3. RESULTS

3.1 Physical and chemical parameters of soil

The water moisture content in soil samples studied ranged from 0.8 to 3.2 per cent. Because soil was air-dried for one day before analysis, these results do not reflect the actual soil moisture in the field but for calculation of PAH concentrations in soil expressed as ng/g dry soil. Organic matter content in soil was from 2.3 to 9.1 per cent. Most soils were sandy loam or loamy sand (Table 3.1).

Table 3.1 Some physical and chemical parameters of soil studied.

Site	Texture	Moisture (%w/w)	Organic content (%w/w)
M1	Loamy sand	0.7	9.1
M2	Loamy sand	2.7	7.2
M3	Sandy loam	2.6	4.5
M4	Sandy loam	2.9	5.3
M5	Sandy loam	2.0	3.7
M6	Sandy loam	1.3	4.0
M7	Loamy sand	1.0	2.5
S1	Sandy loam	1.3	3.8
S2	Silty loam	3.4	5.1
S3	Sandy loam	1.2	4.4
S4	Sandy loam	1.1	3.9
S5	Silty loam	1.5	3.2
S6	Sandy loam	0.8	4.1
S7	Loamy sand	3.0	4.3
S8	Loamy sand	1.7	3.0
S9	Loamy sand	1.3	7.2
D1	Granite	0.9	2.3
D2	Sandy loam	3.0	5.0

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.2 Occurrence of PAHs in soil

3.2.1 The retention times of 16 EPA-PAHs

Under the HPLC conditions employed, the 16 EPA-PAHs showed the retention times from 16.03 to 38.68 min. The retention times of each component may shift in a small range for different injections. Attempts were made to separate ACE from FLU, and CHR from BaA by changing gradient elutions and HPLC columns. However, it was still difficult to separate these compounds by HPLC in this work. Therefore, their retention times are reported together. Figs. 3.1 and 3.2 show a typical chromatogram of 16 PAHs standard mixture and a chromatogram of a soil sample, M4. Table 3.2 lists typical retention times with standard deviation (SD) of the compounds analyzed and each retention time was based on four runs.

Table 3.2 Retention times of 16 EPA-PAH investigated.

Compound	Retention time(min) Mean (SD)	Compound	Retention time(min) Mean (SD)
NAP	16.02 (0.02)	CHR+BaA	29.72 (0.04)
ACY	17.87 (0.02)	BbF	33.20 (0.04)
ACE+FLU	20.77 (0.02)	BkF	33.74 (0.04)
PHE	22.36 (0.02)	BaP	34.99 (0.05)
ANT	23.41 (0.03)	DBA	36.06 (0.05)
FLA	25.49 (0.03)	BPE	38.22 (0.06)
PYR	26.78 (0.03)	IND	38.60 (0.07)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

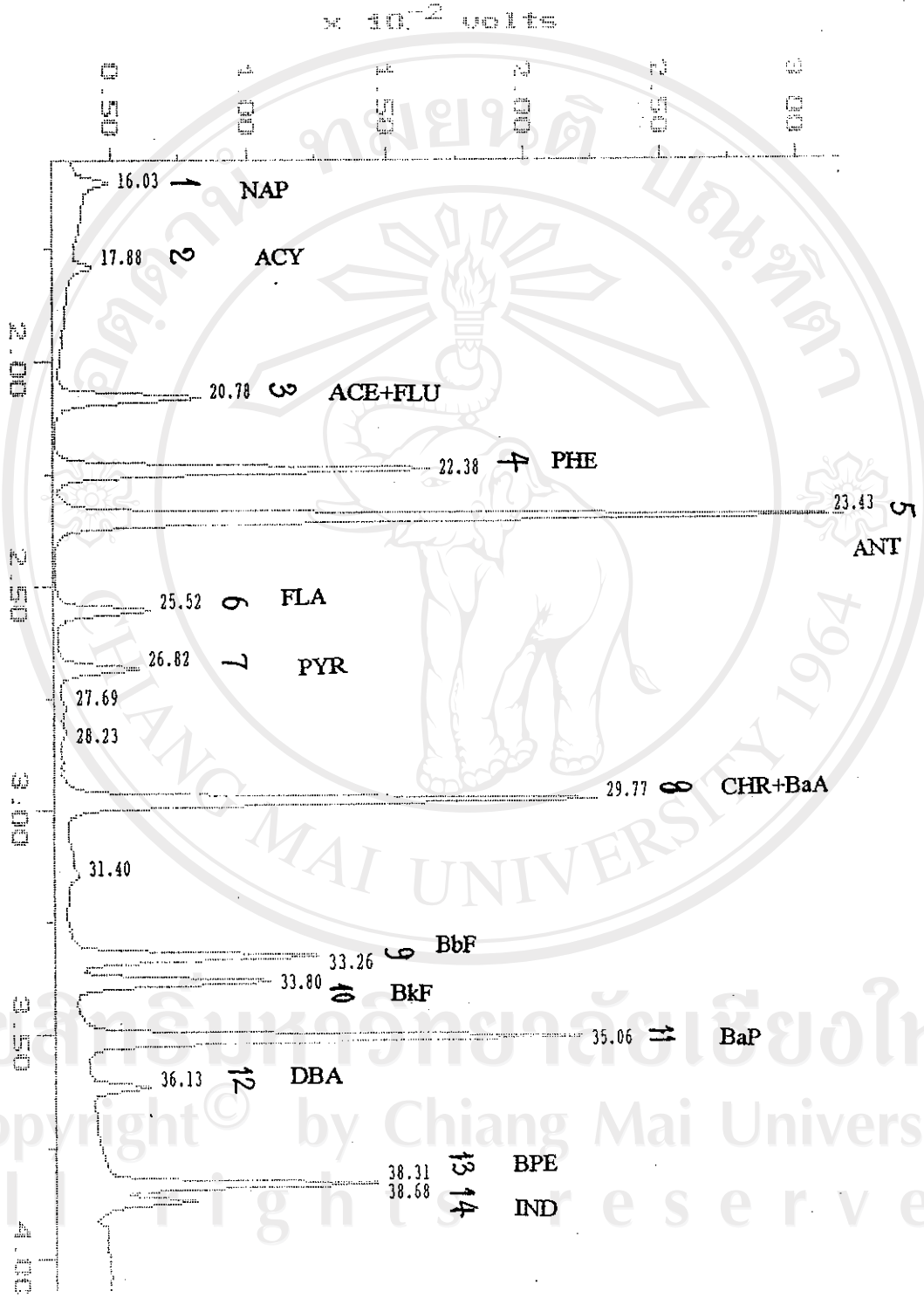


Fig. 3.1 Chromatogram of standard 16 EPA-PAH mix (conc.= 0.5 ppm)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

