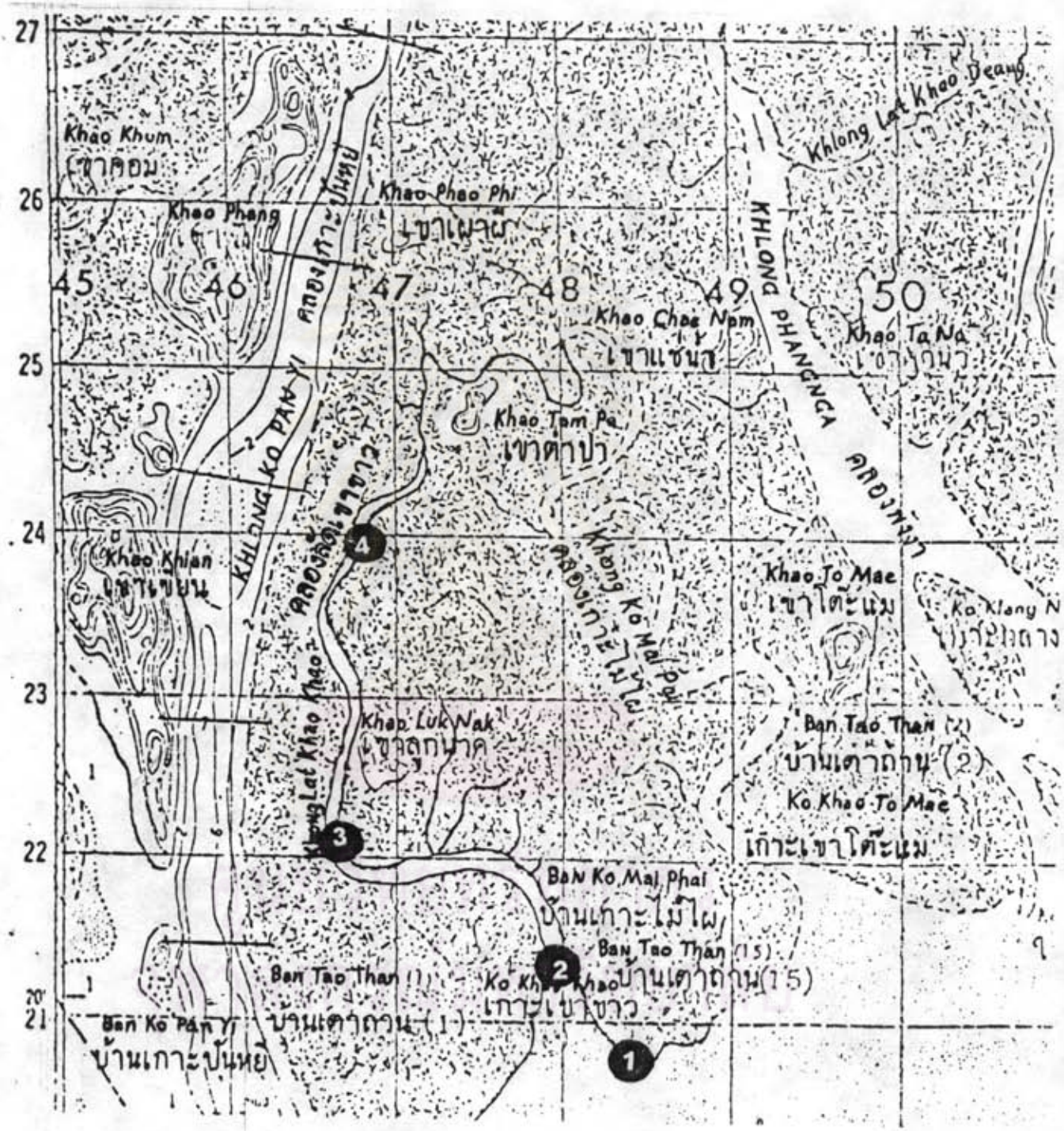
Chapter 3
Materials and Methods

3. Study site

The study was undertaken in the mangrove forest at Klong Lad Khao Kao (Klong = canal), Phang-nga Bay, Changwat Phang-nga, on the Andaman sea coast between latitudes 98°30′E and 98°33′E, and longitudes 8°19′N and 8°28′N. The study was conducted between March 1990 and March 1991. Four stations were established (Figure 3.1). The area is a protected zone dominated by *Rhizophora mucronata*, *Rhizophora apiculata*, *Avincennia* spp., *Sonneratia griffithir*, and *Ceriops tagal*. Other mangrove species are also found (Angsupanich and Aksornkoae, 1993). The type of tidal inundation in the area is semidirunal. The average tidal range is 1.3 m at neap tides and 3.5 m at spring tides. The area is flushed twice daily by the tide. The salinity of the sea water flushing through the mangrove was 26-28% in the wet season and 31-33% in the dry season (Angsupanich and Aksornkoae, 1993).

