CHAPTER 3
RESULT AND DISCUSSION

3.1 Sample Preservation and Storage

In this work, the water sample was preserved with 2 ml phosphoric acid/ liter of water and stored immediately at 4°C in the field, this would bring pH of water to about 2. Stability of phenolic compounds in several waste water was studied[56] as shown in Figure 3.1.

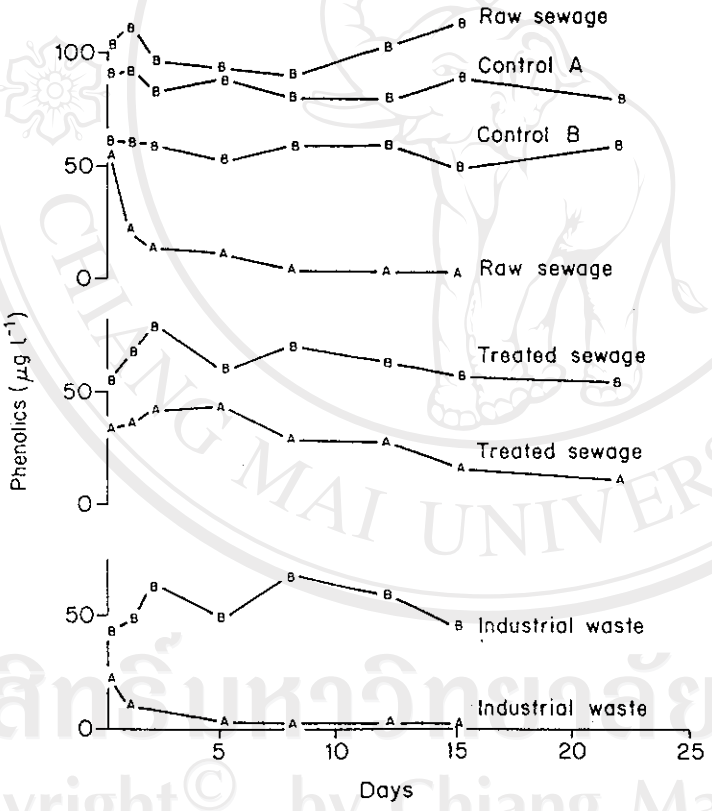


Figure 3.1: **Plot of Stability of Phenolic Compounds in Several Waste Waters with Time.**[56] All samples with points plotted as 'B' preserved with 1.0 g $CuSO_45H_2O$/l, pH brought to 4.0 with phosphoric acid, and then stored at 4°C. Samples plotted as 'A' stored at 4°C with no chemical preservatives. Both industrial waste, raw and treated sewage samples spiked with phenol to bring their initial concentrations to 50, 100, and 60 μg /l respectively.

The rapid loss of phenolic compounds from the sample at 4°C with no addition of any chemical preservative was evidenced. However loss of phenolic compounds occurred rapidly unless the preservative was added immediately after sampling. A correlation found between loss of phenolic compounds and microbial activity suggests that the latter is dominant in determining sample stability. The study[56] which can showed the microbiological effect through phenol degradation was shown in Figure 3.2.

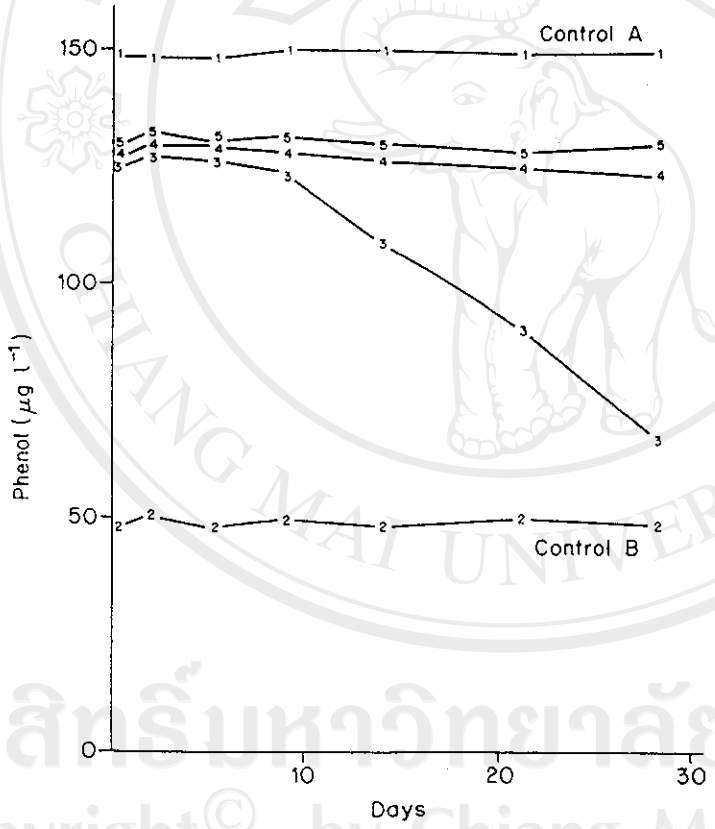


Figure 3.2: **Plot of Stability of Phenolic Compounds in Raw Sewage Sample Preserved with Several Concentrations of Sulfuric Acid.**[56] Aliquots 3 and 5 preserved with 2 ml conc. H_2SO_4/l and stored at 25°C and 4°C respectively. Aliquot 4 preserved with 4 ml conc. H_2SO_4 and stored at 25°C. All samples were spiked with phenol to bring there initial concentration to 130 μg /l.

From this investigation, it is excellent evidence that the enhanced stability of the samples preserved with higher acid concentration and that the greatest cause of sample instability is microbiological activity, not chemical activity.

3.2 Physico-Chemical Parameters

Physico-chemical parameters for each sampling time are shown in Tables 3.1-3.6.

Table 3.1: Physico-Chemical Parameters for Different Sites Collected on the 26th May 1994 (First screening)

Station	Odor	Color	Standing / Flowing	Secchi depth (cm)	Depth (cm)	pH	Conduc -tivity (µs/cm)
1.Mae Kuang Dam	no	greenish	F	115	115	7.1	140
2.MP-A (5 m)	no	turbid-brown	F	34	110	7.3	121
3.CT	bad	dark grey	F	26	32	7.0	280
4.RK	bad	dark grey	F	11	45	6.9	354
5.NW	no	turbid brown	F	37	127	7.5	116
6.WSK	no	turbid brown	F	43	164	7.5	116
7.Tantrapan	fishy	greenish	S	37	83	9.0	167
8. C.M.Ram Hospital	no	greenish	F-slow	47	146	9.1	164
9.Buak-Had	no	greenish	F-slow	39	146	9.0	141
10.Wang Bua-Ban	no	greenish	F	-	-	6.9	32.7

Table 3.2: Physico-Chemical Parameters for Different Sites Collected on the 27th June 1994 (Second screening)

Site	Odor	Color	Standing / Flowing	Secchi depth (cm)	Depth (cm)	Temp (°C)	DO (mg/l)	Sat. O_2 (%)	pH	Conduc -tivity (µs/cm)
1.Mae Kuang Dam	no	greenish	F	180	285	29.3	0.8	11	6.6	173
2.INM-B	no	yellowish br.	S	200	210	29.5	0.8	11	6.7	164
3.MP-A	no	brownish	F-fast	20	180	28.4	5.9	78	7.2	134
4.MK	bad	black	F	33	33	28.9	1.7	25	7.1	267
5.MP-B	no	brownish	F-fast	30	185	28.5	4.7	59	6.6	128
6.CT	bad	greyish-gr.	F	40	40	30.5	1.0	14	7.2	239
7.RK	bad	greyish	F-slow	35	65	30.7	0.5	7	7.2	308
8.NW	no	brownish	F	40	157	29.2	4.7	64	6.4	125
9.WSK	no	brownish	F	23	100	28.7	5.8	78	7.1	132
10.HR	no	greenish	F	28	63	28.9	4.7	63	7.1	132
11.KH	no	greenish	F	130	150	29.6	4.8	65	7.0	124
12.WW	no	greenish	F	40	40	29.6	3.3	45	7.0	176

Table 3.3: Physico-Chemical Parameters for Different Sites Collected on the 26th October 1994

Site	Odor	Color	Standing/ Flowing	Secchi depth (cm)	Depth (cm)	Temp (°C)	DO (mg/l)	Sat. O_2 (%)	pH	Conduc -tivity (µs/cm)
1. INM-B	no	greenish brown	F	58	264	25.1	6.5	81	7.8	184
2. INM	no	greenish brown	F-slow	47	170	24.6	5.3	67	7.7	200
3. INM-A	no	greenish brown	F	60	130	25.0	6.7	83	7.9	196
4. WSK	no	brown	F-fast	55	380	25.4	5.1	65	7.9	177
5. NW	no	brown	F-fast	31	164	25.7	5.5	68	7.8	176
6. MP-B	no	reddish brown	F-fast	61	300	25.0	5.4	71	7.8	176
7. MP-A	no	reddish brown	F-fast	40	250	24.6	5.4	75	7.8	180
8. RK	bad	greyish green	F-slow	48	109	28.1	0.1	3	7.3	528
9. CT	bad	greyish green	F	37	37	27.4	0.7	9	7.4	337
10. MK	bad	grey	F	46	55	25.0	0.8	10	7.5	358
11. HR	no	brownish	F-slow	50	50	25.6	5.3	66	7.8	-
12. SP	no	green	S	72	192	26.4	6.0	71	8.4	192
13. KH	no	greyish green	S	50	320	27.3	5.2	65	7.9	105
14. WW	no	brownish	F-slow	48	48	26.3	3.7	49	7.6	-
15.DS	no	clear	F	-	-	-	-	-	-	-

Table 3.4: Physico-Chemical Parameters for Different Sites Collected on the 24th November 1994

Site	Odor	Color	Standing/ Flowing	Secchi depth (cm)	Depth (cm)	Temp (°C)	DO (mg/l)	Sat. O_2 (%)	pH	Conduc -tivity (µs/cm)
1. INM-B	no	greenish brown	F-slow	55	250	25.5	7.0	75	6.1	239
2. INM	no	greenish brown	F-slow	52	255	25.7	7.3	91	6.7	244
3. INM-A	no	greenish brown	F-slow	70	113	26.1	6.3	75	7.0	255
4. WSK	no	brown	F-fast	32	294	27.4	-	-	7.0	205
5. NW	no	brown	F-fast	30	135	26.1	-	-	6.8	203
6. MP-B	no	brown	F-fast	20	247	25.7	10.7	136	7.3	206
7. MP-A	no	brown	F-fast	35	125	24.6	7.8	96	7.2	201
8. RK	bad	greenish grey	F-slow	45	81	28.1	1.9	24	6.3	655
9. CT	bad	greenish grey	F	46	46	28.0	1.2	14	7.2	630
10. MK	bad	dark grey	F-slow	35	125	26.2	1.4	15	7.2	666
11. HR	no	greenish brown	F-slow	48	75	26.9	-	-	7.3	188
12. SP	no	green	S	56	182	28.7	-	-	8.8	210
13. KH	no	brownish green	F-slow	100	112	27.7	-	-	7.4	184
14. WW	no	turbid white	F-slow	37	38	28.5	-	-	7.7	368
15.DS	no	clear	F	-	-	-	-	-	-	-

Table 3.5: Physico-Chemical Parameters at Different Sites Collected on the 22nd December 1994

Site	Odor	Color	Standing/ Flowing	Secchi depth (cm)	Depth (cm)	Temp (°C)	DO (mg/l)	Sat. O_2 (%)	pH	Conduc -tivity (µs/cm)
1. INM-B	no	greenish	S	45	158	25.6	4.9	62	7.6	254
2. INM	no	brownish green	S	27	147	25.2	4.7	62	7.6	247
3. INM-A	no	greenish	S	47	80	25.4	4.0	48	7.2	258
4. WSK	no	brown	F	22	270	25.6	5.4	67	8.0	212
5. NW	no	brown	F	33	145	25.6	5.9	75	7.8	210
6. MP-B	no	brown	F	27	170	24.9	4.8	61	7.9	213
7. MP-A	no	brown	F	14	109	24.4	5.4	67	7.9	213
8. RK	bad	greenish grey	F-slow	17	30	27.3	1.5	19	7.4	686
9. CT	bad	greenish grey	F	24	45	27.2	1.0	13	7.5	650
10. MK	bad	dark grey	F	4	55	25.1	1.1	14	7.5	688
11. HR	no	greenish grey	F-slow	31	45	25.6	3.9	51	7.5	185
12. SP	no	green	S	19	210	27.5	13.5	175	9.3	257
13. KH	no	greenish	S	42	250	26.7	4.5	60	8.4	213
14. WW	no	greenish grey	F-slow	14	41	26.4	2.6	34	7.9	394
15.DS	no	clear	F	-	-	-	-	-	-	-

Table 3.6: Physico-Chemical Parameters for Different Sites Collected on the 18th January 1995

Site	Odor	Color	Standing/ Flowing	Secchi depth (cm)	Depth (cm)	Temp .(°C)	DO (mg/l)	Sat. O_2 (%)	pH	Conduc -tivity (µs/cm)
RK	bad	greenish grey	F-slow	44	84	22.8	2.7	33	7.5	338
CT	bad	greenish grey	F	45	54	23.1	2.1	26	7.4	361
MK	bad	dark grey	F	34	61	22.8	1.7	20	7.4	412
HR	no	greenish brown	F	47	59	21.1	6.5	77	8.1	174
WW	no	greenish brown	F-slow	51	58	23.5	5.2	63	7.7	236
DS	no	clear	F	-	-	19.4	7.5	94	8.1	32

3.3 Spectroscopic Method

The basic chromatogenic reaction for this standard method, spectrophotometry, in this experiment is shown in topic 1.3.1. Because phenolic type wastes usually contain a variety of phenols, it is not possible to duplicate a mixture of phenols to be used as a standard[54]. Phenol has been selected as a standard and any color produced by the reaction of other phenolic compounds was required as phenol. Consequently, the results are expressed as phenol contents.

