml ammonia solution (25%) and 54 g ammonium chloride diluted to 1 L with distilled water.

(c) Potassium hexacyanoferrate(III), $K_3Fe(CN)_6$, solution

A potassium hexacyanoferrate solution was prepared weekly by dissolving 8g of $K_3Fe(CN)_6$ in distilled water of a final volume of 100 ml.

2.3.2.2 Preparation of Phenol Standard Solutions

The phenol stock standard of 1000 mg/l was prepared by dissolving 0.5 g phenol in distilled water of a final volume of 500 ml. Working standard solutions were prepared by successive dilution of the phenol stock solution as follow:

Stock standard ⟶	Intermediate standard ⟶	Standard solution
1000 mg/l	100 mg/l	10 mg/l

Consequently the phenol working solutions in the range of 4 to 200 μg/l were prepared in 250 ml from the standard solution.

2.3.3 Procedure

The procedure was adapted from the German DIN method (DIN 38409).[30] 250 ml filtered water sample was transferred to 1000 ml separatory funnel, then 40 ml buffer and 2 ml 4-AAP solution were added and mixed well. After 2 min, 5 ml $K_3Fe(CN)_6$ solution was added and mixed well. Then after 15 min, the colored complex formed and was extracted into 30 ml chloroform. The shaking time of extraction was 2 min and standing for 15 min to get the complete separation between organic and aqueous layers. Consequently the chloroform layer was collected by filtering through filter paper topped with 5 g anhydrous sodium sulphate. Absorbance was measured immediately at 470 nm. The absorbance for each concentration was plotted against concentration (0, 4, 8, 20, 80 and 200 μg/l). The procedure is summarized as flow diagram in Figure 2.18.

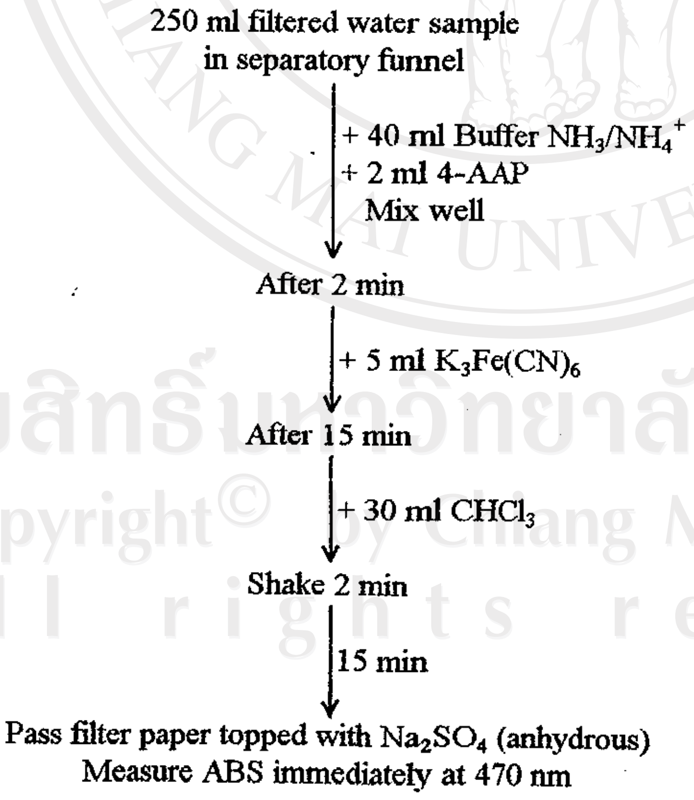


Figure 2.18: Flow Diagram of the Spectrophotometric Procedure

2.3.4 Assessing Phenol Recovery and Data Quality

Laboratory fortified sample matrix (LFM) was conducted to assess the phenol recovery. Two of each phenol concentration, 4 μg/l, 8 μg/l, and 20 μg/l, were added in the water sample and blank. These fortified water samples were analysed similarly to the procedure for solution.

