CHARPTER 2
EXPERIMENTAL

2.1 Apparatus and chemicals

2.1.1 Apparatus

1. Centrifuge, Gemmy Industrial Crop., Model PLC-012E, Taiwan
2. Fluoride combination electrode, Cole-Parmer, Model 5800-06, U.S.A.
3. Hotplate & Stirrer, Harmony, Model HTS-1003, Japan
4. Incubator shaker, Memmert, Model WB 22, Germany
5. Micropipette, Gilson, Model SL-5000, France
6. pH-meter, Metrohm, Model 744, Switzerland
7. Sonicator, Branson, Model 3510-DTH, U.S.A.

2.1.2 Chemicals

1. Ammonium acetate, CH_3COONH_4, A.R., Univar, Australia
2. Calcium Chloride, $CaCl_2.2.H_2O$, RPE, Carlo Erba, Italy
3. Ethylenediamine tetraacetic acid, disodium salt (EDTA), ($C_{10}H_{14}N_2Na_2O_8.2H_2O$), A.R., Aldrich, U.S.A.
4. Glacial acetic acid, CH_3COOH, A.R., BDH, England
5. Hydrochloric acid, HCl, A.R., J.T. Baker, Canada
6. Sodium chloride, NaCl, RPE, Carlo Erba, Italy
7. Sodium fluoride, NaF, Pure, BDH, England
8. Sodium hydroxide, NaOH, RPE, Carlo Erba, Italy
9. Sodium sulphate, Na_2SO_4, RPE, Carlo Erba, Italy

2.2 Preparation of the standard solutions and reagents

All solutions were prepared in deionized distilled water

2.2.1 Stock standard fluoride solution, 1000 mg/l

An accurate weight of 2.21 g NaF was dissolved and diluted to 1000 ml with deionized distilled water.

2.2.2 Standard fluoride solutions, 0.5, 1, 2, 4, 6, 8, 10, 50 and 100 mg/l

The fluoride standard solutions were prepared from 1000 mg/l stock standard fluoride solution to obtain different concentrations in 250 ml volumetric flasks. The desired volumes are listed in Table 2.1

Table 2.1 The desired volume of 1000 mg/l stock standard fluoride solution for diluting to get working standard fluoride solutions

Fluoride concentration (mg/l)	Pipetted volume (ml)
0.5	0.12
1.0	0.25
2.0	0.50
4.0	1.00
6.0	1.50
8.0	2.00
10.0	2.50
50.0	12.50
100.0	25.00

2.2.3 Total ionic strength adjustment buffer; TISAB

Placed a 1 liter beaker containing 500 ml of deionized distilled water on a hotplate-magnetic stirrer, added 57 ml of CH_3COOH, 58 g of NaCl, and 4 g of EDTA respectively and heated the mixture until the solids dissolved. Then the solution was cooled down to room temperature and adjusted the pH to 5.25-5.30 with 5M NaOH. Transferred the solution to a 1 liter volumetric flask and diluted with deionized distilled water.

2.2.4 Sodium hydroxide, 6 M

Prepared 1000 ml of 6 M sodium hydroxide by weighing 240 g of NaOH, dissolved and made up to 1000 ml in a volumetric flask with deionized distilled water.

2.2.5 Hydrochloric acid, 1 M

Pipetted 16.60 ml of concentrated hydrochloric acid into a 200 ml volumetric flask and diluted to the volume with deionized distilled water.

2.2.6 Ammonium acetate, 1 M

Prepared 1 M ammonium acetate by weighing 15.416 g of CH_3COONH_4, dissolved and made up to 200 ml in a volumetric flask with deionized distilled water.

2.2.7 Calcium chloride, 0.01 M

0.01 M calcium chloride was prepared by dissolving 0.294 g $CaCl_2.2H_2O$ in deionized distilled water and diluting to 200 ml in a volumetric flask.

2.3 Experimental method

2.3.1 Soil sample preparation

To a 20 g soil sample collected in Chiang Mai University campus, added 10 ml of 100 mg/l standard fluoride solution and mixed them to obtain homogeneous mixture. Soil sample mixture was air-dried and ground, then extracted the fluoride in the soil with different kinds of extractants as described below.