It has been recommended[55] that the best spectrophotometric method for the determination of phenol in unchlorinated water samples was the 4-AAP method using conditions of pH 10 similar to this work. For chlorinated waste supplied the AAP method performed under condition of pH 7.9. Sample is extracted with diethylether then back extracted with sodium hydroxide from the extract, followed by color development in the aqueous phase after being adjusted to pH 7.9 with 4-AAP and potassium hexacyanoferrate. Although the methods are sensitive they cannot differentiate between substituted phenols which show less reactivity, and are used primarily for determining total phenol concentrations. Also, the color-forming reagents

do not react completely with most p-substituted phenols and thus the results represent minimum concentrations of phenolic compounds in the sample.[29, 54]

3.3.1 Calibration Curve

An example of a standard calibration curve for the determination of phenolic compounds by the spectrophotometric method is shown in Figure 3.3 (a).

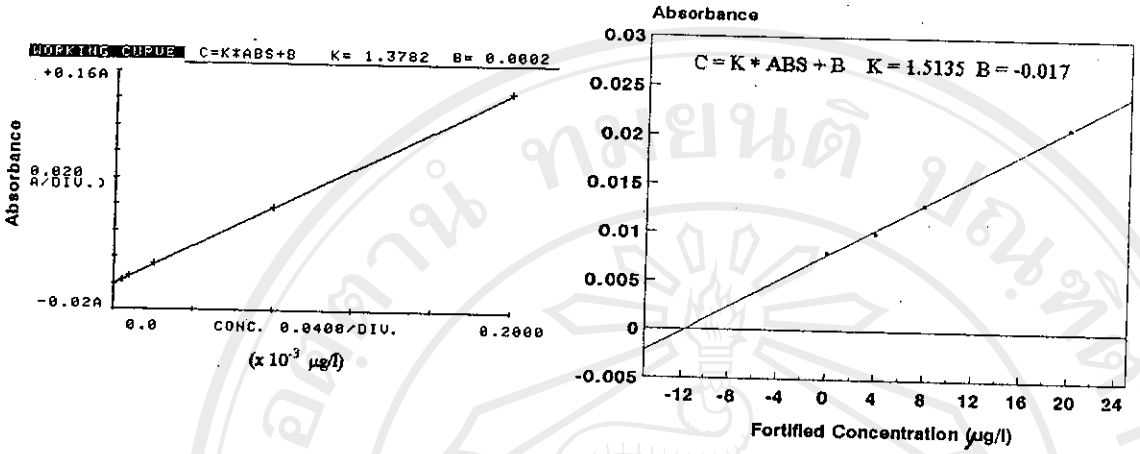
3.3.2 Recovery and Data Quality

Percent recoveries of fortified sample onto environmental sample are shown in Table 3.7. It was found that in the LFM method, the lower fortified concentration the higher deviation. The highest recovery was observed for the highest fortified concentration.

Table 3.7: %Recovery from Laboratory Fortified Sample Matrix (LFM) Method

Fortified Conc. (μg/l)	Recovery (%)		
	1st	2nd	Average
4	86.2	91.2	88.8
8	86.2	86.9	86.6
20	93.8	92.5	93.1

The calibration curve used and the curve of absorbance against the fortified concentrations are in Figure 3.3.



a) Calibration Curve

(b) Curve of Fortified Sample Series against Absorbance

Figure 3.3: (a) Calibration Curve and (b) Curve of Fortified Sample Series against Absorbance

Slopes of standard curve is a little bit less than the one of the fortified series (1.3782 and 1.5135 respectively). This might possibly be due to that the interfering compounds which could react as phenol causing the higher value. However the phenol concentrations found by the calibration curve and curve of fortified sample were close to each other, e.g. 10.8 and 11.7 µg/l.

3.3.3 Precision

Ten replicate determinations of a water sample were performed. The results obtained are in Table 3.8.

Table 3.8: Precision

Replication	Conc.(µg/l)
1	50
2	47
3	46
4	50
5	50
6	47
7	51
8	50
9	51
10	49

x = 49
SD = 1.8
%RSD = 3.7

Precision was found to be 3.7% RSD.

3.3.4 Stability Testing of Standard Series

Results of this investigation are shown in Table 3.9. The relationship between absorbance, concentration and time are illustrated in Figure 3.4.

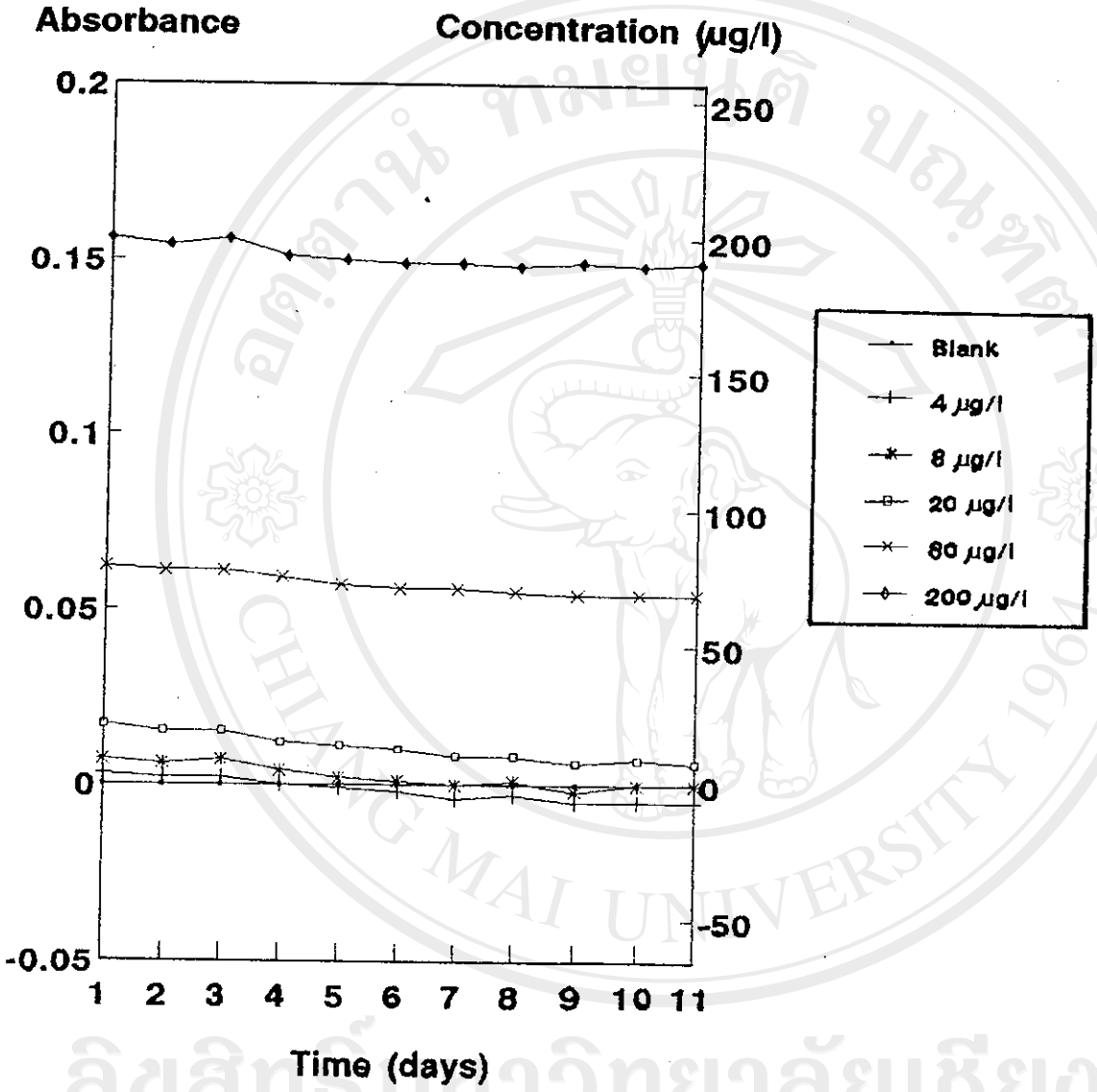


Figure 3.4: Effect of Standing Time on the Stability of Phenol and 4-AAP Product

Table 3.9: Absorbance/ Concentration (μg/l) of the Phenol-4-AAP with Time

Conc. (μg/l) / Day	0	4	8	20	80	200	Slope
1	0.000/-0.5	0.003/3.4	0.007//8.5	0.017/21.4	0.062/79	0.156/200	1.28
2	0.000/-0.5	0.002/2.4	0.006/7.5	0.015/18.4	0.061/78	0.154/197	1.30
3	0.000/0.6	0.002/2.2	0.007/8.6	0.015/18.2	0.061/78	0.156/200	1.28
4	0.000/2.8	0.000/-0.5	0.004/4.5	0.012/15.2	0.059/75	0.151/194	1.31
5	0.000/-0.3	-0.001/-2.0	0.002/2.5	0.011/13.5	0.057/73	0.150/193	1.31
6	0.000/-0.5	-0.002/-3.5	0.001/1.1	0.010/11.8	0.056/72	0.149/191	1.31
7	0.000/-0.2	-0.004/-0.6	0.000/-0.1	0.008/9.5	0.056/72	0.149/192	1.30
8	0.000/-0.3	-0.003/-4.8	0.001/1.2	0.008/10.3	0.055/70	0.148/190	1.32
9	0.000/-0.5	-0.005/-6.6	-0.002/-3.1	0.006/7.6	0.054/69	0.149/191	1.29
10	0.000/-0.5	-0005/-6.7	0.000/-0.5	0.007/8.6	0.054/69	0.148/190	1.31
11	0.000/-0.5	-0.005/-6.8	0.000/-0.8	0.006/8.6	0.054/69	0.149/191	1.30

A very small decrease on absorbances was observed but slopes of the re-construction of calibration curves were still close to each other (1.28-1.32). So the solutions can be used within 10 days if the re-construction of calibration curves are made every time of analysis. Also, the new blank should be prepared.

3.3.5 Phenol Contents of Water Samples Taken from Various Sites at Different Time of Collection

Using the condition of procedure already described in 2.3.3, water samples were analysed for phenol content (Table 3.10).

Table 3.10: Phenol Contents of Water Sample Taken from Various Sites at Different Time of Collection by Spectrophotometric Method

Site	Phenol Contents(μg/l)					
	26May94	27Jun94	26Oct94	24Nov94	22Dec94	18Jan95
1.INM-B	-	2.7	N.D.	2.1	-	-
2.INM	-	-	N.D.	2.0	2.0	-
3.Mae Kuang Dam	29.1	0.5	-	-	-	-
4.INM-A	-	-	2.3	7.0	3.4	-
5.WSK	0.4	6.5	N.D.	2.1	3.1	-
6.NW	21.5	9.3	5.4	2.5	1.9	-
7.MP-B	-	2.3	1.1	2.1	1.7	-
8.MP-A (5m)	22.8	-	-	-	-	-
9.MP-A (1km)	-	1.0	-	2.1	6.3	-
10.RK	36.4	19.1	19.5	20.1	29.7	9.3
11.CT	20.4	28.6	11.3	23.0	20.9	12.5
12.MK	-	14.8	5.1	20.0	36.4	14.7
13.Ram Hospital	1.6	-	-	-	-	-
14.HR	-	10.1	3.9	7.1	11.9	0.5
15.Mr.Donut	8.4	-	-	-	-	-
16.SP	-	-	3.8	10.2	5.3	-
17.KH	-	15.4	6.2	2.9	2.5	-
18.WW	-	-	7.4	13.5	8.5	5.8
19.Wang Bua Ban	4.1	4.1	-	-	-	-
20.DS	-	-	N.D.	1.3	1.1	N.D.

3.4 Flow Injection Analysis (FIA) Technique

3.4.1 Conditions of the FIA System Used

The following conditions were studied.

3.4.1.1 Sample Loop, Flow Rate, and Reaction Column

Calibration curves for phenol standard in the range of 0-100 μg/l were investigated by using different conditions namely sample volumes, reactor units and flow rate. The results are presented in Figure 3.5. Slopes of the calibration curves are summarized in Table 3.11 and Figure 3.6. Effects of flow rates are summarized in Figure 3.7 and Table 3.11.