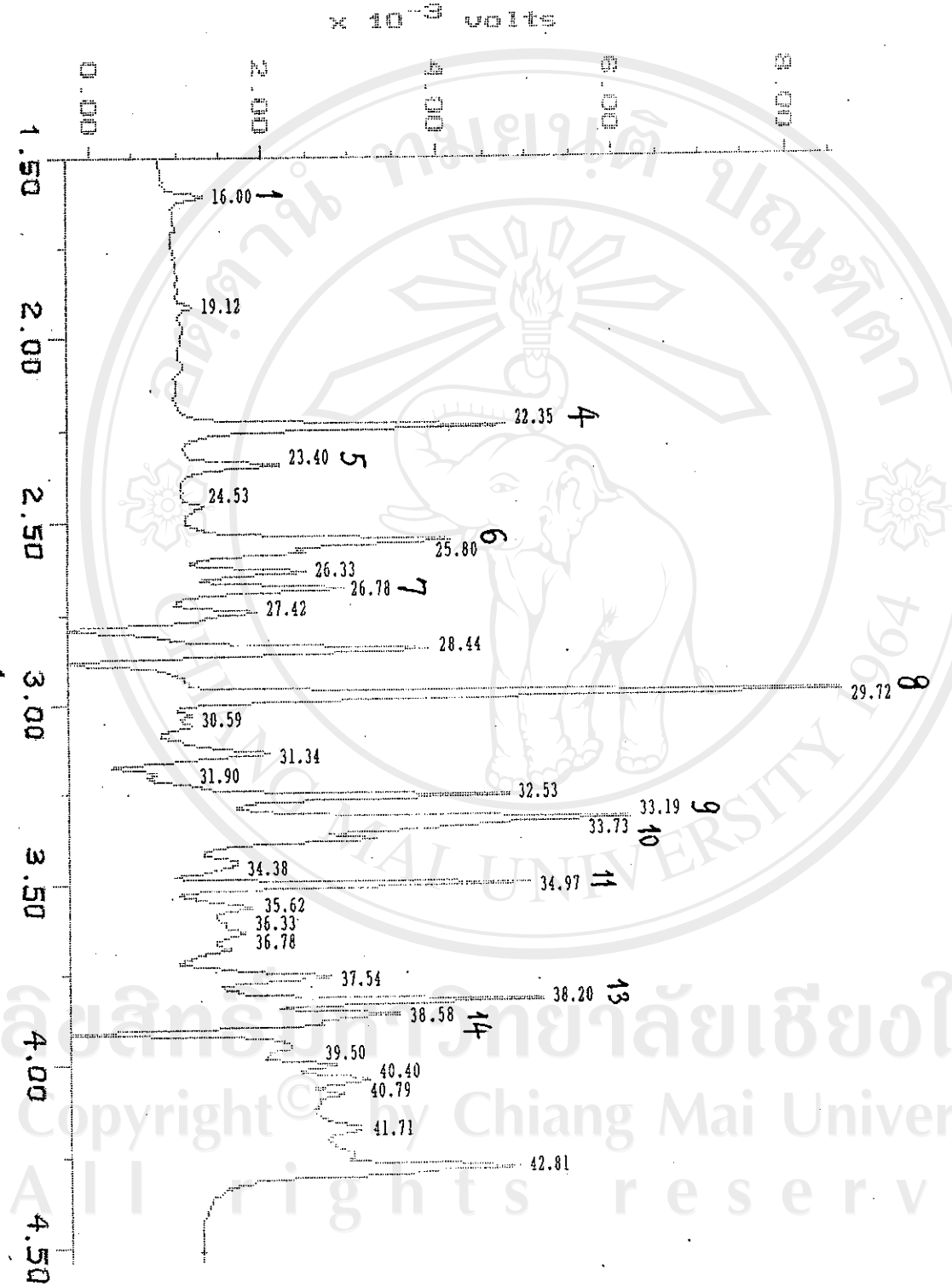


Fig. 3.2 Typical chromatogram of soil samples (sample M4)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.2.2 The ratio of peak heights of PAHs at wavelengths 297 and 254 nm

For confirmation of PAH compounds, the second information is needed. In this study, confirmation of 16 PAHs was carried out by comparing the ratio of peak heights at two different wavelengths, 297 and 254 nm (Table 3.3). For the PAH compounds to be reported in soil, both the specific retention times and the ratios of them were consistent well with both sample chromatograms and standard mixture chromatograms.

Table 3.3 The ratios of peak heights of 16 PAHs at wavelengths 297 and 254 nm.

PAHs	Ratio (H_{297}/H_{254})	PAHs	Ratio (H_{297}/H_{254})
NAP	-	CHR+BaA	0.20
ACY	2.15	BbF	0.62
ACE+FLU	0.55	BkF	1.12
PHE	0.11	BaP	0.96
ANT	0.005	DBA	9.54
FLA	0.18	BPE	0.58
PYR	0.41	IND	2.21

- less than 0.001.

H_{297} = peak height at wavelength 297 nm.

H_{254} = peak height at wavelength 254 nm.

3.2.3 Detection limit and lowest determination limit

The detection limit is expressed as 2 times of the noise level. These HPLC conditions are sensitive to BaP, ANT, CHR, and BaA, with the detection limits at 0.02 ng for each injection solution, but relatively non-sensitive to ACY, with the detection limit at

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

0.89 ng. From the detection limit, the lowest determination limit for each compound of 16 PAHs by this method could be calculated (Table 3.4) by the following equation:

$$\text{Determination limit} = \frac{\text{Detection limit (ng)} \times V_{final}\,(\text{ml}) \times 1{,}000}{V_{inj}\,(\mu\text{l}) \times W_{soil}\,(\text{g})}$$

where V_{final} = final volume of soil residue in actonitrile for HPLC injection (1 ml),

V_{inj} = injection volume (20 μl),

W_{soil} = soil weight for analysis (about 20 g).

Table 3.4 Detection limits and determination limits of PAHs.

Compound	Detection limit (ng)	Determination limit (ng/g dry soil)
NAP	0.47	1
ACY	0.89	2
ACE+FLU	0.09	0.2
PHE	0.04	0.1
ANT	0.02	0.05
FLA	0.14	0.4
PYR	0.16	0.4
CHR+BaA	0.02	0.05
BbF	0.06	0.2
BkF	0.07	0.2
BaP	0.02	0.05
DBA	0.15	0.4
BPE	0.04	0.1
IND	0.13	0.3

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.2.4 Construction of calibration curves

The standard reference material of 16 EPA-PAH mixture, in which all PAHs were present at concentrations 0.1, 0.2, 0.5, and 1 ppm was injected. The calibration curves of 16 PAHs were obtained from the computer systems by plotting peak height vs. concentration. Table 3.5 gives the calibration equation and correlation coefficient of each compound.

Table 3.5 Calibration equation and coefficient for each PAH compound.

Compound	Calibration equation	Coefficient (r^2)
NAP	$C = -0.0411 + 5.89\times10^{-4} R$	0.9950
ACY	$C = 0.0089 + 7.10\times 0^{-4} R$	0.9997
ACE+FLU	$C = -0.0027 + 9.70\times10^{-5} R$	0.9996
PHE	$C = -0.0033 + 3.76\times10^{-5} R$	0.9998
ANT	$C = -0.0079 + 1.79\times10^{-5} R$	0.9998
FLA	$C = 0.0021 + 1.47\times10^{-4} R$	0.9999
PYR	$C = 0.0029 + 1.69\times10^{-4} R$	0.9997
CHR+BaA	$C = -0.0145 + 2.54\times10^{-5} R$	0.9973
BbF	$C = 0.0082 + 5.36\times10^{-5} R$	0.9997
BkF	$C = 0.0112 + 6.67\times10^{-5} R$	0.9995
BaP	$C = 0.0210 + 2.39\times10^{-5} R$	0.9935
DBA	$C = 0.0099 + 2.03\times10^{-4} R$	0.9903
BPE	$C = 0.0070 + 5.02\times10^{-5} R$	0.9998
IND	$C = 0.0222 + 1.44\times10^{-4} R$	0.9990

C = concentration.

R = peak height.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.2.5 Percentage of recovery

A standard PAH solution containing ANT, CHR, BaA, BaP, and DBA with the concentration of 1 ppm each was surrogated into the soil. After repeating all the procedures for HPLC analysis for soil, the peak height of each compound surrogated was recorded, and then compared with the peak height of the standard solution. The percentage recovery was calculated. Because of the low PAH contents in real soil samples, the standard solution with 0.2 ppm of each PAH compound, which was in the neighborhood of PAH concentrations in real soil sample, was repeated for calculation of the percentage of recovery. The ranges for these five compounds at concentration 0.2 ppm and 1 ppm are from 72.9% to 84.0%, and from 79.9% to 89.3%, respectively (Table 3.6).

Table 3.6 Percentage recovery of 5 PAHs at concentration of 1 and 0.2 ppm.

Compound	1 ppm			0.2 ppm		
	H_{std}	H_{srg}	Recovery%	H_{std}	H_{sug}	Recovery%
ANT	56445	50405	89.3	13017	10934	84.0
CHR+BaA	37868	30248	79.9	7747	5598	72.3
BaP	29264	24480	83.7	5851	4264	72.9
DBA	3521	2930	83.2	806	646	80.1

H_{std} = peak height of standard PAHs.

H_{srg} = peak height of PAHs after being surrogated to a soil sample.

3.2.6 The concentration of PAHs in soil

With the exception of acenaphthylene which could not be detected in all samples, the other 15 EPA-PAHs could be found in the soil samples. The concentrations of PAHs

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

are expressed as ng/g dry soil (ppb). The concentrations of each PAH compound from Muang, Sarapee, and Doi Suthep areas are shown in Tables 3.7-9. PYE, FLA, and PHE are three dominant PAHs in Muang District. In Sarapee District, the distributions of individual PAHs are more uniform, FLA, BPE, and PHE with higher concentrations.

Table 3.7 PAH concentrations at study sites in Muang District (ng/g dry soil).

