

Chapter 2

Methods and Materials

The Mae Klong River is one of four major rivers flowing into the Upper Gulf of Thailand. At present, due to the three dams built up river, the flow of this river is partially regulated. In addition, The Kwae Noi and the Kwae Yai rivers flow through the watershed which is rich in limestone, thus, making the Mae Klong River water to be high in alkalinity.

Sampling Timescale

The field surveys were done twice in two seasons, which are dry season and wet season. They are as follows;

1. The First Trip on Dry Season (24-27th February, 1986).

Water samples were collected along the Mae Klong River and its estuary totally 10 samples. Each sample represented members of a mixing series between the Mae Klong River water and seawater which were 0, 2, 4, 6, 8, 10, 15, 20, 25, and 30 ppt. Salinity measurement in the field was performed using a YSI SCT meter. These 10 sampling locations spreaded out over a distance of about 40 kms. to the place located just below the Ratchaburi province. Only the sample of 25 ppt. was in the estuary itself. Other additional 3 samples were collected from Photharam, Ban Tharua, and the Vachiralongkorn Dam respectively (fig. 2). Other 8 samples were taken from the Srinakarindra Dam (fig. 3), and also other 8 samples were collected from Khoa Laem Dam (fig. 4).

2. The Second Trip on Wet Season (7-9th October, 1986).

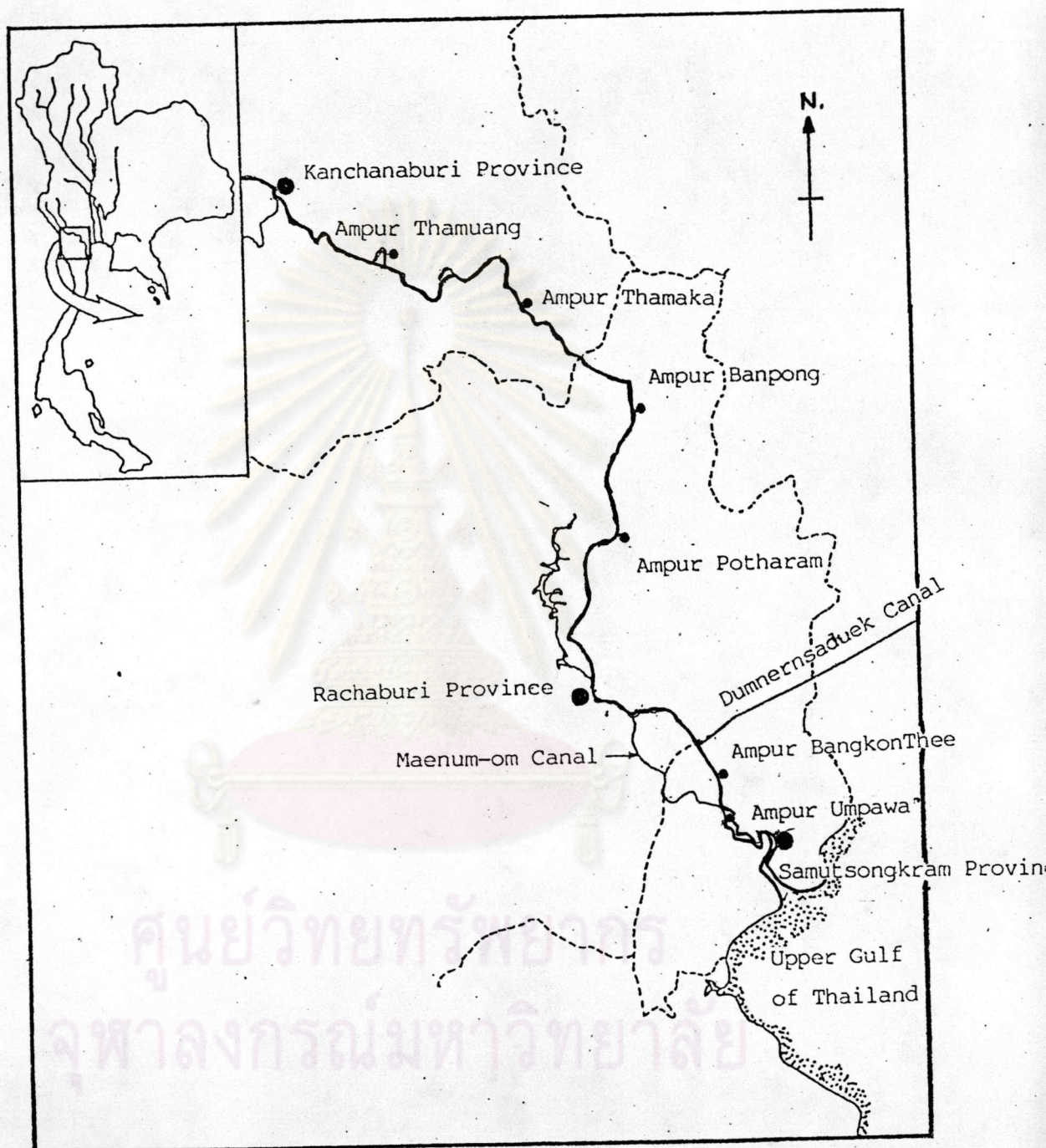


Fig. 2 Map of the Mae Klong River.