It can be seen that the bigger sample volume could reach the lower detection limit regarding to the low phenol concentrations found in water samples. In addition there are no peak broadening found in the biggest sample volume (loop 2). Consequently loop 2 (565μl) was selected. By comparison of reaction coil with glass bead column (see Table 3.11, Figure 3.6-3.7), the higher peak height the less slope in glass bead system. Using glass bead column as the mixing part results less dispersion of sample zone in reagent current, causing higher efficiency for chemical reaction (no peak splitting). Residence time when using glass bead column would also be minimized.[5]

The conditions chosen for this work were the injection loop 2 (565 μl), glass bead and pump speed of 14. The flow rate investigation of carrier (He-degassing water), reagent (4-AAP) and buffer ($K_3Fe(CN)_6$) at pump speed of 14 were 1.83, 0.69 and 0.69 ml/min respectively as presented in Table 3.11.

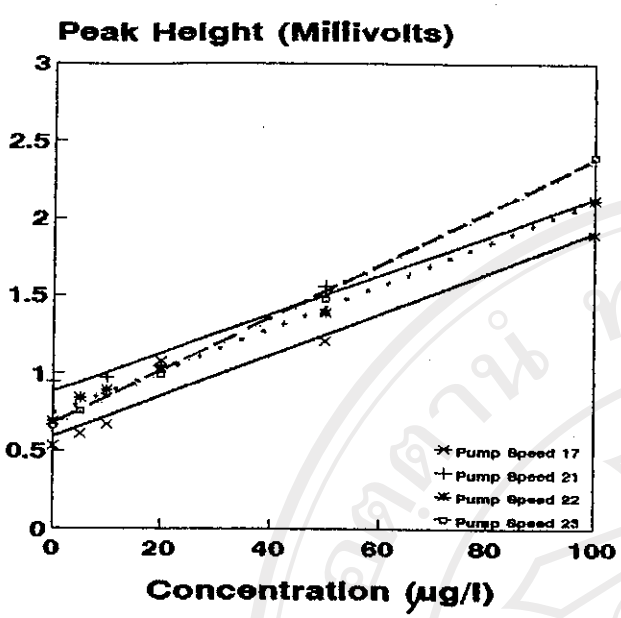
Table 3.11: Calibration Curves Equations and Peak Heights Obtained at Different Conditions

Condition		Peak Height of 100 µg/l (millivolts)	Calibration Curve Equation*
Sample Vol.(µl)	Pump Speed		
Reaction Coil			
20	17	1.896	y = 0.01322x + 0.58805
20	21	2.123	y = 0.01253x + 0.87957
20	22	2.128	y = 0.01379x + 0.73763
20	23	2.040	y = 0.01716x + 0.66408
175	20	4.019	y = 0.04534x - 0.46717
175	22	3.959	y = 0.04159x - 0.20335
175	23	4.171	y = 0.04845x - 0.67392
175	25	3.789	y = 0.04332x + 0.04332
175	28	4.034	y = 0.04778x - 0.74426
175	30	4.291	y = 0.04376x - 0.10769
565	13	6.892	y = 0.10840x - 1.67284
565	14	8.104	y = 0.11219x - 1.59124
565	15	10.646	y = 0.11516x - 1.04207
565	17	10.646	y = 0.11590x - 0 .87955
Glass Bead Column			
565	14	9.471	y = 0.10558x - 1.09980
565	22	9.889	y = 0.11348x - 1.50693

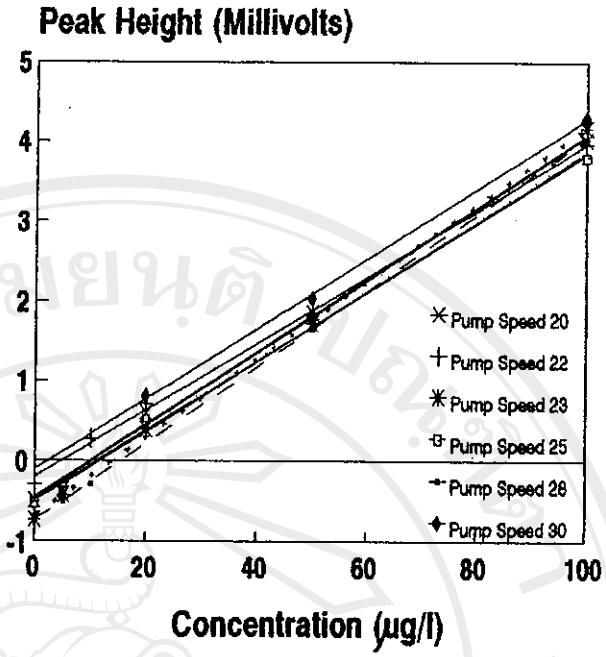
* Calculated by using Lotus 123 package program (PC)

y = peak height (millivolts)

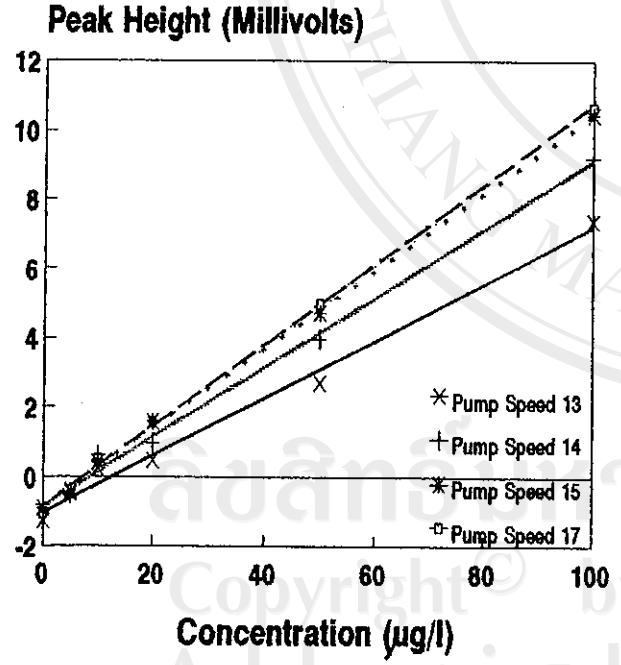
x = concentration (µg/l)



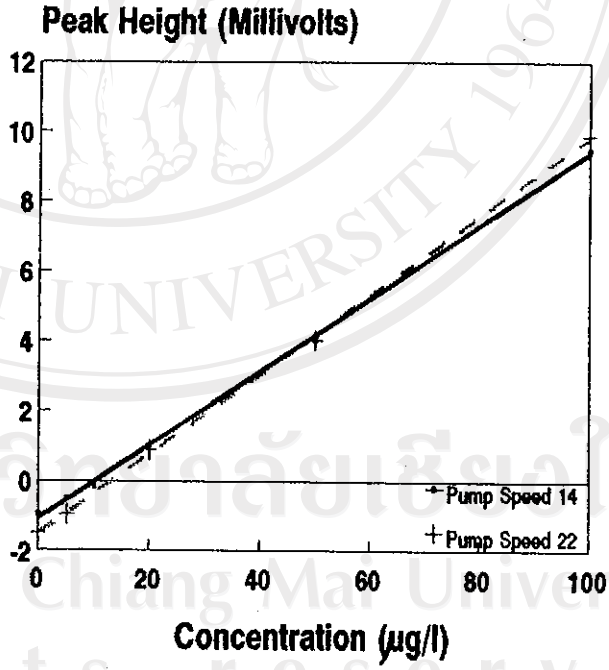
(a) Microloop/Coil



(b) Loop1/Coil



(c) Loop2/Coil



(d) Loop2/Bead

Figure 3.5: Comparisons of Calibration Curves at Different Systems Using FIA Technique

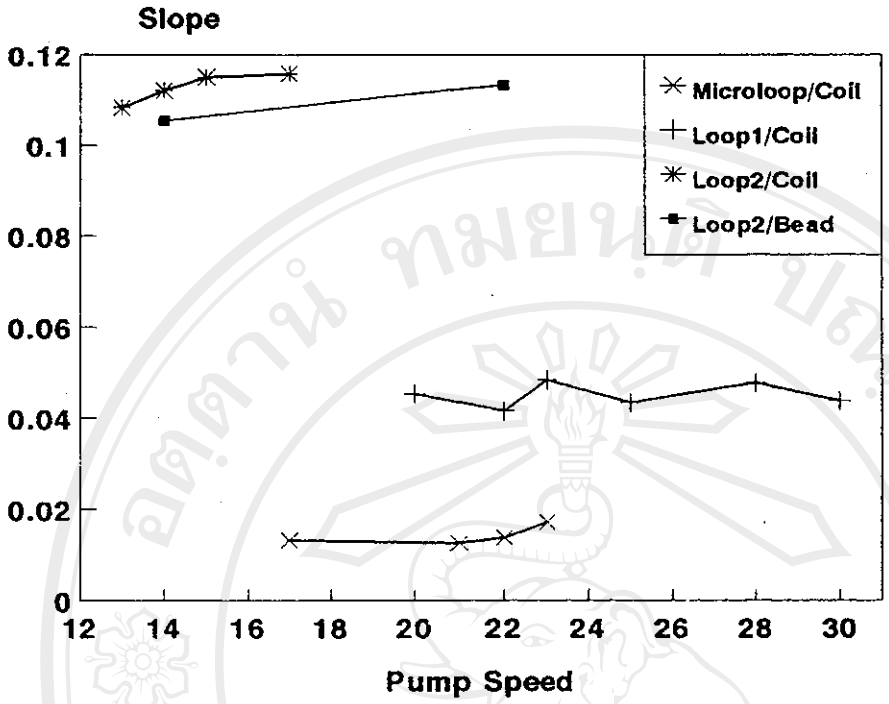


Figure 3.6: Effect of Flow Rates on Slopes of Calibration Curves

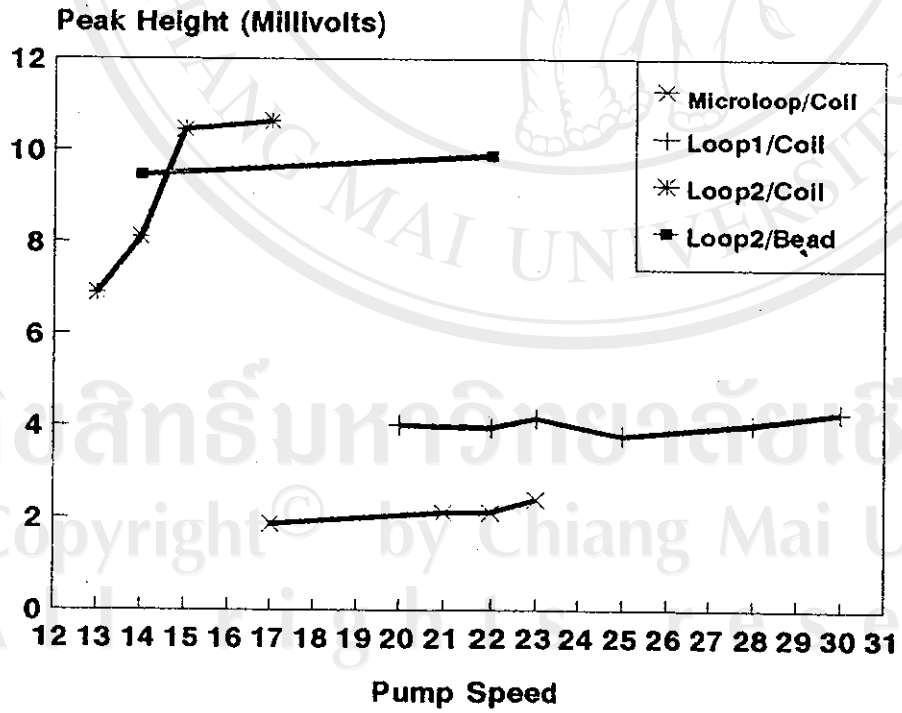


Figure 3.7: Effect of Flow Rates on Peak Heights

Table 3.12: Flow Rate of Carrier, Reagent and Buffer Solutions at Pump Speed of 14

Solution	Time (second)*				Flow Rate (ml/min)
	1st	2nd	3rd	average	
Carrier	65.43	65.69	65.71	65.61	1.83
Reagent	173.63	174.72	173.60	173.98	0.69
Buffer	172.64	174.66	172.80	173.37	0.69

Note: *Time recorded for 2 ml solution passing through the system

3.4.1.2 Wavelengths

The calibration curve of 2 wavelengths (480,520 nm) employed were compared in Figure 3.8. Measurement of colored products at 520 nm is more sensitive.

