



CHAPTER III

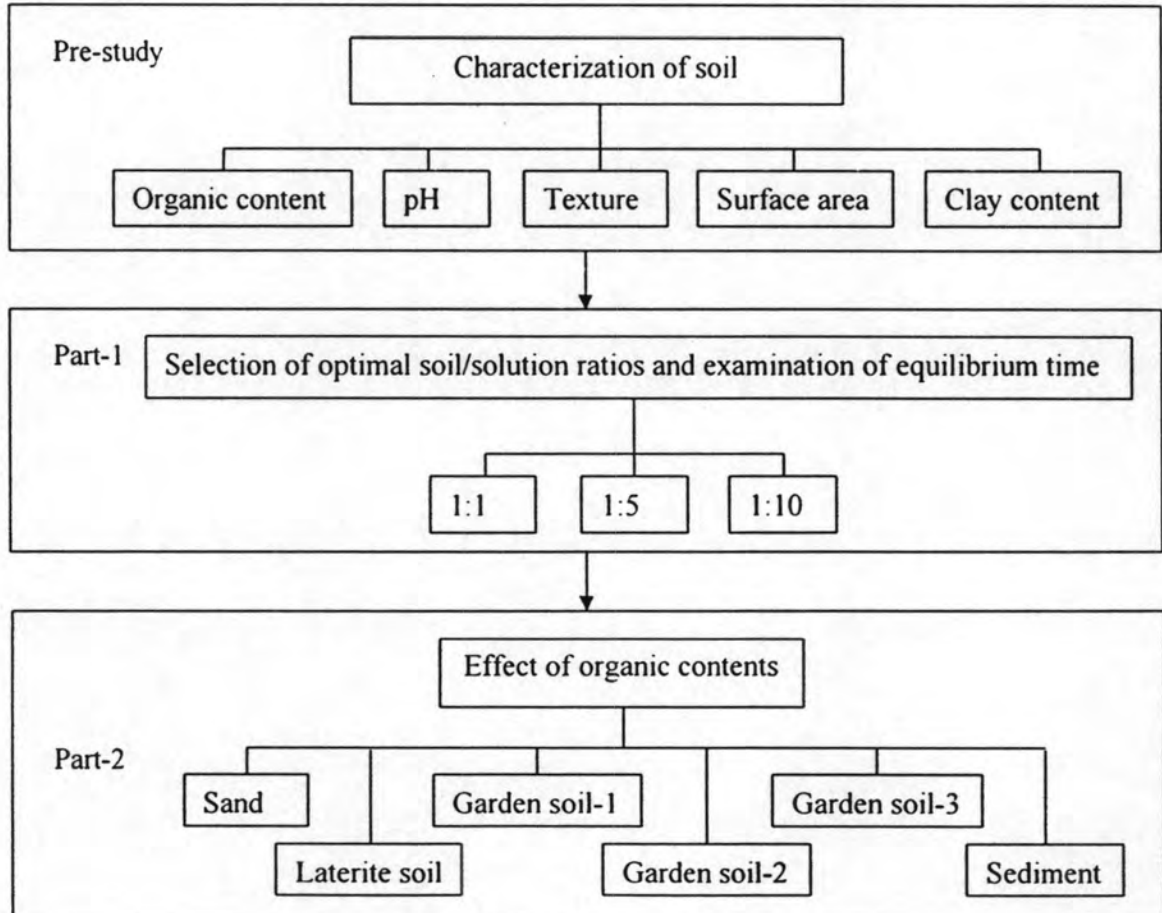
METHODOLOGY

3.1 Experimental framework

The main part of this study is concerned with the sorption of MT onto various soils and sediment by studying the effect of organic content, pH and salinity. The experiment was divided into 5 parts:

1. Characterization of soils and sediments
2. Selection of optimal soil/solution ratios and measurement of equilibrium time
3. Effect of organic content on sorption under original conditions
4. Effect of pH on sorption
5. Effect of salinity on sorption

The experimental framework is shown in Figure 3.1.



