CHAPTER 3

SAMPLES AND SAMPLE PREPARATION

This chapter describes how the samples for the palynological study in the field investigation were collected. The descriptions include where the samples came from. After that, chemical treatments to extract sporomorphs from the samples collected as well as chemical procedure for the modern pollen preparation are explained step by step. Subsequently, slide making, stub preparation, and microscopy are described.

3.1 SAMPLE COLLECTING

Over three hundred samples were collected from outcrops in the mine quarries from the five basins. The samples were mostly greenish to dark gray to dark brown gray oil shale and claystone, and coal. However, brownish gray shale to silty shale were also collected where some parts of outcrops are commonly silty and sandy. Each bed within the stratigraphic succession was described and the stratigraphic levels of the samples collected were determined by using measuring tape. Each sample was collected in such a manner to avoid as much as possible contamination from other samples and modern pollen and spores. Trenching to expose a fresh surface was always done before collecting a sample. The outer outcrop surface was always removed in order to obtain fresh inner material. Packing in a plastic bag, sealing, and labelling with the necessary information was always done in the same manner for the each sample including sample number, location, and date of sampling.

3.1.1 Li basin

Two coalfields in the Li basins were chosen for this study namely the Ban Pa Kha coalfield and Na Sai coalfield. Each coalfield provided different information in term of details in sample collecting.

3.1.1.1 Ban Pa Kha coalfield

The Tertiary sediments in Ban Pa Kha coalfield, including almost the whole sequence from the lowermost part up to the uppermost part of the Ban Pa Kha Formation excepted the underburden unit, are exposed. Sample collection was available from the lower coal zone up to the overburden unit with a brief description in Table 3-1 and sample locations are shown in Figure 3-1. The samples from the lower coal zone were encoded by using LS for samples obtained from the lower split coal sub-unit and using LM for samples obtained from the lower massive coal sub-unit. The samples from the lower split coal sub-unit included both claystone and coal from sample number LS-1 to LS-9 whilst the samples from the lower massive coal sub-unit were only coals from sample number LM-1 to LM-3. The samples from the interburden unit were from the lower half of the unit comprising oil shale and claystone, and include nine samples coded IB from IB-1 to IB-9. The nine samples were oil shale in the lower part and gradually changed upward to claystone and capped by white sandstone. The white sandstone is barren of sporomorphs and was not collected in this study. The twenty-nine samples from the upper coal zone were only coals, represented by the code "U" as sample numbers from U-1 to U-29. The samples from the overburden unit were coded into two different codes, OB representing oil shale and claystone in the lower part, and OU representing sandstone in the upper part including two samples from the thin coal layer. The samples from the OU part were normally from thin lens of claystone or sandy claystone. The upper part of the overburden was not collected for technical reasons.

Table 3-1 Description of samples collected from Ban Pa Kha coalfield.

Sample No.	Distance from previous sample (metre)	Sample Type	Colour	Description	Remarks
OU-26	25.0	coal	black	silty to sandy	Silty to sandy is typical
OU-25	24.0	coal	black	silty to sandy	characteristic of samples
OU-24	23.0	claystone	pale gray	silty to sandy	encoded as OU.
OU-23	22.0	claystone	greenish gray	silty to sandy	
OU-22	21.0	claystone	greenish gray	silty to sandy	
OU-21	20.0	claystone	greenish gray	silty to sandy	
OU-20	19.0	claystone	greenish gray	silty to sandy	
OU-19	18.0	claystone	greenish gray	silty to sandy	
OU-18	17.0	claystone	greenish gray	silty to sandy	
OU-17	16.0	claystone	greenish gray	silty to sandy	
OU-16	15.0	claystone	greenish gray	silty to sandy	
OU-15	14.0	claystone	greenish gray	silty to sandy	
OU-14	13.0	claystone	greenish gray	silty to sandy	
OU-13	12.0	claystone	greenish gray	silty to sandy	
OU-12	11.0	claystone	greenish gray	silty to sandy	
OU-11	10.0	claystone	greenish gray	silty to sandy	
OU-10	9.0	claystone	greenish gray	silty to sandy	
OU-9	8.0	claystone	greenish gray	silty to sandy	
OU-8	7.0	claystone	greenish gray	silty to sandy	
OU-7	6.0	claystone	greenish gray	silty to sandy	
OU-6	5.0	claystone	greenish gray	silty to sandy	
OU-5	4.0	claystone	greenish gray	silty to sandy	
OU-4	3.0	claystone	greenish gray	silty to sandy	
OU-3	2.0	claystone	greenish gray	silty to sandy	
OU-2	1.0	claystone	greenish gray	silty to sandy	
OU-1	0.0	claystone	greenish gray	silty to sandy	
OB-17	16.0	claystone	brownish gray		Samples encoded as OB
OB-16	15.0	claystone	brownish gray		are gradation from oil shale
OB-15	14.0	claystone	brownish gray		to claystone from base to
OB-14	13.0	claystone	brownish gray		top.
OB-13	12.0	claystone	brownish gray		
OB-12	11.0	claystone	brownish gray		
OB-11	10.0	oil shale	brownish gray		
OB-10	9.0	oil shale	brownish gray		
OB-9	8.0	oil shale	brownish gray		
OB-8	7.0	oil shale	brownish gray		
OB-7	6.0	oil shale	brownish gray		
OB-6	5.0	oil shale	brownish gray		
OB-5	4.0	oil shale	brownish gray		
OB-4	3.0	oil shale	brownish gray		
OB-3	2.0	oil shale	brownish gray		
OB-2	1.0	oil shale	brownish gray		
OB-1	0.0	oil shale	brownish gray		
U-29	28.0	coal	black		Samples encoded as U
U-28	27.0	coal	black		are from main coal seam.
U-27	26.0	coal	black		
U-26	25.0	coal	black		
U-25	24.0	coal	black		
U-24	23.0	coal	black		
U-23	22.0	coal	black		
U-22	21.0	coal	black		
U-21	20.0	coal	black		

Table 3-1 Continue.