2.3.2 Selection of the extractants

Fluoride was extracted from soil samples using four different extractants i.e. deionized distilled water, ammonium acetate (CH_3COONH_4), calcium chloride ($CaCl_2$) and hydrochloric acid (HCl). One gram of soil sample was weighed into a 250-ml Erlenmeyer flask. Then mixed with 15 ml of the selected extractant and shook for 1 h at room temperature. The soil sample solution was filtered with a Whatman No.1 filter paper and centrifuged the supernatant for 5 min (at about 4000 rpm). Then the fluoride was analyzed.

2.3.3 Optimization of extraction conditions

Optimization of conditions for extracting fluoride from the soil sample using the suitable extractant determined from 2.3.2 was carried out by varying the parameters i.e. temperature, extraction time and extractant volume. These parameters were varied in the following manner; temperature, 50-90 °C; extraction time, 30-120 min and extraction volume, 15-30 ml. Five milliliters of the supernatant was added to

5 ml of TISAB (total ionic strength adjusting buffer) and the electrical potential of solution was measured with a F-ion-sensitive electrode.

2.3.4 Sampling of the soil

Soil sampling in the areas where the reverse osmosis drinking water vending machines are installed were done. The soil samples were collected, from five villages in Amphoe Mueang Lamphun and Amphoe Ban Thi of Lamphun province, in the systematic grid-sampling pattern and three spots were selected in each block for random soil collection. The block size was 5 m × 5m (Table 2.2 and Fig. 2.1-2.5) and the collection was done at three consecutive depths, i.e., 0-20, 20-40 and 40-60 cm. Soil samples were air-dried, sieved and kept in the plastic containers.

2.3.5 Soil analyses

An amount of 1 g of each soil sample (air-dried and sieved to pass a 1.5 mm sieve) was weighed into a 250 ml Erlenmeyer flask. Then added 15 ml of deionized distilled water, shook for 90 min at 80°C and filtered through a Whatman No.1 filter paper and centrifuged the filtrate for 5 min (at about 4000 rpm). Pipetted 15 ml of each supernatant into plastic bottles and mixed with 5 ml TISAB and the solution was measured with a F-ion-sensitive electrode.

Table 2.2 The description of the soil sampling sites

Site		Global Positioning System (GPS)	Soil Type	Topography	No. of block	Block size (m^2)
1	Ban Luk Tambon San Pa Sak Amphoe Mueang	X (503711-504055) Y (2051026-2051176)	Chiang Rai (silt and clay)	flat + slopy	7	25
2	Ban Mae San Pa Daed Tambon Wiang Yong Amphoe Mueang	X (503182-503191) Y (2052736-2052756)	Chiang Rai (silt and clay)	flat	6	25
3	Ban San Makrut Tambon Ban Pan Amphoe Mueang	X (494950-494968) Y (2048503-2048519)	Alluvial (loamy and sandy)	small waterway	4	25
4	Ban San Phrachao Daeng Tambon Huai Yap Amphoe Ban Thi	X (514738-514749) Y (2063788-2063797)	San Sai (loamy and sandy)	small waterway	4	25
5	Ban Thi Tambon Ban Thi Amphoe Ban Thi	X (513362-513371) Y (2061880-2061890)	San Sai (loamy and sandy)	small waterway + flat	5	25

