

CHAPTER 3



METHODOLOGY

3.1 Materials

3.1.1 Soil and WWTS sample collection and preparation

Soil samples used in these experiments were from a tangerine orchard in Mae-ai District, Chiang Mai. Surface soil were collected from 0-20 cm depth from four locations of the orchard and mixed before used. Samples were air-dried and sieved with sieve no. 200 before analyzing their the chemical and physical properties.

WWTS were collected from sand filter bed of 3 wastewater treatment plants in Chiang Mai, which included;

- (1) WWTS from pig farm (HUASB-High suspension solid upflow anaerobic sludge blanket WWT plant).
- (2) Suan-dok municipal wastewater treatment plant (Activated sludge WWT plant).
- (3) WWTS from food industrial-sweet corn canning (Activated sludge WWT plant).

WWTS were dried at 75 °C for 3 days, sieved with sieve no. 200 and then analyzed for their physical and chemical properties.

3.1.2 Pesticides and chemicals

99.5 % α -, β -endosulfan and 97.5% sulfate-endosulfan analytical grade (Dr. Ehrenstorfer) were used in batch experiments to determine the sorption coefficient of soil and each sludge sample. There are 2 isomers; α -endosulfan, β -endosulfan and 1 derivative; sulfate endosulfan. In the column experiment, commercial endosulfan were used. It is Pesticide-Red Sun with 35% W/V EC (Hitech group chemical supply Co., Ltd) at recommended dose (20 mL pesticide with 20 L fresh water). Hexane, acetone and triton x-100 (Fluka Chemical Industrial) were used for the extraction of endosulfan from liquid and solid samples. All solvents were reagent grade. Other chemicals such as sodium sulphate (Na_2SO_4), and calcium chloride (CaCl_2) were purchased from Fluka Chemical Industrial.

3.2 Methods

3.2.1 Determination of soil and WWTS characteristics

The soil and WWTS samples were analyzed for physiochemical properties including soil texture, pH, moisture content, organic carbon content, cation exchange capacity, and electroconductivity (Black *et al.*, 1969; Tan, 1996). In addition, environmental concerned metals such as copper (Cu), manganese (Mn), nickel (Ni),

and zinc (Zn) were analyzed. The methods of determination of soil and WWTS characteristics were presented in Table 3.1 and the details of the methods were presented in Appendix A.

Table 3.1 The methods of determination of soil and WWTS characteristics

Parameters	Methods
Soil texture	Sieve analysis
pH	pH meter (soil:water 1:1)
Moisture content	Gravimetric method
Organic carbon content	Wet oxidation method
Cation exchange capacity	Ammonium saturation method
Electroconductivity	EC meter (soil:water 1:5)
Metals	Atomic absorption spectrophotometer (AA)

3.2.2 Endosulfan extraction

Solvent extraction was used to extract endosulfan from both liquid and solid samples. The solvent here was a mixture of hexane and acetone at 9:1 ratio. For liquid samples, 2 mL solution samples were transferred into a new 22-mL vial and then 2 mL of solvent were added. For soil samples, 2 g of soil samples were mixed with 4 mL of solvent and 750 μ L of 10% Triton x-100. For sludge samples, 1 g of sludge samples was mixed with 4 mL of solvent and 1,500 μ L of 10% Triton x-100. The

samples were mixed and shaken at 250 rpm for 4 hrs. After shaken, the vials were frozen at $-4\text{ }^{\circ}\text{C}$ to solidify the lower aqueous layer, and then the solvent was transferred to next vial where 2-3 g of anhydrous sodium sulfate was added to dewater the sample. Endosulfan dissolved in solvent fraction was transferred to gas chromatography (GC) auto sample vials for analysis using gas chromatography (GC).