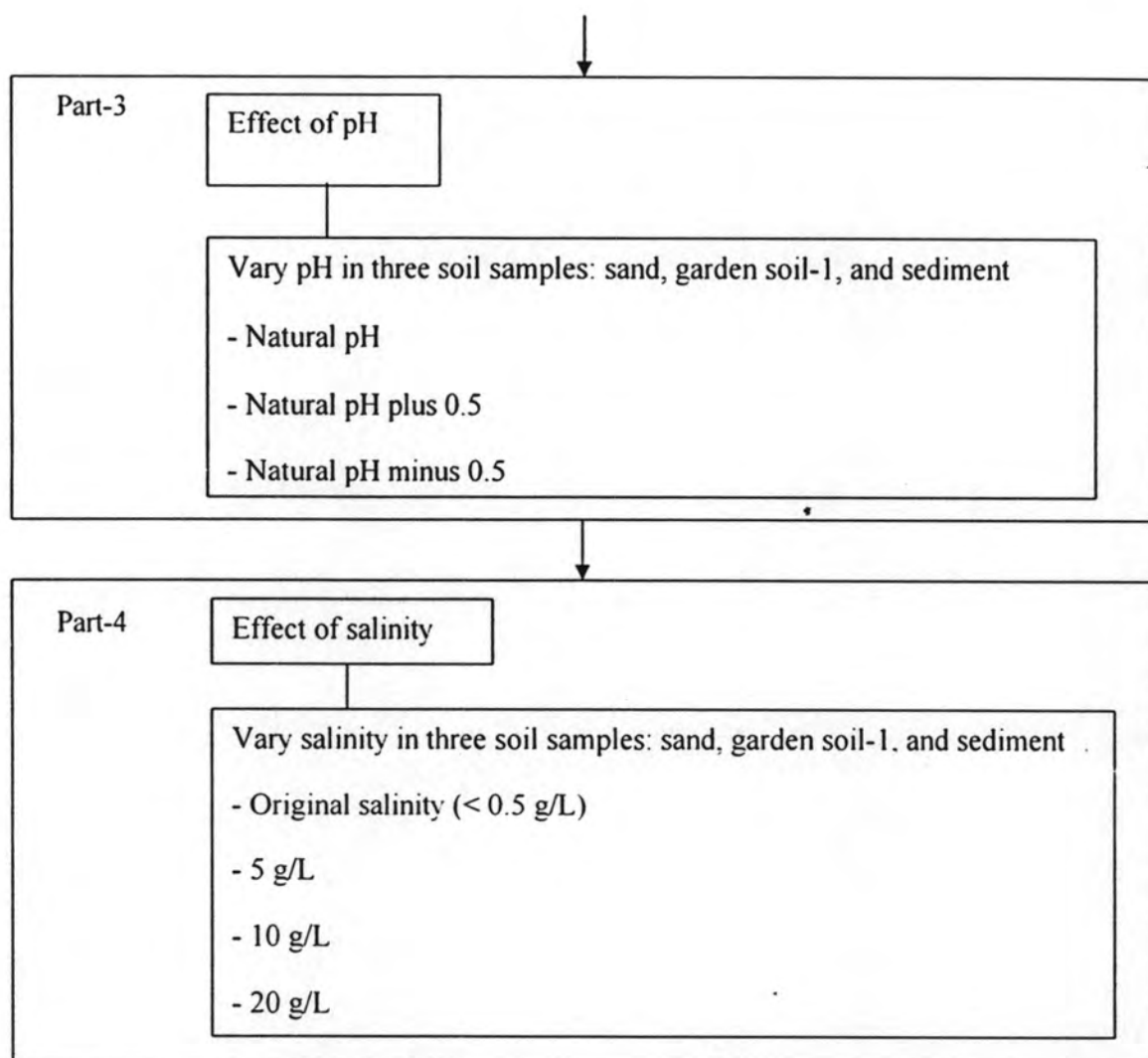


Figure 3.1 Experimental frameworks thesis

3.2 Materials

3.2.1 Soils

Six types of soil were used consisting of sand, laterite soils, garden soil-1, garden soil-2, garden soil-3, and sediments from the muscunization pond of Nile tilapia fry. All soils were air dried at ambient room temperature. Disaggregation was performed with minimal force, so that the original texture of the soil was changed as little as possible. The soils were then sieved to a particle size of less than 2 mm.

3.2.2 Chemicals

Methyltestosterone (HPLC grade), sodium chloride (analytical grade), calcium chloride (analytical grade), sodium hydroxide (analytical grade), hydrochloric acid (analytical grade), sodium azide (analytical grade), and methanol (HPLC grade) was purchased and used without further purification. 18m Ω milli-Q water, produced from ELGA pure lab ultra system, was used.

3.3 Soil and MT analysis

The list of soils parameters and their methods of characterization are presented in Table 3.1. The organic content and soil texture was determined at Agricultural chemistry division, the Department of Agriculture, Bangkok, Thailand. The surface area of the soils using the BET method was determined at the Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology of Ladkrabang. The pH of soil was analyzed using a pH meter (HACH, Sens ion2, Loveland, Colorado, U.S.A.).