Sample No.	Distance from previous sample (metre)	Sample Type	Colour	Description	Remarks
U-20	19.0	coal	black		
U-19	18.0	coal	black		
U-18	17.0	coal	black		
U-17	16.0	coal	black		
U-16	15.0	coal	black		
U-15	14.0	coal	black		
U-14	13.0	coal	black		
U-13	12.0	coal	black		
U-12	11.0	coal	black		
U-11	10.0	coal	black		
U-10	9.0	coal	black		
U-9	8.0	coal	black		
U-8	7.0	coal	black		
U-7	6.0	coal	black		
U-6	5.0	coal	black		
U-5	4.0	coal	black		
U-4	3.0	coal	black		
U-3	2.0	coal	black		
U-2	1.0	coal	black		
U-1	0.0	coal	black		
IB-9	8.0	claystone	yellowish brown		Samples encoded as IB
IB-8	7.0	claystone	yellowish brown		are gradation from oil shale
IB-7	6.0	claystone	yellowish brown		to claystone from base to
IB-6	5.0	claystone	yellowish brown		top.
IB-5	4.0	oil shale	brownish gray		
IB-4	3.0	oil shale	brownish gray		
IB-3	2.0	oil shale	brownish gray		
IB-2	1.0	oil shale	brownish gray		
IB-1	0.0	oil shale	brownish gray		
LM-3	3.0	coal	black		
LM-2	1.5	coal	black		
LM-1	0.0	coal	black		
LS-9	8.0	claystone	brownish gray		
LS-8	7.0	coal	black		
LS-7	6.0	claystone	brownish gray		
LS-6	5.0	coal	black		
LS-5	4.0	claystone	brownish gray		
LS-4	3.0	coal	black		
LS-3	2.0	claystone	brownish gray		
LS-2	1.0	coal	black		
LS-1	0.0	claystone	brownish gray		

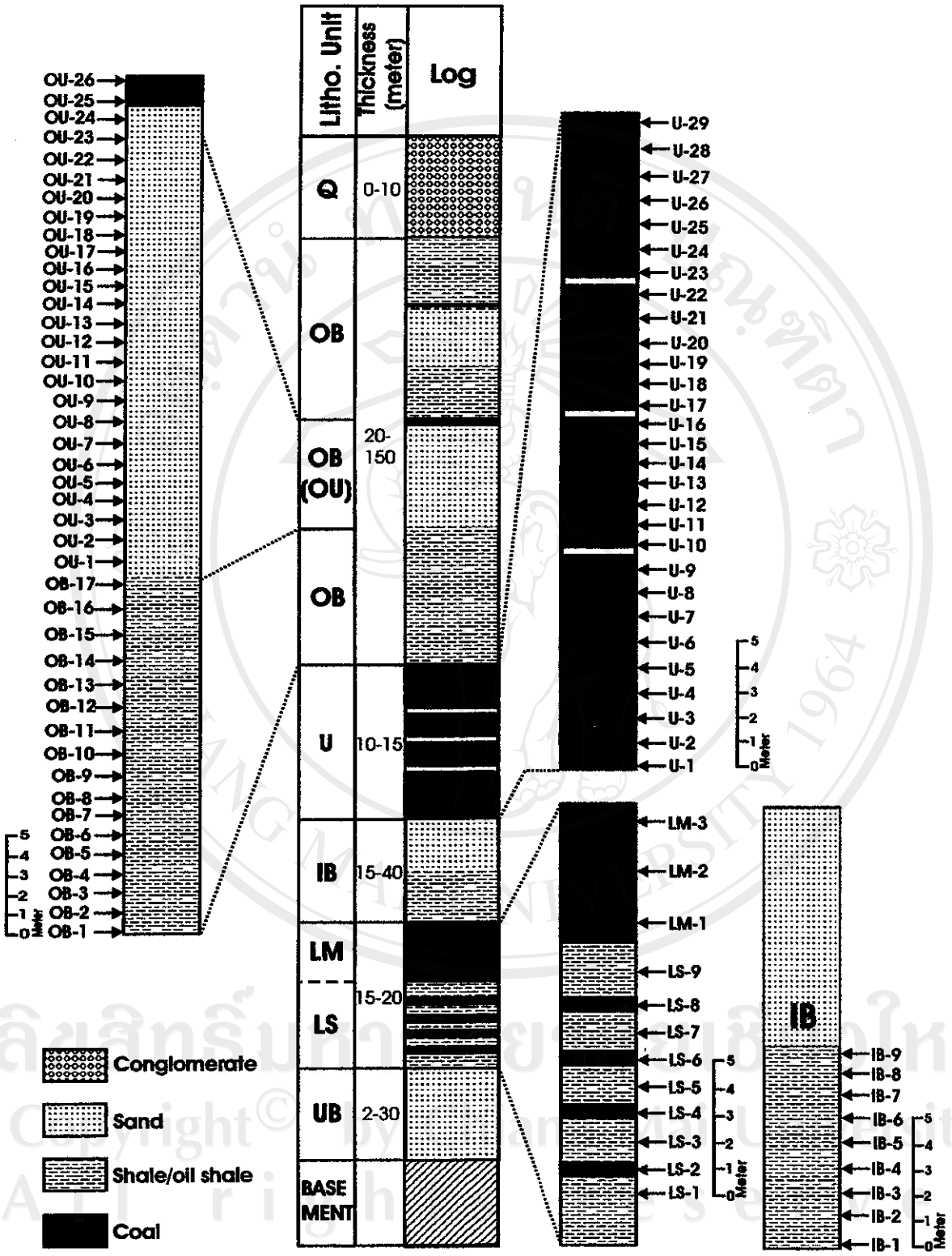

Figure 3-1 Schematic stratigraphic succession of Ban Pa Kha Formation showing stratigraphic levels of the sample collected.

3.1.1.2 Na Sai coalfield

The samples collected from the Na Sai coalfield came from a less than 15 meter-thick section of which were briefly described in Table 3-2 and sample locations are shown in Figure 3-2. Twelve samples were collected from four different parts. One sample (NS-1) was from the underburden claystone and four samples (NS-2 to NS-5) were from the main coal seam. In the overlying fossiliferous bed, three samples (NS-6 to NS-8) were collected from carbonaceous claystone and claystone. Four samples were collected from the claystone overburden part (NS-9 to NS-12).

3.1.2 Chiang Muan basin

Eighty-two samples were collected from the Chiang Muan coalfield from almost every unit except the underburden unit (Figure 3-3). Samples were shortly described as in Table 3-3. Three samples of coal and one of claystone were from lower split coal sub-unit with "LS-B" as sample number from LS-B-1 to LS-B-4. In the lower massive coal zone, only coals were collected from sample LM-A-1 to LM-A-6 and LM-B-1. About eight-meters thick U-2 unit consists of coal and claystone. The five samples of claystone with four coal samples were collected (U2-1 to U2-9). The IB-1 unit is light greenish green claystone containing common fossil leaves and fruits and three samples (IB-1 to IB-3) were collected. Unit U-1 is dominated by ligneous claystone with light greenish gray claystone intercalated. The six samples from unit U-1 were from ligneous clay (U1-1-B1 to U1-6-B1). The overburden unit is a somewhat homogenous greenish gray claystone with some very thin ligneous clay layers intercalated. The samples were collected in intervals of 0.5 meters totally 52 samples (CM-17 to CM-69).

3.1.3 Mae Moh basin

Table 3-2 Description of samples collected from Na Sai coalfield.