3.2.3 Gas chromatographic analysis

Gas chromatographic analysis was performed with a Hewlett Packard 6890 equipped with an electron capture detector (GC-ECD) and a HP-5 (5% Phenyl Methyl Siloxane) fused-silica capillary column (30 m x 0.32 mm ID; thickness, 0.25 μm). The following operating conditions were used: injector temperature $250\text{ }^{\circ}\text{C}$, detector temperature $250\text{ }^{\circ}\text{C}$, initial column temperature $120\text{ }^{\circ}\text{C}$ then, programmed at $120\text{ }^{\circ}\text{C}$ to $250\text{ }^{\circ}\text{C}$ at a rate of $10\text{ }^{\circ}\text{C min}^{-1}$ (3 min), and $250\text{ }^{\circ}\text{C}$ to $300\text{ }^{\circ}\text{C}$ at a rate of $50\text{ }^{\circ}\text{C min}^{-1}$ (4 min). A post column temperature of $325\text{ }^{\circ}\text{C}$ was held for 3 min. The carrier gas was helium with gas flow 20 mL min^{-1} , and a 5: 1 injector split ratio. The make-up gas was N_2 at 60 mL min^{-1} .

Under these conditions, endosulfan compounds had a retention time for α - , β -, and sulfate-endosulfan at 12.060, 13.167, and 14.100 min, respectively.

3.3 Method evaluation

3.3.1 Background contamination in soil and WWTS

Background contamination of soil and WWTS were analyzed. Soil and WWTS samples were checked for endosulfan contamination prior to use. Three blank analyses were carried out in each soil and WWTS analyses.

Two g soil and 1 g WWTS were used for background analysis. Then the samples were extracted and prepared for GC analysis.

3.3.2 Calibration

(1) Stock standard solutions

α -, β -, and sulfate-endosulfan were dissolved in solvent (hexane: acetone 9:1) at a concentration of 10,000 ppm in hexane. The stock solutions were kept in 22 mL-vial and stored in the dark at 4 °C.

(2) Calibration curve

The calibration curve was developed using six concentrations of endosulfan. This was carried out with 10, 25, 50, 100, 250, and 500 ppb (0.02S, 0.05S, 0.1S, 0.2S,

0.5S, and 1S) (S = water solubility of endosulfan). The analysis was carried out in triplicate. The samples were injected and analyzed by GC.

3.3.3 Percent recovery

Recoveries of the endosulfan were determined and repeated three times to determine the extraction efficiencies. Percent recovery was determined according to the following

$$\text{Percent recovery} = \frac{\text{Amount recovered} \times 100}{\text{Original amount spiked}}$$

As the result, the recovery of endosulfan in water and soil were 90 and 75 %, respectively.

3.4 Batch partitioning experiment

3.4.1 Sorption equilibrium of endosulfan

Sorption equilibrium of endosulfan were conducted using 0.5 g dried soil (or sludges) with 5 mL of 100 ppb endosulfan in 0.01 M of CaCl₂. Then, the samples were shaken for 0-6 hr before extracted and analyzed by GC. The samples were collected at time interval as shown in Table 3.2. The equilibrium time of endosulfan sorption was determined from a plot between % sorption and time (hr).

Table 3.2 Time interval for determination of equilibrium

Item	Description	Time interval (hr.)						
		0	0.5	1	1.5	2	4	6
1	Soil 0.5g + endosulfan 5 mL							
2	Pig farm sludge, 0.5g + endosulfan 5 mL							
3	Municipal sludge, 0.5g + endosulfan 5 mL							
4	Food ind. sludge, 0.5g + endosulfan 5 mL							

Remark: Pig farm sludge = Wastewater treatment sludge from pig farm.

Municipal sludge = Municipal wastewater treatment sludge.

Food ind. sludge = Wastewater treatment sludge from food industrial-sweet corn canning.