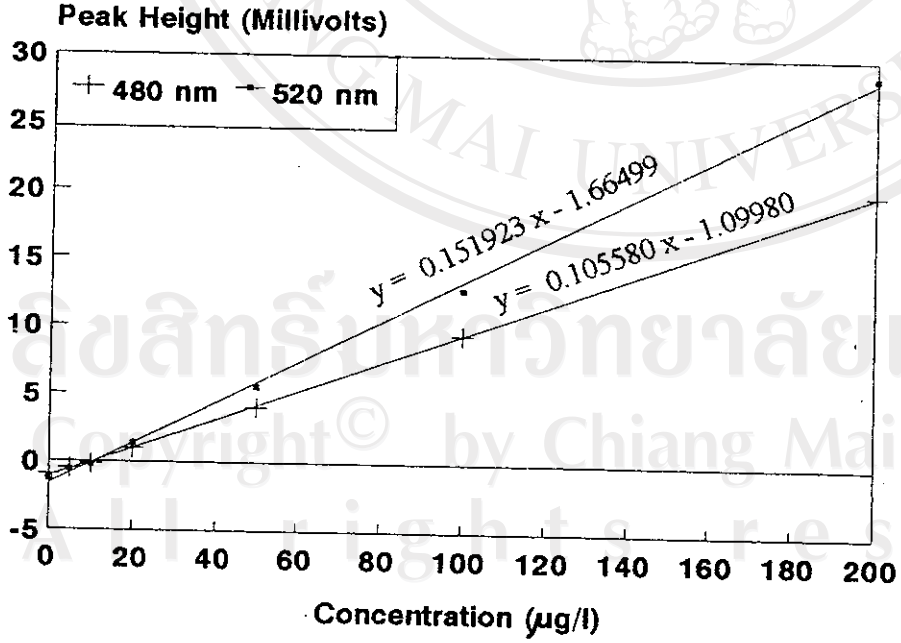


Figure 3.8: Calibration Curve Using the Wavelength at 480 and 520 nm

3.4.2 Calibration Curve

The FIAgram of phenol standards for calibration curve, 0-200 μg/l at loop2/ bead/ pump speed 14 at 480 nm is illustrated in Figure 3.9. The calibration curve is presented in Figure 3.10.

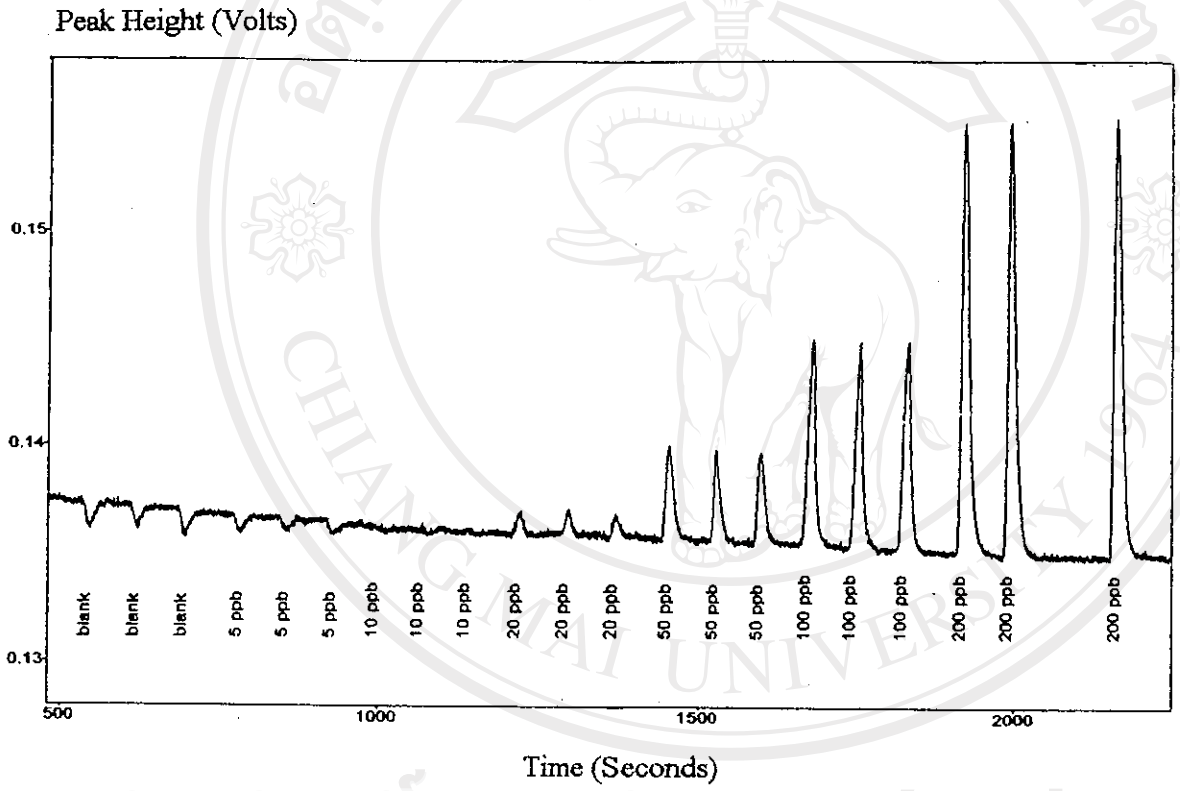


Figure 3.9: FIAgram of Standard Phenol (Triplicate Injections)

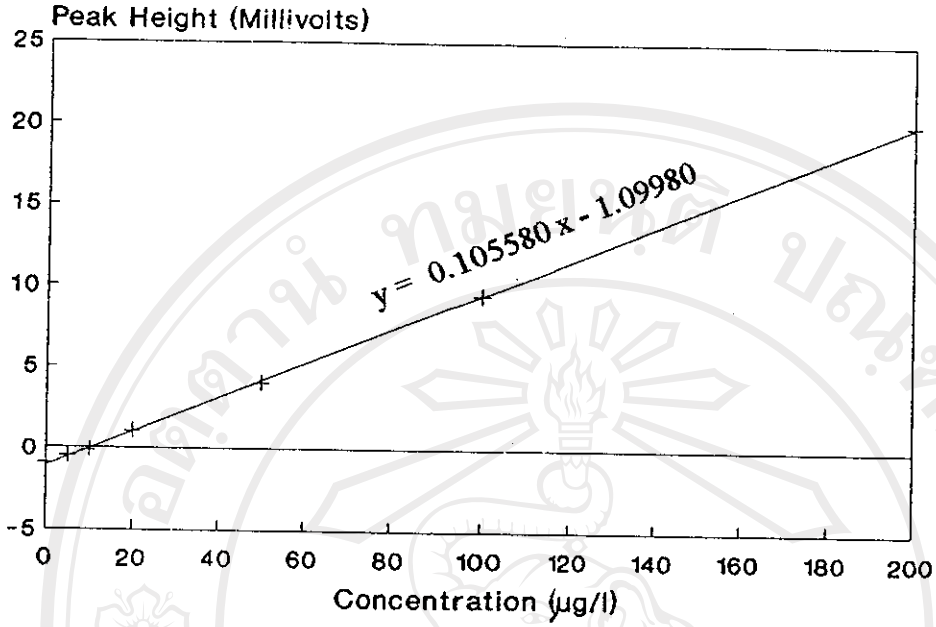


Figure 3.10: Calibration Curve for Phenol Determination Using Loop2 (565 µl)/ Glass Bead/ Pump Speed 14 at 480 nm

3.4.3 Precision Checking

FIAgram of 12 replicate of injections of water sample(pH4) at Chao Tung site is depicted in Figure 3.11. Peak height and concentration of each injection were shown in Table 3.13. Precision was found to be 1.31% RSD.

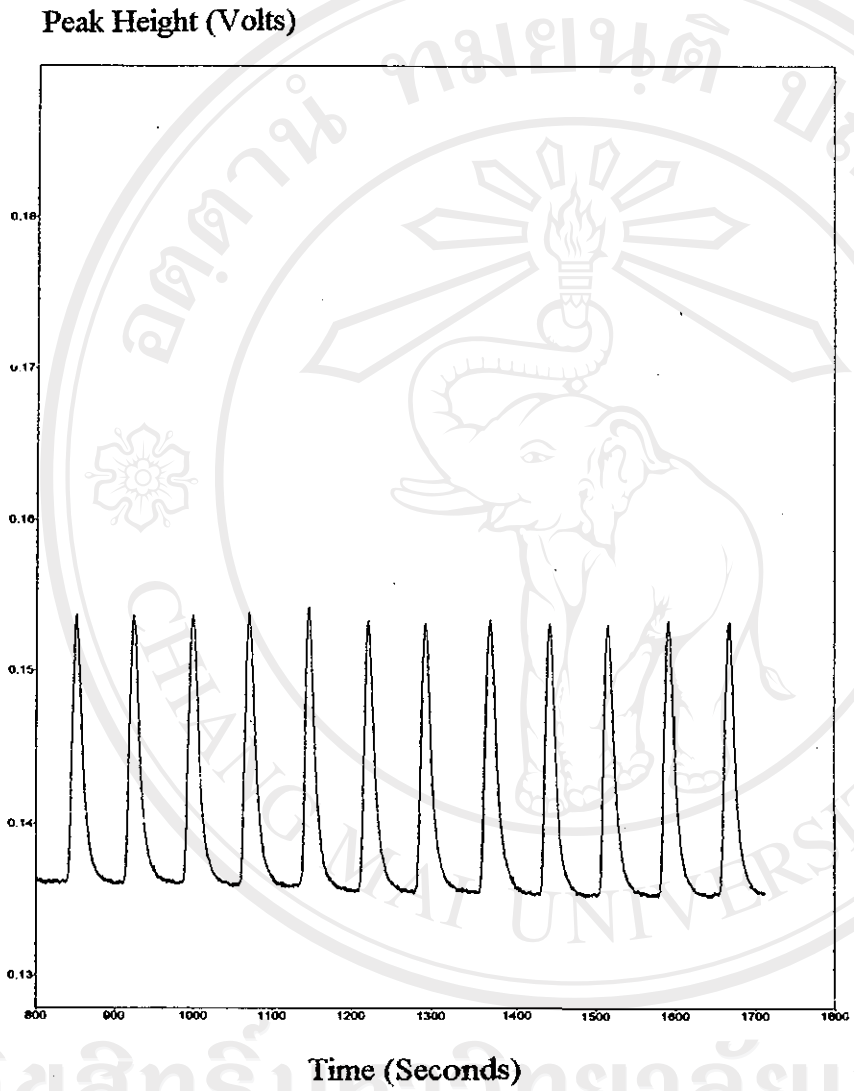


Figure 3.11: FIAgram of 12 Replicate Injections of Water Sample (pH 4) at Chao Tung Site

Table 3.13: Phenol Contents of 12 Replicate Injections of Water Samples at Chao Tung Site

No.	Peak Height (millivolts)	Phenol Content (µg/l)
1	172.29	175
2	174.63	177
3	174.23	176
4	175.14	177
5	180.59	182
6	174.31	176
7	174.98	177
8	178.76	181
9	174.09	176
10	176.80	179
11	178.64	181
12	177.03	179

$\bar{x} = 178$
SD = 2.3355
%RSD = 1.31

3.4.4 Phenol Contents in Water Sample

FIAgram of triplicate injections of each water sample collected on January 1995, at pH 4 and pH 5, were attached as Figure 3.12. Phenol contents of the water samples each sites are shown in Table 3.14.

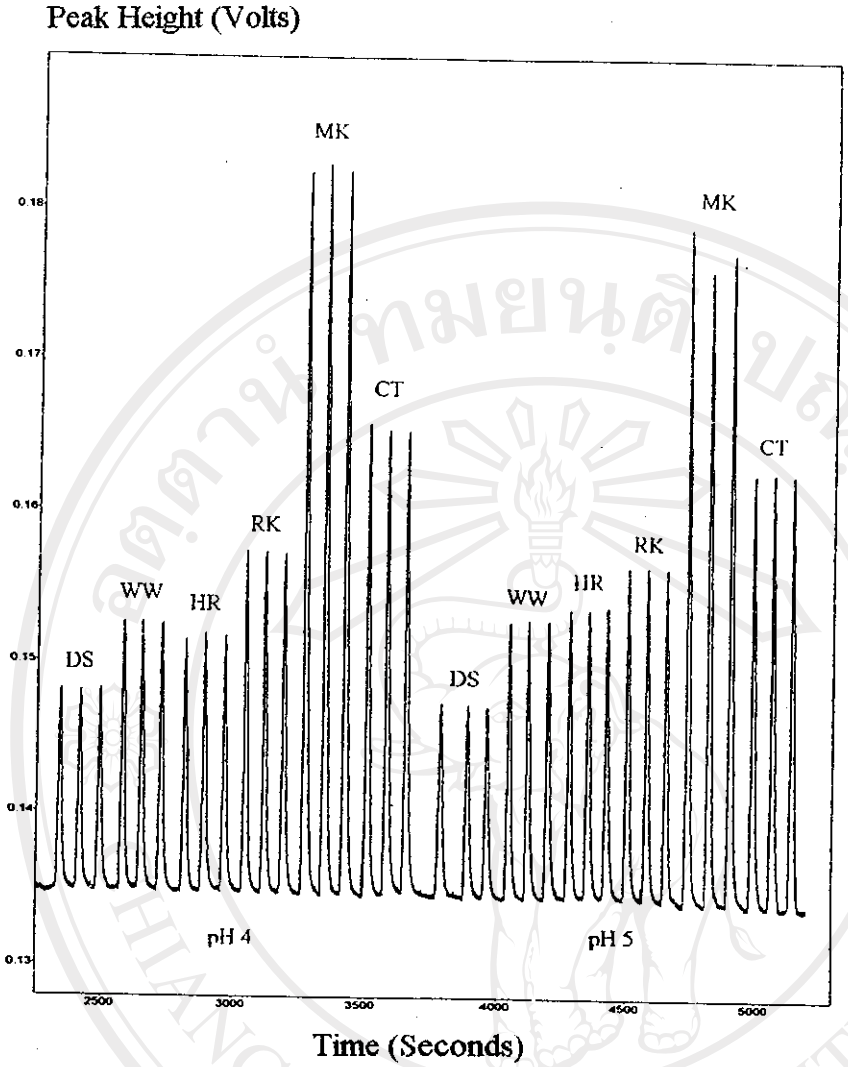


Figure 3.12: FIAgram of Phenol in Water Sample at pH 4 and pH 5.

