Chapter 4. Results

4. 1. Soil Analysis

Table 4.1 shows the results of soil parameter analysis.

Table 4.1. Some of the physical , chemical and biological parameters of soil analysis.

Parameter	ST	SS	VT	VS
pH	7.07	7.03	7.26	7.33
Moisture (%/g dry soil)	18.1	17.2	15.4	18.2
WHC (%/g dry soil)	40.51	36.23	41.82	43.81
O.M. (%/g dry soil)	2.71	2.51	3.87	4.02
% N	0.134	0.127	0.198	0.211
P (ppm)	70.0	87.5	62.5	95.0
K (ppm)	186.15	206.55	188.70	183.60
Texture analysis				
% sand	34.00	33.40	39.40	34.40
% silt	29.24	25.04	34.84	41.64
% clay	36.76	41.56	25.76	23.96
texture	Clay loam	Clay	Clay loam	Loam
Pour Plate Count				
$\times 10^4$ cfu / g dry soil	19	15	77	77

ST: Top 10 cm layer sample from soybean field;

SS: Sub 10 cm layer sample from soybean field;

VT: Top 10 cm layer sample from vegetable field;

VS: Sub 10 cm layer sample from vegetable field.

WHC: Water holding capacity;

O.M.: Organic matter content.

N: nitrogen; P: phosphorus; K: potassium.

The samples from vegetable field showed higher amount of organic matter content, so do the phosphorus and pour plate count results.

4.2. Enrichment , Isolation and Purification

During the first enrichment, the samples were withdrawn every 24 hours, the concentration of carbaryl in the medium are shown in table 4.2.

Table 4.2. The concentration of carbaryl in the enrichment medium. (mg/l)

Sample	Day1	Day2	Day3	Day4
Blank	16.5	21.8	20.3	21.9
ST	14.1	0.04	ND	ND
SS	14.0	21.6	2.48	ND
VT	19.8	20.1	1.60	ND
VS	15.7	22.1	20.7	0.04

ND: not detectable.

The concentration of carbaryl in the medium decreased in all the samples, however it remained more or less the same level in the control flask, which was not inoculated with soil. The same thing happened in the second enrichment, the carbaryl concentration decreased in all the samples, and was undetectable after 4 days. The medium was then serially diluted and spread on the nutrient agar plate. After two days, 7 different colonies appeared on the agar surface, among which the isolates 1-5 showed up in all the samples but isolates 6 and 7 appeared only in the samples taken

from both layers of the soybean field. The colony morphology was described in table 4.3.

Table 4.3. Colony characteristics on agar media

Isolate	Form	Elevation	Margin	Optical features	Chromogenesis
1	circular	convex	entire	opaque	pink
2	circular	raised	entire	opaque	yellow
3	circular	raised	entire	opaque	yellow in edge
4	irregular	umbonate	filamentous	translucent	dark gray
5	circular	convex	entire	opaque	white
6	irregular	flat	curled	translucent	white
7	irregular	raised	entire	opaque	gray and dark gray edge

The gram stain result showed that isolates 1 ,6 and 7 were gram positive rods, isolate 2 and 3 were gram positive cocci, isolate 4 was gram positive actinomycete like cells, and isolate 5 was gram negative short rod.

All the isolates were tested for their ability to degrade carbaryl, and the concentration of carbaryl decreased only in the flask which was inoculated with isolate 5, but maintained the same level after 4 days in the flasks inoculated with other isolates. The cultural characteristics of isolate 5 were checked again. It was found that the

characteristics of the colony varied with the incubating time. Table 4.4 shows the results.

Table 4.4. Colony characteristics of isolate 5.

1. Colonies on agar media		
	1-6 days	after 6 days
Form	spindle	circular
Elevation	convex	umbonate
Margin	entire	lobate
Optical features	opaque	opaque in the middle, translucent on edge
2. Growth on agar slant		
Amount	moderate	
Form	filiform	
Consistency	butyrous	beads on the surface
Chromogenesis	white	gray
3. Growth in nutrient broth		
Surface	ring	
Subsurface	turbid, cloudy with flocculent particles	
Amount	moderate	
Sediment	flocculent	

[The characteristics was described according to the Microbiology (Pelczar and Reid,1958)]

4.3. Degradation Kinetics

Table 4.5 shows the absorbance which represented the growth of bacteria and the concentration of carbaryl in the medium at different time intervals in the control flasks.

Table 4.5. The absorbance and concentration of carbaryl in the medium in the control flasks.

Time (hr.)	Control 1		Control 2
	Ab.	Conc.	Ab.
0	0.017	-	0.013
6	-	-	0.014
12	0.009	10.3	0.008
18	-	-	0.008
24	0.008	9.97	0.012
48	0.003	10.8	0.015
72	-	10.9	-

Ab. : absorbance at 600 nm. - : not measured.

Conc. : concentration of carbaryl in the medium (mg/l).

Control 1: the minimum mineral medium with carbaryl but no inoculum.

Control 2: the minimum mineral medium with acetonitrile and inoculum but without carbaryl.

The results from the control 1 flask showed no degradation of carbaryl when there is no microorganisms in the medium. The possibility of contamination was also eliminated. The results from the control 2 flask showed that the microorganisms were not able grow in the presence of with acetonitrile only, the possibility that they might use acetonitrile as carbon source was eliminated.

Table 4.6 shows the absorbance and carbaryl concentration change in the minimum mineral medium with carbaryl (MMM) and inoculum.

Table 4. 6. The absorbance and carbaryl concentration in the medium with carbaryl and inoculum.

Time (hr.)	MMM1		MMM2	
	Ab.	Conc.	Ab.	Conc.
0	0.104	-	0.104	-
3	0.135	-	0.094	-
6	0.154	8.48	0.125	10.2
9	0.160	-	0.109	-
12	0.269	8.26	0.141	8.96
15	0.402	7.96	0.179	8.30
18	0.796	7.58	0.429	8.51
21	1.201	6.97	0.779	8.37
24	1.359	4.78	1.179	8.14
30	1.545	2.14	1.483	4.41
48	1.567	0.07	1.794	1.46
72	ND	ND	ND	ND

MMM1 and MMM2: duplicate 1 and 2.