3.4.2 Sorption coefficient and desorption efficiency of soil and WWTS

For sorption coefficient (K_d), the experiments were conducted using 0.5 g dried soil (or sludge) with 5 mL of endosulfan solutions in 0.01 M of CaCl_2 as shown in Figure 3.1. The initial concentrations of endosulfan were ranged from 50 to 300 ppb as shown in Table 3.3. Shaking period for the samples was equal to the equilibrium time (4 hrs). The sorbed concentration of soil (or sludge) was determined by GC analysis. Then, the sorption coefficient of soil (or sludges) was calculated from sorption isotherm. Desorption efficiency were measured immediately after completed sorption by successive dilution (in 0.01 M CaCl_2) of the sorped samples from the 50,

150, and 300 ppb initial endosulfan concentration points. The 5 mL of supernatant removed for sorption analysis were replaced with 5 mL of 0.01 M CaCl_2 . The samples were resuspended and shaken at 20 °C for another 24 hr. Equilibrium concentration of desorped endosulfan in the supernatant were determined.

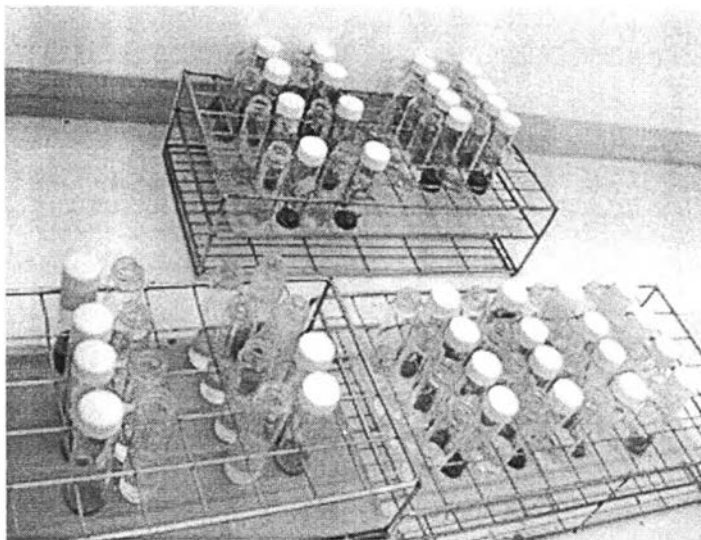


Figure 3.1 Soil sample vials in batch partitioning experiment

The sorption coefficient and desorption efficiency of each WWTS were compared to each others. The more sorption coefficient would represent the more pesticide that is sorped to sludge. Thus, WWTS with the highest value of sorption coefficient was chosen for the following soil column experiment.

Table 3.3 Concentration of endosulfan for determination of sorption coefficient

Item	Description	Concentration of endosulfan (ppb)					
		50	100	150	200	250	300
1	Soil 0.5g + endosulfan 5 mL	50	100	150	200	250	300
2	Pig farm sludge, 0.5g + endosulfan 5 mL						
3	Municipal sludge, 0.5g + endosulfan 5 mL						
4	Food ind. sludge, 0.5g + endosulfan 5 mL						

3.5 Soil column experiment

Soil columns were prepared by connecting five 5-cm glass rings and sealed with para film and plastic tape. The length and diameter of soil columns are 25 cm and 3.8 cm, respectively. The bottom ring was filled with coarse sand and glass wool, to prevent losses of soil. The upper three rings were hand-packed with soil sample (density for soil compaction 1.4 g/L, moisture content 14%). Finally, the top ring was covered with 1 or 2 cm depth of WWTS as shown in Figures 3.2 and 3.3.

Then, endosulfan were applied. Endosulfan stock solution was prepared by mixing 0.2 mL endosulfan commercial grade (Red Sun Pesticide-Red Sun with 35% W/V EC) with 200 mL Deionized water. The stock endosulfan was applied on the soil surface at 650 $\mu\text{L}/\text{column}$ (double amount of the recommended dose) for preliminary study and 1,000 $\mu\text{L}/\text{column}$ (triple amount of the recommended dose) in soil column

experiment. Then, 30 ml. CaCl_2 were poured into the column to simulate the worst case scenario of heavy rainfall after pesticide application. The columns were poured with 0.01 M CaCl_2 solution everyday after application to maintain soil moisture content. columns were collected and separated into five rings at given time period as shown in Table 3.4. Soil and sludge samples from each depth were extracted and analyzed for the amount of endosulfan. The column test were conducted in triplicate.