Table 3.14: Phenol Contents Found in Water Samples of Various Sites at pH 4 and pH 5

Site	Phenol Content (μg/l)*	
	pH 4	pH 5
RK	219	215
CT	289	280
MK	459	413
HR	165	189
WW	174	186
DS	133	128

* Averaged of triplicate determinations

Highest difference in phenol concentrations obtained at pH 4 and pH 5 was noted in the sample from Mae Kha site. The possible reason might be that at the Mae Kha site, biological activity might cause more phenol degradation[56] at pH 5 than at pH 4(the more acidic, the less biological activity). In addition the time after adjusted pH until analysis time was quite long, around 3 hrs, affected significantly concentration change in the sites which have high biological activity, Mae Kha. Mae Kha is well known as domestic wastewater collection canal in the city center[21, 22]where presumably more biological activities should taken place than other sites.

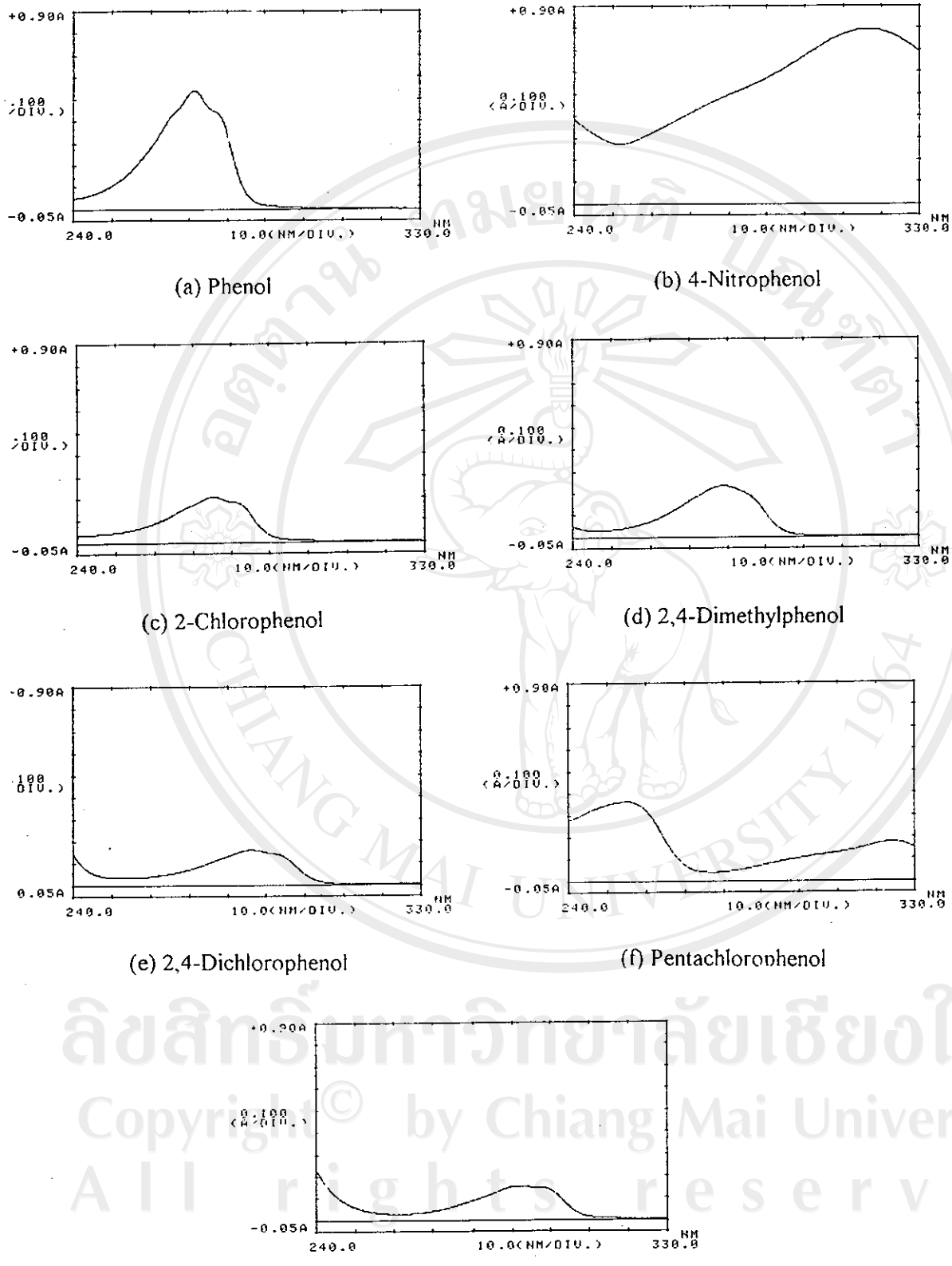
3.5 HPLC

3.5.1 Optimization of the HPLC System

3.5.1.1 Optimal Wavelengths for the Determination of 11 Priority Phenols Mixture Using UV-VIS Spectrophotometer as Detector

Various substituted phenols are pollutants of interests particularly in water. Maximum absorption wavelengths of those compounds may not be the same. Therefore the analytical wavelength chosen for all 11 phenolic compounds analysis, should be suitable (sensitive enough) to those compounds of interest, although this wavelength may not be the most sensitive for every compound.

UV spectra (240-330 nm) of 7 individual phenolic compounds are illustrated in Figure 3.13. The optimal wavelength was chosen at 280 nm where absorptivities of compounds were reasonably high.



(a) Phenol

(b) 4-Nitrophenol

(c) 2-Chlorophenol

(d) 2,4-Dimethylphenol

(e) 2,4-Dichlorophenol

(f) Pentachlorophenol

(g) 2,4,5-Trichlorophenol

Figure 3.13: UV-VIS Spectra of 7 Individual Phenolic Compounds at 240-330 nm

3.5.1.2 Chromatographic Systems and Mobile Phase Optimization

(1) Gradient System

Solvent gradients were used in order to decrease analysis time and optimize separation with respect to time and resolution. Shape of the eluting peaks can be much improved, thus yielding the better detectability. Different mobile phases: solvent A, 0.5% H_3PO_4 acid in water: solvent B, 0.5% H_3PO_4 acid in methanol were tried. At first the gradient was first started from 60%B to reach 95%B in 10 min[5]. In optimizing a gradient system, it was worked systematically from the front of the chromatogram to the back. This was because what happens in the beginning may have a corresponding influence on later peaks.[36] Separation of 6 phenolic compounds under the gradient system for C_{18} column which was programmed for mobile phase composition as mentioned in Table 2.2 is illustrated in Figure 3.14.

With this gradient program the mixture of phenolic compounds could be completely separated. However the baseline stability was difficult to obtain, even for the standards, this effect will be more pronounced in the real sample determination. The isocratic system was later applied to get over this problem.

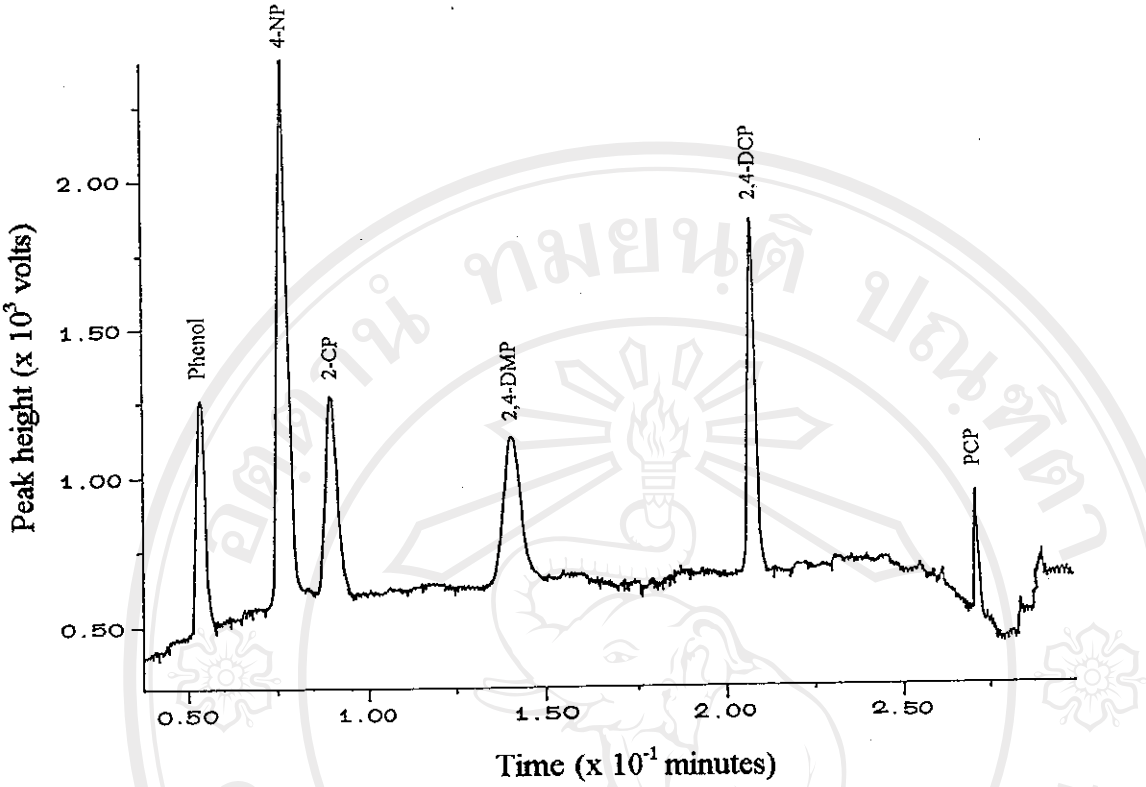


Figure 3.14: Separation of 6 Phenolic Compounds C_{18} Column Under the Gradient System

(2) Isocratic System

The column for isocratic system employed was Spherisorb ODS-2 under the conditions summarized in Table 2.3. An aliquot (20 μl) of 11 phenolic compounds mixture standard was injected onto this column. The separation of the mixture compounds is illustrated in Figure 3.15.

An isocratic system showed completely separation of all 11 phenolic compounds. Baseline obtained in isocratic system was quite stable. Thus should led to the better sensitivity for the low contents. Therefore isocratic system was chosen for the determination of phenolic compounds through out this work.

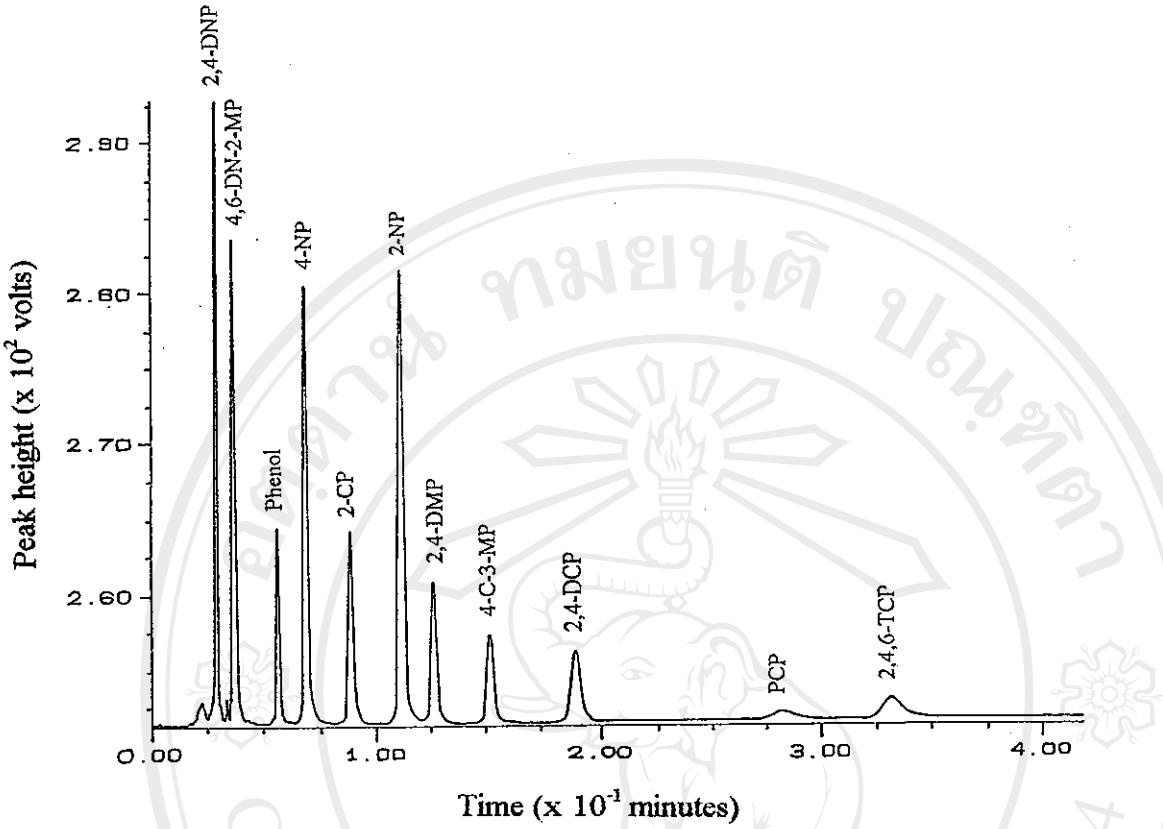


Figure 3.15: Separation of 11 Phenolic Compounds Mixture Under the Isocratic System Using Spherisorb ODS-2 Column

The elution orders of phenolic compounds (concerning t_R for single standard) via those columns employed showed the same sequence for the available standards checking. Apart from this considering, in case of 11 phenols mixture, the chromatogram was compared with Alarcon *et al.*'s study[34] which was similar in elution order of available standards, consequently the elution order for the others were made. Due to the systems employed were reverse phase which had the nonpolar sorbent and polar eluent, the elution depends on the polarity of the compounds of interest. The higher polar would come out first. Peak intensity of each phenolic compounds at one concentration in chromatogram was respected to its molar absorptivity and solvent used.(see Appendix 3.1)

3.4.1.3 Calibration Curve

Due to wide ranges of phenolic compound found in the sample, 2 ranges of calibration curves, 0.25-1 mg/l and 20-200 µg/l were conducted to cover the concentration in water sample as shown in Table 3.15. Calibration curve of phenol in the range of 0.25-1 mg/l is illustrated in Figure 3.16.