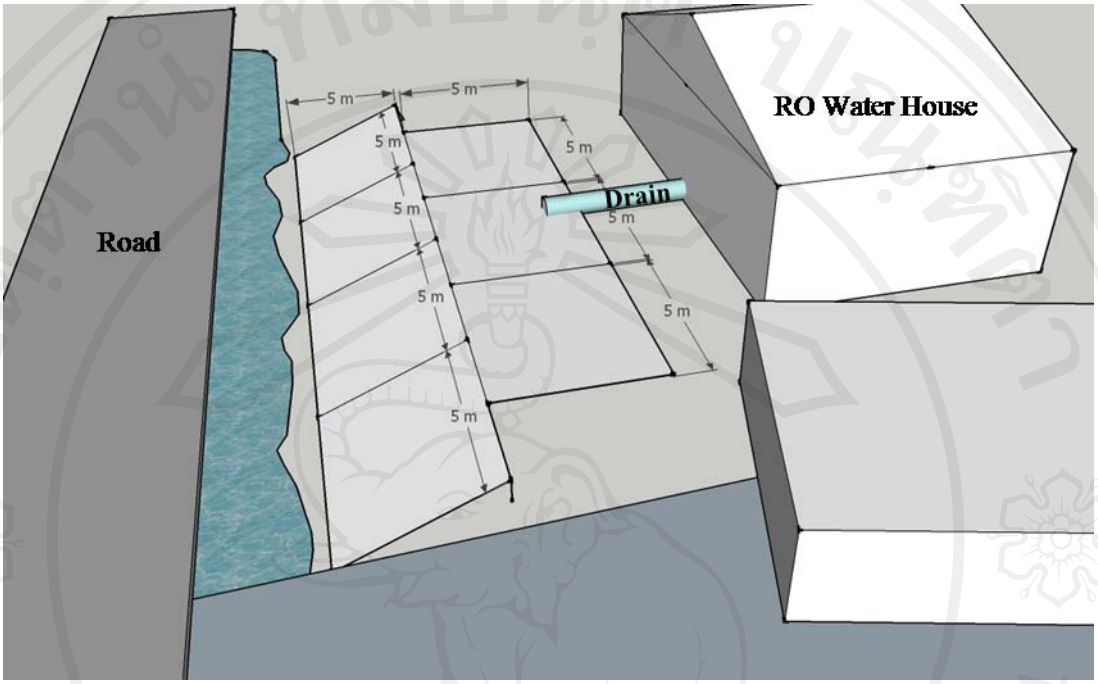

Fig. 2.1 Soil sampling location of Ban Luk

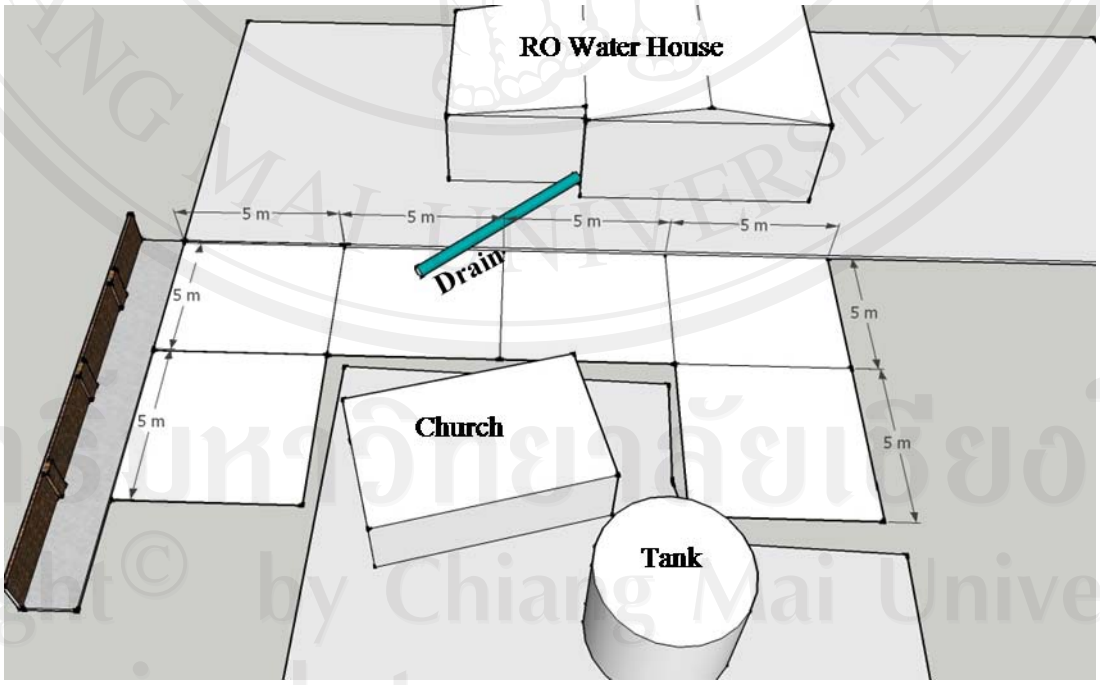

Fig. 2.2 Soil sampling