CHAPTER III

Materials and methods

Materials and equipments

Meterials :

- Sampling peat core (obtained from Associate Professor Kosum Pyramarn and Assistant Professor Dr. Thanawat Jarupongsakul)
- Distilled water
- HCl 10 %
- KOH 10%
- 70% , 95% , and ethanol-absoluted alcohol
- HF 40 %
- Glacial acetic acids
- Acetic anhydride(CH_3 CHOH)
- Conc. H_2SO_4
- Silicone oil (No. AK 2000)
- Benzol (Benzene)
- Solid paraffin

Equipments :

- Centrifuge and centrifuge tubes
- Test tubes and test tube-rack
- Water bath
- Small sieves (200 microns)
- Warm plate
- Glass stirring rods
- Beaker glass 25, 50, 100, 250 and 500 cm^3
- Fume cupboard

- Residue tubes with corks
- Stove
- Microscopic slides and cover glasses
- Dissected needles
- Microscopes
  - Light microscope (LM)
  - Electron microscope (SEM)
- Vials 5 ml
- Double-sided adhesive tape
- Stubs

Methods of investigations

1) Peat core sampling

The peat core sampling was conducted by Associate Professor Kosum Pyramarn and Assistant Professor Dr. Thanawat Jarupongsakul during 29 April to 3 May, 1996 at Ang-Ka intramontane peat bog of the peak of Doi Inthanon National Park.

The five sample cores (Fig. 2) had been taken by hand-auger. All sample cores were sealed by plastic sheet. Sampling cores were stored in the refrigerator to prevent the possible contamination and oxidation.

The stratigraphic core of Ang-Ka 1A was selected by auther for studying (Fig. 2 and Fig. 3)

2) Laboratory investigations

For laboratory method, each two-centrimetre of peat core was subsampled in one-centrimetre cube for analysis. These 76 peat cubes were treated by the method of Jarupongsakul (1987) to extract the spores and pollen grains. Then, the obtained grains were preserved in silicone oil-AK2000 (Andersen, 1960) and mounted on the microscopic slides. The identification and the analytical investigation were done through light microscope (LM) and scanning electron microscope (SEM) in order to confirm that the recently collected pollen grains and spores and those collected from living-plants in the area of the sampling site are of the same vegetation type. The counting of spores and pollen grains was done following the system of Moore, Webb and Collinson (1991).

2.1) Laboratory investigation for preparing permanent slide and taking photograph.

Table 1 Flow chart for processing the spore and pollen samples

Pretreatment	1) Sample 1 centimetre-cube
↓	2) Washing with H_2O (in centrifuge tube, 10 ml)
To remove eventual $CaCO_3$ and for dispersal	3) 10 % HCl at room tenperature
↓	4) Washing with H_2O (1-2 times)
Separation of coarse grain sediments and large wood debris	5) Sieving over 6-8 mm
↓	
To dissolve trace of clay or sand	6) 30-40 % HF 10 ml (12 hr at room temperature)
↓	7) Washing with H_2O (1-2 times)
To remove humic acids	8) 1% KOH (10 min at 50-60 °C)
↓	9) Washing with H_2O (1-2 times)
To remove cellulose and protein	10) (conc.) CH_3COOH 4 ml + 33 % $NaClO_3$ 3-4 drops + (conc.) HCl 1-2 ml
↓	11) Washing with H_2O (1-2 times)
	12) Washing with (conc.) CH_3COOH
To remove organic constituents without destracting	13) Mix acids at 80 °C, 10 min. (conc.) $(CH_3CO)_2$: (conc.) H_2SO_4 in ratio 9:1

of spores and pollen grains

↓

14) Washing with H_2O (1-2 times)

For dehydration

15) Dehydrate by 70 %, 95 % and absoluted alcohol.

↓

16) Remove from test tube to vial by using benzol.

For microscopic-slide preparation

17) Add 2-3 drops of silicone oil (AK2000) as mounting media

↓

For identification, grain counting and taking photographs

18) Microscopic-slide was made and sealed withparaffin whenever the benzol had completely evaporated.

Grain counting were performed according to system of Moore, Webb and Collinson (1983)

2.2) Laboratory investigation for scanning electron microscope and taking photograph.

2.2.1) The acetolyzed and non-acetolyzed samples, spores and pollen grains, were fixed on stubs with double-sided adhesive tape.

2.2.2) The samples on stubs were coated with gold in a Bazer-sputter coater for a total of 5 minutes depositing about 30 nm of metal.

2.2.3) Scanning electron microscope was performed and photographed by using JOEL, model JSM-5410LV.

2.2.4) The samples were examined with 1,000 to 2,000 magnification scanning electron microscope, at 15 kV.

2.3) Laboratory investigation for macroremains through LM. Each 10-cm core was dissolved with distilled water and put on slide microscope to examine. The course debris of each subsamples were determined by the remains of plant tissue and other micro-organisms.

3) The survey of present plants

Two surveying trips on the living plants in the vicinity of sampling bog were done in November 1997 and Febuary 1998. The plants specimens as well as pollen materials were collected during the trips. Plant identification and palynological study of the dominant plants around the sampling area was consequently done.