Sample No.	Distance from previous sample (metre)	Sample Type	Colour	Description	Remarks
NS-12	1.0	claystone	greenish gray		
NS-11	1.0	claystone	greenish gray		
NS-10	1.0	claystone	greenish gray		
NS-9	0.6	claystone	greenish gray		
NS-8	0.5	claystone	greenish gray	fossiliferous	
NS-7	0.3	claystone	greenish gray	fossiliferous	
NS-6	0.4	claystone	greenish gray	fossiliferous	
NS-5	1.8	coal	brownish black		
NS-4	1.5	coal	brownish black		
NS-3	1.7	coal	brownish black		
NS-2	1.5	coal	brownish black		
NS-1	0.0	claystone	dark gray		

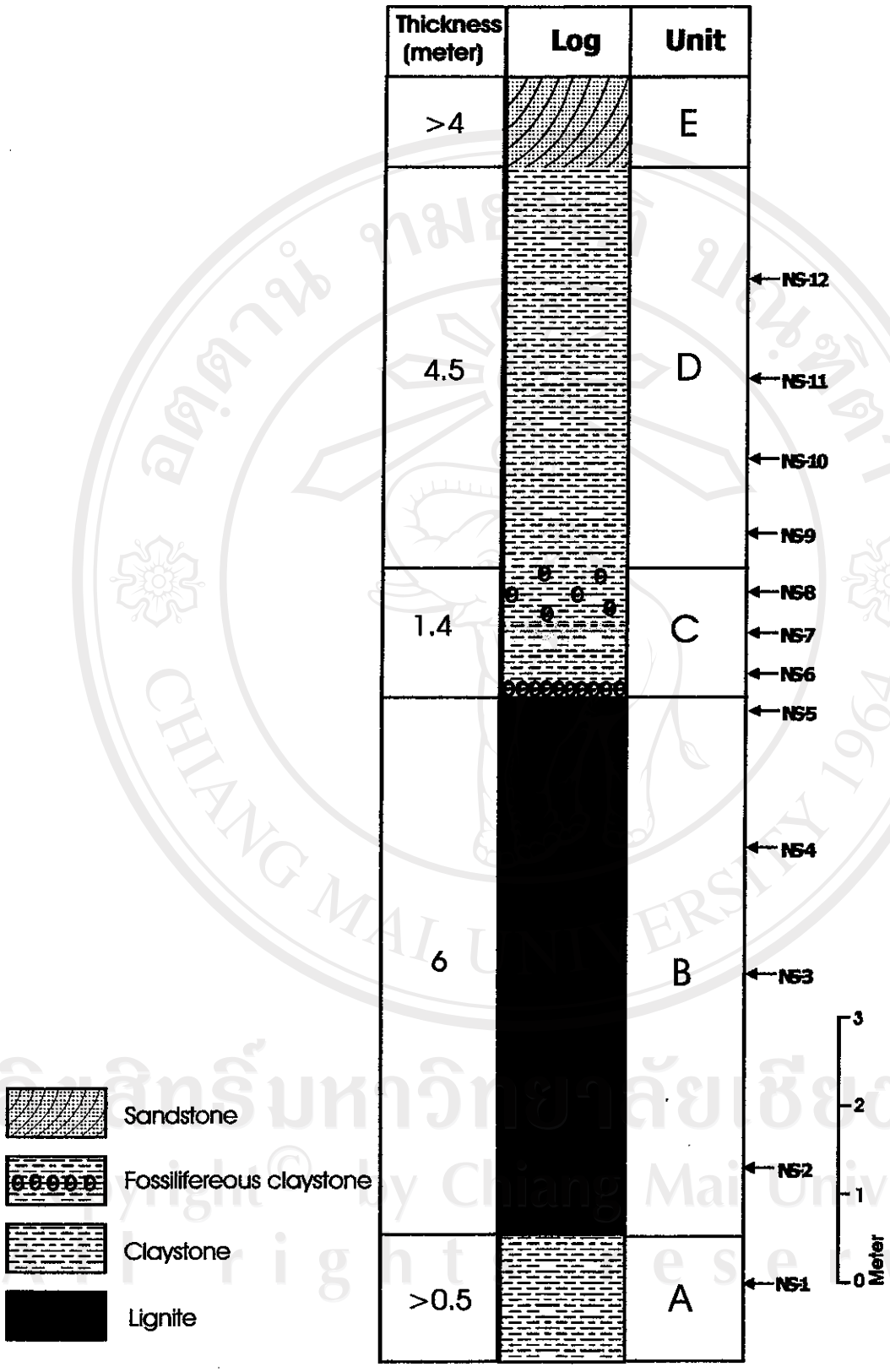

Figure 3-2 A partial schematic stratigraphic succession of Na Sai coalfield showing stratigraphic levels of the sample collecting..

Table 3-3 Description of samples collected from Chiang Muan coalfield.

Sample No.	Distance from previous sample (metre)	Sample Type	Colour	Description	Remarks
CM-69	26.0	claystone	greenish gray		
CM-68	25.5	claystone	greenish gray		
CM-67	25.0	claystone	greenish gray		
CM-66	24.5	claystone	greenish gray		
CM-65	24.0	claystone	greenish gray		
CM-64	23.5	claystone	greenish gray		
CM-63	23.0	lig. Claystone	dark gray		
CM-62	22.5	claystone	greenish gray		
CM-61	22.0	claystone	greenish gray		
CM-60	21.5	claystone	greenish gray		
CM-59	21.0	claystone	greenish gray		
CM-58	20.5	claystone	greenish gray		
CM-57	20.0	lig. Claystone	dark gray		
CM-56	19.5	lig. Claystone	dark gray		
CM-55	19.0	claystone	greenish gray		
CM-54	18.5	claystone	greenish gray		
CM-53	18.0	claystone	greenish gray		
CM-52	17.5	claystone	greenish gray		
CM-51	17.0	lig. Claystone	dark gray		
CM-50	16.5	claystone	greenish gray		
CM-49	16.0	claystone	greenish gray		
CM-48	15.5	claystone	greenish gray		
CM-47	15.0	claystone	greenish gray		
CM-46	14.5	claystone	greenish gray		
CM-45	14.0	claystone	greenish gray		
CM-44	13.5	lig. Claystone	dark gray		
CM-43	13.0	claystone	greenish gray		
CM-42	12.5	claystone	greenish gray		
CM-41	12.0	claystone	greenish gray		
CM-40	11.5	claystone	greenish gray		
CM-39	11.0	lig. Claystone	dark gray		
CM-38	10.5	lig. Claystone	dark gray		
CM-37	10.0	claystone	greenish gray		
CM-36	9.5	claystone	greenish gray		
CM-35	9.0	claystone	greenish gray		
CM-34	8.5	claystone	greenish gray		
CM-33	8.0	claystone	greenish gray		
CM-32	7.5	lig. Claystone	dark gray		
CM-31	7.0	claystone	greenish gray		
CM-30	6.5	claystone	greenish gray		
CM-29	6.0	claystone	greenish gray		
CM-28	5.5	claystone	greenish gray		
CM-27	5.0	claystone	greenish gray		
CM-26	4.5	lig. Claystone	dark gray		
CM-25	4.0	claystone	greenish gray		
CM-24	3.5	claystone	greenish gray		
CM-23	3.0	claystone	greenish gray		
CM-22	2.5	lig. Claystone	dark gray		
CM-21	2.0	lig. Claystone	dark gray		
CM-20	1.5	claystone	greenish gray		
CM-19	1.0	claystone	greenish gray		
CM-18	0.5	claystone	greenish gray		

Table 3-3 Continue.