Table 3.15: Calibration Curve Equations of the 11 Phenolic Compounds in the Mixture Standard

Component	Equation* C(mg/l) = (slope) R + constant	Correlation Coefficient (r^2)
2,4-DNP	3.873E-5(R) - 2.001E-2	0.9949
4,6-DN-2-MP	2.982E-5(R) + 2.387E-2	0.9992
Phenol	6.914E-5(R) + 4.597E-2	0.9978
4-NP	2.467E-5(R) + 4.000E-3	0.9976
2-CP	4.984E-5(R) + 1.342E-2	0.9962
2-NP	1.745E-5(R) + 8.373E-3	0.9988
2,4-DMP	5.483E-5(R) + 3.386E-3	0.9969
4-C-3-MP	7.118E-5(R) + 6.171E-3	0.9973
2,4-DCP	7.103E-5(R) - 2.465E-3	0.9979
PCP	1.921E-4(R) + 8.637E-2	0.9978
2,4,6-TCP	1.250E-4(R) - 1.790E-2	0.9940
phenol*	7.980E-5(R) - 2.1375E-2	0.9934
2-NP*	2.0559E-5(R) -1.4887E-3	0.9985

Note: * Equations obtained from Maxima 820 (chromatography workstation), and averaged from 3 injections of 11 mixture standard in the range of 0.25-1 mg/l.

** Calibration curve was constructed in the range of 0.0125-0.250 mg/l

R = Response (peak area)

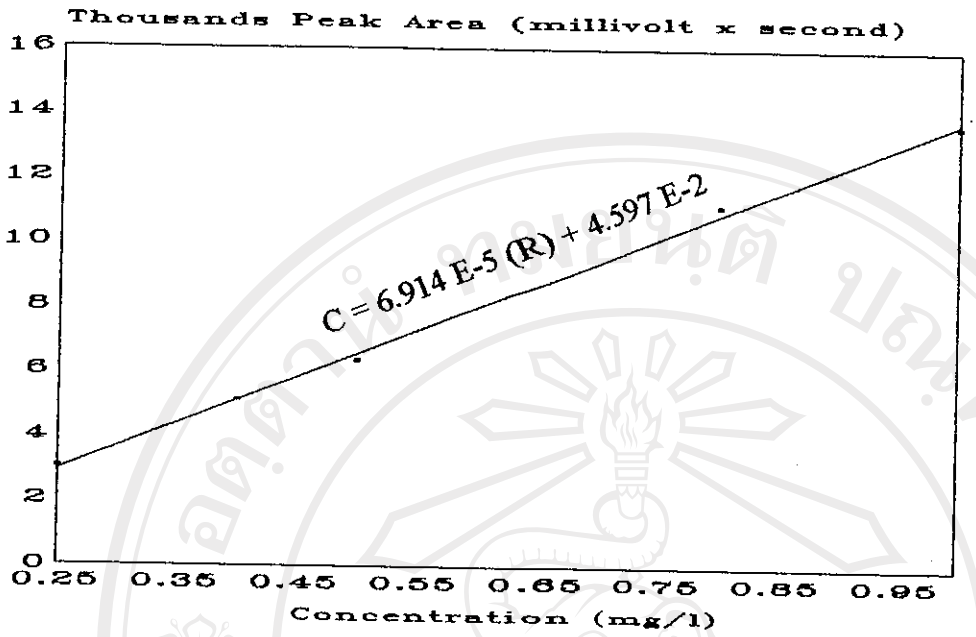


Figure 3.16: Calibration Curve of Phenol in the range of 0.25-1 mg/l

3.5.3 Detection Limit and Limit of Determination

Figure 3.18 depicts a chromatogram for the investigation of detection limit. Limit of determination was calculated by concerning the enrichment factor being 250. Table 3.16 summarizes the results of the investigation.

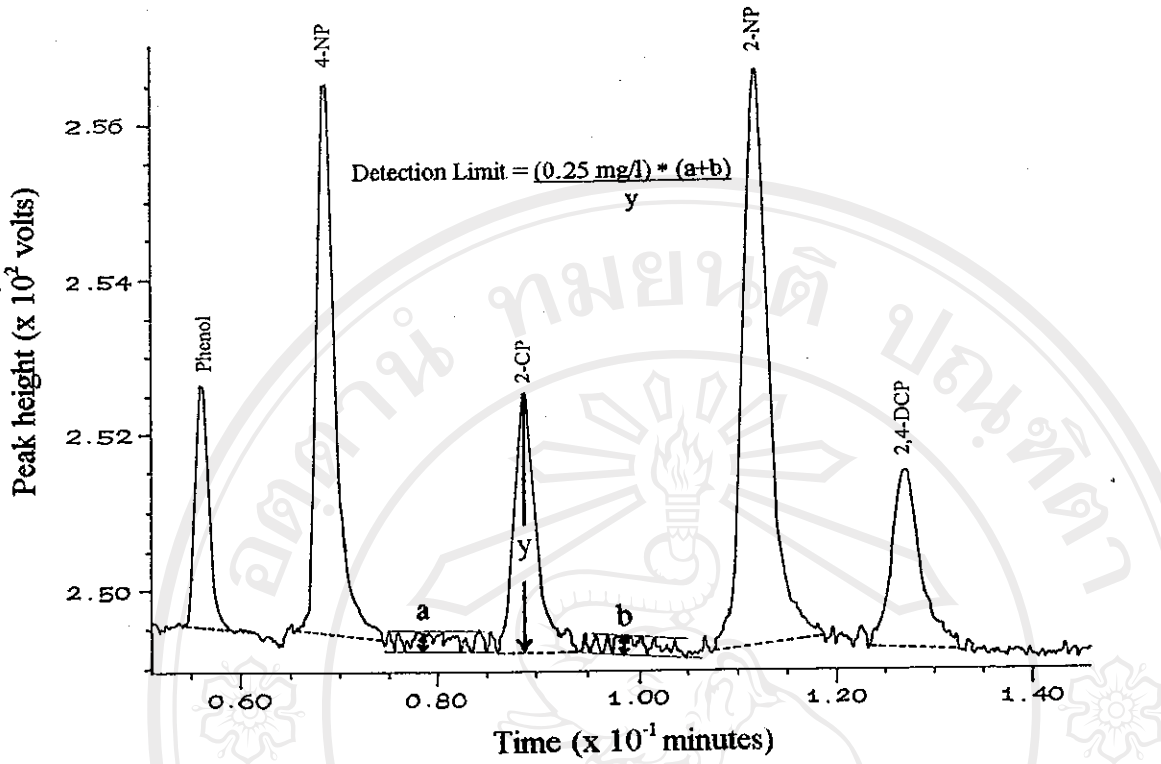


Figure 3.17: Chromatogram for the Detection Limit Determination Employing 0.25 mg/l of Standard Mixture

Table 3.16: Detection Limit and Limit of Determination of 11 Phenolic Compounds Mixture

Component	Detection Limit (µg/l)	Limit of Determination (ng/l)
2,4-DNP	4.4	17.6
4,6-DN-2-MP	5.0	20.0
Phenol	21.2	84.8
4-NP	13.4	53.6
2-CP	31.1	124.4
2-NP	13.3	53.2
2,4-DMP	40.3	161.2
4-C-3-MP	39.6	158.4
2,4-DCP	52.1	208.4
PCP	251.1	1004.4
2,4,6-TCP	149.1	596.4

Note: Calculated from 3 chromatograms of 11 mixture standard (0.25 mg/l.)

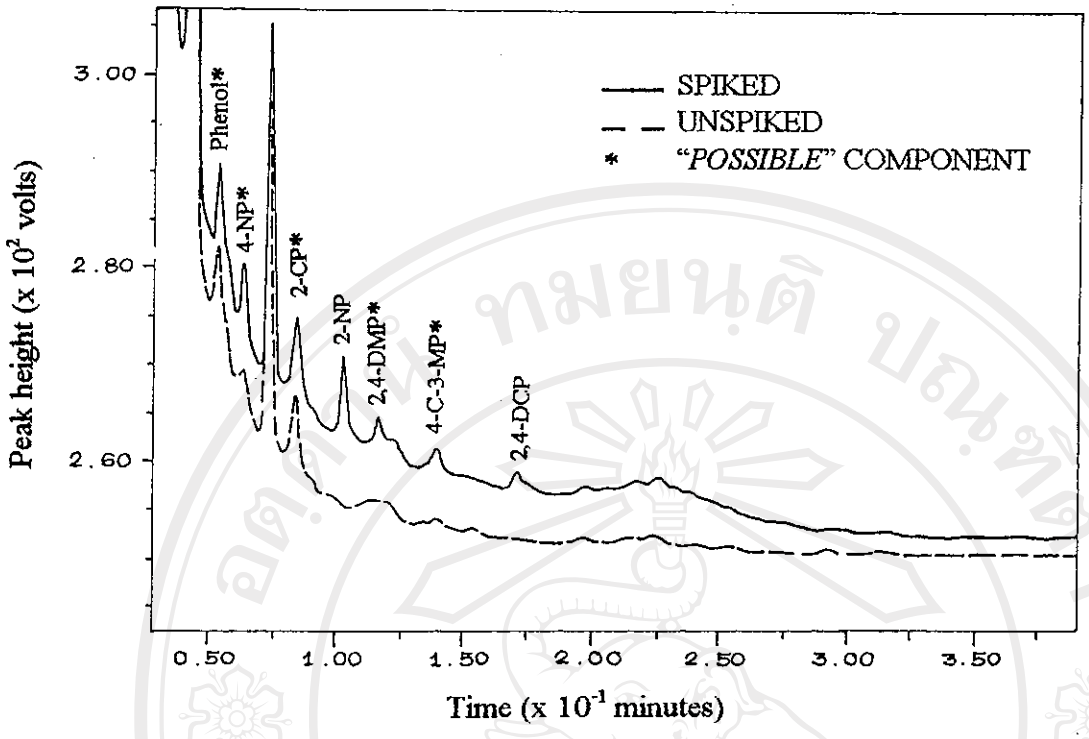
Phenolic compounds showed different sensitivity for this system resulting in different detection limits and different limit of determination. At the same concentration, the higher response the lower detection limit and the lower limit of determination. For this conditions, the highest sensitive compound (lowest detection limit) was 2,4-dinitrophenol, the lowest one was pentachlorophenol. The preparation step could help to detect the phenolic compounds at the low concentration in a water sample down to ng/l level. In this experiment the final concentration of phenolic compounds in water was 250 times higher than original one.

3.5.4 Confirmation Analysis

(a) Spiking Technique

This was undertaken for more information of peak identification since the retention time of interesting compounds sometimes were effected by matrix resulting in retention time shifting. Spiking of appropriate amounts of compounds of interest (approx. 2 times for the expected compounds) was conducted. An example of confirmation by spiking technique is depicted in Figure 3.18.

Considering the higher peak intensity of interesting peak, the information of peak position is cleared. As the result, phenol, 4-NP, 2,4-DMP and 4-C-3-MP were noted as "possible" components. In addition the interesting peaks were small and disturbing components could be found in environmental samples. More confirmation analysis is needed.