Compound	M1	M2	M3	M4	M5	M6	M7	Mean
NAP	8	1	2	11	7	1	-	4.3
ACY	-	-	-	-	-	-	-	-
ACE+FLU	-	1	1	-	1	-	-	0.4
PHE	11	5	4	7	15	3	18	9.0
ANT	2	1	-	1	1	-	1	0.9
FLA	22	11	9	10	24	7	20	14.7
PYR	23	12	4	16	18	6	26	15.0
CHR+BaA	9	5	2	9	10	4	12	7.3
BbF	13	5	2	13	11	-	5	7.0
BkF	12	3	2	7	7	-	9	5.8
BaP	5	1	2	6	5	3	6	4.0
DBA	6	2	-	3	7	-	5	3.3
BPE	4	5	3	9	12	3	14	7.1
IND	7	4	3	11	9	7	9	7.1
TOTAL	122	56	34	103	127	34	125	85.9
4-PAHs[a]	20	8	4	18	22	7	23	14.6

- not detected.

[a] 4-PAHs : BaP + DBA + BaA + CHR.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.8 PAH concentrations at study sites in Sarapee District (ng/g dry soil).

Compound	S1	S2	S3	S4	S5	S6	S7	S8	S9	Mean
NAP	-	-	-	5	15	1	3	2	-	2.9
ACY	-	-	-	-	-	-	-	-	-	-
ACE+FLU	-	-	-	-	-	-	2	-	2	0.4
PHE	1	3	1	1	1	2	23	3	6	4.6
ANT	-	-	-	-	-	-	9	-	2	1.2
FLA	2	3	2	2	2	5	34	3	7	6.7
PYR	1	4	2	1	1	4	14	5	6	4.2
CHR+BaA	1	5	1	1	1	2	5	5	2	2.6
BbF	1	6	2	-	2	3	5	12	5	4.0
BkF	1	3	2	-	1	3	6	6	8	3.3
BaP	2	2	2	-	2	3	2	3	2	2.0
DBA	0	2	4	2	0	0	4	2	2	1.8
BPE	1	6	2	1	2	7	8	14	4	5.0
IND	-	2	3	-	2	2	2	4	2	1.9
TOTAL	10	36	21	13	29	32	117	59	48	40.6
4-PAHs[a]	3	9	7	3	3	5	11	10	6	6.3

-, [a], see Table 3.7.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.9 PAH concentrations at study sites in Doi Suthep areas (ng/g dry soil).

Compound	D1	D2	Compound	D1	D2
NAP	-	-	CHR+BaA	-	-
ACY	-	-	BbF	-	-
ACE+FLU	-	-	BkF	-	-
PHE	-	-	BaP	-	-
ANT	1	-	DBA	-	-
FLA	-	-	BPE	-	-
PYR	-	-	IND	-	-

- not detected.

The amounts of 16 PAHs in soil samples from Muang and Sarapee ranged from 34 to 127, and 10 to 117 ng/g dry soil, respectively. In Doi Suthep area, only anthracene was detected at 1 ppb in soil. The average of total PAHs in Muang and Sarapee Districts is 85.9 and 40.6 ppb, respectively. By statistical analysis, the difference is significant ($p<0.05$, Table 3.10 and Fig. 3.3). Most of PAHs present at higher concentration in Muang District (Fig. 3.5). PAHs in the samples collected from roadsides normally were from motor exhaust, while the main sources of PAHs in garden sites are burning of garbage and cooking. M1, M4, M5, M7, and S9 were collected from roadsides, while M2, M3, S1, S2, S3, S5, S6 and S8 were from gardens. The difference of total PAHs between roadsides and gardens in Muang District, with higher amount of PAHs in soils from roadsides, reflects that traffic emissions are the main sources of PAHs in this district (Fig. 3.3). In contrast, the total PAHs from a road in Sarapee District did not demonstrate higher concentration than those from gardens. This suggests that both traffic emission and burning of home wastes are likely the main contributors to PAHs in this district (Fig. 3.3).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.10 The comparison of total PAHs between Muang District and Sarapee District.

District	Sample size (n)	Total PAHs (Mean ± SD)	*t* value	Probability (p)
Muang	7	85.9 ± 43.0	2.40	0.03
Sarapee	9	40.6 ± 32.7		

BaP, BaA, and DBA are considered to be probably carcinogenic to humans on the basis of experimental data obtained from both whole animals and *in vitro* test systems [9,10]. Among all of the PAHs, BaP is one of the most studied for its carcinogenicity. It is used as the indicator compound for the presence of PAH in environmental material [9]. The comparison of these compounds between different districts and exposure types is shown in Fig. 3.4. It is consistent with the trend of total PAHs. The concentrations in Muang District are higher than those in Sarapee District, and samples collected from roadside sites have higher contents than those from garden sites in Muang District. The correlation between the amount of total 16 PAHs analyzed and the amount of the sum of BaP, BaA, DBA, and CHR are significant, with the correlation efficient 0.92 ($p < 0.05$).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