Percent recovery for each fortified sample, was calculated using the following equation:[54]

$$R = \frac{C_s - C}{S} \times 100$$

where, R = Percentage of recovery

C_s = Fortified sample concentration

C = Sample background concentration

S = Concentration equivalent of analyte added to sample

A plot of the absorption at 470 nm against the fortified concentration employed is illustrated in Figure 3.3. The recovery results of each fortified concentration are shown in Table 3.7.

2.3.5 Precision

10 aliquots of the water sample at Jang Hua Rin site collected on 26th October 1994 were analysed and compared with obtained concentration. Precision of the method was evaluated in term of the mean value, standard deviation and % relative standard deviation.

2.3.6 Stability of Standard Series

Stability testing of standard series was investigated by measuring absorbance of each concentrations everyday for 11 days. The calibration of first day employed was fixed in order to investigated the changing of obtained concentration and absorbance at different period of time.

2.4 Flow Injection Analysis(FIA)

2.4.1 Apparatus and Chemicals

2.4.1.1 Apparatus

(a) FIA Instruments, Lachat, model Quick-chem 8000, USA

(b) pH meter, Cole-Parmer chemcadet 5986-25 pH/mv meter, USA

(c) Lachat mixing coil for Cl^- analysis

(d) Glass bead column; Silicone tubing 7.5 cm, i.d. 3.5 mm, 4.5 cm long containing glass beads of 3 mm diameter

(e) Sample loops;

- Microloop (20 μl)
- Loop1 (175 μl)
- Loop2 (565 μl)

2.4.1.2 Chemicals

(a) Phenol, A.R. grade, BDH chemicals, England

(b) 4-Aminoantipyrine, A.R. grade, Fluka, Switzerland

(c) Potassium hexacyanoferrate(III), A.R. grade, Fluka, Switzerland

(d) Boric acid, A.R. grade, Merck, Germany

(e) Potassium chloride, A.R. grade, Merck, Germany

(f) Sodium hydroxide, A.R. grade, Merck, Germany

(g) Phosphoric acid 85%, A.R. grade, Carlo Erba, Italy

2.4.2 Methodology

The method used was adapted from the method provided by Lachat Instrument (Quickchem Method 10-210-00-1-A).[54]

2.4.2.1 Preparation of Reagents

(a) 4-Aminoantipyrine Color Reagent

In a 250 ml volumetric flask, 0.16 g 4-AAP was dissolved in deionised water and stored in the flask. Fresh reagent was prepared daily.

(b) Buffer Potassium hexacyanoferrate(III), pH 10.3

In a 1 l volumetric flask, 2.0g potassium hexacyanoferrate, 3.1 g boric acid , 3.75 g potassium chloride and 47 ml 1 M sodium hydroxide were dissolved in deionised water. This reagent was stored in glass container and prepared fresh weekly.

2.4.2.2 Preparation of Phenol Standard

The phenol stock standard of 500 mg/l was prepared by dissolving 0.5 ml 85% phosphoric acid and 0.250 g phenol in deionised water in 500 ml volumetric flask. Intermediate stock standard of 2.50 mg/l was prepared by 200 times dilution of stock standard. Consequently, working standards in the range of 5-200 μg/l were prepared by successive dilution of the intermediate stock solutions.

2.4.2.3 Sample Preparation

Water samples were adjusted pH to 4 and 5 (by 85% phosphoric acid) prior analysed with FIA instruments.

2.4.3 Condition of the FIA system

In this experiment, the instrument employed was Lachat FIA Instruments is shown in Figure 2.19. The manifold which was modified from the one for chloride determination for the use of this phenol study, is shown in Figure 2.20. Some of the system conditions were then re-investigated.

Figure 2.19: Lachat FIA Instruments

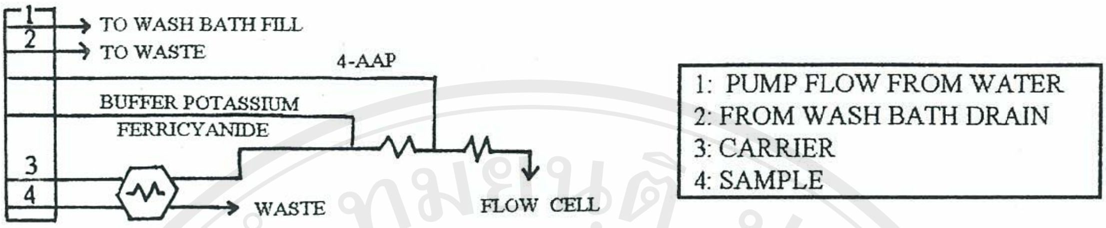
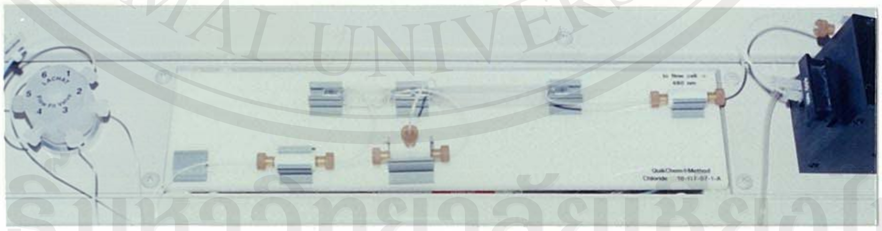


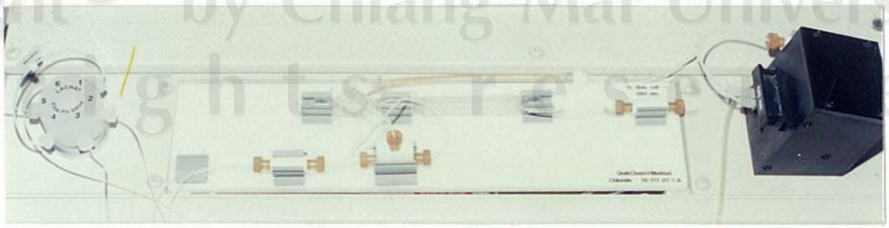
Figure 2.20: Manifold Diagram for FIA Procedure[54]

2.4.3.1 Sample Loop, Flow Rate and Reaction Coil

The sample loops (microloop (20 μl), loop1 (175 μl) and loop2 (565 μl) and reaction column (mixing coil and glass bead column) were tested together with variation of flow rate via changing of rotator pump speed. Comparisons of peak heights and slopes of each set of conditions of analysis were carried out for suitable condition. Flow rate of carrier (He degassed water), reagent (4-AAP) and buffer ($K_3Fe(CN)_6$) were measured by recording the time for 2 ml of solutions to pass the system. The reaction columns were shown in Figure 2.21.



(a) Reaction Mixing Coil



(b) Reaction Bead column

Figure 2.21: Mixing Coil and Bead Column

2.4.3.2 Summary of Condition Used

The condition of FIA used are listed in Table 2.1.

Table 2.1: FIA Conditions

DESCRIPTION:
Determination of phenols/ Loop2 (565 μl)/ Bead/480 nm

ANALYTE DATA:

Analyte Name:	phenols
Concentration Units:	μg/l
Chemistry:	Bipolar

CALIBRATION DATA:

Levels:	1) 0	2) 5.0	3) 10.0	4) 20.0
	5) 50.0	6) 100.0	7) 200.0	

SAMPLING TIMING:

Method cycle Period:	70.0 second
Min. Probe in Wash Period:	20.0 second
Probe in Sample Period	30.0 second

2.4.4 Calibration Curve Construction

Phenol standard series were prepared according to 2.4.2.2. The phenol standard was injected to FIA system. The calibration curve was obtained by plotting peak height against concentration.

2.4.5 Precision

The water sample of Chao Tung site collected on 26th October 1994, was injected 12 times under the same condition. The responses obtained were used for calculations of mean, standard deviation and % relative standard deviation.

2.5 Chromatographic Method

2.5.1 Apparatus and chemicals

2.5.1.1 Apparatus

(a) High performance liquid chromatographic system, Waters, U.S.A., consisting of:

- (1) Autosampler, Waters 717
- (2) System Controller, Waters 600E and Fluid Handling Units connection
- (3) 10 μm μBondapak C_{18} Column (300 x 3.9 mm)
- (4) Bondclone 10, C_{18} (300 x 3.9 mm)
- (5) Spherisorb ODS-2, 5 micron (4.6 x 250 mm)
- (6) Guard-Pak HPLC Precolumn
- (7) Tunable UV-Visible Absorbance Detector, Water 486
- (8) Chromatography Workstation, Maxima 820, NEC, Power Mate SX/16 and Printer, NEC Pinwriter P6200

(b) GC-MS system

- (1) DB1 column, 30 m, 0.25 mm i.d., film thickness 0.25 micron
- (2) GC-MS, Shimadzu QP2000A, Japan

(c) Air pump, , Waters, U.S.A.

(d) Filter unit, 0.2 μm Polyamid, Sartorious, Germany

(e) Ultrasonicator, model 2200, Branson, U.S.A.

(f) Extraction glass columns capacity of 8 ml

(g) Frits (PTFE) for 8 ml glass columns, Merck, Germany

(h) SPE Manifold, Merck, Germany

(i) pH meter, Cole-Parmer chemcadet 5986-25 pH/mv meter, Germany

(j) Rotary Evaporator, model R-114, Buchi, Switzerland

(k) UV-VIS Spectrophotometer, Shimadzu UV-1604, Japan

2.5.1.2 Chemicals

(a) Acetronitrile, A.R. grade, Riedel-de Haen, Germany

(b) Methanol, HPLC grade, J.T. Baker, U.S.A.

(c) Phosphoric acid, 85%, A.R. grade, Carlo Erba, Italy

(d) Ammonium acetate, 97%, Lab grade, Fluka, Switzerland

(e) Sodium hydroxide, A.R. grade, Merck, Germany

(f) Sodium chloride, A.R. grade, Merck, Germany

(g) Octadecyl(C_{18}), 40 μm Prep LC Packing, J.T. Baker, U.S.A.

(h) Phenol, general grade, BDH Chemicals, England

(i) 4-Nitrophenol, A.R. grade, Fluka, Switzerland

(j) 2-Chlorophenol, A.R. grade, Prolabo, Italy

(k) 2,4-Dimethylphenol, A.R. grade, Fluka, Switzerland

(l) 2,4-Dichlorophenol, A.R. grade, Fluka, Switzerland

(m) Pentachlorophenol, 99%, A.R. grade, Germany

(n) 2,4,5-Trichlorophenol, A.R. grade, Germany

(o) EPA Phenols mixture, 20 μg/L in methanol, ULTRA Scientific, 401-294-9400 (Lot No. E-0679), Germany

2.5.2 Preparation of Solutions

2.5.2.1 Reagent Preparation

(a) 0.0033 M (0.01 N)Phosphoric acid

0.0033 M Phosphoric acid was prepared by dissolving 0.3843 g, 85% phosphoric acid in 1 l of deionised water.

(b) 0.5% (v/v) Phosphoric acid in water

5 ml of 85% Phosphoric acid was dissolved by deionised water to 1 L volumetric flask. The solution was passed through 0.2 μm polyamid following with the degassing step by ultrasonic bath for 30 min.

(c) 0.5%(v/v) Phosphoric acid in methanol

Similarly to (b) but phosphoric acid in methanol was used instead of deionised water.

(d) 30 mM Ammonium acetate buffer solution

2.3124 g ammonium acetate was dissolved in 1 L of deionised water. Then, the buffer was adjusted to pH 5.0 by 85% phosphoric acid.

(e) 30 mM Ammonium acetate:ACN:methanol (56:34:10)(v/v)

30 mM Ammonium acetate, ACN and methanol were mixed in the ratio of 56:34:10 (v/v). After that it was filtrated and degassed for 30 min.

2.5.2.2 Phenols Standard Preparation

(a) Standard mixture of 11 priority substituted phenols solution

A stock standard of 11 phenols mixture, 20 mg/l, was successive diluted to the range of 250-1000 μg/l. Similarly, the standard phenols mixture in the range of 50-200 μg/l was also prepared because of a wide range of phenols concentrations found in water samples.

(b) Standard solution of individual phenolic compounds

0.01 g of each phenol was dissolved in mobile phase(according to system used) in individual 10 ml volumetric flask to obtain 1000 mg/l stock solution. Working standard solutions were prepared by successive dilution of each phenol stock solution as follows:

$$1000 \text{ mg/l} \longrightarrow 100 \text{ mg/l} \longrightarrow 10 \text{ mg/l}$$

(C) Standard mixture of 11 priority phenols and mixture of 6 phenols solution (1 mg/l)

1 ml of each phenol or standard mixture of 11 phenols at concentration of 10 mg/l was taken into 10 ml volumetric flask and adjusted to the mark with an appropriate mobile phase.

Note: All standards were kept at 4 °C in a refrigerator.

2.5.3 Optimization of HPLC System

The procedure has been adapted from the previous report.[34] This optimization was carried out to obtain an adequate separation and short analysis time for phenolic compounds determination employing an UV detector. Composition of mobile phase and flow rate were investigated.

2.5.3.1 Optimal Wavelength of 11 Priority Substituted Phenol

Absorption spectra (240-330 nm) of the following phenolic compounds: phenol, 4-NP, 2-CP, 2,4-DMP, 2,4-DCP, PCP and 2,4,5-TCP(to compare with 2,4,6-

TCP since no standard available) were recorded. The suitable analytical wavelength was considered from the absorption spectra.

2.5.3.2 Mobile Phase Optimization

(a) Gradient System

This investigation was carried out initially by injecting 6 phenols mixture of 1 mg/l onto the C_{18} Column under the gradient system. Various mobile phase compositions were employed with the program as shown in Table 2.2. The sequence of the phenolic compounds in the chromatograms were determined by running individual compounds.

Table 2.2: Gradient Program for Phenolic Compounds Separation

Time (min)	Mobile Phase		
	A	B	Curve@
Initial	60	40	*
10:00	60	40	6
18:00	20	80	6
22:00	5	95	6
25:00	5	95	6
30:00	60	40	6
35:00	60	40	11

Note: A = 0.5% Phosphoric acid in deionised water
B = 0.5% Phosphoric acid in methanol
@ = see Appendix 2.1[55]

(b) Isocratic System

The mobile phase used in this system was 30 mM ammonium acetate: ACN: methanol (54:34:10, v/v). Standard mixture of 11 phenolic compounds were injected to the system with Spherisorb ODS-2 column. Sequence of phenols in the chromatograms was checked by running the individual compounds. The others which no standard available in this laboratory were identified by using the literature.[34]

2.5.3.3 Summary of Optimized HPLC Conditions

The optimized HPLC condition established in this work are summarized in Table 2.3. Unless otherwise stated, the optimized conditions presented in Table 2.3 were used through out this work for determination of phenolic compounds in water samples.

Table 2.3: Optimized Isocratic HPLC Conditions for Analysis of Phenolic Compounds

Operation	Optimal condition
Stationary Phase	C_{18}
Mobile Phase	30mM CH_3COONH_4/ACN/MeOH (56:34:10, v/v)
Flow Rate	1.0 ml/min
Column Temperature	25°C
Wavelength	280 nm
Helium Sparge	20 ml/min
Injection Volume	20 μl
Detector Sensitivity	0.01 AUFS

2.5.3.4 Calibration Curve

The external standard method was constructed for determination of phenolic compounds in water sample. Calibration curves for each phenols at concentrations of an appropriate range were constructed by plotting the peak area which obtained from standard mixture chromatograms against concentrations. In this work, 2 ranges of calibration curve for the ranges of the phenols concentrations in water sample were constructed.

2.5.3.5 Detection Limit and Limit of Determinations

The detection limits were determined and averaged from 3 times injection of phenolic compound mixture at 0.250 mg/l, using as criterion signal of twice the background noise (nearby the peak), or cumulative of both noise signals besides peak of interest. The limit of determination was investigated by considering the enrichment factor from preparation step which was 250 times for this experiment.

2.3.3.6 Confirmation Analysis

Due to a low concentration of the interests and matrix effects of environmental samples, confirmation analysis was needed to identify each phenolic compound. In this study, most of the phenolic compounds were found in low concentrations. Three kinds of confirmation analysis were conducted.

(a) Spiking Technique

The spiking technique was employed to get the primary data for confirmation of the retention time. An appropriate amount of 11 standards mixture (20 mg/l) was spiked into the extracted sample (approx. 2 times for the expected compounds).

(b) Wavelength Ratio Technique

The 11 phenolic compounds standard mixture was injected onto HPLC column at three wavelengths (270, 280 and 295 nm). The standard ratio values were obtained by dividing peak height at different pair wavelengths ($\lambda_{295}/\lambda_{270}$, $\lambda_{295}/\lambda_{280}$ and $\lambda_{280}/\lambda_{270}$).

(C) Gas Chromatography-Mass Spectrophotometry (GC-MS) Analysis

The additional confirmation technique in this experiment was GC-MS for consideration of fragmentation pattern (m/z ratio) of a specific compound. The instrument conditions are summarized in Table 2.4.

Table 2.4: GC-MS Conditions Used in Confirmation Analysis

Instrument: GC-MS, shimadzu QP2000A
Column: DB1, 30 m, 0.25 mm i.d., film thickness 0.25 micron
Injector Temperature: 250°C
Column Temperature: $\underset{2\ \text{min}}{75^{\circ}\text{C}} \xrightarrow[15^{\circ}\text{C / min}]{} \underset{0\ \text{min}}{150^{\circ}\text{C}} \xrightarrow[8^{\circ}\text{C / min}]{} \underset{7\ \text{min}}{230^{\circ}\text{C}}$
Ion Source Temperature: 250°C
Helium Carrier Gas Pressure: 0.75 kg/cm^2
Injection Volume: 1 μl

2.5.4 Solid Phase Extraction (SPE) for Sample Preparation

1 g of 40 μm average particle size octadecyl (C_{18}) endcapped sorbent was packed in an 8 ml extraction glass column ended with polytetrafluoroethylene (PTFE) frit.(Figure 2.22) The water sample was filtrated and pretreated by dissolving 50 g sodium chloride in 250ml water sample, and adjusted to pH 2 with phosphoric acid. The column was first conditioned by 10 ml of ACN followed with 10 ml 0.01 N phosphoric acid. The column was taken care not to run dry during this step. Then the pretreated sample was forced through the conditioned SPE-column at the rate of 10 ml/min by using the tap water suction system. The SPE column containing the retained analyte was subsequently washed with 5 ml 0.01 N phosphoric acid before dried the column by passing nitrogen gas through the column (5 to 10 min). The phenolic compounds were finally eluted with 6 ml ACN. The extracted was

concentrated to about 1 ml (determining by weight) using the rotary evaporator. The sample was analyzed by HPLC (UV detection at 280 nm). The solid phase extraction process is summarized in Figure 2.23.