ND : not detectable.

- : not measured.

After 48 hours, the turbidity of the medium already exceeded the detecting range (0-2) and no peaks of carbaryl were identified from the chromatogram at 72 hour.

The pattern of growth and degradation is shown in Fig 4.1 (a and b). The patterns are similar in the duplicate flasks.

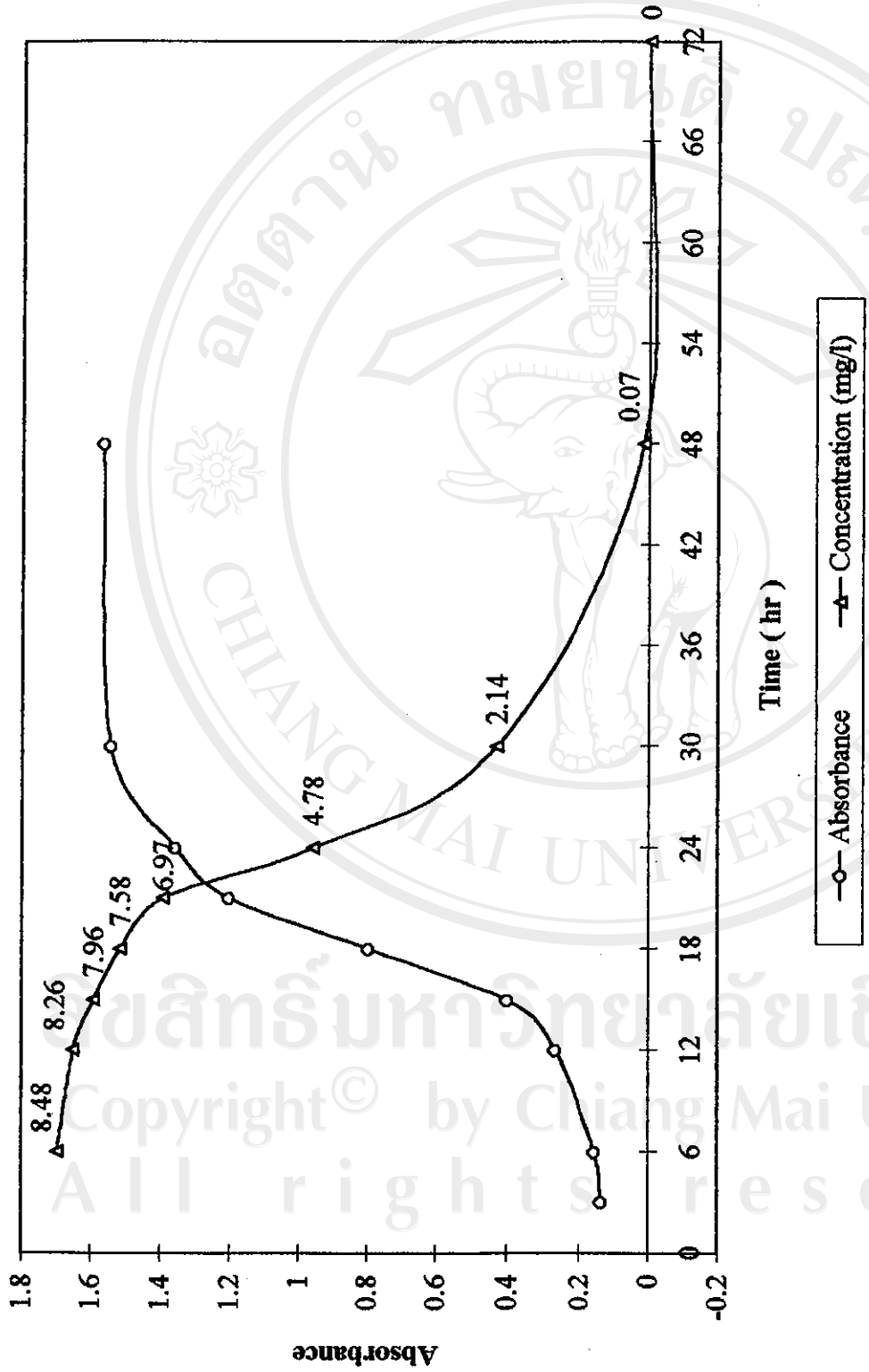

Fig 4.1.a. Bacterial growth and carbaryl degradation in the minimum mineral medium.