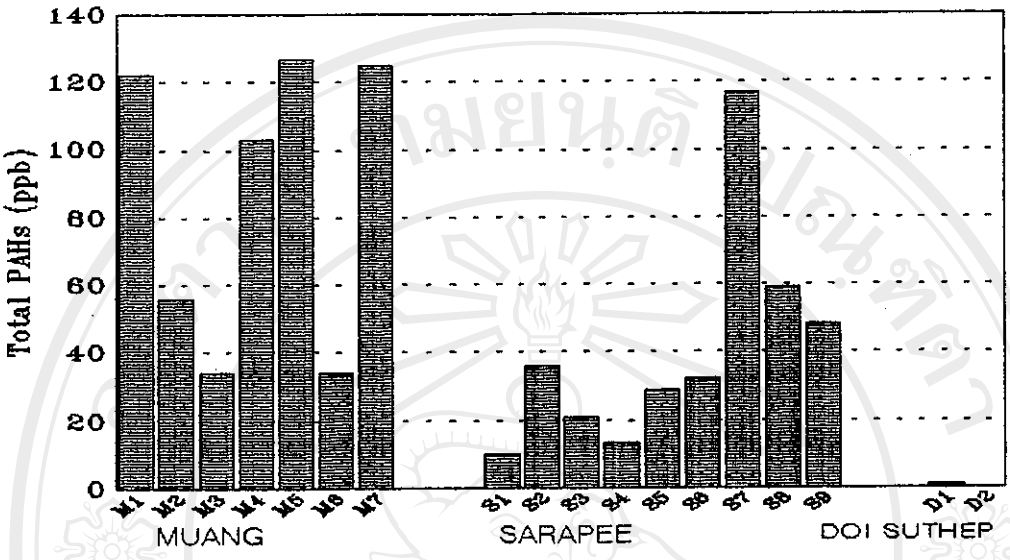


Fig.3.3 Comparison of total PAHs at each site

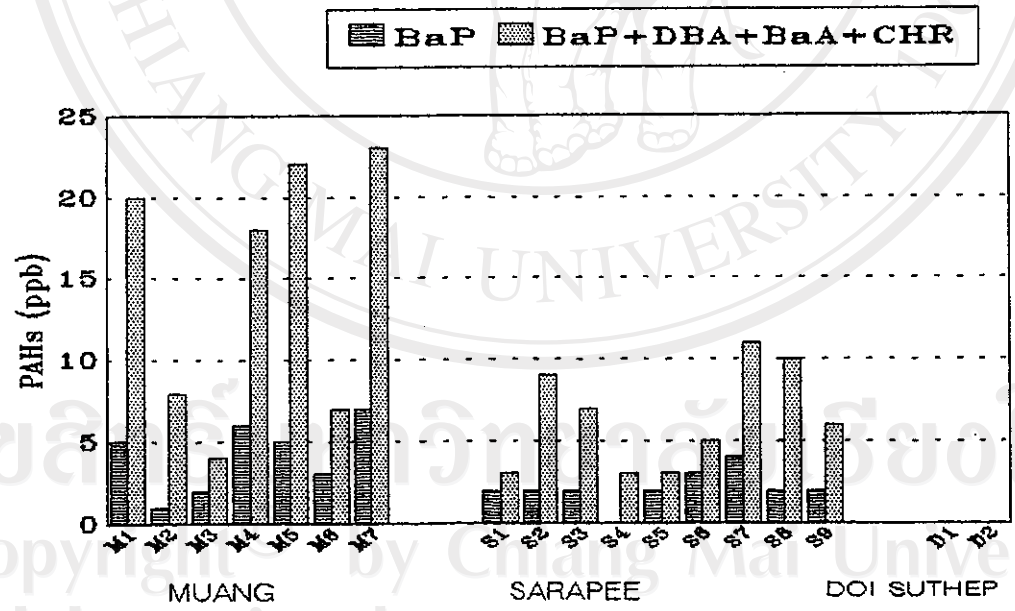


Fig.3.4 Comparison of strongly carcinogenic PAHs at each site

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

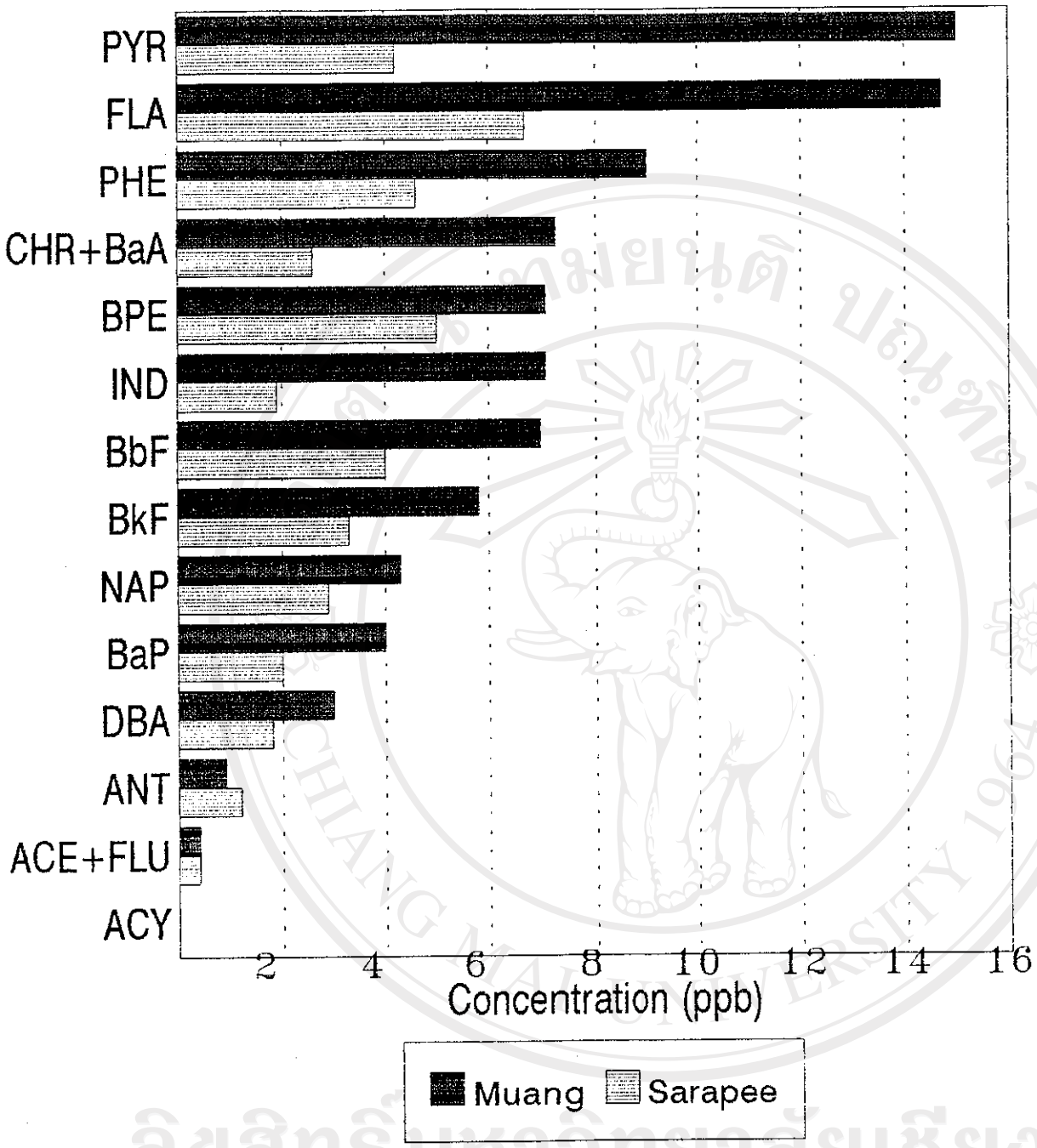


Fig. 3.5 Mean of individual PAH in Muang and Sarapee

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.2.7 Comparison between GC/MS and HPLC analysis

Three samples, M4, M7, and S9, were conducted GC/MS analysis in addition to HPLC analysis. Most GC/MS results are in accordance with HPLC (Table 3.11). Because GC/MS has lower detection limit than HPLC in this study, ACY could be detected by GC/MS in one sample, with a concentration less than 1 ppb.

Table 3.11 Comparison of results from GC/MS and HPLC analysis for M5, M7, and S9.

	M4		M6		S9	
Compound	GC/MS	HPLC	GC/MS	HPLC	GC/MS	HPLC
NAP	15	11	4	0	3	0
ACY	0	0	<1	0	0	0
ACE+FLU	1	0	2	0	2	2
PHE	6	7	15	18	5	6
ANT	2	1	3	1	3	2
FLA	15	10	19	20	5	7
PYR	14	16	21	26	4	6
BaA+CHR	16	9	9	12	5	2
BbF	16	13	5	5	3	5
BkF	6	7	3	9	2	8
BaP	20	6	5	6	4	2
DBA	3	0	4	5	3	2
BPE	15	9	15	14	4	4
IND	8	11	5	9	2	2

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.3 Mutagenicity of soil extracts in *Salmonella typhimurium* strains

3.3.1 The amounts of extractable matter of soil

Most of the organic matters in soil are not extractable by dichloromethane. Table 3.11 shows the amounts of dichloromethane extractable matter, ranged from 0.07 to 3.73 mg/g soil. Soil sample S9 from the main road in Sarapee District gave the highest amount. The soils collected from garden sites generally contain less extractable organic matter.

Table 3.12 The amounts of extractable organic matter (EOM) in soil (mg/g soil).

Site	EOM	Site	EOM	Site	EOM
M1	0.62	S1	0.13	D1	0.07
M2	0.39	S2	0.22	D2	0.20
M3	0.39	S3	0.08		
M4	0.90	S4	0.08		
M5	1.45	S5	0.25		
M6	0.77	S6	0.18		
M7	1.89	S7	1.23		
		S8	0.21		
		S9	3.73		

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

3.3.2 Known mutagens as positive control in the test

In each experiment, the known mutagens were tested in order to calibrate the results. BaP and 2-aminoanthracene (2AA) were selected as positive control for TA100 and TA98 with metabolic activation, respectively. Positive control for both TA100 and TA98 without metabolic activatin was 2-(2-furyl)-3-(5-nitro-2-furyl)furamide (AF-2).

Table 3.12 gives the doses of mutagens used in *Salmonella* assay and the ranges of revertants yielded from this experiment.

Table 3.12 Known mutagens and their ranges of revertants in this experiment.

Name	Dose (μg/plate)	Strain	S9 mix	His^+ revertants[a]
BaP	5	TA100	+	1,431 - 1,591
2AA	0.5	TA98	+	381 - 512
AF-2	0.1	TA98	-	350 - 447
	0.01	TA100	-	765 - 905

[a], Spontaneous reverts have not been subtracted yet.

3.3.3 Mutagenicity of soil extracts in *Salmonella typhimurium* TA98

Because it is difficult to obtain sufficient extracts from soil D1, S3, and S4, they were not tested for mutagenic activity by *Salmonella typhimurium* TA98. The rest of the samples were all subject to mutagenicity assay at dose levels 0.5, 1.0, 2.0, and 4.0 mg/plate in addition to the solvent control and positive control (Tables 3.14-16).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.14 Mutagenicity of soil from Muang District in *S. typhimurium* TA98.

Soil	Dose (mg/plate)	His^+ revertants/plate[a] + S9 mix	- S9 mix
M1	0	34 ± 8.4	25 ± 4.8
	0.5	87 ± 11.7	35 ± 4.5
	1.0	159 ± 21.4	46 ± 5.4
	2.0	187 ± 9.9	59 ± 8.1
	4.0	242 ± 17.7	63 ± 10.3
M2	0	50 ± 11.5	35 ± 3.6
	0.5	118 ± 20.2	62 ± 11.3
	1.0	143 ± 25.5	89 ± 9.6
	2.0	184 ± 29.6	127 ± 10.8
	4.0	254 ± 40.8	T[b]
M3	0	50 ± 11.5	35 ±3.6
	0.5	92 ± 13.6	54 ± 8.3
	1.0	133 ± 13.1	76 ± 9.2
	2.0	197 ± 19.8	105 ± 10.2
	4.0	273 ± 35.2	167 ± 11.1
	8.0	NT[c]	T
M4	0	34 ± 8.4	25 ± 4.8
	0.5	32 ± 5.8	37 ± 6.1
	1.0	61 ± 7.0	34 ± 5.1
	2.0	61 ± 10.0	38 ± 9.5
	4.0	47 ± 26.9	44 ± 6.9

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.14 (continued).