One mL of the supernatant from the sorption experiment was filtered through a 0.45 μ m syringe filter and 50 μ L uL was analyzed immediately using a High Performance Liquid Chromatography (HPLC) (Shimadzu, Model No. 228-34356-38, Tokyo, Japan) with a *Inertsil ODS-3 C-18* column (GL sciences Inc., Tokyo, Japan) and a *SPD-10AV VP* UV-vis detector (Shimadzu, Model No. 228-40001-38, Tokyo, Japan) at 240 nm system. The mobile phase used was 65% methanol/ 35%milli-Q water. The flow rate was at 0.8 mL/min and the column temperature was 40.0 \pm 0.5 $^{\circ}$ C.

Table 3.1 Methods for soil and chemical analysis.

Parameters	Method/ instrument	References
Organic content	Loss on ignition	Konen et al. 2002
Soil texture	Hydrometer	Ashworth et al., 2001
Clay content	Hydrometer	Ashworth et al., 2001
Surface area	BET	Bowman et al., 2002
Methylestosterone	HPLC	-
pH	pH meter	Bank's thesis book

3.4 Experimental Procedures

Part 1: Selection of optimal soil/solution ratios and measurement of equilibrium time

To examine the suitable soil/solution ratio for the sorption experiments, 3 soil samples were used. These were sand, garden soil-1, and sediment. Three soil/solution ratios (gm:mL) were used at 1:1, 1:5, 1:10, for sand and garden soil-1 and 1:5, 1:10, and 1:30 for the sediments. The salinity or ionic strength used were equal to the solution containing 0.1 M CaCl_2 and a 0.01 M NaN_3 in milli-Q water..

Batch sorption experiments were carried out by adding 1 g soil and varying solution volumes of MT solutions in 16 mL amber vials with Teflon screw cap. In the case of sediments, 60 ml amber vials were used. The slurry was mixed with a vortex mixer for 20 s, before shaking at 170 rpm. The procedure is presented in Figure 3.2.

MT solution was prepared by diluting MT stock solution of 10,000 mg/L into a solution containing 0.1 M CaCl_2 and a 0.01 M NaN_3 in milli-Q water. The methanol in the final MT solution was kept to less than 0.5% in order to prevent desorption of MT during the sorption experiments (Kim et al., (2007). The CaCl_2 solution was used as the aqueous solvent phase to minimize cation exchange. The NaN_3 solution was used as an inhibitor of microbial biodegradation. The initial MT concentration is 2 mg/L. This solution was added in to the soil samples. For the control test, a 0.1 M CaCl_2 and a 0.01 M NaN_3 in milli-Q water was used without the sediments or soils. The experiment time set as 72 hr to observe the equilibrium. All experiments were done at least in duplicate to allow estimation of the variance of the results.