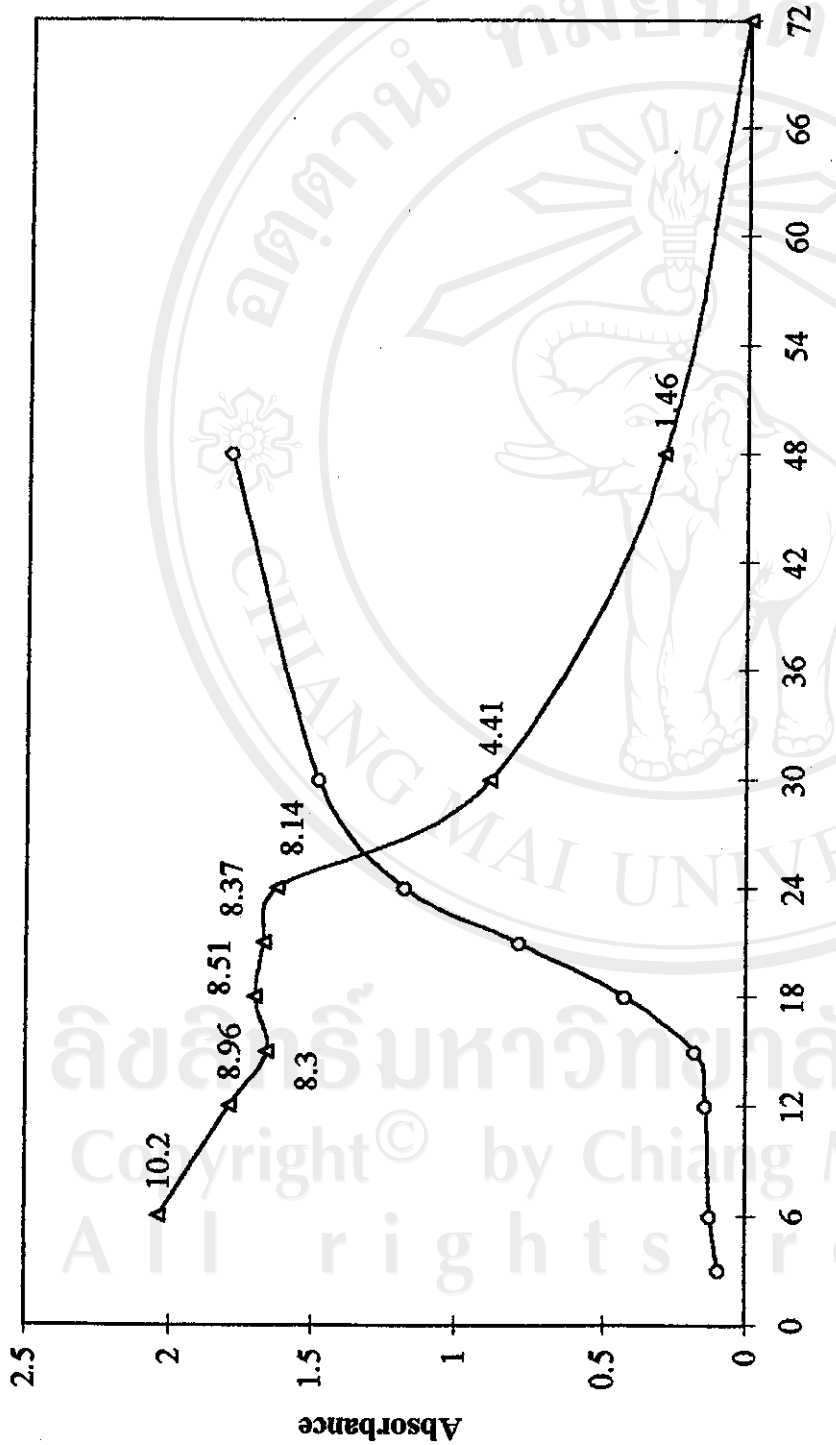

Fig 4.1.b. Bacterial growth and carbaryl degradation in the minimum mineral medium.

4.4. Degradation Kinetics in the Presence of Glucose, Sucrose and Starch

Tables 4.7, 4.8 and 4.9 show the change of absorbance and concentration of carbaryl with time in the presence of 1 % glucose, sucrose and starch respectively. The control flasks are the same as mentioned in 4.3. The pattern of degradation are shown in Figs 4.2, 4.3 and 4.4. respectively.

Table 4.7. The absorbance and carbaryl concentration (mg/l) in the presence of glucose.

Time (hr.)	G1		G2	
	Ab.	Conc.	Ab.	Conc.
0	0.146	-	0.146	-
3	0.075	-	0.111	-
6	0.120	9.18	0.160	9.48
9	0.128	-	0.175	-
12	0.247	9.20	0.279	9.50
15	0.895	9.15	0.745	9.46
18	1.545	9.34	1.216	9.50
21	1.790	9.24	1.543	9.34
24	1.823	9.34	1.644	9.47
30	1.955	8.57	1.675	8.80
48	ND	8.72	1.849	8.88
72	ND	8.61	ND	8.93

G1 and G2: duplicate 1 and 2 .

ND: not detectable. - : not measured.

The growth continued up to 48 hours but already exceeded the detecting range (0-2), and after 72 hours, the carbaryl remaining in the medium was still high in both duplicate flasks.