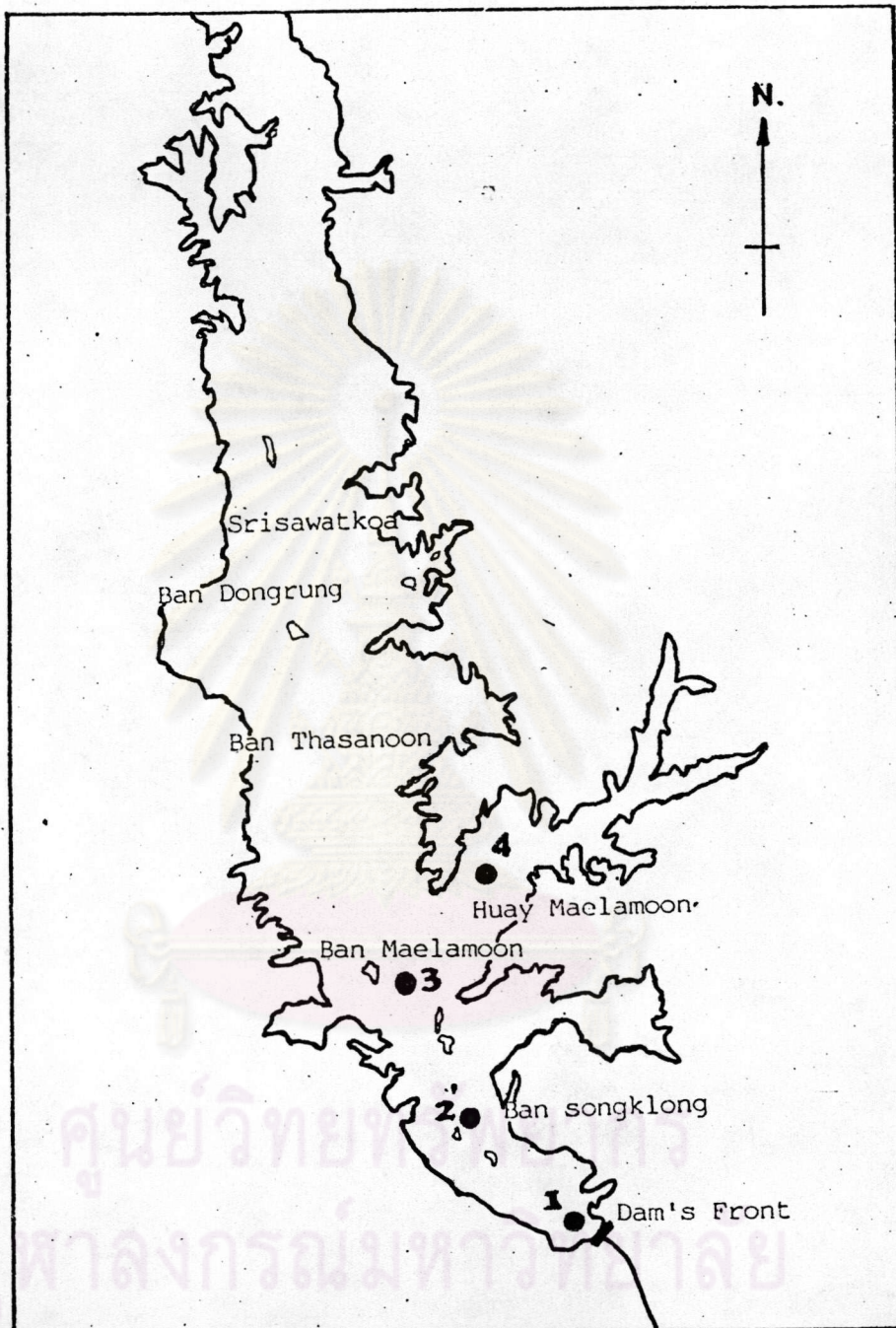


Fig. 3 Sampling stations of the Srinakarindra Dam

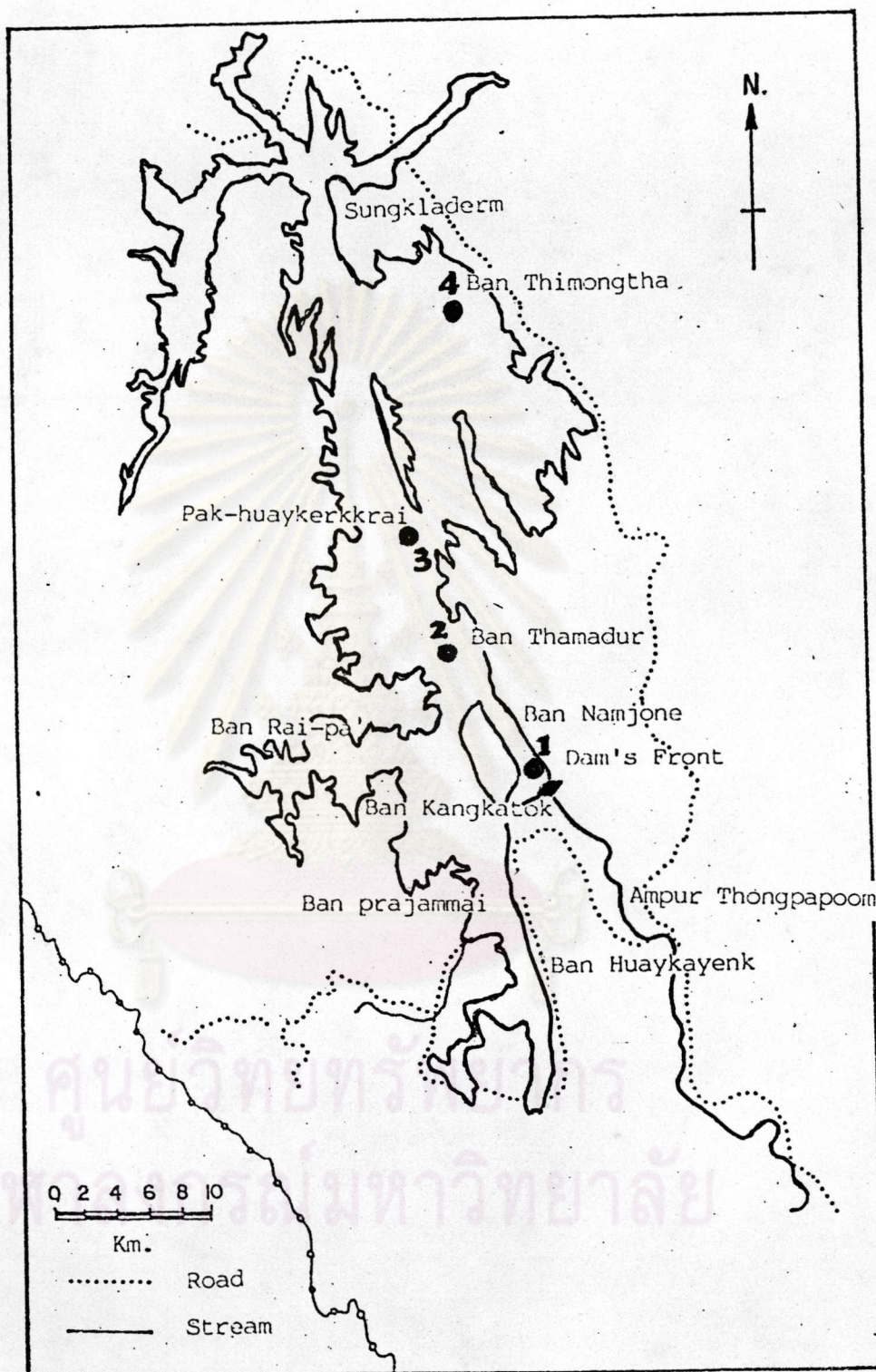


Fig. 4 Sampling Stations of Koa Laem Dam

On this trip, samples from the Mae Klong River were collected with respect to salinity of 0, 2, 4, 6, 8, 10, 15, 20, 25 ppt., which were measured by a YSI SCT meter. Because of strong wind condition and the use of small boat in sampling, it was not safe enough to go further away from the estuary in order to collect seawater endmember. However, samples collected would represent the actual mixing products. And the results could be compared with those from the mixing experiments in the laboratory.

Because of the higher discharge in this season, these 9 samples were collected mostly within the estuary itself. Only the sample of salinity 2 and 0 ppt. were collected in the river. The sampling site of the 0 ppt. was located at Ampur Muang, Samut Songkram province. In comparison to the dry season, sampling locations in this season spreaded out over a relatively short distance.

At Srinakarindra Dam and Khoa Laem Dam, samples were collected on 8th October, 1986 and 9th October, 1986 respectively. Only 4 stations were selected at each dam, one station was the most upper station collected on the first trip, and other 3 stations located along the length of the dam with distance of 150 m. from the dam. Samples were collected at 2 depths from each station, 1 and 10 m., in order to determine the extent of vertical of water.