Soil	Dose (mg/plate)	His^+ revertants/plate[a] + S9 mix	- S9 mix
M5	0	34 ± 8.4	25 ± 4.8
	0.5	46 ± 8.2	38 ± 6.8
	1.0	50 ± 7.8	32 ± 8.9
	2.0	72 ± 16.6	36 ± 8.1
	4.0	88 ± 11.9	49 ± 11.6
M6	0	50 ± 11.5	35 ± 3.6
	0.5	65 ± 10.5	28 ± 2.9
	1.0	85 ± 18.2	32 ± 5.9
	2.0	77 ± 19.1	31 ± 5.7
	4.0	86 ± 11.8	41 ± 11.4
M7	0	36 ± 3.1	31 ± 2.3
	0.5	101 ± 6.1	40 ± 10.3
	1.0	131 ± 16.1	50 ± 16.7
	2.0	153 ± 8.9	58 ± 25.4
	4.0	182 ± 15.2	64 ± 21.1

[a], average of 2 independent tests with 2 or 3 plates per dose in each test. Standard deviation are included. Spontaneous revertants have not been subtracted yet. Zero concentration indicates spontaneous revertants (only solvent control 50 µl DMSO).
[b] T = toxic effects.
[c] NT = not tested.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.15 Mutagenicity of soil from Sarapee District in *S. typhimurium* TA98.

Soil	Dose (mg/plate)	Total his$^+$ revertants/plate[a] + S9 mix	- S9 mix
S1	0	34 ± 5.2	25 ± 5.2
	0.5	74 ± 10.1	78 ± 14.9
	1.0	122 ± 15.4	137 ± 13.3
	2.0	302 ± 43.6	284 ± 28.6
	4.0	524 ± 25.4	505 ± 10.7
S2	0	36 ± 3.06	31 ± 2.3
	0.5	82 ± 8.6	49 ± 4.0
	1.0	111 ± 18.2	70 ± 14.9
	2.0	152 ± 26.5	T[b]
	4.0	178 ± 26.0	T
S5	0	34 ± 5.2	25 ± 5.2
	0.5	70 ± 8.3	61 ± 16.8
	1.0	147 ± 10.7	170 ± 11.7
	2.0	283 ± 29.9	202 ± 31.3
	4.0	462 ± 23.7	253 ± 18.6
S6	0	39 ± 7.6	32 ± 8.0
	0.5	75 ± 15.0	45 ± 6.0
	1.0	109 ± 14.7	58 ± 13.5
	2.0	133 ± 17.9	71 ± 15.7
	4.0	148 ± 18.8	77 ± 10.5

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.15 (continued).

Soil	Dose (mg/plate)	Total his$^+$ revertants/plate [a]	
		+ S9 mix	- S9 mix
S7	0	39 ± 7.6	32 ± 8.0
	0.5	48 ± 11.7	29 ± 6.8
	1.0	62 ± 17.7	35 ± 8.5
	2.0	72 ± 13.6	42 ± 8.9
	4.0	119 ± 18.0	48 ± 13.6
	8.0	116 ± 12.7	77 ± 8.5
S8	0	34 ± 5.2	25 ± 5.2
	0.5	94 ± 7.4	47 ± 8.3
	1.0	159 ± 22.2	73 ± 12.6
	2.0	286 ± 24.0	63 ± 73
	4.0	416 ± 44.4	T
S9	0	39 ± 7.6	32 ± 8.0
	0.5	37 ± 2.3	32 ± 7.2
	1.0	48 ± 12.3	37 ± 9.7
	2.0	49 ± 14.9	35 ± 5.9
	4.0	62 ± 13.0	37 ± 5.8
	8.0	85 ± 7.1	51 ± 2.8

[a], [b], see Table 3.14.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.16 Mutagenicity of soil from Doi Suthep in *S. typhimurium* TA98.

Soil	Dose (mg/plate)	His^+ revertants/plate [a]	
		+ S9 mix	- S9 mix
D2	0	36 ± 3.1	31 ± 2.3
	0.5	67 ± 17.6	49 ± 12.3
	1.0	124 ± 17.1	65 ± 21.7
	2.0	190 ± 9.4	73 ± 13.9
	4.0	275 ± 20.2	89 ± 7.3

[a], see Table 3.14.

Twelve samples, except M4, M6, and S9 showed mutagenicity towards TA98 with metabolic activation. Among them, M1, M2, M3, S1, S2, S5, and D2 also showed mutagenicity to TA98 without activation activation. Soil samples M7 and S7 demonstrated a dose-response relationship without metabolic activation, and S9 also showed a dose-response relationship with metabolic activation. However, because the dose levels were not increased after 4.0 mg/plate for M7, and 8.0 mg/plate for S7 and S9, the positive response were not observed in this experiment (Figs. 3.6-10). Some samples, M2, M3, S2, and S8, demonstrated toxic effect to bacteria when they were not activated. However, after having been activated, all the samples showed mutagenic activity instead of toxicity.

The specific activity of soil, which is defined as the net revertants at a specific dose level of soil extract, provides a qualitative measurement of the mutagenic potential of a specific soil sample. And the weighted activity, expressed as net revertants per gram soil, gives a quantitative measure of mutagenic potential [8]. If a sample fails to induce a doubling revertants in at least two consecutive dose levels, both the specific activity and weighted activity are considered as below detection [8] (Table 3.17).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.17 The specific activity at a dose level 1 mg/plate and weighted activity in *S. typhimurium* TA98.

	Specific activity (Net his$^+$ revertants/mg extract)[a]		Weighted activity (Net his$^+$ revertants/g soil)	
Sample	+ S9	-S9	+ S9	- S9
M1	125	21	78	13
M2	93	54*	36	21*
M3	83	41*	32	16*
M4	BD[b]	BD	BD	BD
M5	16	BD	23	BD
M6	BD	BD	BD	BD
M7	95	BD	180	BD
S1	88	112	11	15
S2	75	39*	17	9*
S5	113	145	28	36
S6	70	BD	13	BD
S7	23	BD	28	BD
S8	125	BD	26	BD
S9	BD	BD	BD	BD
D2	88	34	18	7

[a] Spontaneous revertants have been subtracted already.

[b] BD = below detection.

* at higher dose levels, toxic effects were observed.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