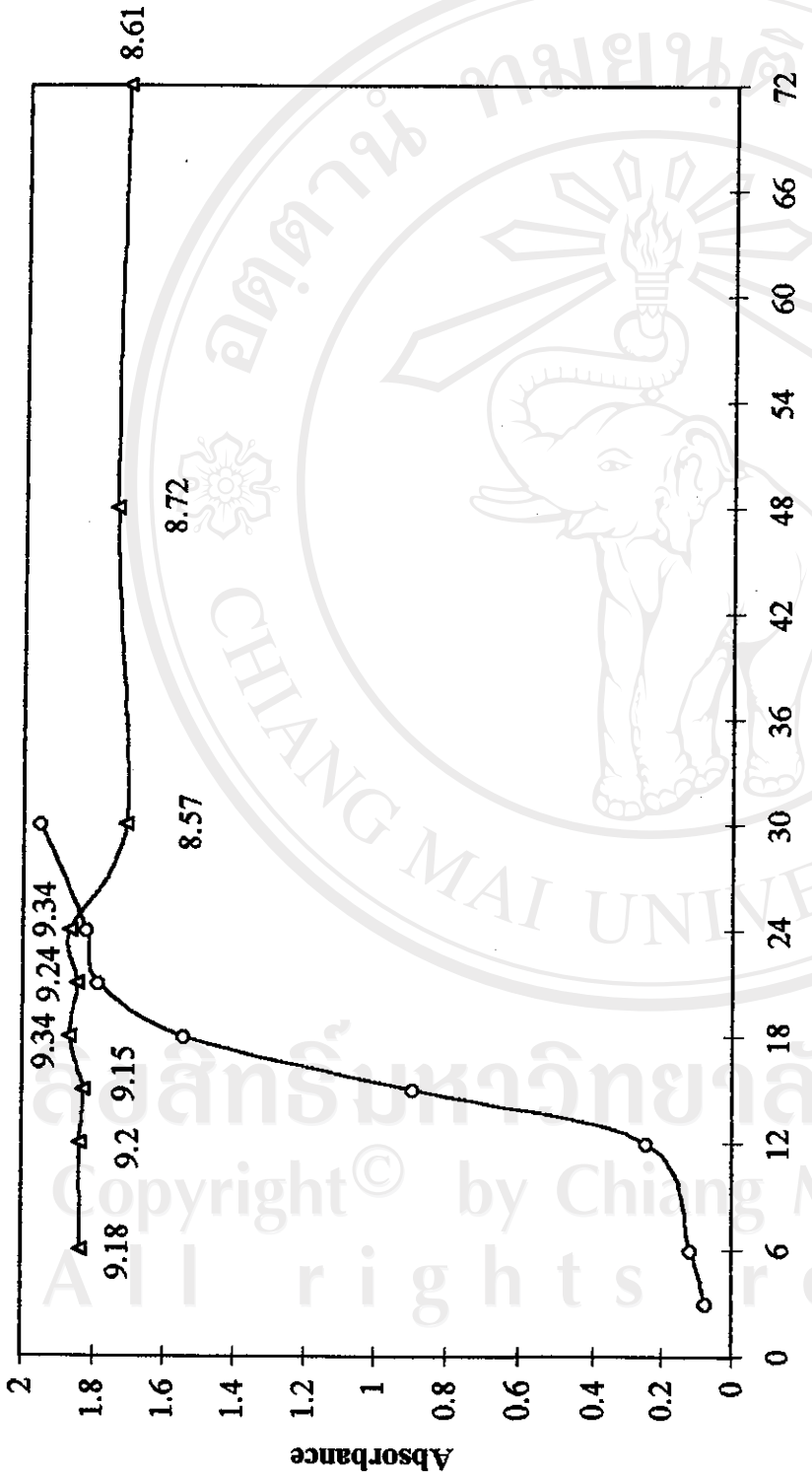

Fig 4.2.a. Bacterial growth and carbaryl degradation in the presence of glucose.

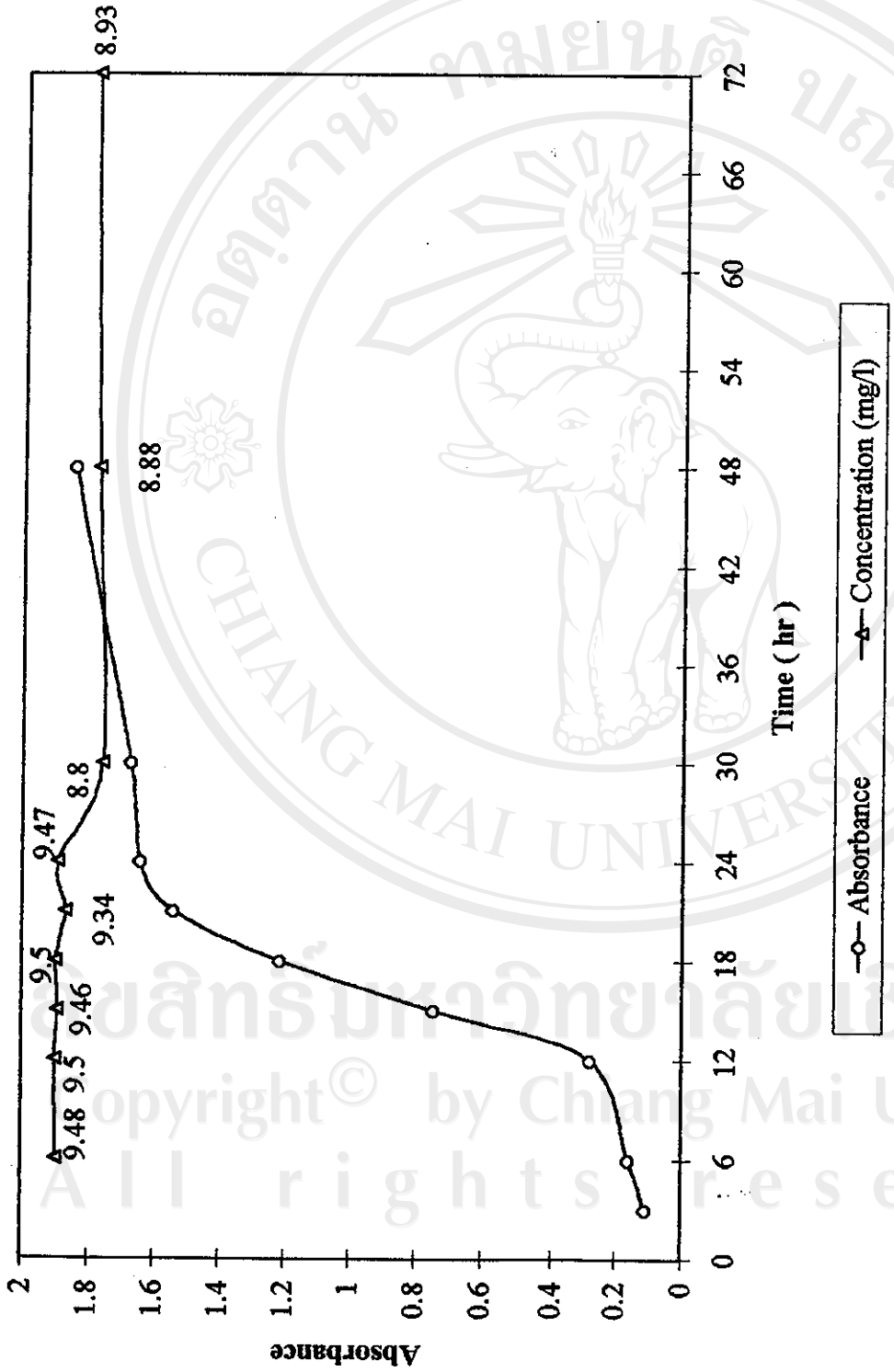

Fig 4.2. b. Bacterial growth and carbaryl degradation in the presence of glucose.