The stations' code number were given as MK1 to MK(n). The MK1 represents the sample of highest salinity and the number is increased as the salinity decreases. It should be noted that the station numbers do not represent the sampling location but represent the salinity instead. The same coding system was also applied to the mixing experiment by changing MK(n) to MX(n).

3. Mixing Experiments

In order to understand the chemical behaviours of trace metals (Fe, Pb, Cu) during estuarine mixing. A mixing experiment was carried out together with each field sampling. In this study, the Mae Klong River water with salinity 0 ppt. (measured in the field with a YSI SCT meter).

Methods of Analysis

Contamination is always serious problems in trace metal analysis. Therefore, great care should be given to every step of analysis. It is recommended that experiment must be conducted under a clean atmosphere, e.g. in a laminar flow bench. All plasticwares or glassware must be thoroughly cleaned. For example, all plastic and glassware were previously washed with 10% nitric acid solution and rinsed with double deionized water (Karim, et al, 1975), all reagents were analytical grade. All cleaned are manipulated using plastic gloves. The sample bottles must be kept in plastic bags at all times to prevent contamination. In addition, all reagents used should be purest. In all experiments, several blanks were treated as samples with the reagents used, in order to check for possible contamination.

1. Analysis of Dissolved Metals

In natural water, trace metal concentrations are very low, thus, making a difficulty for direct analysis. Practically, dissolved trace metals should be preconcentrated in order to raise the concentration to a more suitable level for analysis. There are many methods of preconcentration. The method chosen for this study is called coprecipitation method which was firstly proposed by Boyle and Edmond (1977) and later modified by Huizenga (1981). The dissolved trace metals

are complexed with APDC and later coprecipitated with Co-APDC in an acid solution. A brief summary of the Huizenga (1981)'s method is as follows ;

The filtered water sample is acidified to pH 3 immediately after filtration. Cobalt reagent is added to the sample followed by the APDC reagent. The cobalt is precipitated with the APDC and in the process, other trace metal-APDC complexes are broughtt down with the precipitate. The APDC concentration is kept high so that the metals do indeed coprecipitate with the cobalt.

The precipitate is then separated from the solution by centrifugation at 3000 rpm. for 30 minutes. Discard the supernatant. The precipitate is then resuspended in distilled water to wash it free of seasalt. Repeat the centrifugation. Again, discard the supernatant. The precipitate is taken up in concentrated nitric acid and diluted with distilled water to desired volume. The solution is then stored in the same sample bottle until analysis.

The standards should be prepared in medium containing the same acid, cobalt and APDC residue concentrations. The amount of cobalt in the concentrate is measured and the loss of cobalt in the sample preparation procedure is assumed to be matched by an equal loss of the trace metals. All metal concentrations and concentration factors are calculated on a weight basis so that sample concentration can be determined on a weight basis.