Sample No.	Distance from previous sample (metre)	Sample Type	Colour	Description	Remarks
CM-17	0.0	claystone	greenish gray		
U1-6-B1	4.0	coal	dark gray		
U1-5-B1	3.0	claystone	pale gray		
U1-4-B1	2.5	coal	dark gray		
U1-3-B1	1.0	claystone	pale gray		
U1-2-B1	0.5	coal	dark gray		
U1-1-B1	0.0	coal	dark gray		
IB-3	2.0	claystone	pale gray		
IB-2	1.0	claystone	pale gray		
IB-1	0.0	claystone	pale gray		
U2-9	6.0	coal	black		
U2-8	5.5	coal	black		
U2-7	5.0	coal	black		
U2-6	4.0	claystone	dark brown-gray		
U2-5	3.5	claystone	dark brown-gray		
U2-4	2.5	claystone	dark brown-gray		
U2-3	2.0	claystone	dark brown-gray		
U2-2	1.0	coal	black		
U2-1	0.0	claystone	dark brown-gray		
LM-B-1	6.0	coal	black		
LM-A-6	5.0	coal	black		
LM-A-5	4.0	coal	black		
LM-A-4	3.0	coal	black		
LM-A-3	2.0	coal	black		
LM-A-2	1.0	coal	black		
LM-A-1	0.0	coal	black		
LS-B-4	3.0	coal	black		
LS-B-3	2.0	coal	black		
LS-B-2	1.0	claystone	dark brown-gray		
LS-B-1	0.0	coal	black		

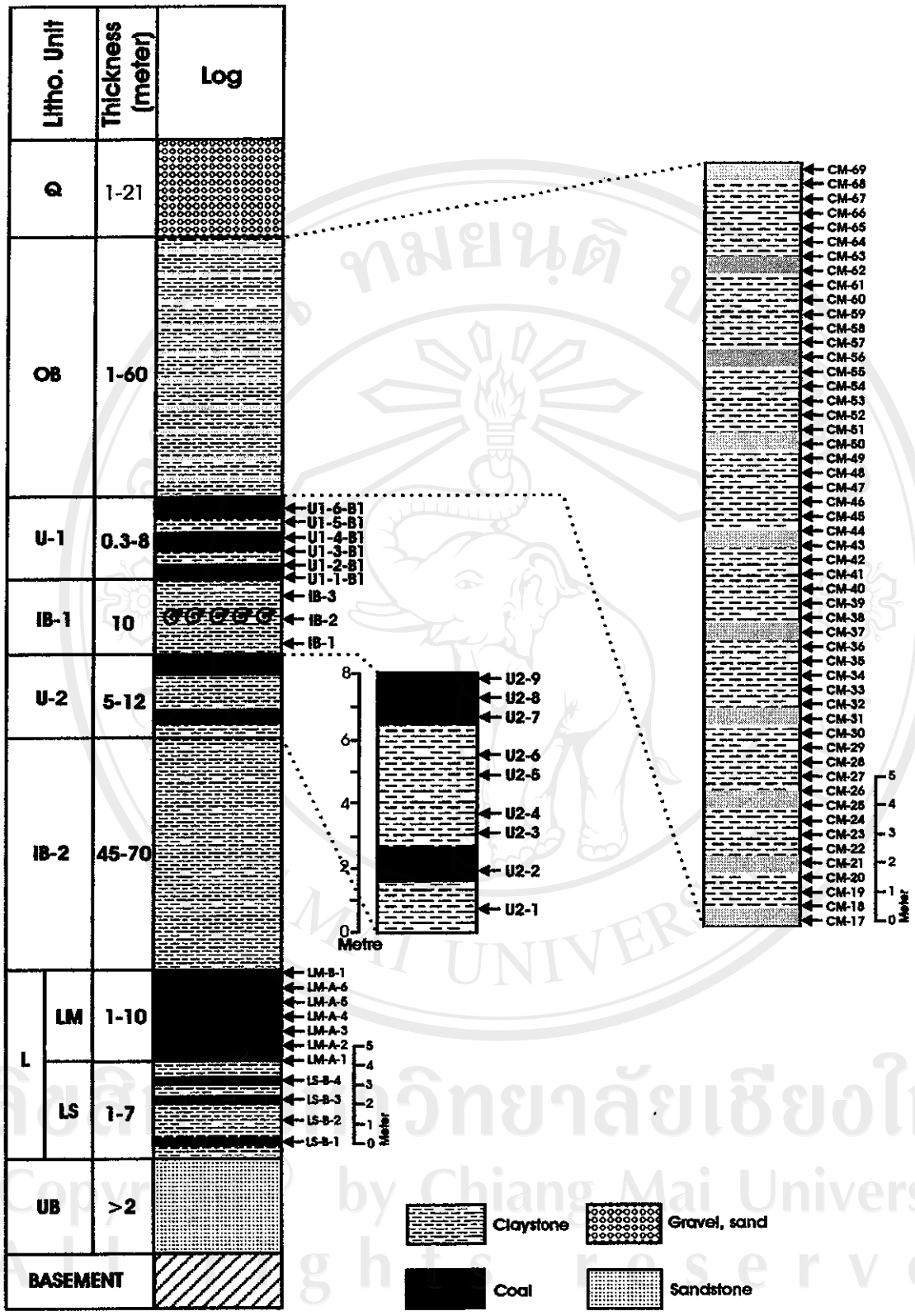

Figure 3-3 Schematic stratigraphic succession of Chiang Muan Formation showing stratigraphic levels of the sampls collected.

The exposure Tertiary sediments in the Mae Moh coal mine include only part of the whole sequence. Only the Na Khaem Formation and Huai Luang Formation were observed (Figure 3-4). The samples collected were briefly described in Table 3-4. The Huai King Formation and almost all of Member III of the Na Khaem Formation was unexposed. Only one sample from Member III of the Na Khaem Formation was collected (UB-1). Nine coal samples from the Q coal zone and eleven coal samples from the K coal zone were collected (Qm-1 to Qm-9 and Km-1 to Km-11). Unit IB, sandwiched between Q and K zones, is a claystone unit and only five claystone samples were collected (IB-1 to IB-5). Only coal samples from J coal zone were collected from each of the six coal seams, J-6 to J-1 from the bottom to top. The code "Jm" was applied to the whole J zone. Accordingly, the samples Jm-1 to Jm-14 (14 samples) were collected. For the I zone, 20 samples were collected from both coal and claystone, The samples were coded as Im-1 to Im-20. No sample was collected from the Huai Luang Formation.

3.1.4 Mae Lamao basin

Samples collected from the Mae Lamao mine are illustrated in Figure 3-5. The samples collected were briefly described in Table 3-5. Eleven samples from unit A were greenish gray claystone (MLM-4-1 to MLM-4-11). The gap between unit A and unit B was a steep cliff where it was impossible to collect any samples. Four samples of coal and claystone were from the thin horizon of unit B (MLM-2-1 to MLM-2-4). Unit C is a conglomerate and no sample was collected. Fifteen samples were collected from unit D (MLM-1-1 to MLM-1-15). The samples were greenish gray claystone.