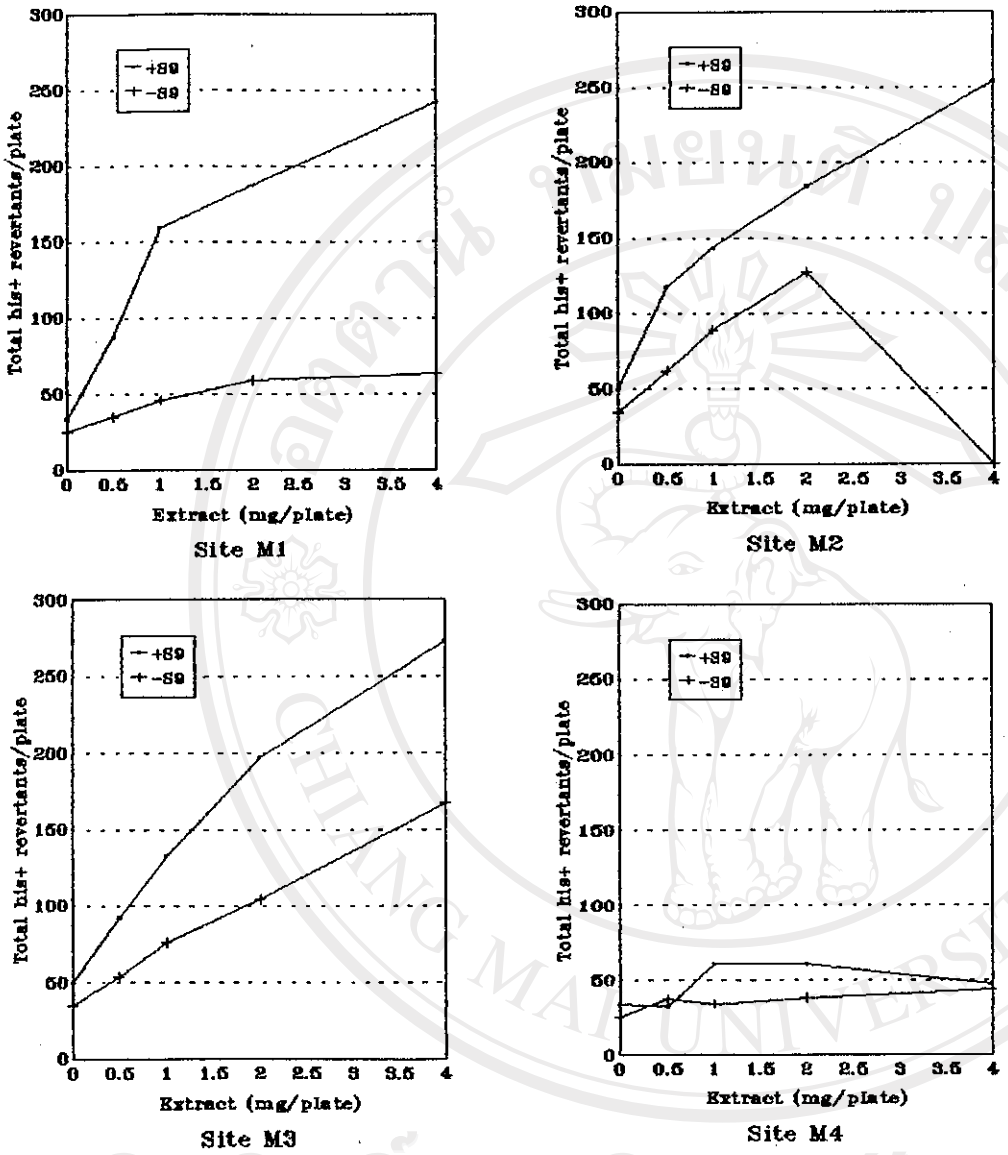


Fig. 3.6 Mutagenicity of soil extracts M1, M2, M3, and M4 in TA 98 with and without metabolic activation

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

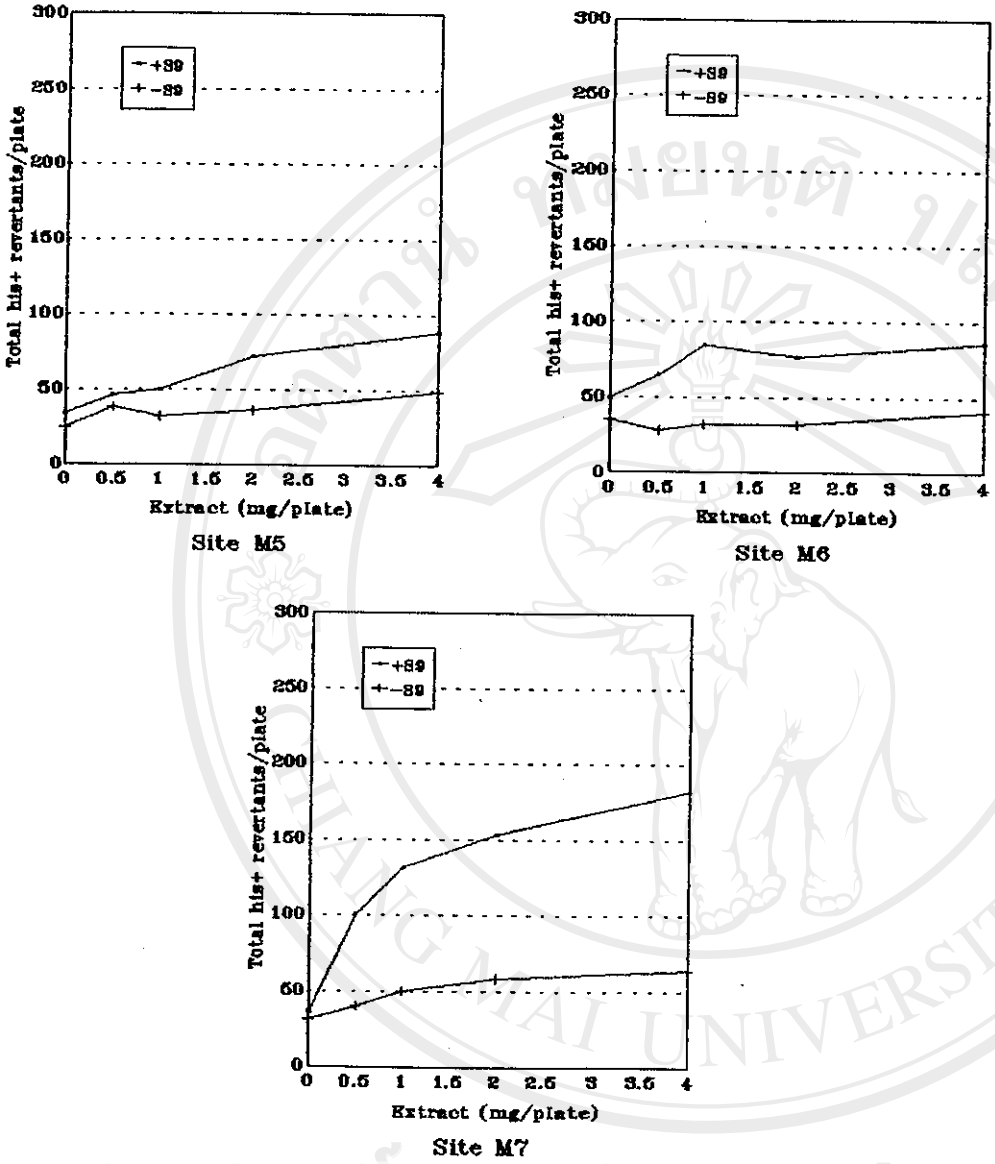


Fig. 3.7 Mutagenicity of soil extracts M5, M6, and M7 in TA 98 with and without metabolic activation

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

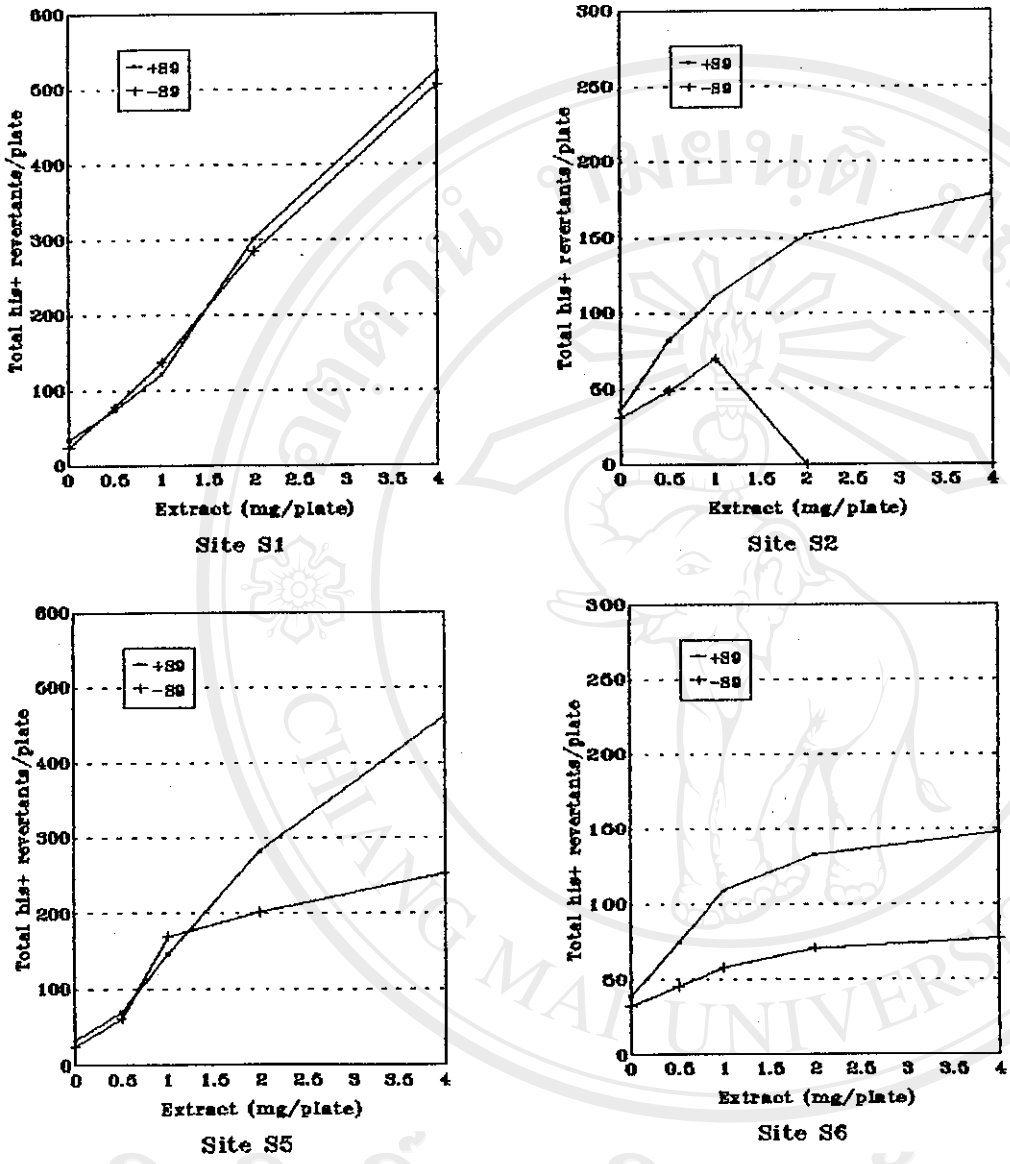


Fig. 3.8 Mutagenicity of soil extracts S1, S2, S5, and S6 in TA 98 with and without metabolic activation