Table 4. 8. The absorbance and carbaryl concentration (mg/l) in the presence of sucrose.

Time (hr.)	Su1		Su2	
	Ab.	Conc.	Ab.	Conc.
0	0.036	-	0.036	-
3	0.145	-	0.098	-
6	0.300	9.16	0.134	9.42
9	0.377	-	0.195	-
12	0.416	9.04	0.257	9.12
15	0.503	8.52	0.368	8.92
18	0.690	8.52	0.487	9.00
21	1.321	8.70	0.938	8.34
24	1.540	7.54	1.154	8.46
30	1.815	5.06	1.440	7.13
48	ND	1.52	ND	6.32
72	ND	0.01	ND	4.06

Su1 and Su2: duplicate 1 and 2.

Table 4.9. The absorbance and carbaryl concentration (mg/l) in the presence of starch.

Time (hr.)	St1		St2	
	Ab.	Conc.	Ab.	Conc.
0	0.101	-	0.101	-
3	0.132	-	0.110	-
6	0.176	10.2	0.150	10.7
9	0.179	-	0.158	-
12	0.179	10.6	0.187	10.5
15	0.273	10.5	0.336	8.83
18	0.561	10.1	0.532	10.3
21	0.786	10.3	0.948	11.0
24	0.931	9.90	1.068	9.20
30	1.103	8.60	1.220	8.51
48	1.229	0.42	1.401	0.74
72	ND	ND	ND	ND

St1 and St2: duplicate 1 and 2.

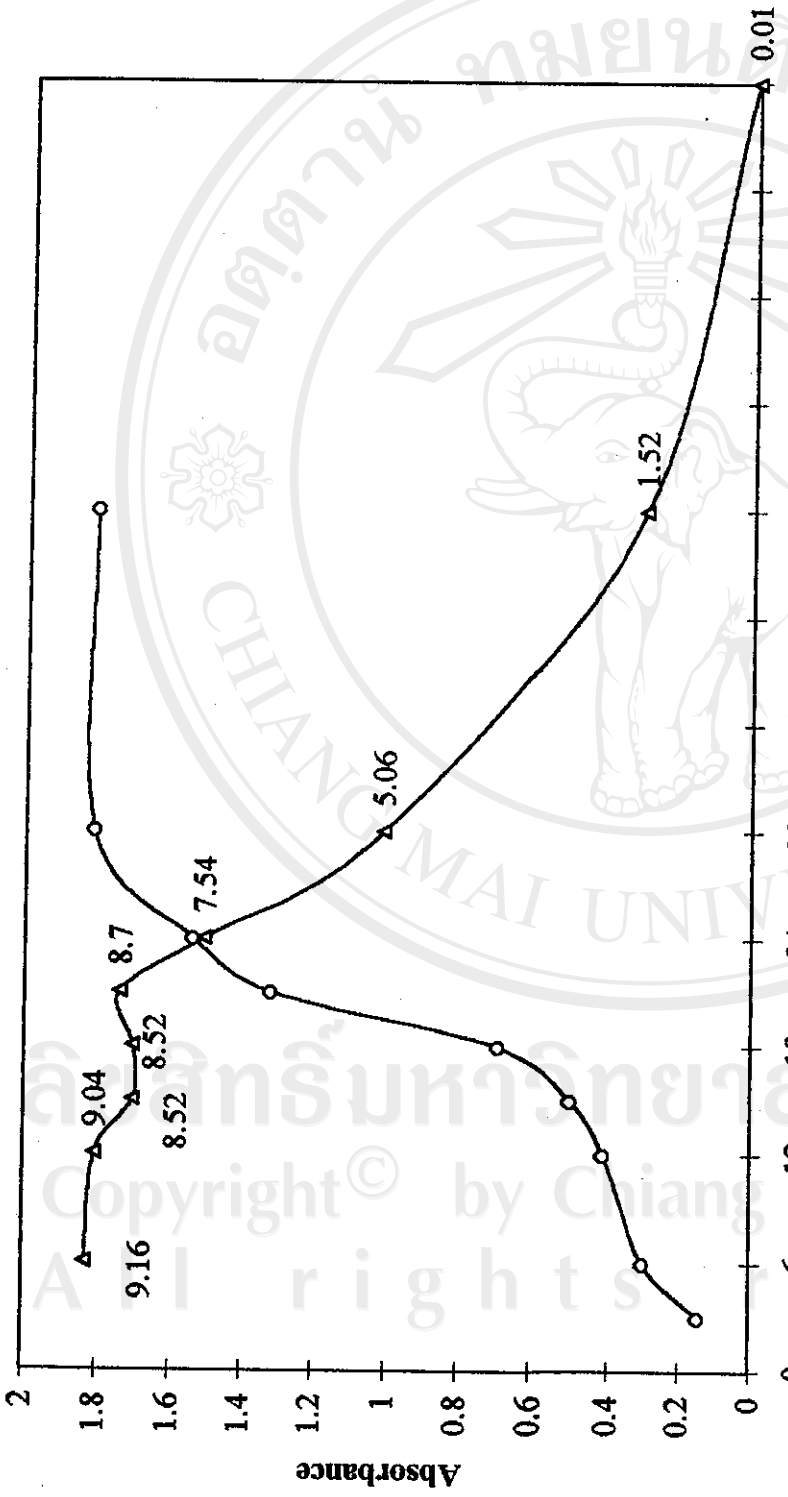

Fig 4.3.a. Bacterial growth and carbaryl degradation in the presence of sucrose.