3.1.5 Na Hong basin

Table 3-4 Description of samples collected from Mae Moh coalfield.

Sample No.	Distance from previous sample (metre)	Sample Type	Colour	Description	Remarks
Im-20	29.0	claystone	brown- gray		
Im-19	28.0	claystone	brown- gray		
Im-18	27.0	claystone	brown- gray		
Im-17	16.0	claystone	brown- gray		
Im-16	15.0	claystone	brown- gray		
Im-15	14.0	claystone	brown- gray		
Im-14	13.0	claystone	brown- gray		
Im-13	12.0	claystone	brown- gray		
Im-12	11.0	claystone	brown- gray		
Im-11	10.0	claystone	brown- gray		
Im-10	9.0	coal	black		
Im-9	8.0	coal	black		
Im-8	7.0	coal	black		
Im-7	6.0	coal	black		
Im-6	5.0	claystone	brown- gray		
Im-5	4.0	claystone	brown- gray		
Im-4	3.0	claystone	brown- gray		
Im-3	2.0	claystone	brown- gray		
Im-2	1.0	coal	black		
Im-1	0.0	coal	black		
Jm-14	11.2	coal	black		
Jm-13	10.7	coal	black		
Jm-12	9.3	coal	black		
Jm-11	7.5	coal	black		
Jm-10	7.0	coal	black		
Jm-9	5.0	coal	black		
Jm-8	4.5	coal	black		
Jm-7	4.0	coal	black		
Jm-6	3.5	coal	black		
Jm-5	3.0	coal	black		
Jm-4	2.5	coal	black		
Jm-3	1.0	coal	black		
Jm-2	0.5	coal	black		
Jm-1	0.0	coal	black		
Km-11	10.0	coal	black		
Km-10	9.0	coal	black		
Km-9	8.0	coal	black		
Km-8	7.0	coal	black		
Km-7	6.0	coal	black		
Km-6	5.0	coal	black		
Km-5	4.0	coal	black		
Km-4	3.0	coal	black		
Km-3	2.0	coal	black		
Km-2	1.0	coal	black		
Km-1	0.0	coal	black		
IB-5	11.0	claystone	brown- gray		
IB-4	10.0	claystone	brown- gray		
IB-3	2.0	claystone	brown- gray		
IB-2	1.0	claystone	brown- gray		
IB-1	0.0	claystone	brown- gray		
Qm-9	9.0	coal	black		
Qm-8	8.0	coal	black		

Table 3-4 Continue.

Sample No.	Distance from previous sample (metre)	Sample Type	Colour	Description	Remarks
Qm-7	7.0	coal	black		
Qm-6	6.0	coal	black		
Qm-5	5.0	coal	black		
Qm-4	4.0	coal	black		
Qm-3	3.0	coal	black		
Qm-2	2.0	coal	black		
Qm-1	1.0	coal	black		
UB-1	0.0	claystone	brown- gray		

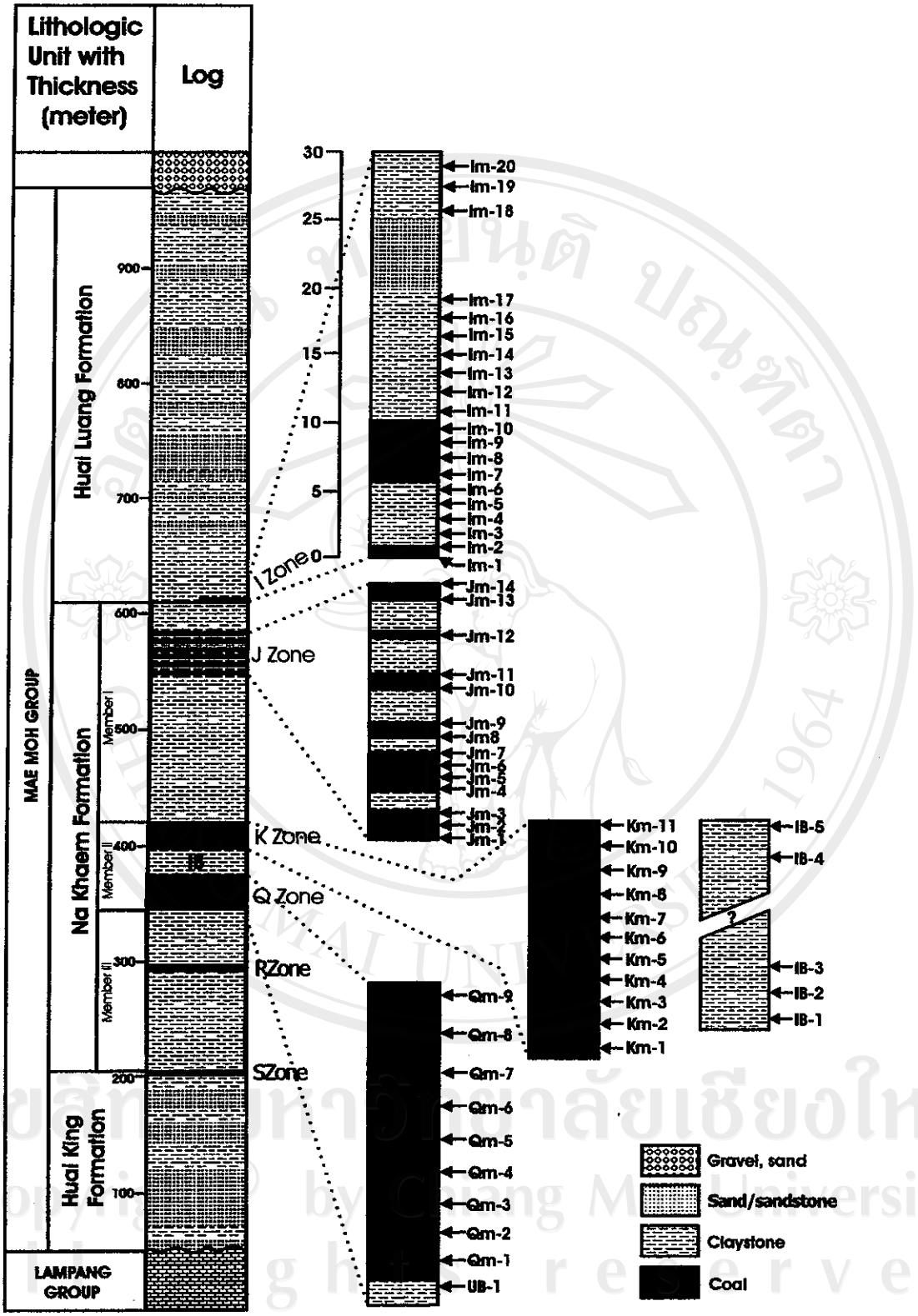

Figure 3-4 Schematic stratigraphic succession of Mae Moh Group showing stratigraphic levels of the samples collected.