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

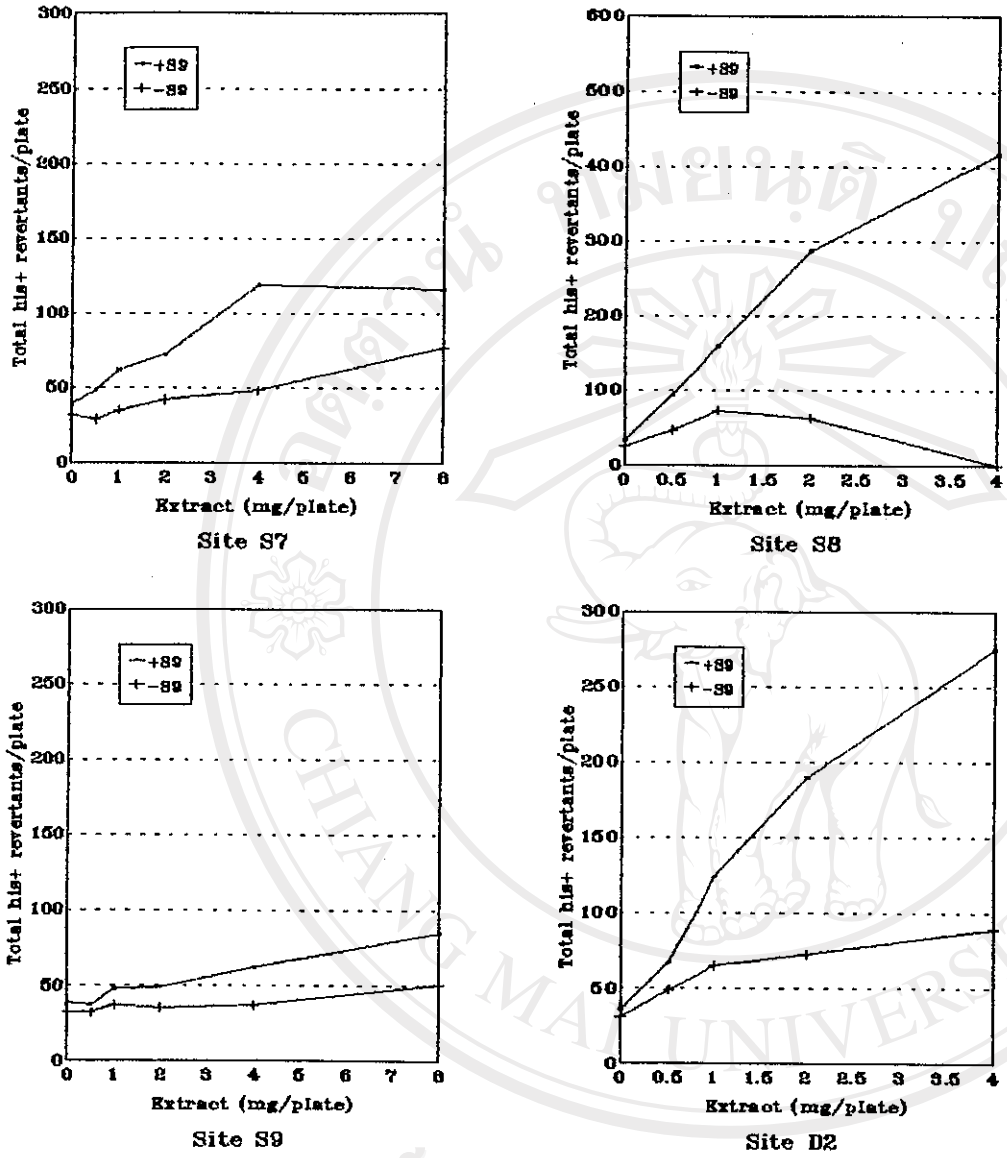
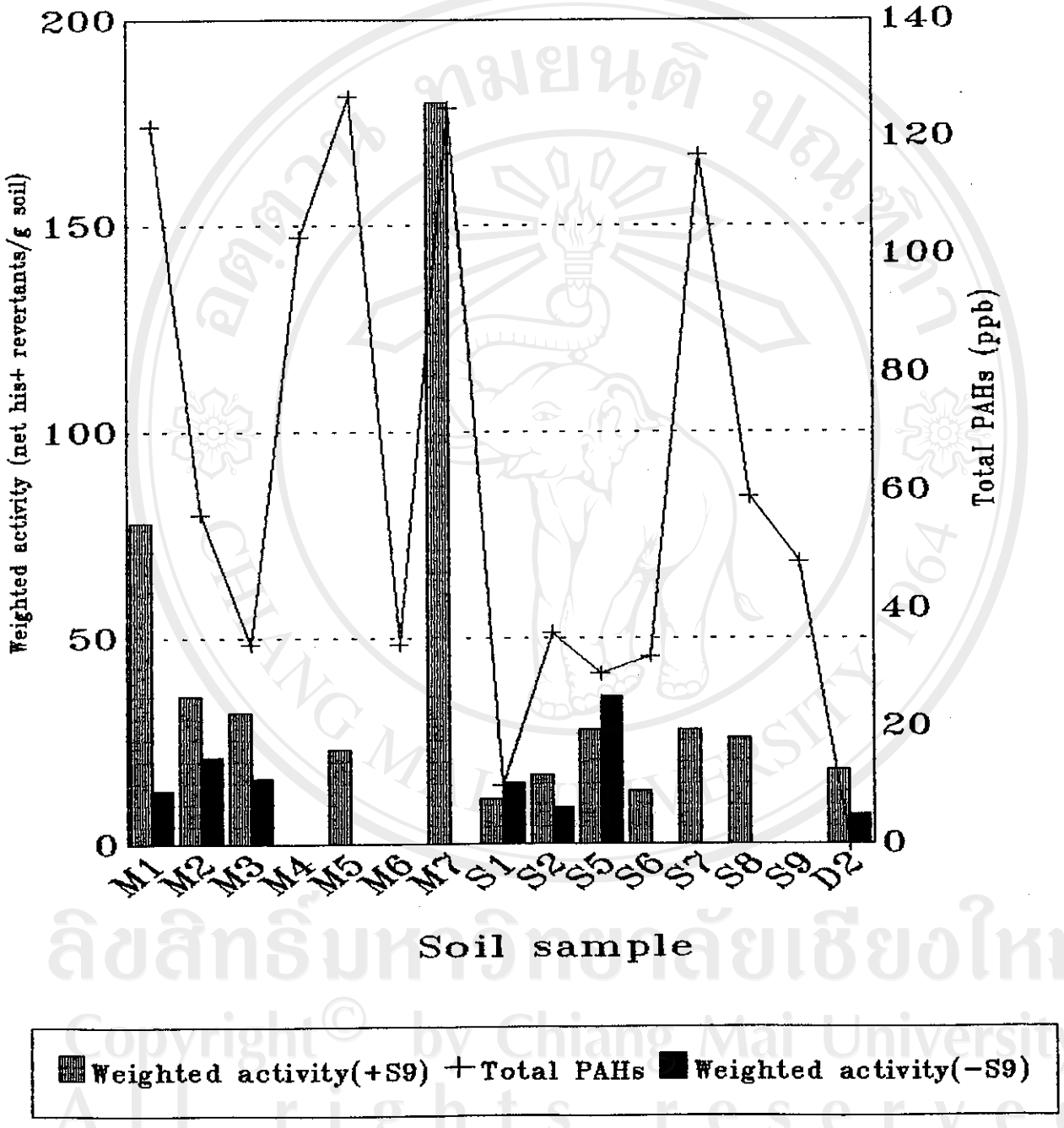


Fig. 3.9 Mutagenicity of soil extracts S7, S8, S9, and D2, in TA 98 with and without metabolic activation

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Fig. 3.10 Total PAHs and the weighted activity in TA 98

With metabolic activation, the specific activities at a dose level of 1 mg/plate of soil extracts from Muang District did not show higher values than those from Sarapee distict. Howerver, the weighted activities of soil from Muang were relatively high because more extractable organic matter in soils. Soil extracts from Sarapee induced more his^+ revertants without metabolic activation. This suggests more direct acting mutagens in soil extract. The relationship between total PAHs and weighted activity in *S. typhimurium* TA98 is showed in Fig. 3.10. With metabolic activation, the correlation efficient is 0.51 ($p = 0.05$); this indicates weak correlation between mutagenicity and PAH levels with metabolic activation. No correlation was demonstrated between total PAHs and weighted activity in TA98 without metabolic activation.