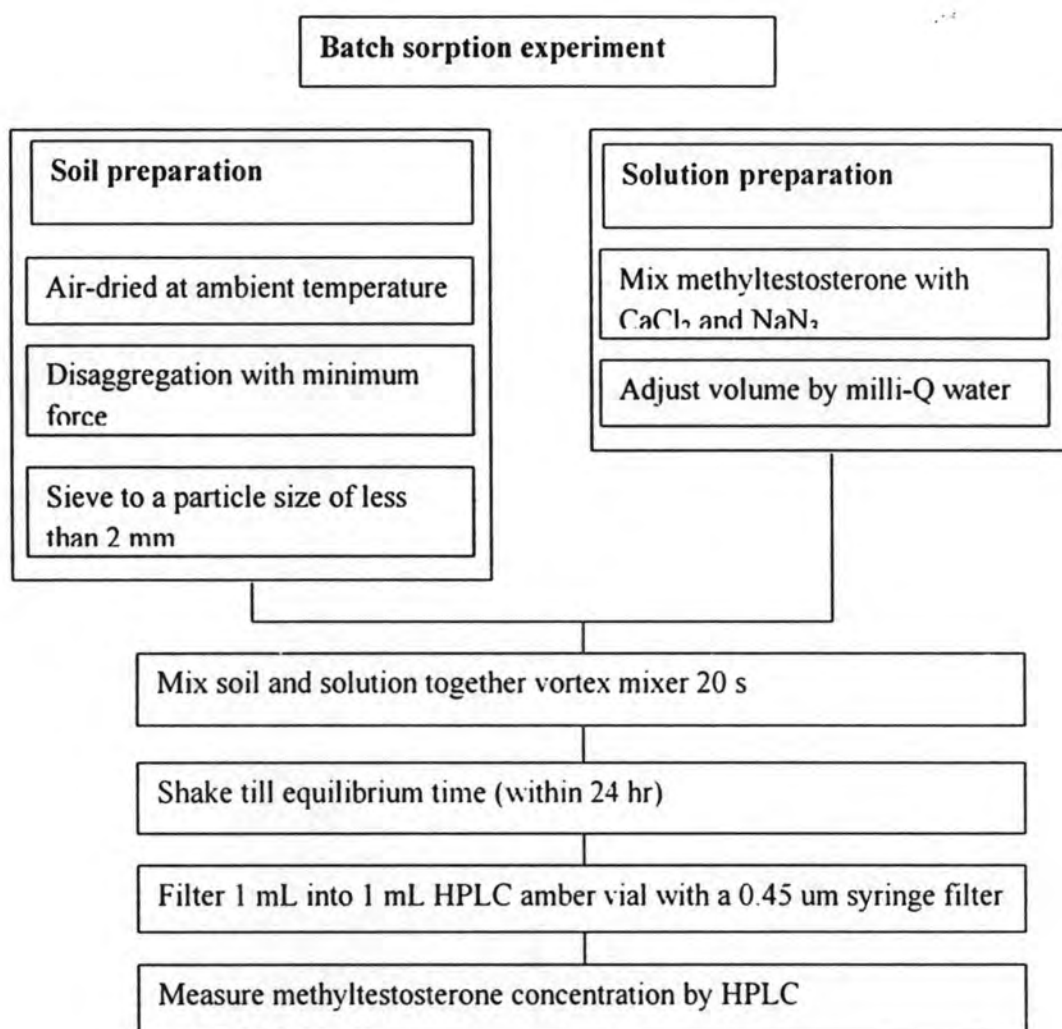


Figure 3.2 Overall methodologies for batch sorption experiment preparing

Part 2: Effect of organic content on sorption under original condition

Six types of soil were used in this study. They were sand, laterite soils, garden soil-1, garden soil-2, garden soil-3, and sediments from the masculinization pond of Nile tilapia fry. Five concentrations of MT between 1-3 mg/L were used. The pH of the slurry was as it was and the salinity or ionic strength used was equal to the salt solution added (i.e., 0.1 M CaCl_2 and a 0.01 M NaN_3). The measured initial pH of the slurry was: sand pH 7.2, laterite soils pH 7.1, garden soil-1 pH 7.5, garden soil-2 pH 7.5, garden soil-3 pH 7.4, and sediments pH 5.8.

The sorption experiments were prepared and conducted similar to Part 1. Initially, 1 g of sample and 10 mL of MT solution was added in 16 mL amber vials with Teflon screw cap, except in case of sediment, 30 mL of MT solution was added into 60 mL amber vials. The experiment was done at least in duplicate sample for

each sample and collected twice at time 14 hours and 16 hours after the start of the experiments.

Sorption isotherms were constructed accordingly. The sorption coefficient together with soil characteristic was used to identify the sorption behavior of MT.

Part 3: Effect of pH on sorption

To investigate the effect of pH on sorption, 1 g of sand or garden soil-1 was mixed with 10 mL of MT solution in 16 mL amber vials with Teflon screw cap. In the case of sediments, 1 g of sediment was added to 30 mL of MT solution in 60 mL amber vials. Five concentrations of MT in range of 1-3 mg/L were used. The experiment was conducted in the same manner as part 2. The measured initial original pH of the sand, garden soil-1 and sediments were 7.1, 7.3, and 5.9, respectively.

Before mixing with the vortex mixer for 20 s, the pH of samples was adjusted by HCl and NaOH. The pH in the vials were adjusted by + 0.5 pH units or -0.5 pH units. The experiment was done at least in duplicate sample for each sample and collected twice at time 14 hours and 16 hours after the start of the experiments.

Part 4: Effect of salinity on sorption

Sand, garden soil-1 and sediment were used in this study. Five concentrations of MT between 1-3 mg/L were used. The experiment was conducted in the same manner as the part 3.

The salinity was varied using NaCl. The salinity used in the study were initial salinity or ionic strength equal to the salt solution added (i.e., 0.1 M CaCl_2 and a 0.01 M NaN_3), 5 g/L of NaCl, 10 g/L of NaCl, and 20 g/L of NaCl which may be the salinity for Nile tilapia's breeding conditions. The pH of the slurry was the actual soil pH. The initial pH for the sand, garden soil-1 and sediment were 6.8-7.3, 6.9-7.2, and 5.4-5.9, respectively. The experiment was done at least in duplicate sample for each sample and collected twice at time 14 hours and 16 hours after the start of the experiments.