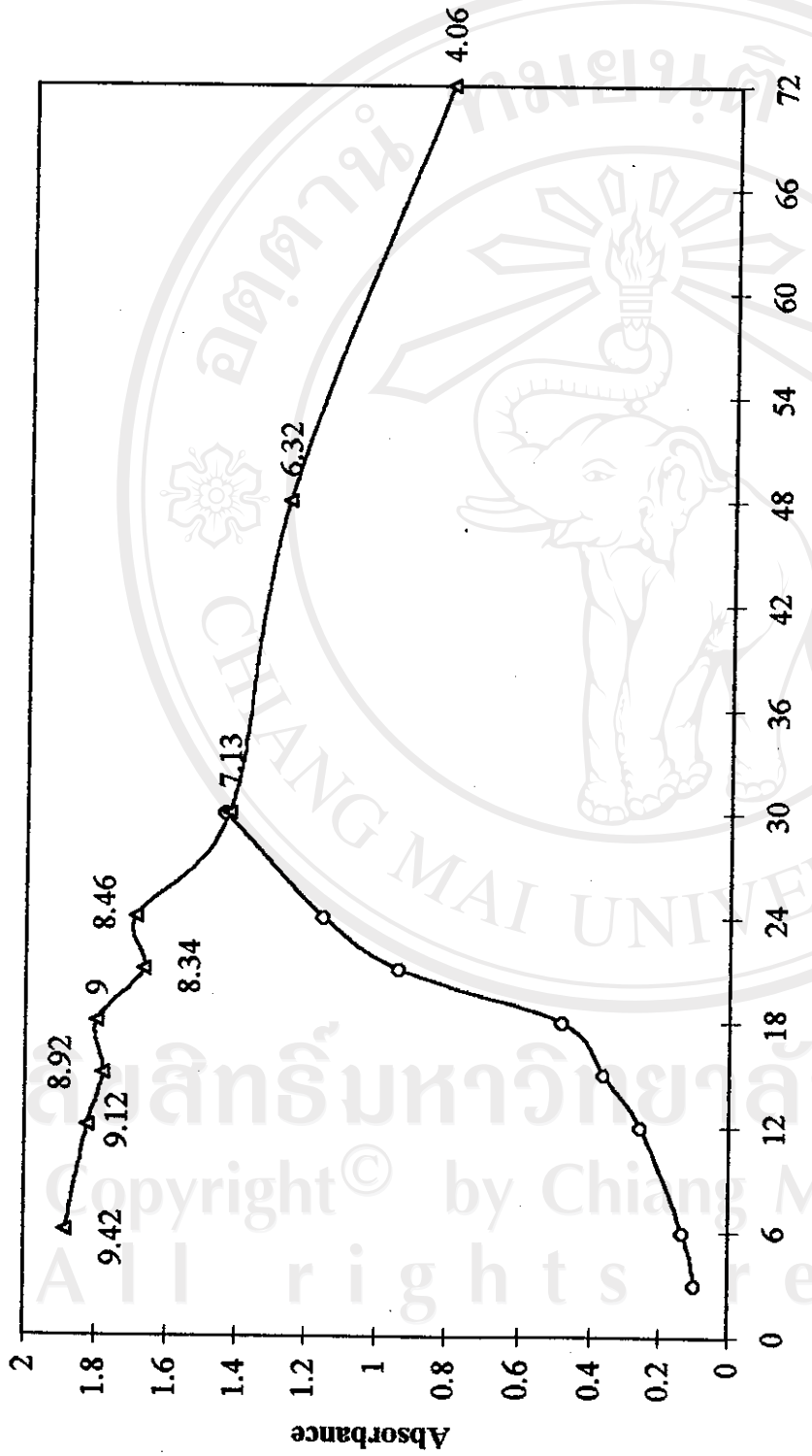

Fig 4.3.b. Bacterial growth and carbaryl degradation in the presence of sucrose.