[Note: Spiked 0.125 μg of each compound in 0.5 ml extracted sample]

Figure 3.18: Chromatograms Showing the Confirmation by Spiking Technique of CT Site Collected on 18th January 1995

(b) Wavelength Ratio Technique

The responses in chromatograms (295, 280 and 270 nm) were compared to find the ratio of each corresponding peak obtained at the wavelengths of 295 and 270, and the ones of 295 and 280 and the ones of 280 and 270 nm. The ratio values are used as the criteria for confirmation analysis. Since each phenolic compound has its characteristic spectrum (see Figure 3.13), the selected wavelengths for this confirmation were chosen according to the sensitive wavelengths of three groups of compounds. These were at 270, 280 and 295 nm. Peak height was used to calculate the ratio due to the peak of interest in environmental samples were always small and sometimes were susceptible to have more than one component in one peak (overlapping). The ratio of peak height used for confirmation analysis is shown in

Table 3.17. The result of wavelength ratio confirmation for CT site (collected on 18th January, 1995) is summarized in Table 3.18.

Table 3.17: Ratios of Peak Heights at Different Pairs of Wavelengths

compound	Ratio of Peak Heights of		
	λ295/ λ270	λ295/ λ280	λ280/ λ270
2,4-DNP	0.42	0.67	0.63
4,6-DN-2-MP	0.56	0.76	0.75
Phenol	0	0	0.63
4-NP	2.83	1.85	1.53
2-CP	0.056	0.023	1.07
2-NP	0.67	0.66	1.01
2,4-DMP	0.24	0.17	1.43
4-C-3-MP	0.63	0.39	1.60
2,4-DCP	2.20	1.04	2.12
PCP	1.12	2.02	0.55
2,4,6-TCP	3.30	1.60	2.07

(c) Gas Chromatography-Mass Spectrometry (GC-MS) Technique

GC-MS was the technique chosen for this work for more confirmation analysis because of its sensitivity and its ability to identify compounds from their retention times and fragmentation patterns. GC-MS is the most reliable technique by considering the characteristic m/z patterns of the compound. Mass spectrometry is an effective means to determine an analyte component of interest in a high concentration matrix containing sample.[58]

11 Priority pollutant phenols (PPP) were injected onto DB1 column. The separation is shown in Figure 3.19. Chromatogram of CT site, collected on 18th January 1995, in GC-MS system is depicted in Figure 3.20. An example of mass spectrum for phenol (from GC-MS technique) is shown in Figure 3.21.

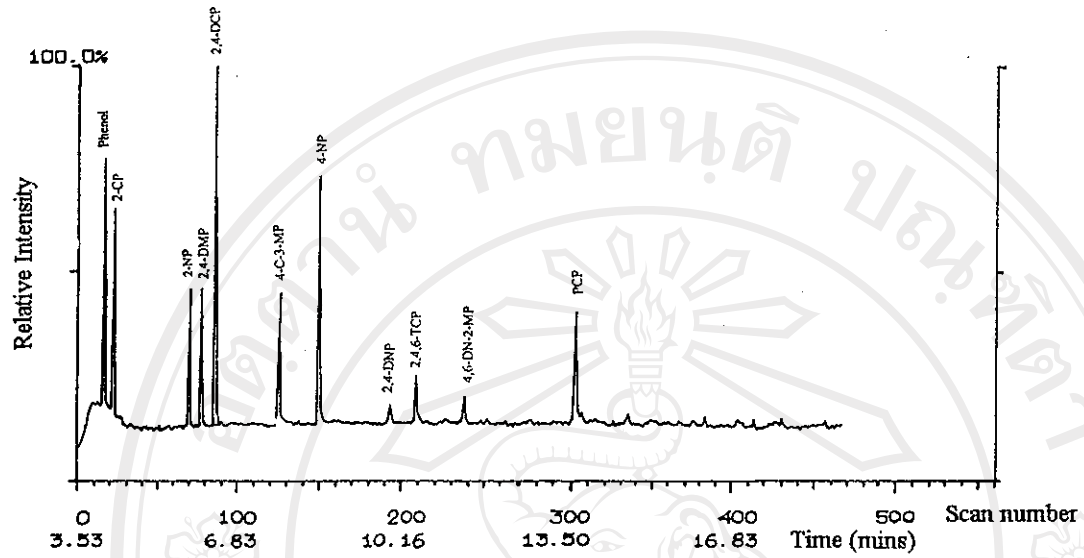


Figure 3.19: Chromatograms of 11 Priority Pollutant Phenols (PPP) (20 mg/l) by GC-MS Technique

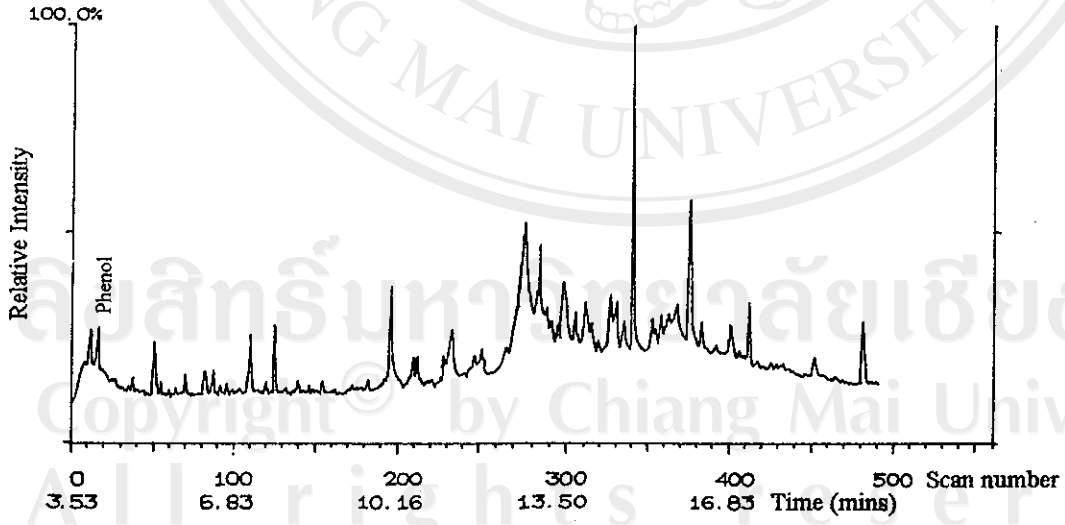


Figure 3.20: Chromatograms from GC-MS Technique for CT Site Collected on 18th January, 1995

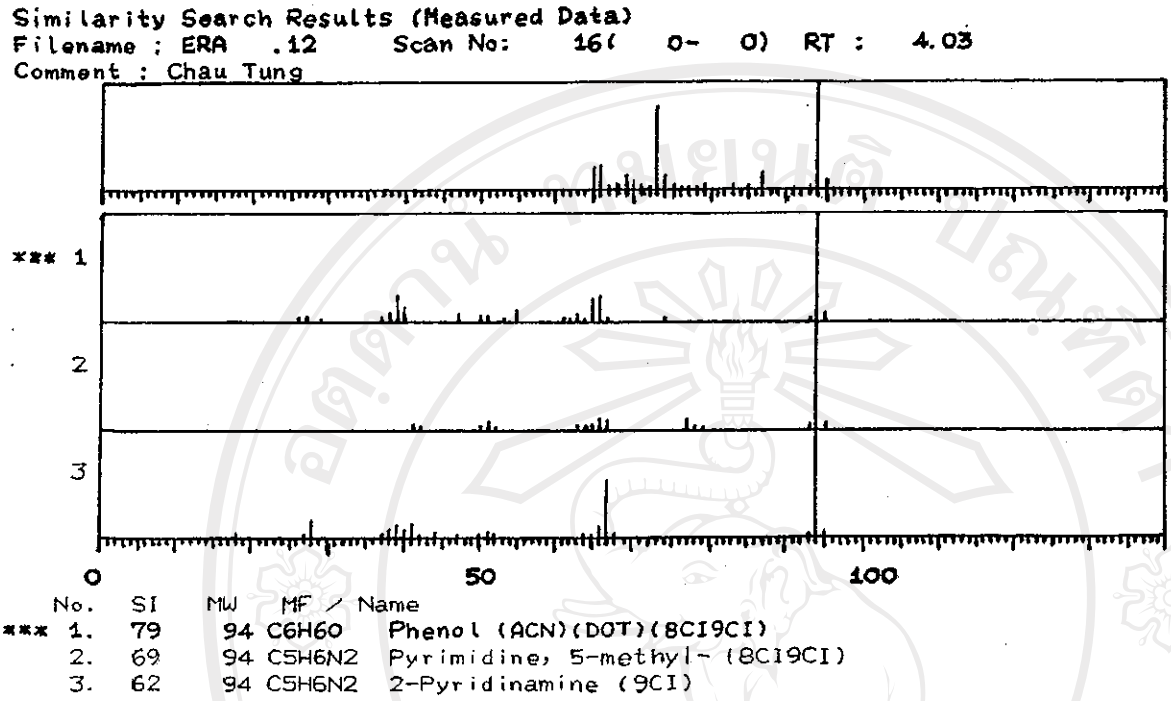


Figure 3.21: Mass Spectrum of Phenol Peak from CT Site Collected on 18th January 1995

The identification of individual phenolic compounds could be done by comparing the retention times (Figure 3.20) and fragmentation (m/z) patterns of standard and sample (Figure 3.21). The fragmentation pattern is the characteristic for each compound, as shown for the 11 PPP in Appendix 3.1. The result of GC-MS confirmation technique showed that there is only phenol in water sample in CT site. (see Table 3.18)

Table 3.18: Confirmation Result from Three Techniques

Compo-nent	t_{R}-spiking (min)	1° Result	Wavelength ratio (295/270, 295/280, 280/270)		2° Result	GC-MS Result	Final Result
			Standard	Sample			
Phenol	5.25	P	0, 0, 0.63	0.38, 0.66, 0.57	N	P	Y
4-NP	6.25	P	2.83, 1.85, 1.53	2.00, 1.25, 1.60	N	N	N
2-CP	8.30	P	0.056, 0.023, 1.07	0.33, 0.50, 0.67	N	N	N
2-NP	10.10	N	----------	----------	-------	N	N
2,4-DMP	11.65	P	0.24, 0.17, 1.43	0.38, 0.35, 1.06	N	N	N
4-C-3-MP	14.00	P	0.63, 0.39, 1.60	0.63, 0.38, 1.63	P	N	N
2,4-DCP	17.15	N	----------	----------	--------	N	N

Note: "P" = possible

"N" = negative

"Y" = yes (positive)

From the results obtained from the water sample of CT site taken on 18th January 1995 using the three confirmation techniques, it could be concluded that only phenol contains in this water sample. The "negative" result for phenol from wavelength ratio technique could be due to small and/or overlapped peak from matrix in the sample. This "negative" result (wavelength ratio for phenol component) happened to water sample for all sites. All water sample showed the similar peak pattern which could be noted as "phenol fingerprint" for this area.

3.5.5 Determination of Phenolic Compounds in Water Samples In and Around Chiang Mai City

For qualitative analysis, all information from result of confirmation techniques (retention time, spiking technique, wavelength ratio and GC-MS results) were combined. For quantitative analysis, the concentration of each compound was calculated using the suitable range of calibration curve according to its concentration. (see Table 3.15) The results were shown in Table 3.19.

Table 3.19: Phenolic Compounds Found in Water Samples

Compound	Phenol Contents (μg/l)									
	November 1994			January 1995						
	RK	CT	HR	RK	CT	MK1	MK2	HR	WW	DS
2,4-DNP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4,6-DN-2-MP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Phenol	2.94	3.47	0.75	2.17	4.26	2.18	3.08	N.D.	1.18	N.D.
4-NP	N.D.	N.D.	0.16	0.08	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2-CP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2-NP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4-DMP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4-C-3-MP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4-DCP	0.22	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4,6-TCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Note: Duplicate sample analyses (extractions and injections) of MK1 and MK2

3.5.6 Solid Phase Extraction (SPE) Results

3.5.6.1 Optimization of SPE System and Recovery Test

Using the conditions described in section 2.5.3, the results are represented in Table 3.20.

Table 3.20: Recovery Test of Phenolic Compounds by Varying the Amount of Sorbent and Type and Amount of the Eluent

Component	Recovery (%)								
	Methanol			Acetronitrile					
	3g/ 10ml	3g/ 10 ml	Ave.	2g/ 10 ml	2g/ 5 ml each fraction		1g/ 5 ml each fraction		1g/ 6ml
					1st	2nd	1st	2nd	
Phenol	70	55	63	-	-	-	-	-	0093
4-NP	-	-	-	106	101	-	100	-	100
2-CP	-	-	-	96	97	-	96	-	98
2,4-DMP	106	83	94	87	80	-	94	2.2	98
2,4-DCP	91	78	84	79	80	-	90	-	90
PCP	-	-	-	100	93	1.8	88	5.6	95
2,4,5-TCP	-	-	-	70	84	3.3	87	-	88

Methanol and acetronitrile were tested as the eluent for the extraction of phenolic compounds by octadecyl (C_{18}) sorbent. The polarity of octadecyl (C_{18}) was weak, while methanol and acetronitrile were polar solvents. They can elute the polar compounds from the sorbent according to the "*like dissolves like*" principle.

Acetronitrile was selected to be the eluent due to that it can easily be evaporated in a rotary evaporator in order to reduce the volume to about 1 ml. This concentration step is also highly recovered (88-100%).

% Recoveries for 1g or 2g of the sorbent used are found to be non-significantly different for both applications. Eluent volume was applied at 6 ml for the January sample because there are very small amount of phenols found in the second 5 ml fraction (<6%). Reducing eluent volume should be preferred due to the less effect of matrix or contaminant towards interested peaks in chromatogram. The optimized condition for SPE method is shown in Figure 2.23.