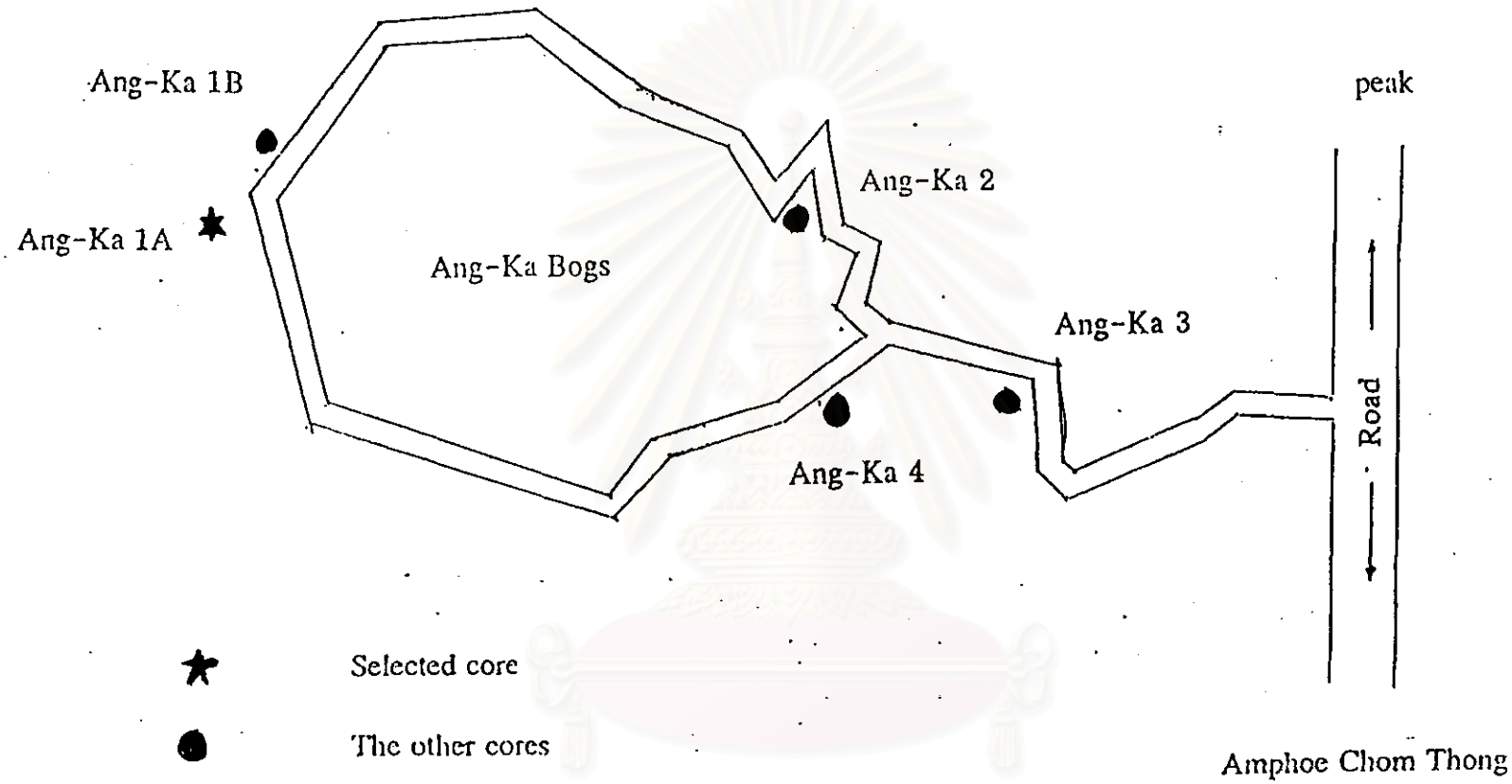

Fig. 2 Route map of Ang-Kha peat bog area around Doi Inthanon' s highest peak and location of drilling core.
(No fixed scale)

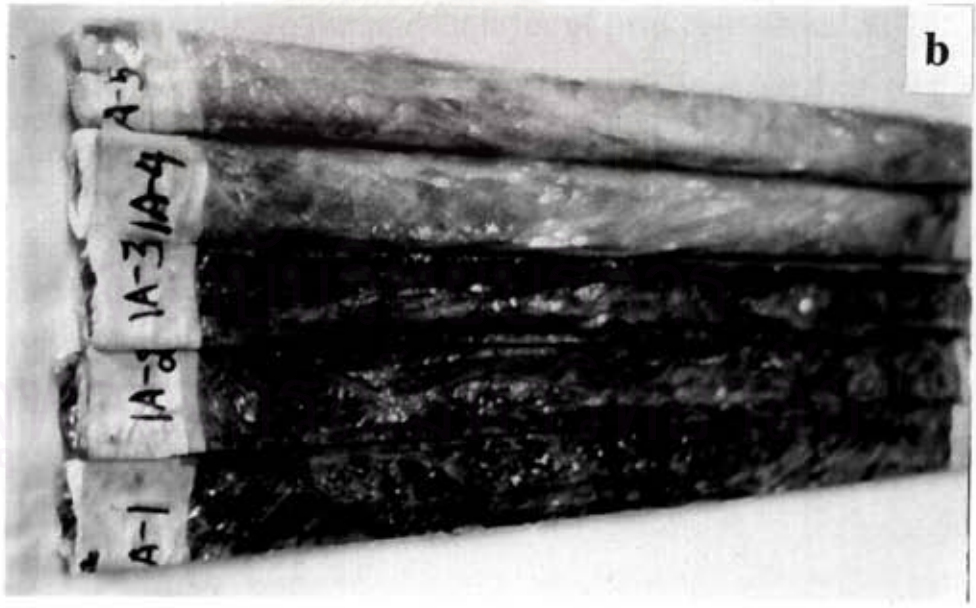

Fig. 3 a, Sampling area.

b, Sampling core, 1A.