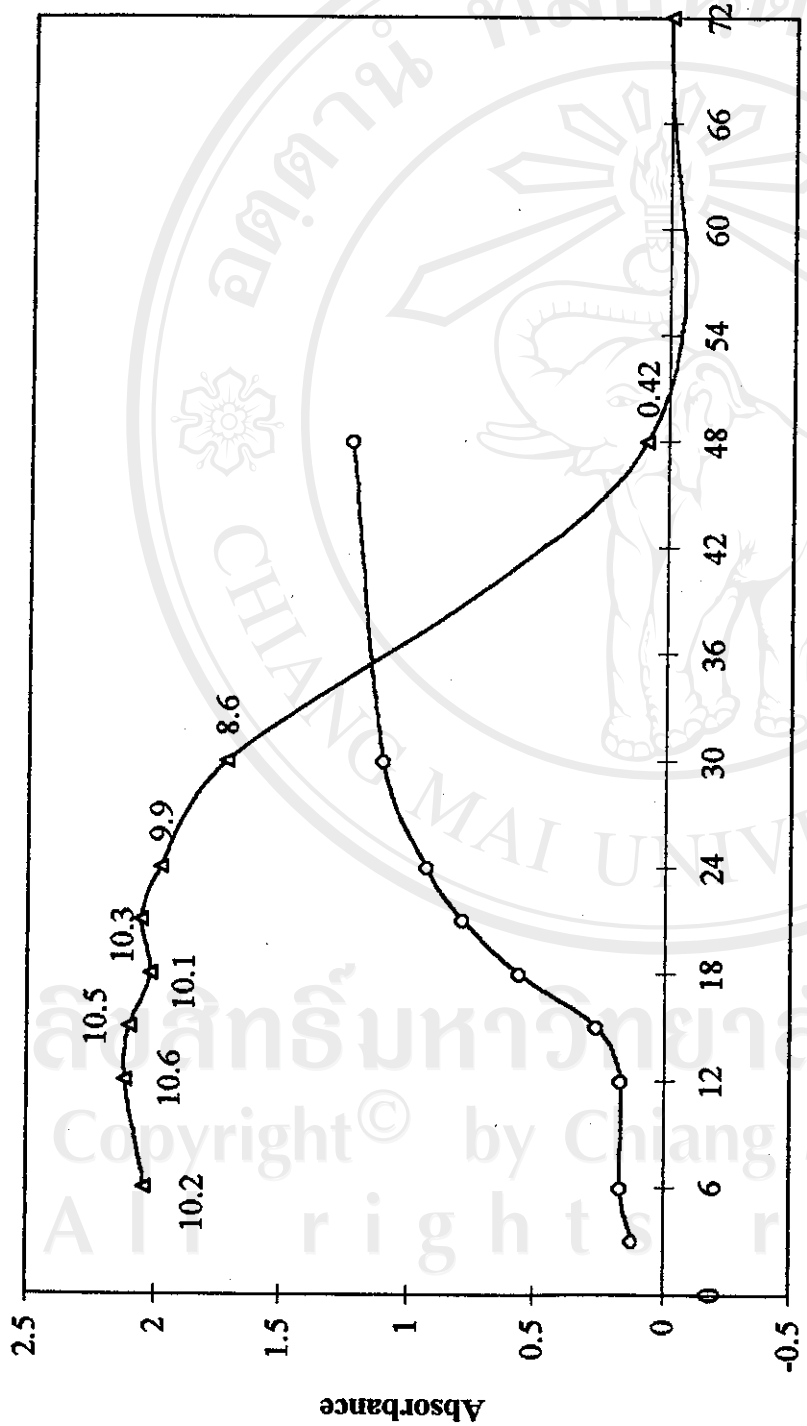

Fig 4.3.a. Bacterial growth and carbaryl degradation in the presence of starch.

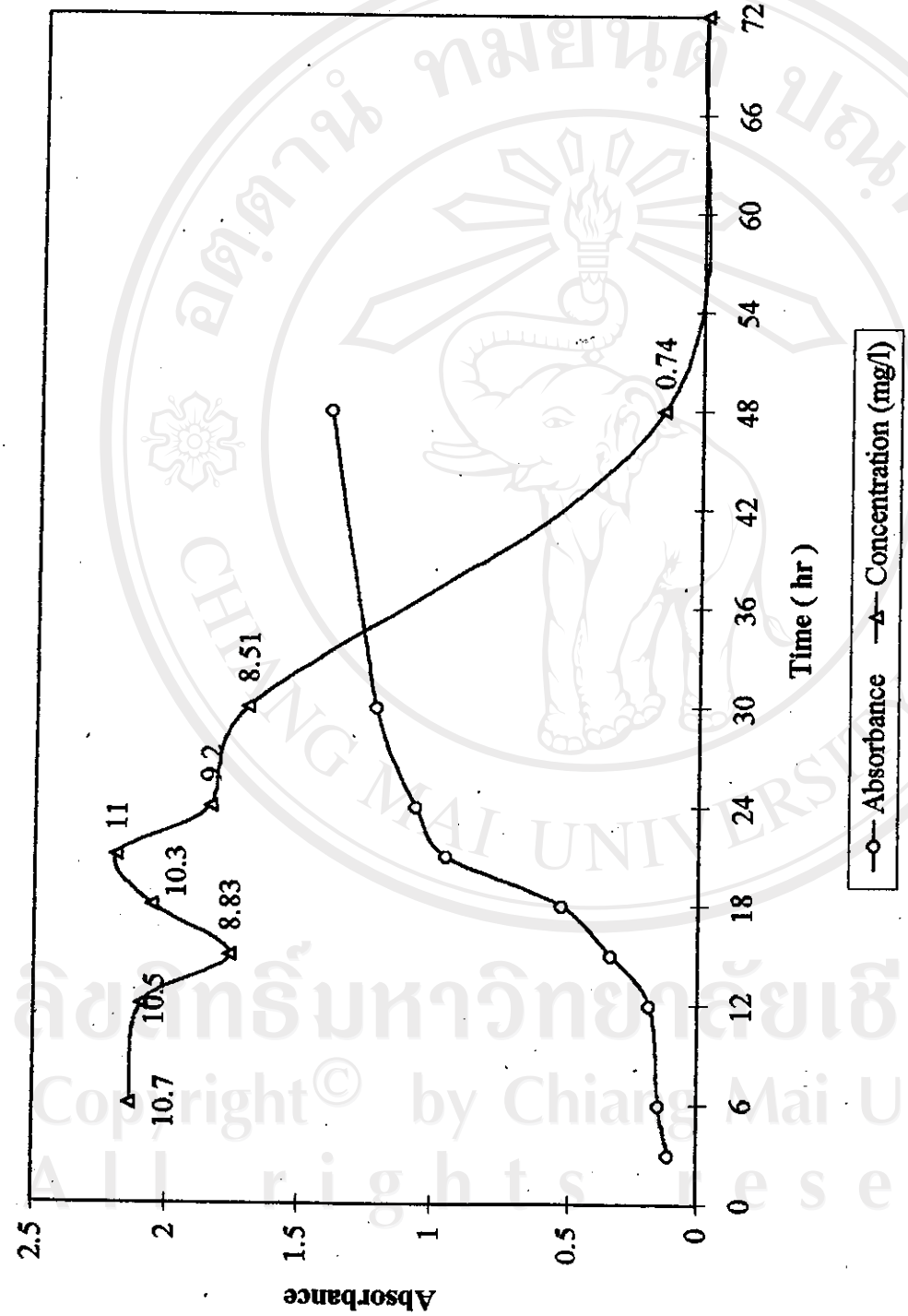

Fig 4.3.b. Bacterial growth and carbaryl degradation in the presence of starch.

4.5 Degradation of Carbaryl in the Presence of Herbicide Paraquat

Table 4.10 shows the concentration of carbaryl in the minimum mineral medium before and after 72 hours incubation in the presence of different concentrations of paraquat. The remaining carbaryl in the medium was expressed as the percentage of the initial concentration. The control flasks were done without the addition of paraquat into the medium. The degradation was completed when the paraquat was present in low concentration, but remained considerably high in the presence of high concentration of paraquat. During the experiment it was found after 1 day of incubation, the medium containing paraquat was less turbid than the ones without paraquat, and it was less turbid in the medium containing high amount of paraquat than the ones containing lower amount of paraquat after 48 hours incubation.