3.6 Comparison of the Techniques Employed

In this work, batch spectroscopic method and flow injection analysis technique were used to investigate for the total phenol contents in water samples using the similar reaction of the phenolic compounds and 4-AAP to form colored complex. In order to identify the most common phenolic compounds which pay an important role on environmental situation in Chiang Mai area, HPLC analysis was applied. Advantages and disadvantages were summarized in Table 3.21.

Table 3.21: Comparison of Technique Employed

Technique	Advantage	Disadvantage
Spectroscopic Method	1) Cheap 2) Fast 3) Easy	1) Waste problem: toxic chemical 2) Open system, health risk 3) Only total Phenol content determined
Flow Injection Analysis	1) Simpler instrument 2) Fast/ high sample through-puts 3) Close system, less risk 4) Possibility on on-line analysis 5) Low reagent consumption 6) Less sample volume used	1) Only total phenol content determined 2) Waste problem
HPLC	1) Can indicate individual phenolic compound	1) Expensive and complicate instrumentation 2) Time consuming for sample preparation 3) Confirmation needed 4) Waste problem of mobile phase and toxic chemical used 5) skill needed

3.7 Phenol Contents

The concentration of total phenols obtained by the FIA technique were higher than the ones obtained by spectrophotometric method. This could be due to that no sample pretreatment prior to analysis with FIA was made. The analogous compounds possibly react with the reagent and the products absorb at the analytical wavelength leading to higher absorbance. The compounds may possible be cresols and other substituted phenols.[4] However the trend of phenol contents found by the FIA method are similar to the one obtained by batch colorimetry. There was a decrease of the contents at the following sites, from MK, CT, RK, HR, WW upto DS. The phenol contents obtained from spectrophotometric method are used further for evaluation.

The sites which were noted as phenol pollution areas were MK, CT, RK, HR and WW with the order of increasing degrees of pollution respectively. The samples collected on 26 October 1994 showed relatively low phenol contents possibly due to dilution effect since heavy rain in August and September, including the flooding events. (see Appendix 3.4) Samples taken on 18th January 1995 showed low contents of phenols for every site, this may due to the changing of drainage system in Chiang Mai area. The water from irrigation canal was turned to the moat, since there was construction for cable-line along the irrigation canal, caused the rising up of water level in all collected sites (see Table 3.6) and consequently affected by dilution. Effect of seasons on the phenol contents could not be observed since the longer period of rainy season provided unusual raining. In comparison to the study of phenol contents for 14-day spot samples during the period 1954 to 1976 (Figure 3.22), the reported values are mainly below 0.05 mg/l similar to this study.[59] It showed similar fluctuation of phenol content for one site at different times.

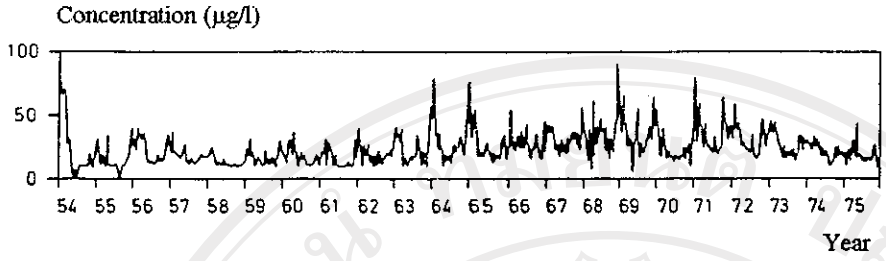


Figure 3.22: Phenols in the Middle Rhine at Branbach, 14 day spot sample[59]

Attempt were made to determine specific individual phenols of known chemical composition (concerning PPP) by HPLC technique. The sum concentrations of the individual species found is compared with the total phenol contents obtained by the spectrophotometric measurement. The results are depicted in Figure 3.23. Figure 3.24 shows the results of the previous study concerning the comparison of colorimetry and gas chromatography.

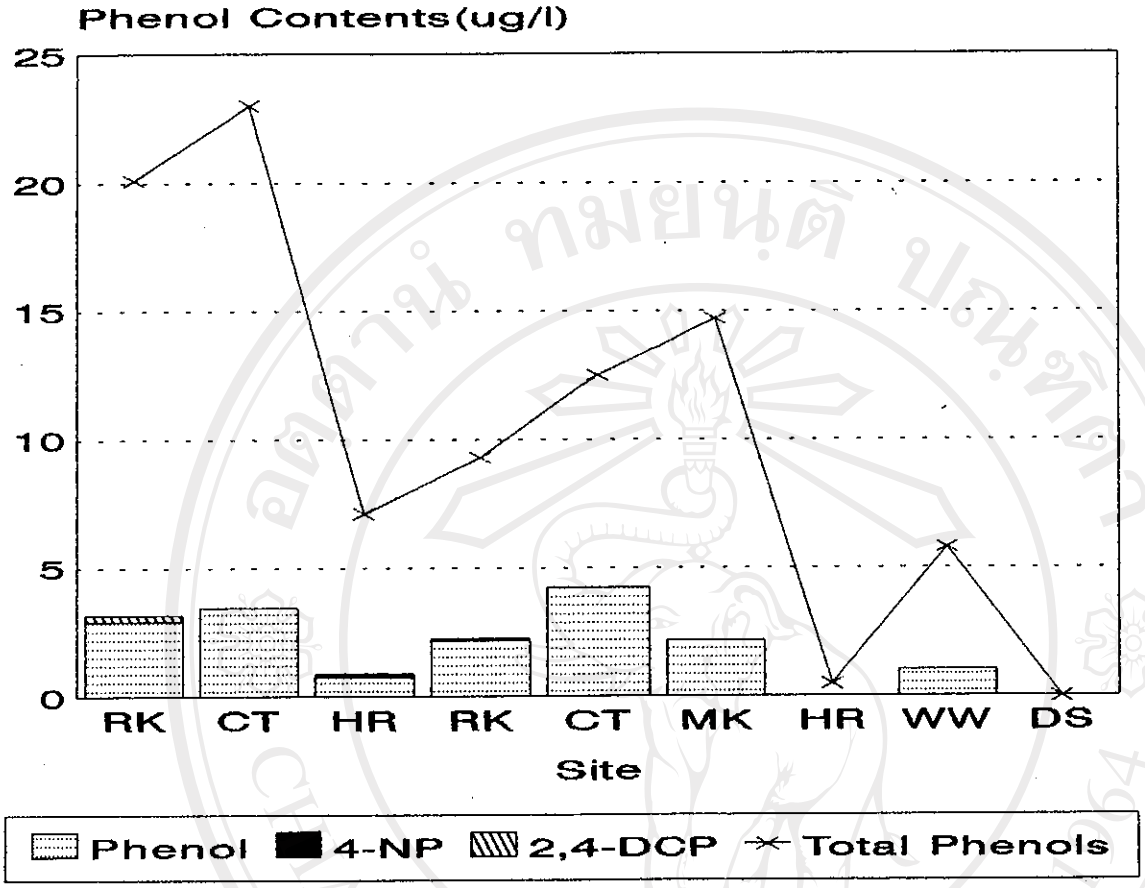


Figure 3.23: Comparison of the Total Phenol Contents and the Sum of the Individual Phenol Contents of Samples Taken from Various Sites on 18th January 1995

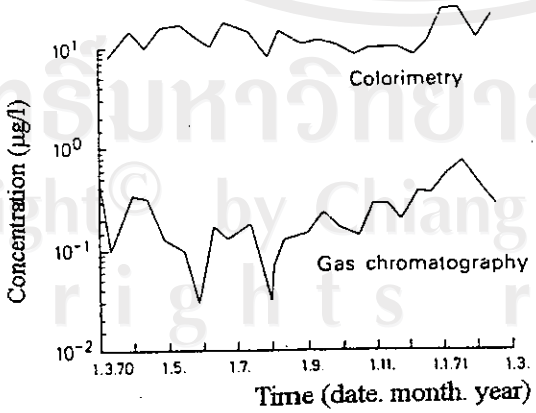


Figure 3.24: Comparison of Phenol Contents Obtained by Colorimetric and Gas Chromatographic Method at the Main Schweinfurt, 1970-1971.[60]

The total phenol contents were higher than the sum concentrations of the individual species. This might be due to the following reasons. Firstly there might be phenolic compounds other than the ones in the EPA list. For some phenolic compounds, the detection limit in HPLC is relatively poor, e.g. PCP, 2,4,6-TCP or others but these compound (and/or other analogous compounds) can react with 4-AAP to yield the products which absorb at the analytical wavelength. 2,4-DNP and 4,6-DN-2-MP were discarded from consideration due to the peaks affected by solvent and/or salt effects in the SPE step. Secondly, there could be the matrix effect in sample which may overlap the peaks of phenols of interest resulted in negative results in wavelength ratio confirmation technique.

Water from most of the sites can not be consumed as drinking water, according to the exceeding of phenol contents from the standard value ($\leq$ 0.001 mg/l) (see Appendix 3.2) except in Doi Suthep in some months. Some sites i.e. MK, CT, RK, WW and HR were also considered as phenol polluted area regarding to the water quality standard for surface water ($\leq$0.005 mg/l). In addition there was no signal for phenol pollution by industrial activities. The possible reason of lower values was that it was diluted by the Mae Kuang river which flow passing the discharging point of the NIE, resulted in no effect of industrial effluent to the natural water. It is recommended to conduct surveying of phenol in the effluent of specific factory or at least before its discharging point to natural water, in order to assess and recommend the relevant official to control and prevent phenol pollution affected by industrial wastewater. There is no significant phenol contents produced by natural process which could be observed in DS site. It could be concluded that in and around Chiang Mai city, the existing pollution due to phenol as the indicator has been resulted from anthropogenic activities. These are dominantly found in domestic area. The phenol pollution sites were shown in Figure 3.25.

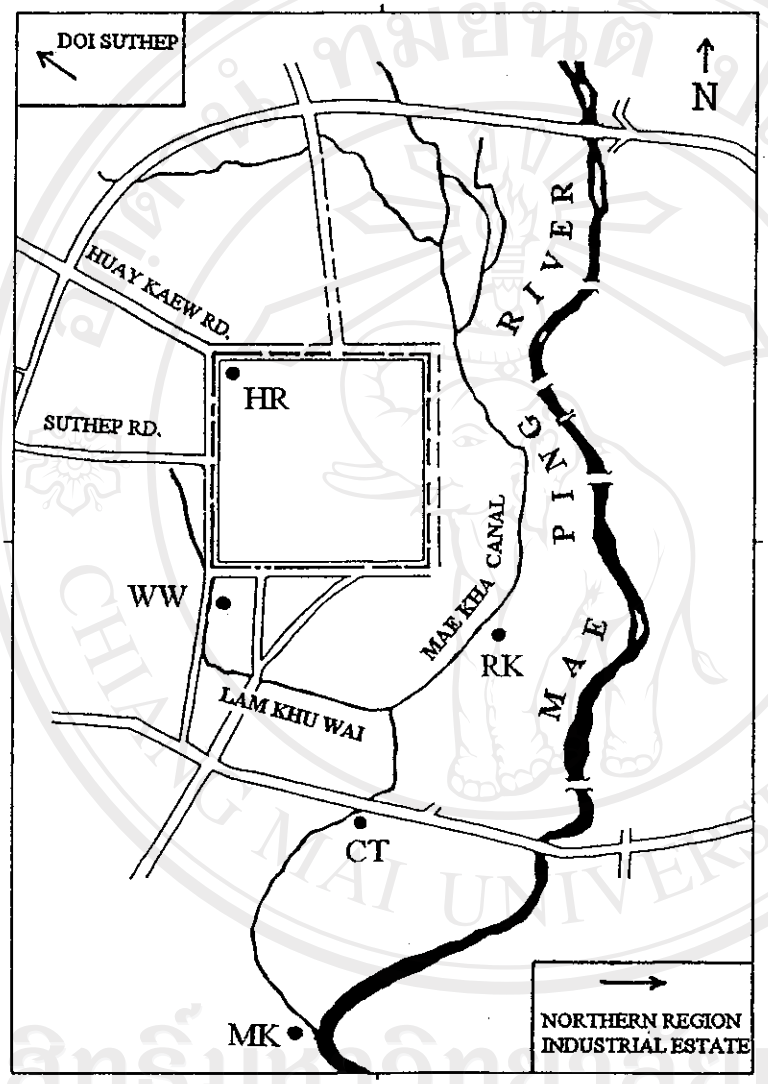


Figure 3.25: Map Showing Locations of Phenols Pollution Sites

According to effect of the pattern of the flow of the natural water passing the city, the Mae Kha canal (east side of the city) was affected more on phenol pollution compared with Lam Khu Wai (west side of the city). The phenol contents was found higher after passing the city center. For the Mae Ping river, it was found for slightly higher of phenol contents in NW, the part of river which passed the city center comparing to WSK, MP-B and MP-A sites. From phenol content at WSK site, which is the upper part of this site namely Pa Tun is used as the raw water for water supply having lower values than standard surface water($\leq$0.005 mg/l) but still higher than standard value for drinking water ($\leq$0.001 mg/l).