3.1.1 Interstitial nutrient concentrations with depth

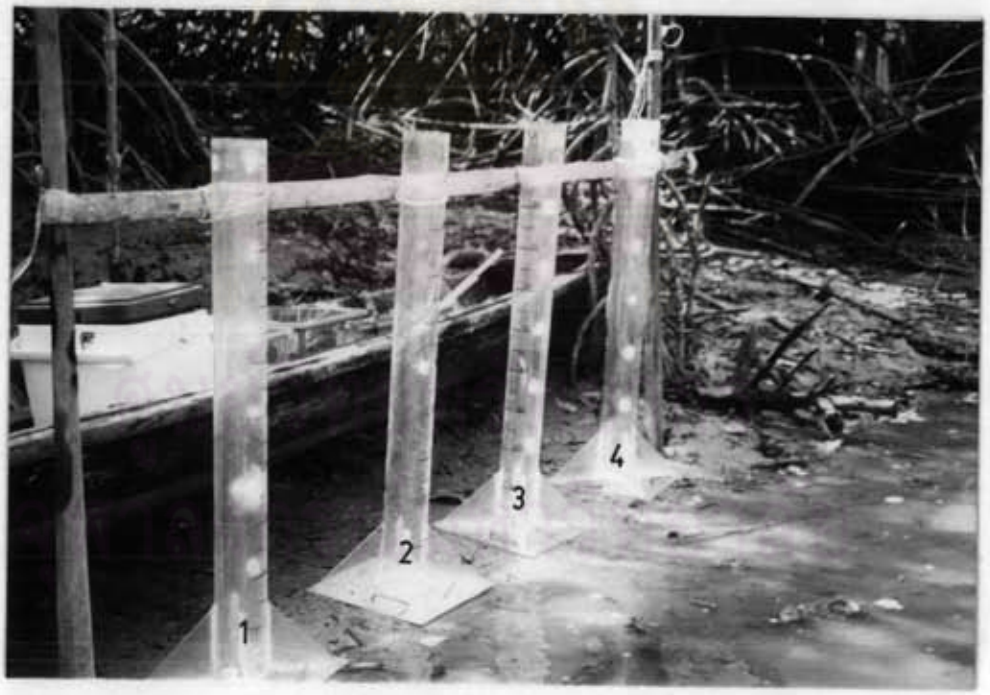
Concentration of plant nutrients in interstitial water was estimated by collecting sediment samples at different depths using a core sampler. The core sampler was made of stainless steel, 100 cm long, 6 cm in diameter, with punctures 2.5 cm in diameter at every 5 cm. The entire body of the core sampler was covered by a plastic tube. Each tube was cut into 5 cm-long sections that could slide to cover the holes of the core sampler (Figure 3.2). Core sampling was taken up to the depth of 60-70 cm. As soon as the core sampler was pulled out of the sediment the pH and Eh probes were inserted through the holes in the core sampler in order to measured pH and redox potential of the sediment. The samples were then separated into 5 cm-thick sections and stored in a cool container for laboratory analysis. The samples were collected from Stations 1, 2, 3, and 4 (Figure 3.1). Three duplicate samples were collected in each station. Soil samples were collected between March 1990 (dry season) and October 1990 (wet season).



Note : 1-4 = Core sediment sampling

3 = Water sampling

Figure 3.1 Study sites



Note : **1-3** **= Experiment**

4 **= Control**

Figure 3.2 *Core sampling device(above) and in situ apparatus for bentric flux studies (below)*

Each 5 cm-thick section was centrifuged at approximately 3000 rpm to extract interstitial water for nutrient analysis. The procedure was as follows:

Soil sample

↓

Measurement pH, water content, Eh

↓

centrifuge

↓

Interstitial water

↓

Filter through Whatman GF/C

↓

Interstitial water

↓

Analysis

- NO_2+NO_3
- NH_4, TN
- PO_4, TP
- SiO_4

Figure 3.3 Procedure for extracting and analyzing nutrients in interstitial water

3.1.2 Nutrients released from the sediment

Concentrations of nutrients released from the sediment were estimated by collecting water in the tubes. The tubes were made of transparent plastic. Each tube was 10 cm in diameter and 100 cm long (Figure 3.2). Four tubes were set up for the experiment. Tubes 1, 2, and 3 were open at the bottom. Tube 4 was closed at the bottom with a plastic plate, and had a hole on the side 5 cm from the bottom.

The tubes were installed during lowest tide on the dry surface. Water will gradually flowing up into the tubes as warer level rise. The water samples were then collected hourly from each tube for nutrients analysis. In October 1990, water samples were collected at the mid-depth of the water level. For example, if the water level was 20 cm, the sample was taken at 10 cm. In March 1991, the samples were collected as the same way as in October, but two points, the first above the bottom 5 cm, and the second 5 cm from the surface of the water instead of only at mid-point.