Table 4.10. The concentration of carbaryl in the presence of different concentrations of paraquat. (mg/l)

Conc.	Control		Paraquat 1ppm		Paraquat 10ppm		Paraquat 100ppm	
	1	2	1	2	1	2	1	2
Initial	9.32	13.90	10.38	14.43	9.99	13.28	10.28	14.59
Final	ND	0.01	ND	ND	1.64	2.28	8.97	6.14
%	0.0	0.0	0.0	0.0	16.4	17.2	87.3	42.0

Conc. : Concentration of carbaryl. (mg/l)

% : percent of carbaryl remaining in the medium.

ND: not detectable.

4.6. HPLC Analysis

Figs 4.5. a, b, and c show the chromatogram of carbaryl standard (1 mg/l), blank sample (medium without inoculum) and sample (medium with inoculum) respectively.

The first calibration curve ranges from 0.1000 to 2.0000 μg/ml, and the calibration equation reported from the instrument is:

Conc.= -2.429976E-2 + 2.475229E-6 * R

R: response of peak area (microvolts).

Correlation coefficient is 0.9996393.

The second calibration curve ranges from 0.0200 to 0.1000 μg/ml. The calibration equation reported from the instrument is:

Conc. = -6.722063E-03 + 2.190259E-6 * R

R: response of peak area (microvolts).

Correlation coefficient is 0.9987530.

Table 4.11 shows the result of the confirmation test. The results confirmed that the peak in the chromatogram of sample which was 0.05min later than the carbaryl standard peak was the carbaryl in the medium. The difference between the retention time was due to the fact that the carbaryl standard was dissolved in the acetonitrile while the sample was mainly water based solution.

Table 4.11. Carbaryl peak data at different wavelengths.

Wavelength	Retention time		Peak height		Peak area	
	standard	sample	standard	sample	standard	sample
220 nm	6.458	6.500	30297	26593	428613	319982
270 nm	6.458	6.517	2438	2114	32974	22238
Ratio (220 /270)	1	0.997	12.43	12.58	13.00	14.39

Fig 4.5.a. Chromatogram of carbaryl standard.

Fig 4.5.b. Chromatogram of blank.

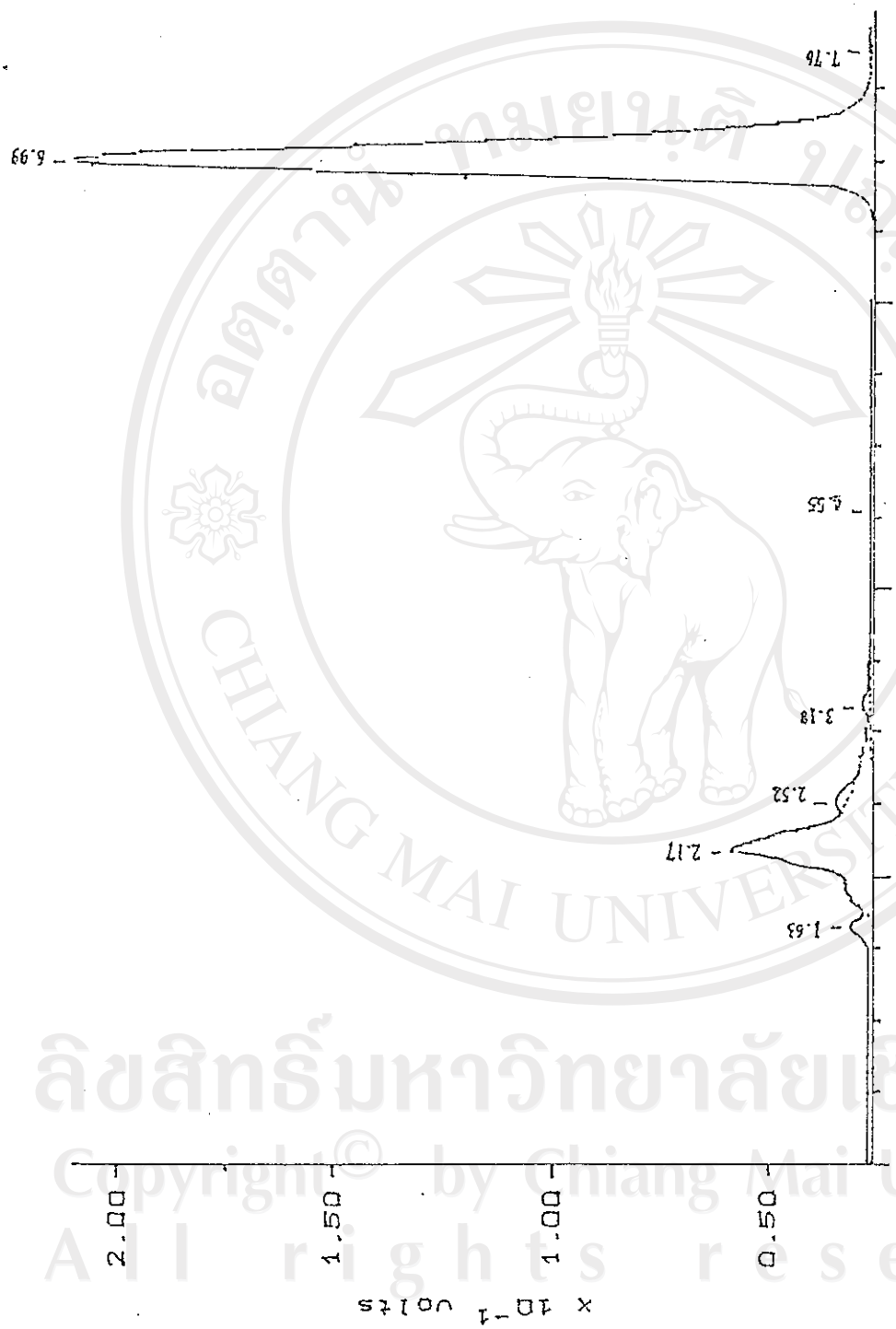

Fig 4.5.c. Chromatogram of sample.