Chapter 3

Materials and Methods

1. Materials and equipments

1.1 Paper bag

1.2 Filter paper (blue ribbon band)

1.3 Soil tube

1.4 Hotplate

1.5 Electronic balance and micro balance

1.6 Dilutor model Gilson 401

1.7 Glass crucible (B extraction)

1.8 Porcelain crucible

1.9 Grinding machine

1.10 Oven and desiccator

1.11 Blender

1.12 Titrator

1.13 Digital refractometer by ATAGO Co., LTD

1.14 pH meter

1.15 Colorimeter instrument model Minolta CR-300

1.16 Nitrogen Analyzer model Elementar Vario Max

1.17 Spectrophotometer model Hitachi U-3300

1.18 Flame photometer model Eppendorf ELEX 6361

1.19 Atomic absorption spectrophotometer model Unicam 939

1.20 Inductively coupled plasma mass spectrometry Analyzer (ICP-MS) model ELAN 6000

1.21 CE – Instrument model CNS 2500

2. Methods

1. Nutrient concentration in soil of lychee orchards at different representative sites

The experiment was conducted on lychee orchard located in different areas of Chiang Mai province in order to obtain sample from different types of soils: granite soil as parent material at Mae Sa Mai village, Mae Rim district, limestone soil as parent material at Nawai village, Chiang Dao district and sandstone soil as parent material at Phae Jadee village, SanSai district. Both leaves and soils were collected to be used in further analyses.

Nine of soil cores were randomly collected from each orchard by using soil tube. The samples were separated and collected at four levels of soil depth: 0 – 15 cm., 15 – 30 cm., 30 – 45 cm. and 45 – 50 cm. Soil samples (composite sample) of each soil depth were air dried at room temperature. The samples were digested and analysed at Institute of Soil Chemistry, University of Hohenheim, Germany.

Data collection

Data collected included:

1. Soil pH was analysed by Electrometric method.
2. Nitrogen was analysed by the Kjeldahl method by Olsen (1929).
3. Phosphorus and Potassium were analysed by using CAL-method by Schueller, (1969)
4. Calcium was analysed with DTPA-extraction method by Lindsay and Norvell (1978)
5. Iron, Manganese, Copper and Zinc were extracted with DTPA-extraction method by Lindsay and Norvell (1978)
6. Magnesium was analysed by $CaCl_2$-extracted method
7. Boron was extracted by using hot water method (Dible *et al*, 1954)