Water properties such as salinity, dissolved oxygen (DO), temperature, and pH were measured during collection. Salinity was measured using an SCT meter (YSI model 33), dissolved oxygen was measured using a DO meter (YSI model 51B), a thermometer for temperature, and pH using a pH meter (model P101). Water samples were placed in plastic bottles and stored under cool conditions. Samples were filtered through a Whatman GF/C filter to separate dissolved nutrients and particulate matter. The filtrate water was analyzed for nitrite, nitrate, ammonia, total nitrogen, phosphate and total phosphorus and silicate using the procedure outlined by Strickland and Parsons (1972). Organic nitrogen and organic phosphorus levels were calculated from total nitrogen and total phosphorus minus inorganic nitrogen and inorganic phosphorus respectively.

3.1.3 Laboratory experiment

Plant nutrients released from unstirred and stirred sediment were studied as follows:

Samples of wet sediment taken from several locations at Klong Lad Khao Kao, to a height of 10 cm were placed in the glass container (25 cm wide x 40 cm long x 30 cm high) and artificial sea water was then added to a height of 25 cm. The experiments were conducted at room temperature. There were two experimental conditions with two replications: 1) unstirred sediment, and 2) stirred sediment. The latter was done by stirring the water sitting on top of the sediment using a small electrical motor to turn a set of blades clockwise. The water samples were collected hourly from each container for nutrient analysis. Dissolved oxygen and pH were

measured every hour for 24 hours during sampling. The water samples were filtered through a Whatman GF/C filter and analyzed for nitrite, nitrate, ammonia, total nitrogen, phosphate and total phosphorus using the procedure outlined by Strickland and Parsons (1972)

Figure 3.4 Sediment containers used in laboratory study

3.3.2 Laboratory conditions

Experiment I

Containers	Sediment levels (cm)	Water levels (AS) (cm)	Condition
A1 (control)	10	15	unstirred
A2	10	15	stirred
A3	10	15	stirred

Experiment II

Containers	Sediment levels (cm)	Water levels (AS) (cm)	Condition
B1(control)	10	15	unstirred
B2	10	15	stirred
B3	10	15	stirred

Note: *AS = Artificial Seawater (NaCl= 27.8 g/l, MgCl.6H2O = 8.4 $MgSo_4.7H_2O$ = 5.53 g/l, $CaSo_4.2H_2O$ = 1.78 g/l, K_2SO_4 = 0.89 g/l);*
Salinity = 33 ppt

3.5 Nutrient analysis

- Ammonium nitrogen (NH_4^+-N): phenol-hypochlorite method (Strickland and Parsons, 1972).
- Nitrite nitrogen (NO_2^--N): Shinn's method (Strickland and Parsons, 1972).
- Nitrate nitrogen (NO_3^-- N): samples passed trough reduction column (Amalgamated Cd) to reduce nitrate to nitrite and then Shinn's method (Strickland and Parsons, 1972).
- Reactive phosphate (PO_4): molybdenum blue method (Murphy and Riley, in: Strickland and Parsons, 1972).
- Organic nitrogen and organic phosphorus: clculated from total nitrogen or total phosphorus minus inorganic nitrogen or inorganic phosphorus.
- Silicate (SiO_4^{2-}): modified Mullin and Riley procedure (Strickland and Parsons, 1972).
- Water content: 1-2 gm soil samples were placed into an oven at 110° C for 3 hr (Lim and Sugahara, 1984), following which the samples were again weighted.

3.6 Data analysis

Data of nutrients in inerstitial water were interpreted by using T-test (Poonlaptavee, K., 1987). The analyses included: range, mean, and T-test: to determine any significant differences in nutrient concentration during different seasons.

Nutrients released from sediment were calculated from concentration changes in the tubes (in the field), and containners (in the laboratory) over time.

Net sediment-water fluxes were calculated using the following formula of Teague et al., (1988):

$$\Delta N/\Delta T_{(net)} = [(\Delta N/\Delta T_{(experiment)} - \Delta N/\Delta T_{(control)}) \times 9.81\ L]/0.0078\ m^2 \quad (\text{In the field})$$

$$\Delta N/\Delta T_{(net)} = [(\Delta N/\Delta T_{(experiment)} - \Delta N/\Delta T_{(control)}) \times 15\ L]/0.1 m^2 \quad (\text{In laboratory})$$

Where

$\Delta N/\Delta T$ = rate of change of nutrient.

9.81 L = Highest volumn of water in the tube

0.0078 m^2 = Surface area of tube

15 L = Highest volumn of water in the container

0.1 m^2 = Surface area of container

Average fluxes were computed by calculating the arithmetic means of all replicate chamber measurements for each period and station. Positive fluxes indicated net release from the sediment to the water column, while negative fluxes indicated net uptake.