Table 3-5 Description of samples collected from Mae Lamao coalfield.

Sample No.	Distance from previous sample (metre)	Sample Type	Colour	Description	Remarks
MLM-1-15	6.9	claystone	pale gray		
MLM-1-14	6.5	claystone	pale gray		
MLM-1-13	6.0	claystone	pale gray		
MLM-1-12	5.5	claystone	pale gray		
MLM-1-11	5.0	claystone	pale gray		
MLM-1-10	4.5	claystone	pale gray		
MLM-1-9	4.0	claystone	pale gray		
MLM-1-8	3.5	claystone	pale gray		
MLM-1-7	3.0	claystone	pale gray		
MLM-1-6	2.5	claystone	pale gray		
MLM-1-5	2.0	claystone	pale gray		
MLM-1-4	1.5	claystone	pale gray		
MLM-1-3	1.0	claystone	pale gray		
MLM-1-2	0.5	claystone	pale gray		
MLM-1-1	0.0	claystone	pale gray		
MLM-2-5	1.4	coal	black		
MLM-2-4	1.1	claystone	dark brown gray		
MLM-2-3	0.7	claystone	dark brown gray		
MLM-2-2	0.4	coal	black		
MLM-2-1	0.0	claystone	dark brown gray		
MLM-4-11	10.0	claystone	greenish gray		
MLM-4-10	9.0	claystone	greenish gray		
MLM-4-9	8.0	claystone	greenish gray		
MLM-4-8	7.0	claystone	greenish gray		
MLM-4-7	6.0	claystone	greenish gray		
MLM-4-6	5.0	claystone	greenish gray		
MLM-4-5	4.0	claystone	greenish gray		
MLM-4-4	3.0	claystone	greenish gray		
MLM-4-3	2.0	claystone	greenish gray		
MLM-4-2	1.0	claystone	greenish gray		
MLM-4-1	0.0	claystone	greenish gray		

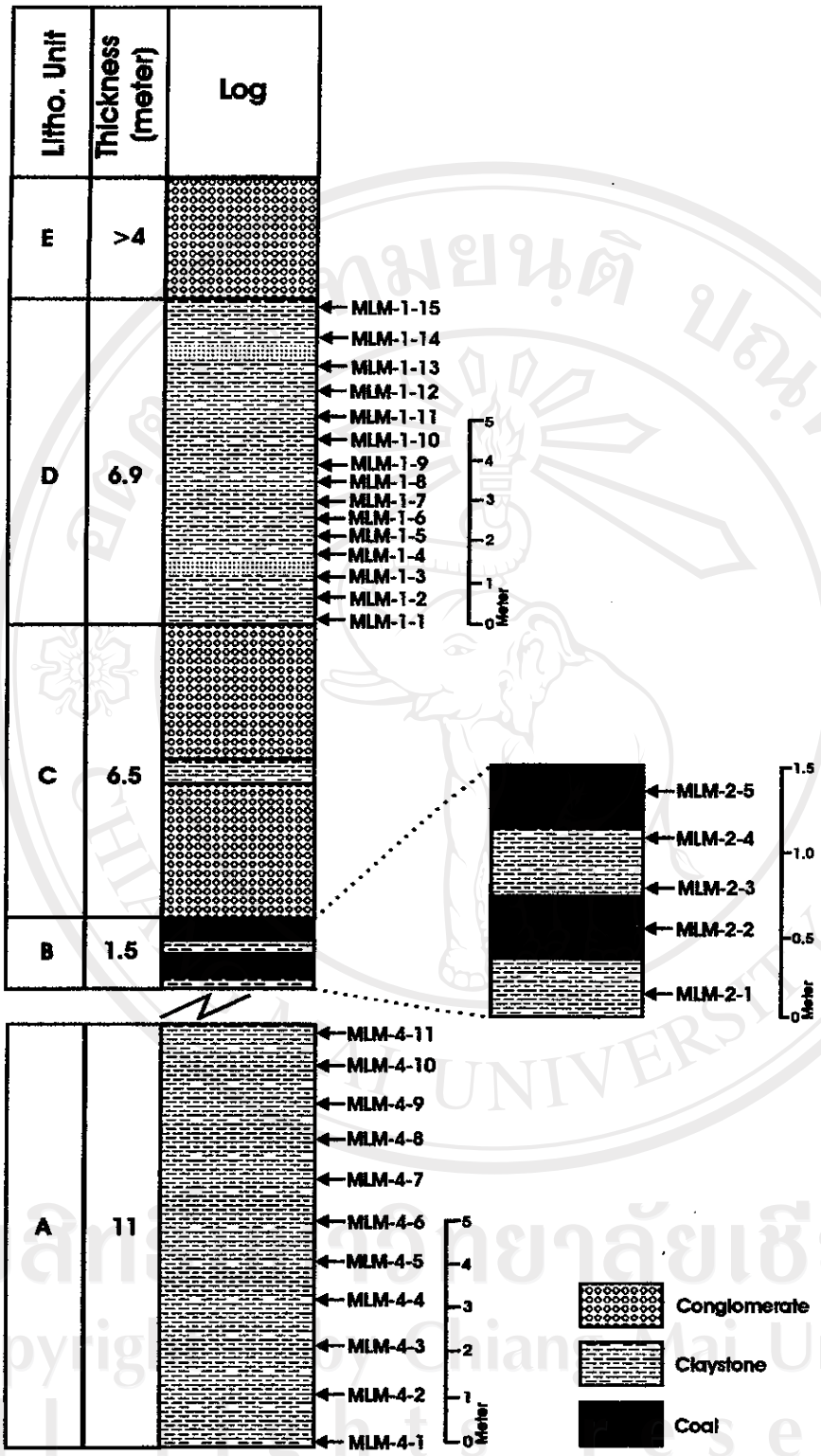

Figure 3-5 A partial schematic stratigraphic succession of Mae Lamao coalfield showing stratigraphic levels of the samples collected.

Only oil shale and shale/claystone samples from the Na Hong mine were from both units (Figure 3-6). There were 21 samples including sample numbers NH-1 to NH-21 from the base and briefly described in Table 3-6.

3.2 SAMPLE PREPARATION

3.2.1 Sedimentary sample preparation

The samples were all treated using a modification and simplification of the general standard technique that was developed at the palynology laboratory of the Bureau of Geological Survey, Department of Mineral Resources. Figure 3-7 shows the flowchart, which is as follows:

1. One piece of sedimentary sample was first cut with a new cutter to eliminate the outer weathered surface in order to reach the inner, uncontaminated fresh material.

2. The sample was gently cut up by a scalpel onto a paper cup and broken into particles about 1-2 millimeters in diameter.

3. About 5 grams of sample were put into a 250 milliliter acid-resistant beaker.

4. If the sample was coal, then proceed to step 8.

5. The sample was tested for the presence of carbonate by the addition of a few drops of dilute hydrochloric acid. If there was no reaction, then proceed to step 7. If a reaction occurred, which was indicated by gas bubbles, the sample was treated with additional dilute hydrochloric acid until the gas bubbles disappeared.