location of Ban Mae San Pa Daed

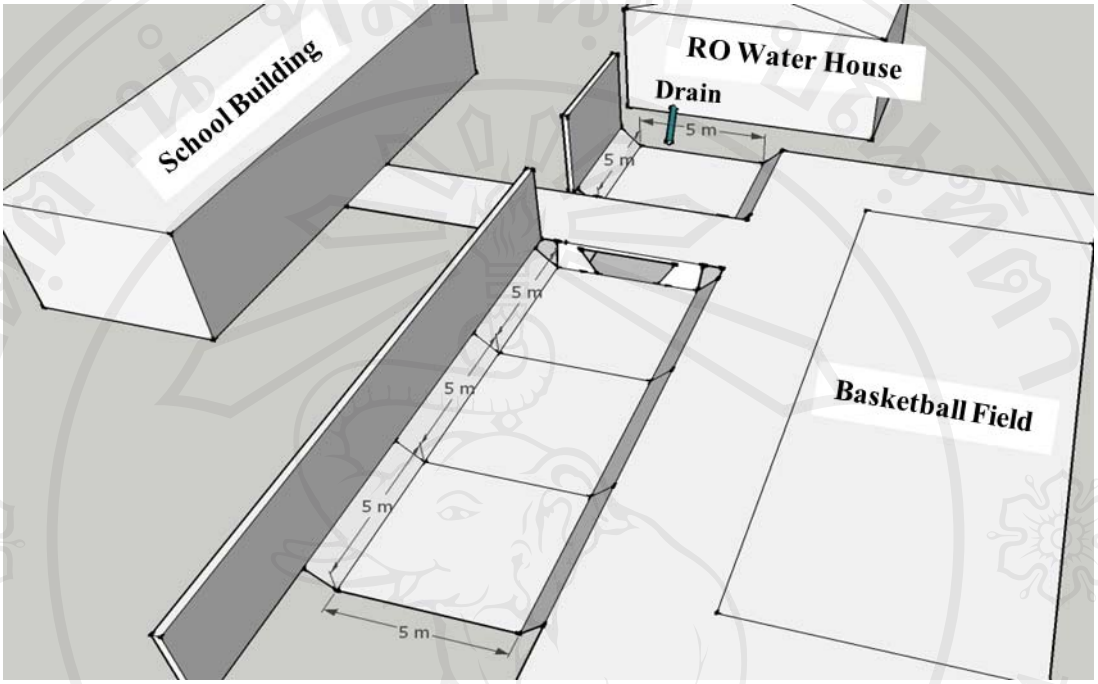

Fig. 2.3 Soil sampling location of Ban San Makrut

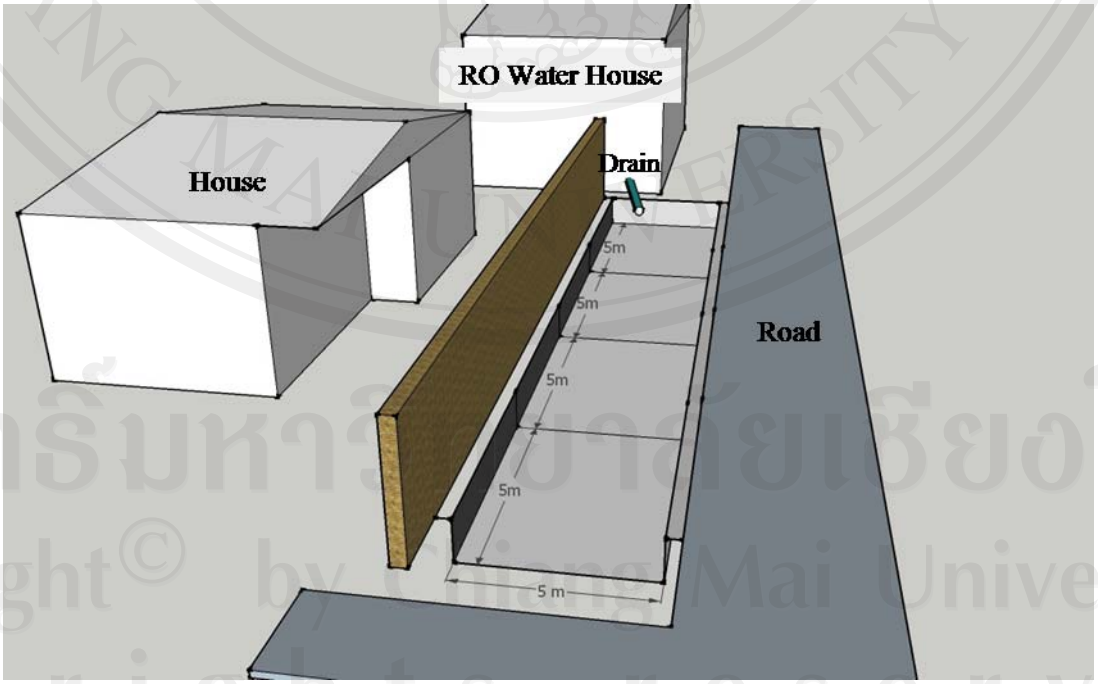