The metal content in the concentrate was analysed by atomic absorpton spectroscopy, using a deuterium background correction. Pb and Cu were measured by electrothermal atomic absorpton spectroscopy while Fe, at higher concentration, was measured by flame atomic absorpton spectroscopy. Comparing the results obtained for Pb analysis by standard addition method and by the direct matching, slope of calibration curves

corresponding to both methods were constructed. Difference in slope of the two methods indicates that a heavy matrix interference would cause negative deviations if the direct methods were used as analytical method. Therefore, the standard addition method was preferred.

1.1 Preparation of Reagents

There are 2 main reagents that have to be prepared for cobalt coprecipitation. They are as follows:

1.1.1 2% APDC solution

Dissolve 2.79 gm. of APDC (A.R. grade) in 100 ml. of super-Q water. Vigorously shaking for 5 minutes, and then let undissolved APDC settle down. Decant the supernatant solution into a separatory funnel. Extract the APDC solution with MIBK (Methyl Isobutyl Ketone) at the ratio of 10:1, shakes well for 5 minutes. Allow the two solutions to separate for at least 1 hour. Discard the MIBK solution. Repeat the extraction twice with MIBK at the ratio of 20:1. Stores the solution frozen until use.

1.1.2 CoCl_2 solution

Dissolve 0.425 gm. of $\text{CoCl}_2 \cdot \text{H}_2\text{O}$ (A.R. grade) in 150 ml. of 9 M. HCl. Prepares a Dowex 1X3 column (size 1 cm.X 22 cm.). Treats the column with 30 ml. 0.01 M. HCl, follows by rinsing the column with 5 ml. of 9 M HCl. Passing the CoCl_2 solution through the column, follow by 40 ml. of 4 M HCl. When the solution flows through the column and changes colour from blue to pink, starts collecting about 8 ml. of the pink solution. Discard the final clear solution that flows through the column. Dilutes the pink solution with 500 ml. of super-Q water.

1.3 Preparation of Samples

Trace metals in seawater exist mainly in two forms; dissolved and particulate. These two forms of metals in samples from

river could be rapidly interchanged, thus, they have to be separated as soon as possible by pressurized filtration under nitrogen gas using an all-Teflon filtering unit under clean atmosphere (Duinker & Nolting, 1977).

In practice, filtration of samples was immediately done within a portable clean bench. Keep the particulate filtered on nucleopore membrane in a flat polypropylene petri-dish which already precleaned with 10% HNO_3 and rinsed twice with super-Q water.

After filtration, the filtrate was stored in a acid-cleaned HDPE bottle. Then, adjust pH to 3 with distilled conc. HNO_3 , and left to stabilize for at least 48 hours before carrying out pre-concentration. Another portion of each filtrate was measured for its salinity by an YSI SCT meter.

2. Analysis of Metals in Particulate Form

Based on the hypothesis that only the metals which could be easily leached with dilute acid would play a major role to living organisms in water. Therefore, metals in particulate form is leached with re-distilled HNO_3 (A.R. grade) in the PTFE bottles which are closed with screw caps loosely. Heating at 110 - 130 °C for 10 minutes. After cooling down to room temperature, separates the undissolved portion off by centrifugation. The supernatant volume is made up to 10 ml. with super-Q water. Then, measure Cu and Pb concentrations by the Graphite Furnance AAS Perkin-Elmer with deuterium background correction and electrothermal atomization and measures Fe concentration by flame atomic absorption spectrophotometry. Resonance lines of 324.8, 283.3 and 248.3 nm. of Cu, Pb and Fe were respectively employed.

3. Method of Mixing Experiment

The river water is mixed proportionally with seawater to

make solutions of 0, 2, 4, 6, 8, 10, 15, 20, 25 and 30 ppt. of seawater. These solutions are stored in polyethylene bottles which have already precleaned with 10% HNO_3 for 3 days and have been rinsed twice with super-Q water. Let these solution stand for 30 minutes with occassionally shaking. Loosen the screw-caps of samples' bottles and leaves them in a laminar flow cabinet for 24 hours. Filter each sample and follow the procedures as described in section 1 and 2.



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