6. The sample was washed (Washing is done by irrigating the sample with distilled water in a centrifugal tube, shaking the sample to mix well, and then centrifuging the sample at approximately 2,500 revolutions per minute for 3 minutes

Table 3-6 Description of samples collected from Na Hong coalfield.

Sample No.	Distance from previous sample (metre)	Sample Type	Colour	Description	Remarks
NH-21	53.0	shale	purplish gray		Samples collected from
NH-20	50.0	shale	purplish gray		Na Hong are well conso-
NH-19	44.5	shale	reddish brown		lidated, rocks. Samples are
NH-18	43.0	shale	greenish gray		always shale and oil shale.
NH-17	32.5	shale	greenish gray		
NH-16	31.0	shale	greenish gray		
NH-15	29.5	shale	greenish gray		
NH-14	23.0	oil shale	greenish gray		
NH-13	21.5	oil shale	greenish gray		
NH-12	19.0	oil shale	greenish gray		
NH-11	17.0	oil shale	greenish gray		
NH-10	15.2	oil shale	greenish gray		
NH-9	14.0	oil shale	greenish gray		
NH-8	12.0	oil shale	greenish gray		
NH-7	11.0	oil shale	greenish gray		
NH-6	9.0	oil shale	greenish gray		
NH-5	8.0	oil shale	greenish gray		
NH-4	5.5	oil shale	greenish gray		
NH-3	3.5	oil shale	greenish gray		
NH-2	2.7	oil shale	greenish gray		
NH-1	0.0	oil shale	dark gray	brittle, hard	

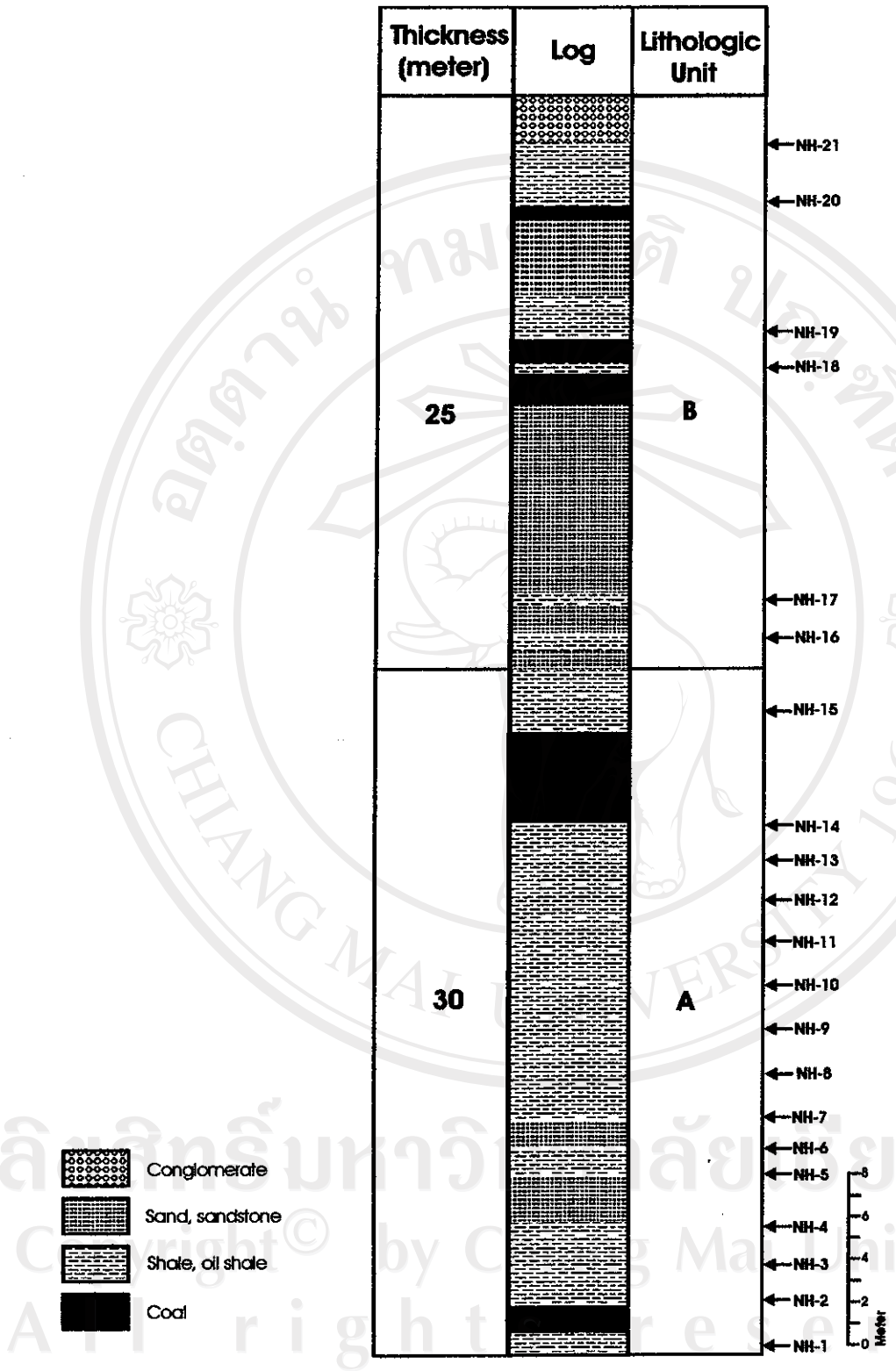

Figure 3-6 A partial schematic stratigraphic succession of Na Hong coalfield showing stratigraphic levels of the samples collected.

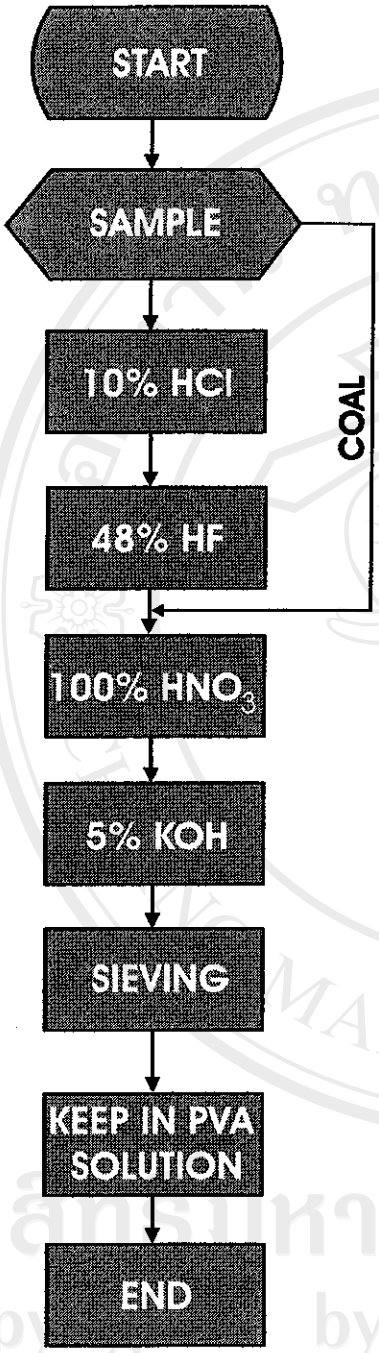

Figure 3-7 Flowchart showing general standard technique of paleopalynological processing procedure.

to separate the residues, following which the top liquid is decanted. Washing is repeated until the residues become neutral, but always at least 3 times).