Fig. 2.4 Soil sampling location of Ban San Phrachao Daeng

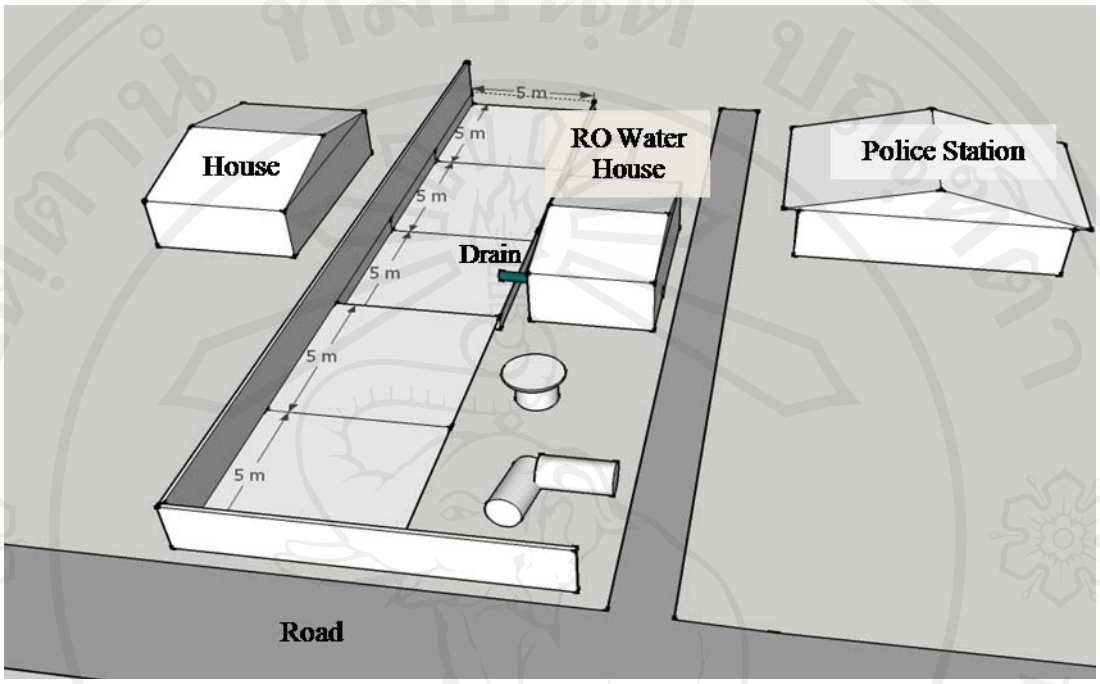

Fig. 2.5 Soil sampling location of Ban Thi