3.3.4 Mutagenicity of soil extracts in *S. typhimurium* TA100

From pre-study, the soil extracts were found not sensitive to TA100, therefore, at first, all the samples were tested at the lowest (0.5 mg/plate) and highest (4 mg/plate) dose levels for screening. If soil extract was likely to induce a positive response, the four dose levels were further tested for confirmationn. The screening test results are showed in Table 3.18.

In screening test, soil samples M2, S1, and S8 were likely to show mutagenic potential. Therefore, these samples were tested at dose levels of 0.5, 1.0, 2.0, and 4.0 mg/plate for further confirmation. M2 and S8 demonstrated positive response both with and without metabolic activation. S1 were toxic both with and without activation at 4 mg/plate, while S8 showed toxic effect only without activation (Table 3.19, Fig. 3.11). The specific activity and weighted activity are listed in Table 3.20.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.18 Screening of soil extracts in *S. typhimurium* TA100.

Soil	Dose (mg/plate)	His^+ revertants/plate[a] + S9	- S9	Soil	Dose (mg/plate)	His^+ revertants/plate + S9	- S9
M1	0.5	216	146	S1	0.5	198	190
	4.0	338	241		4.0	T[b]	T
M2	0.5	196	233	S2	0.5	170	160
	4.0	382	544		4.0	430	300
M3	0.5	221	191	S3	0.5	220	159
	4.0	301	180		4.0	317	196
M4	0.5	185	156	S4	0.5	151	138
	4.0	235	155		4.0	390	215
M5	0.5	201	132	S5	0.5	154	143
	4.0	279	199		4.0	176	140
M6	0.5	202	162	S6	0.04	144	127
	4.0	281	205		0.15	158	122
M7	5.0	268	164		0.5	231	NT[c]
	10.0	318	142		0.6	190	139
D1	0.5	170	124		4.0	306	NT
	4.0	172	172	S7	0.5	216	176
D2	0.5	187	178		4.0	261	221
	4.0	272	226	S8	0.5	314	T
					4.0	944	T
				S9	0.5	195	162
DMSO	50 µl	179	141		4.0	245	195

[a], average of one independent tests with 2 plate per dose level. Spontaneous revertants have not been subtracted yet.

[b] T = toxic effect.

[c] NT = not tested.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

Table 3.19 Mutagenicity of M2, S1, and S8 in *S. typhimurium* TA100.

Soil	Dose (mg/plate)	Total his$^+$ revertants/plate[a] + S9	- S9
M2	0	152 ± 30.9	130 ±18.7
	0.5	183 ± 14.2	213 ± 27.1
	1.0	274 ± 26.5	304 ± 36.6
	2.0	344 ± 47.2	367 ± 49.9
	4.0	401 ± 37.1	464 ± 58.1
S1	0	141 ± 30.8	128 ± 19.2
	0.5	215 ± 16.5	171 ± 22.2
	1.0	264 ± 17.5	272 ± 61.0
	2.0	342 ± 52.8	243 ± 14.5 (T)
	4.0	T[b]	T
S8	0	152 ± 30.9	130 ± 18.7
	0.5	310 ± 16.2	188 ± 29.7
	1.0	521 ± 38.8	324 ± 43.8
	2.0	684 ± 42.0	T
	4.0	872 ± 93.3	T

[a], [b], see Table 3.14.

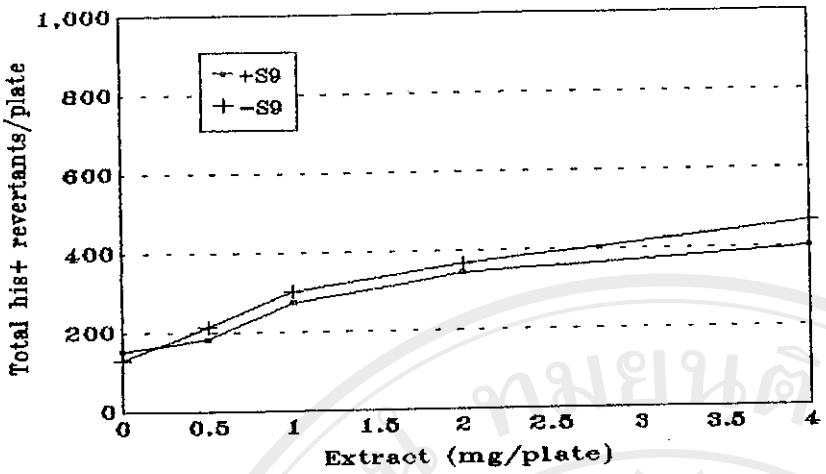
ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3.20 Specific activity at 1 mg/plate and weighted activity of M2, S1, and S8 in *S. typhimurium* TA100.

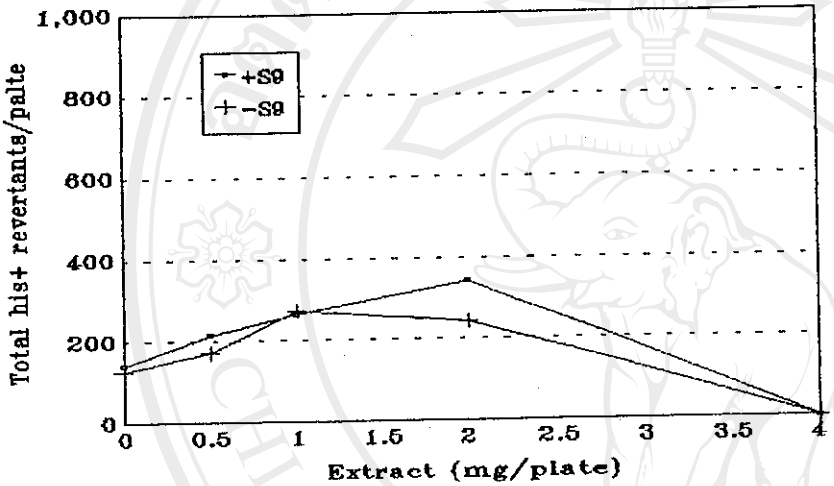
Sample	Specific activity (Net his$^+$ revertants/mg extract)[a]		Weighted activity (Net his$^+$ revertants/g soil)	
	+ S9	- S9	+ S9	- S9
M2	122	174	48	68
S1	BD	BD[b]	BD	BD
S8	369	194*	78	41*

[a], [b], *, see Table 3.17.

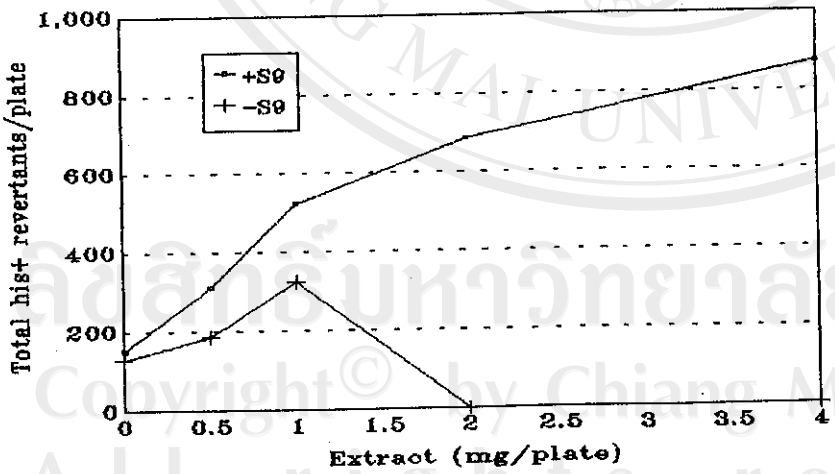
ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved



Site M2



Site S1



Site S8

Fig. 3. 11 Mutagenicity of soil extracts M2, S1, and S2 in TA100 with and without metabolic activation

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved