

CHAPTER 2

MATERIALS AND METHOD

2.1 Materials, instrument and apparatus

2.1.1 Field work

- 1) Plastic tanks (35 liters, 1000 liters)
- 2) Plastic jar with cap (10 liters)
- 3) Plastic pots (top wide 11")
- 4) Watering cans
- 5) Plastic net and plastic bags
- 6) Wood pole
- 7) Polyethylene bottles (100 ml)
- 8) Seed of rice, pole bean, tobacco, tomato, cucumber
- 9) pH meter and conducto-meter
- 10) Pipette, beaker
- 11) Dropper, label paper, permanent pen

2.1.2 Laboratory work

- 1) pH meter and conducto-meter
- 2) Spectrophotometer (Shimatzu)
- 3) Atomic absorption spectrophotometer (Perkin Elmer)
- 4) Ion chromatography (Water)
- 5) Micro Kjeldah and digester (Kjeldahl digestion tubes in heating block)

- 6) Electric balancing
- 7) Dry oven
- 8) Pump and filtering machine
- 9) Blender, sieves
- 10) Burette, beakers
- 11) Volumetric flasks

2.2 Chemicals and others

2.2.1 Field work

- 1) Conc. H_2SO_4
- 2) Deionized water

2.2.2 Laboratory work

- 1) Standard solution 1000 ppm (Na, NH_4 , K, Mg, Ca, Cl, NO_3 , SO_4)
- 2) Conc. H_2SO_4 ,
- 3) Methyl orange
- 4) K_2PO_4 , BaCl_2 , KCl
- 5) Ammonium fluoride, hydrochloric acid
- 6) Ammonium molybdate, antimony
- 7) Potassium tartrate, ascorbic acid
- 8) KH_2PO_4 , selenium powder
- 9) Hydrogen peroxide, sodium hydroxide
- 10) Bromocresol green, methyl red
- 11) Boric acid, ammonium acetate

12) Potassium chloride, barium chloride

13) Calcium sulfate, gum acacia

14) Deionized water and distilled water

15) Filter paper and tissue paper

2.3 Methods

2.3.1 Rainwater collection and preparation

Rainwater was collected on the roof of the Science (SCB) building 1, Science Faculty, Chiang Mai University, far from obstructions such as buildings and trees. Rainwater was collected from 4 points on the roof, 2 in an open area and 2 collecting water draining off of a concrete roof. The rainwater from all sample points was mixed and stored in a 1000-liter plastic tank. Sixty liters of natural rainwater were taken to the green house of the Biology Department everyday and the pH adjusted to pH 2.0, 2.5, 3.0, 4.0 and 5.0 by adding sulfuric acid. Natural rainwater was retained for the controls.

2.3.2 Planting and watering

Seeds of five local plants species namely rice (100 seeds per replicate), tomato (100 seeds per replicate), tobacco (200 seeds per replicate), pole bean (30 seeds per replicate) and cucumber (50 seeds per replicate) were sown in plastic pots with 6 treatments and 5 replicates. The seeds were counted and placed in pots, which were labeled with the species name. Most of the species were planted in loamy soil. Compost fertilizer was added into the soil in every pot. The plants were stood in light and stored in the green house at the Department of Biology, Chiang Mai University (Figures 2.1 and 2.2).

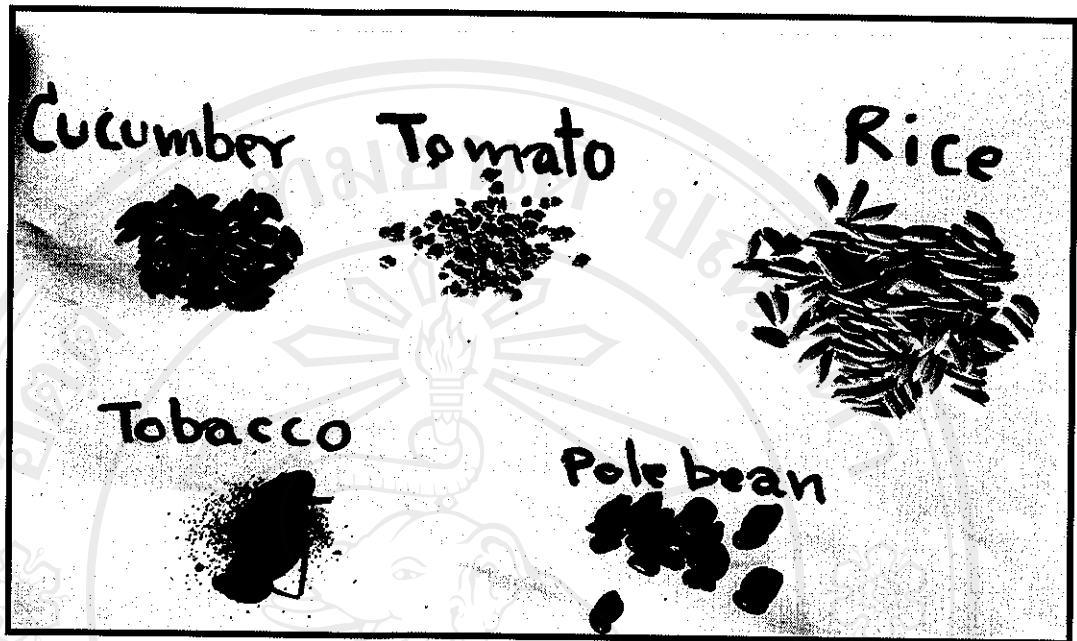


Figure 2.1 Seeds of five plants species in the study



Figure 2.2 Planting in the green house at Chiang Mai University (aged 3 days)

Three hundred milliliters of natural rainwater and simulated rainwater were applied to the plants by watering can, once a day during the germination and seedling periods and once every 2 days after the seedling period. Percentage of seed germination was recorded (Figure 2.3). After germination, all except 5 plants were removed. The height of each of the 5 remaining plants and general appearance, including yield were recorded.



Figure 2.3 Seedlings of study's plants in the greenhouse (aged 14 days)

2.3.3 Sampling

2.3.3.1 Rainwater

After mixing, natural rainwater was sampled for the control treatment. 100 milliliters of the simulated rainwater at pH 2.0, 2.5, 3.0, 4.0 and 5.0 were also collected for the analysis. Total samples for natural rainwater were 69 samples and simulated rainwater were 87 samples.

2.3.3.2 Soil

One hundred and fifty soil samples were taken from the pots in the greenhouse for the analysis in the laboratory. 1 gram of soil in each sample was analysed.

2.3.3.3 Plants

Two plant species with 23 samples namely pole bean (12 samples) and tomato (11 samples) were collected from the pots subjected to each treatment.

2.3.4 Sample analysis

2.3.4.1 Rainwater

Rainwater samples were analyzed by using the standard procedure of the technical manual in EANET, 1999. Measured parameters were pH, conductivity, cations (Na^+ , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+}) and anions (Cl^- , NO_3^- , SO_4^{2-} , PO_4^{2-}).

1) pH

The pH was measured by using a pH-meter. pH probe consisting of electrodes which are sensitive to hydrogen ion concentration (strictly speaking, the hydrogen ion activity).

2) Electrical conductivity (EC)

Conductivity was measured by using a conducto-meter. EC probe consists of electrodes that are sensitive to measures the ability of conductivity in water. The results were expressed in $\mu\text{s/cm}$.

3) Anions (Cl^- , NO_3^- and SO_4^{2-})

Samples were filtrated with $0.45\ \mu\text{m}$ teflon filter and analyzed for Cl^- , NO_3^- and SO_4^{2-} by ion chromatography (Figure 2.4). The results were expressed in ppm.

4) Cations (Na^+ , K^+ , Mg^{2+} and Ca^{2+})

The samples were filtrated with $0.45\ \mu\text{m}$ teflon filter and analyzed for Na^+ , K^+ , Mg^{2+} and Ca^{2+} by ion chromatography. The results were expressed in ppm.

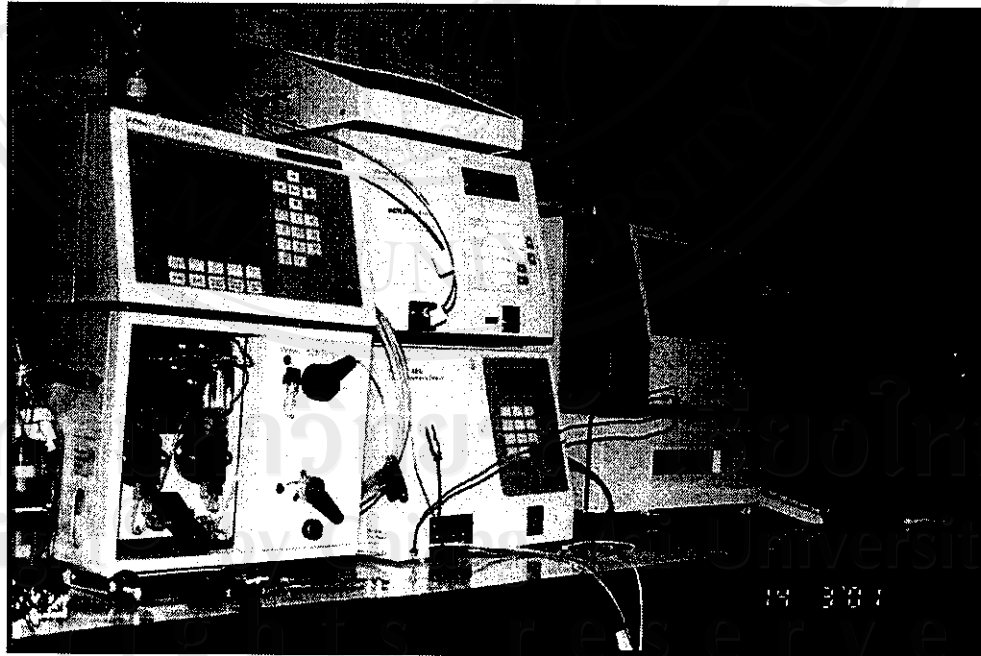


Figure 2.4 The Ion Chromatography

5) Phosphate (PO_4^{2-})

The ascorbic acid method used to measure and analyze for PO_4^{2-} using a spectrophotometer at 880 nm. Ammonium molybdate and potassium antimonyl tartrate were reacted in an acid medium with orthophosphate, forming a heteropoly acid phosphomolybdic acid. This was reduced by ascorbic acid into an intense molybdenum blue solution. The result was calculated in ppm.

2.3.4.2 Soil

Soil samples were analyzed using the standard procedure of the technical manual in EANET, 1999. Measured parameters were pH (KCl), tota-N, Na^+ , K^+ , Mg^{2+} , Ca^{2+} , SO_4^{2-} and PO_4^{2-} .

1) Sample drying and crushing

Samples were air dried, ground and sieved through a 0.5 mm sieve.

2) Soil pH (KCl)

This was determined on a 1:1 (V/V) soil/water mixture (5 g NCR-13 soil scoop/5 ml deionized water). Samples were stirred both before and after a 15 minutes equilibration period. pH was measured on a pH meter with glass electrodes. Standard pH buffers 4.0 and 7.0 were used to calibrate the electrode.

3) Na^+ , K^+ , Mg^{2+} and Ca^{2+}

Samples were extracted with 30 ml of 1 M NH_4OAc (pH 7.0) for 30 minutes, filtered through a 0.45 μm teflon filter and analyzed for Na^+ , K^+ , Mg^{2+} and Ca^{2+} by using atomic absorption spectrophotometer. The results were calculated in ppm.

4) SO_4^{2-}

Samples were extracted with 30 mL of 1 M NH_4OAc (pH 7.0) for 30 min, and filtered through a 0.45 μm teflon filter and analyzed for SO_4^{2-} by using ion chromatography. The results were expressed in ppm.

5) PO_4^{2-}

The ascorbic acid method was used to analyze for PO_4^{2-} by using a spectrophotometer at 880 nm. Ammonium molybdate and potassium antimonyl tartrate were reacted in acid medium with orthophosphate to form a heteropoly acid phosphomolybdic acid. This was reduced by ascorbic acid into an intense molybdenum blue solution. The results were expressed in ppm.