7. The residues were then covered with 48 percent hydrofluoric acid. In the classical process, the residues were soaked at room temperature for more than a week. More silt and sand sample contains, more time would be taken. For practical reasons, 30 minutes in a hot bath was frequently done to simplify the process. However, the full process was more appropriate. Thereafter, the residues were washed.

8. After step 7, the residues generally contained sporomorphs and unwanted amorphous organic debris, such as animal and plant tissue fragments, and particles of uncertain origin. This unwanted organic debris was oxidized by 100% nitric acid for lignite coal samples. In the case of high rank coal and shale, a few drops of saturated potassium chlorate solution ($KClO_3$) would be added for stronger oxidization. After that, the residues were washed. The residues were subsequently cleaned by using a 5 percent potassium hydroxide solution for a few minutes and followed up with further washing.

9. Finally, the residues were sieved by nylon nets to obtain 11 to 133 micron sporomorphs and kept in a small vial with dilute polyvinyl alcohol. The vial was labeled with the sample number and date of preparation. After this, the sporomorphs were stored for the slide making process needed for both light microscopic and scanning electron microscopic purposes.

3.2.2 Modern spore and pollen preparation

Modern spores and pollen were collected from identified plants, normally from botanic gardens, arboreta, botanic parks, and any places where the trees were given names by botanists. The places include localities all over the country such as the

Queen Sirikit Botanic Garden and Huay Kaew Arboretum in Chiang Mai, Queen Sirikit Park, King Rama IX Park, and Royal Forest Department in Bangkok, Mangrove Forest Conservative Center in Chanthaburi and Trat Provinces, and any flowers whose genera and species were recognizable and identifiable. Each species of flower or fertile fronds of fern was kept in a paper envelope labeled with important collection information.

Chemical treatment used in modern pollen and spore preparation in this research followed the acetolysis method first developed by Kurtzch (1960) and was modified for this research as follows:

1. Anthers or sporangia were separated from flowers or from fertile fronds of ferns and put into a centrifugal tube.

2. The pollen or spores were soaked with 10 percent potassium hydroxide for half an hour then washed with distilled water and the water drained out.

3. After that the pollen and spores were covered by glacial acetic acid for a few minutes and the acid drained out.

4. The process proceeded by dropping a milliliter of acetolysis mixture (eight parts of acetic anhydride and two parts of concentrated sulphuric acid). Since this solution mixture is very toxic and dangerous, the operation must be done carefully under strict laboratory instructions.

5. The mixture was then washed with glacial acetic acid with excess volume to neutralize the acetolysis mixture and, subsequently, with distilled water.

6. The pollen or spores were concentrated by sieving and kept in a tertiary butyl alcohol solution in a suitably sized vial and labeled.

7. The pollen and spores were prepared for light and scanning electron microscopy. Six microscopic slides for light microscopy were duplicated from each

species. Each slide was labeled with the Thai common name, scientific name, family name, location and date of collecting. The slides were separated by family and housed at palynology laboratory of the Department of Mineral Resources in Bangkok and the Department of Geological Sciences, Chiang Mai University.

3.2.3 Specimen preparation for microscopy

Specimens were prepared for light microscopy, scanning electron microscopy, and a combination of light and scanning electron microscopy.

3.2.3.1 Slide making for light microscopic study

A few drops of the polyvinyl alcohol solution with suspended sporomorphs were smeared on a cover slip that was placed on a warm plate. This mixture would dispersed evenly over the cover slip surface and adhere as a film to the cover slip after it dried. The cover slip would then be sealed to a slide glass by one or two drops of Eukitt mounting medium solution and allowed to dry at room temperature over night. Each slide was labelled with the sample number and some important identification information.

3.2.3.2 Stub preparation for scanning electron microscopic study.

A small drop of polyvinyl alcohol solution with suspended sporomorphs was applied on a warm one-centimeter diameter rounded cover slip, which was equal to the size of the scanning electron microscope stub being used. This mixture dispersed evenly over the cover slip surface and adhered as a film to the cover slip when it dried. The cover slip was then placed on the scanning electron microscope stub with a small piece of carbon sticky tape. Thereafter, the sporomorphs were coated with gold under vacuum. This coating needed to be as thin as possible and yet effective for scanning electron microscopy. A fifteen to thirty-nanometer coating satisfied this

need. The stub was stored in a desiccator to keep dry the specimens at all times prior to use in the scanning electron microscopy.

3.2.4 Application of the light microscope and scanning electron microscope to the same pollen grain.

The application of the light microscope and scanning electron microscope to the same pollen grain is a very useful technique. The scanning electron microscope provided an detailed view of the external surface sculpture of the grain at a desired high magnification. On the same grain, the light microscope also provided information on the external ornamentation and, additionally, internal features like wall structure and thickness as well as pore structure in some cases. Many features are revealed by the scanning electron microscope but not by the light microscope and vice versa. The combination of the two is, therefore, a very useful approach in sporomorph description and identification. This technique is used today worldwide in paleopalynological work and is deemed to be an astute strategy for the paleopalynologist.

There are many methods in specimen preparation for this approach (Wood and others, 1996). Each method seems to revolve around how to relocate the specimen that was examined with the scanning electron microscope so that it can be re-examined with the light microscope. The method used in this study was the application of a standard microgrid available from a leading scientific instrument agency. After finishing the step of specimen preparation for the scanning electron microscope before gold coating, a microgrid was placed on the cover slip that contained sporomorphs with a pinpoint drop of latex glue. Specimens of sporomorphs examined under scanning electron microscope were recorded their grid coordinates. After scanning electron microscope examination, the cover slip was removed from the

scanning electron microscope stub and a permanent light microscope slide containing the microgrid was made. It was then very easy to re-locate specimens under the light microscope by using the previously recorded grid locations.

3.3 MICROSCOPY

Microscopic work was done using two different microscopes, a light microscope and a scanning electron microscope. The light microscope was used for sporomorph observation and statistical counting. The scanning electron microscope was used for detailed study of features observed with the light microscope. Sporomorphs were, however, described using both light microscope and scanning electron microscope photomicrographs.

The sporomorphs were observed using a Zeiss light microscope, Axiolab, which was usually connected to sophisticated equipment and a computer. The analogue image from the microscope was transferred to digital data by special computer hardware and was then displayed on a computer monitor by specified KS-200 software, version 2.00. This operation made statistical counting easier. This work was done at the Department of Mineral Resources in Bangkok.

A Jeol JSM 5410 model was used as the scanning electron microscope system. This equipment is at the National Science and Technology Development Agency in Pathumthani.