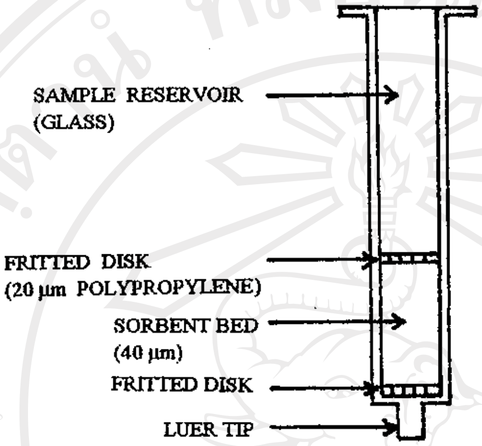


Figure 2.22: Disposable Extraction Column

2.5.4.1 Optimization of SPE System

The optimization of solid phase extraction was investigated in terms of percentage recovery of phenols by varying the amounts of octadecyl (C_{18}) including types and amounts of eluent applied.

The water samples were spiked with standard phenols mixture at known concentration. The spiked sample was extracted using solid phase extraction. Finally, the extract and phenol standard were injected onto HPLC column and calculated the recoveries.

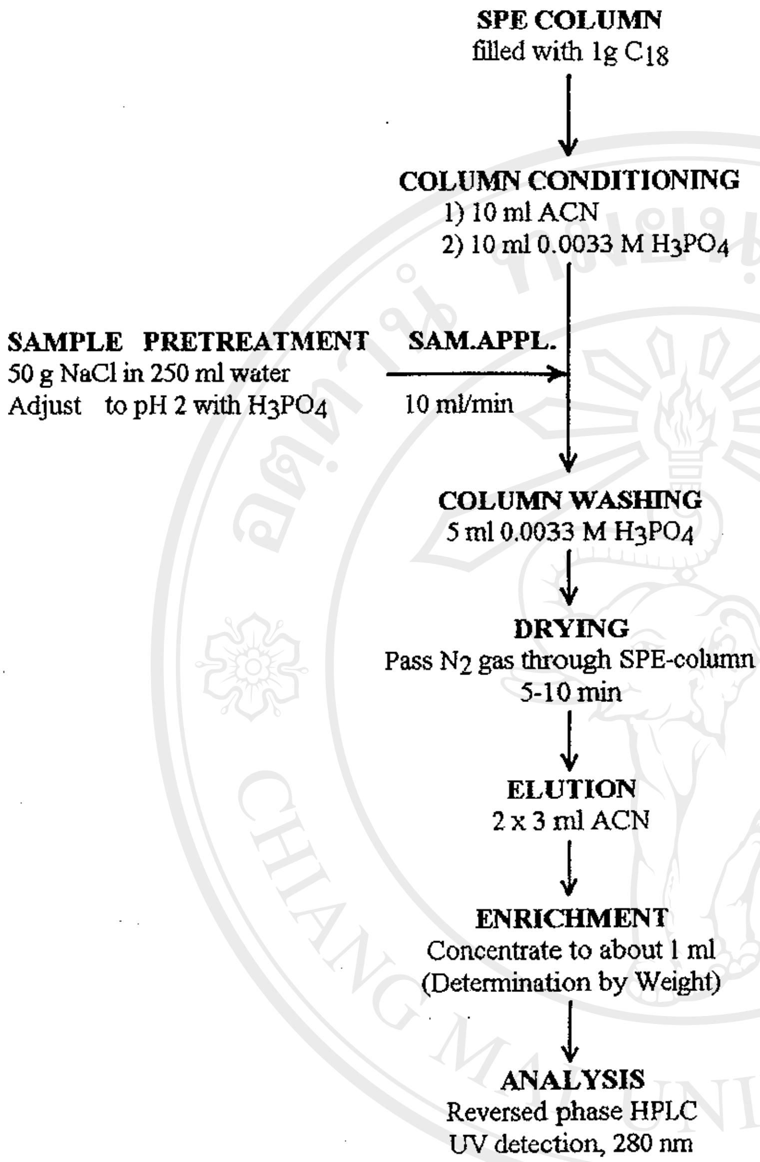


Figure 2.23: **Diagram of Solid Phase Extraction Process for Phenols Determination**