6) Total Nitrogen (N)

Samples were digested with 2.5 ml conc. H_2SO_4 1.5 g K_2SO_4 and 7.5 mg selenium. The mixture was placed in an electrically heated aluminum block at 400°C and digested for 3 hours (Figure 2.5).

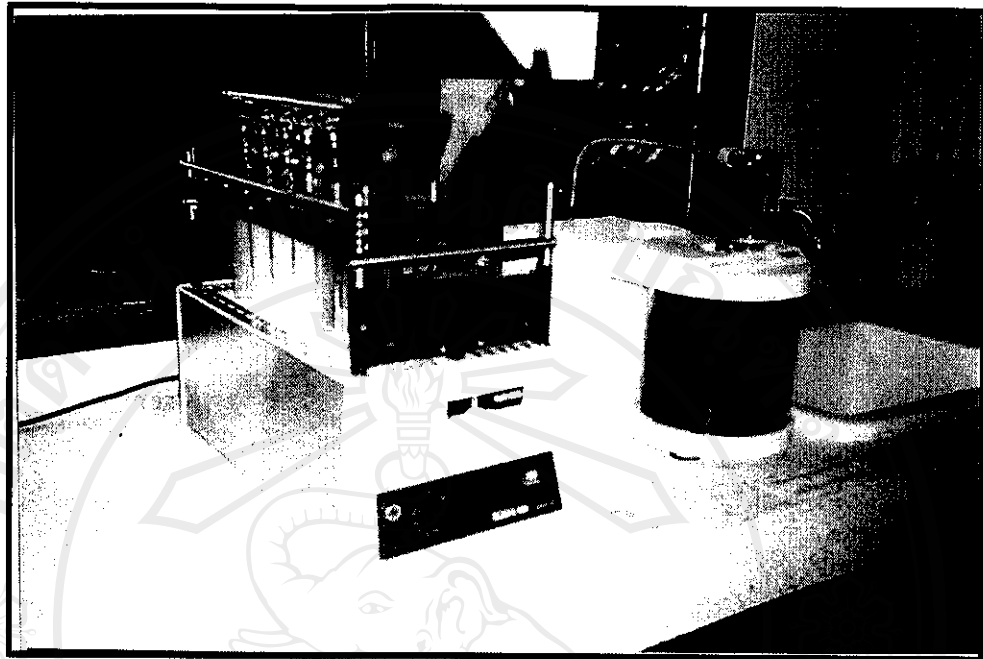


Figure 2.5 Kjeldahl digestion tubes

2.3.4.3 Plants

Samples of two plant species namely pole bean and tomato were analyzed by using the standard procedure of the technical manual in EANET, 1999. Measured parameters were total-N, phosphorus, sulfur, Na^+ , K^+ , Mg^{2+} and Ca^{2+} .

1) Sample drying and crushing

Samples were oven-dried at 80°C for 24 hours. A grinding mill was used and the ground samples were sieved through a 0.5 mm sieve.

2) Total nitrogen (N)

Samples were oven dried and digested with 2.5 ml conc. H_2SO_4 1.5 g K_2SO_4 and 7.5 mg Selenium. This mixture was placed in an electrically heated aluminum block at 400°C and digested for 45 minutes.

3) Na^+ , K^+ , Mg^{2+} and Ca^{2+}

Samples were extracted by 30 ml of 1 M NH_4OAc (pH 7) for 30 min. 0.45 μm teflon filter were used for filtered process and analyzed for Na^+ , K^+ , Mg^{2+} and Ca^{2+} by atomic absorption spectrophotometer. The results were shown in ppm.

4) Phosphorus (P)

Samples were analysed for phosphorus by molybdate-blue/ascorbic acid method at 880 nm wavelength. Orthophosphate was reacted with ammonium molybdate and potassium antimony tartrate were formed phosphomolybdic acid. This complex was reduced by ascorbic acid. The results were shown in ppm.

5) Sulfur (S)

Samples were measured for sulfur by using the colorimetric method at 410 nm wavelength. Sulfur was reacted with barium chloride and ammonium acetate. This complex was produced white sediment as gum acacia. The results were shown in ppm.