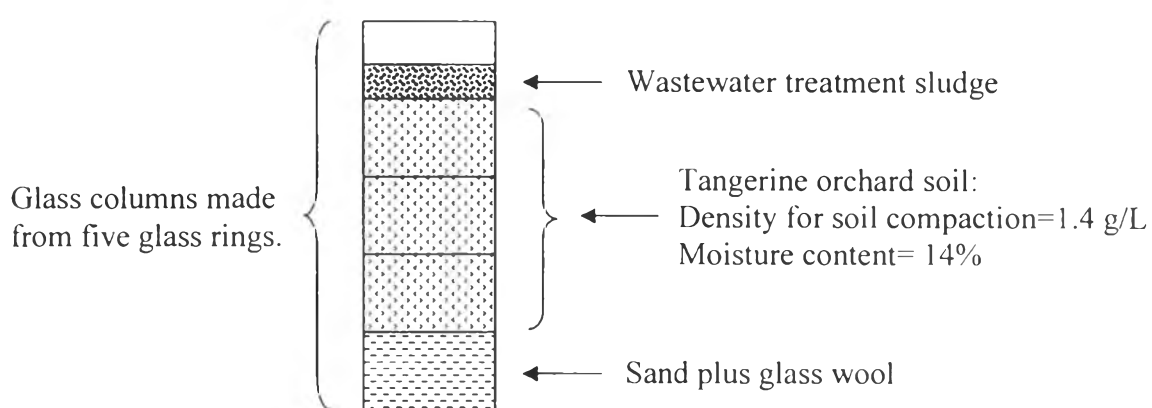


Figure 3,2 Diagram of the constructed soil column.

Comparison of single and weekly endosulfan application was studied. The weekly application experiment was represented the worst case scenario of pesticide application. The amount of endosulfan in each depths of column were determined. The period of sampling soil were the same as one application experiment (Table 3.4).

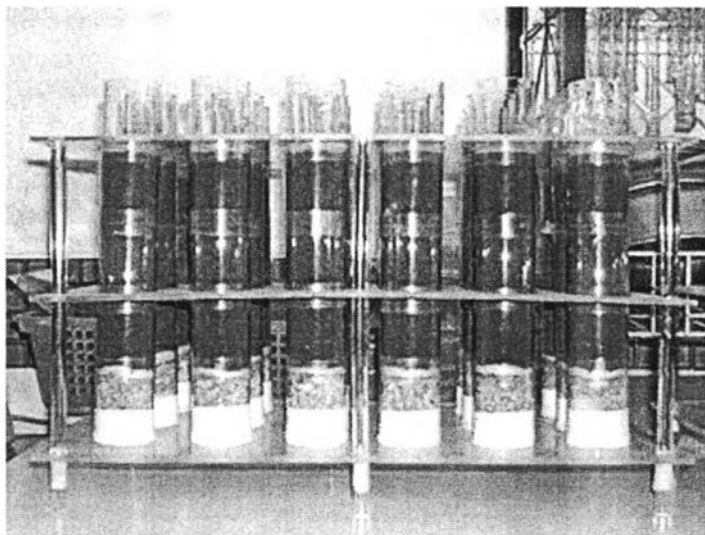


Figure 3.3 Soil column experiment's apparatus.

Table 3.4 Sampling periods after endosulfan application to the soil columns

Item	Description of column	Period after application for sampling (days)					
		0	7	14	21	28	35
1	Soil without Sludge						
2	Soil with 1 cm. depth of sludge	0	7	14	21	28	35
3	Soil with 2 cm. depth of sludge						

Remark: Five soil samples from 0-2.5, 2.5-5, 5-7.5, 7.5-10, 10-15 cm depths and 1 additional for sludge sample.

3.6 Data analysis

Statistical analysis in this study was processed in Microsoft Excel XP. The software provided a wide range of statistical analyses including mean, standard deviation, linear regression, equations and correlative coefficient, that were mostly used in this study.