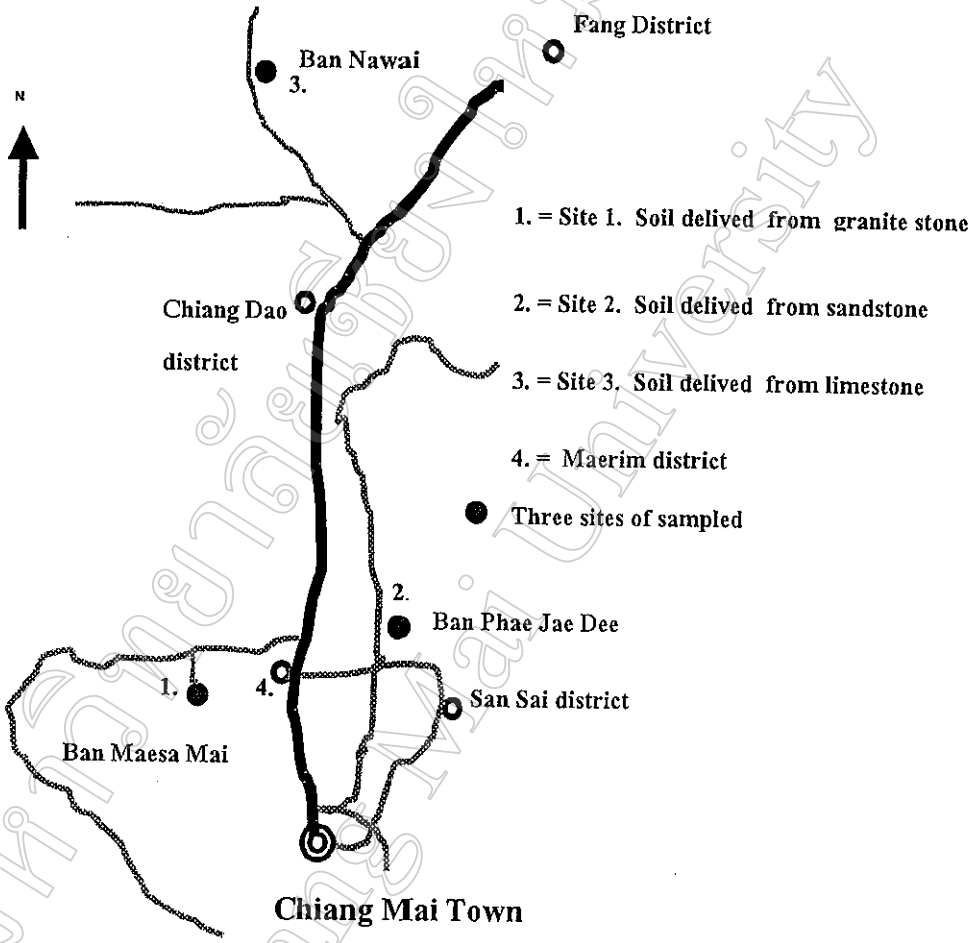

Figure 3.1 Locations where samples were collected in Chiang Mai, Thailand

2. Nutritional concentration in lychee leaf

Nutritional concentration of lychee leaves was conducted on trees closed to the spot of soil sampling

Fully expanded leaves of the 2nd youngest flush of 8 year-old lychee (v. Hong Huay) were randomly sampled from underneathflower panicle or shoot (leaves between the second – the fifth node). Twenty compound leaves were collected around the tree. One tree was used as one replication, 10 replications per site.

Leaf samples were washed in water, rinsed with distilled water, spread in a paper bag then dried at 65 ^{0}C for three days. The drying temperature should not exceed 70 ^{0}C because higher temperature may cause volatilization loss (Oom, 1992).

Before grinding, the samples were redried at 50 ^{0}C and milled with grinding machine. The samples were kept in desicator for digestion process.

Data collection

1. Analysis of Nitrogen

Standard solution was prepared using 200 mg of glutamine in steel crucible. Then, 300 mg of dry samples was weighed in a steel crucible using the micro balance. Samples were held in an automatic sample feeder and transferred into the combustion tube and heated to a temperature of 850 ^{0}C. Quantitative oxidation was achieved by passing the gases over a catalyst (copper oxide and platinum).

Once the combustion of samples had accomplished, the gas mixture was let flowed into a reduction tube which filled with copper powder and heated at 850 ^{0}C. The excess oxygen was eliminated and the nitrogen oxides were reduced to nitrogen. Small packing of silver wool adsorbed any halogen if present. Nitrogen was passed through the columns and measured by the thermal conductivity detector (TCD). In addition, carbondioxide was released from the adsorption column, passed to the TCD for measurement. Analysis time for nitrogen was 7-8 minutes per sample (Institute of Plant Nutrition, University of Hohenheim, 2001).

2. Analysis of Phosphorus, Potassium, Calcium, Magnesium, Manganese, Iron and Zinc

2.1 Preparation of reagents.

1. Dilution of nitrictic acid: 1 part of 65% nitric acid + 2 parts of deionized H_2O
2. Dilution of hydrochloric acid: 1 part of 37% HCl + 2 parts of deionized H_2O
3. Molybdate-Vanadate solution (for P analysis)
   a. Diluted 1:3 HNO_3
   b. Ammonium vanadate solution 1.25%: 2.5 g of ammonium monovanadate was dissolved in 600 ml of boiling deionized water. After cooling, 80 ml conc. HNO_3 was added and increased the final volume to 1 liter with deionized water.
   c. Ammonium molybdate solution 5%: 50 g of ammonium heptamolybdate-tetrahydrate was dissolved in 800 ml deionized water at 60 ^{0}C and diluted to 1 liter with deionized water after cooling.

   The solution from a.- c. were then mixed to 1:1:1 ratio.

2.2 Digestion

Digestion was conducted using 500 mg of dry sample in a porcelain crucible. It was placed in the oven at 500 ^{0}C for at least 4 hours until the ash was light color. Samples were taken out and let them cool down to room temperature, few drops of deionized water were added until the sample was wet, then added with 5 ml HNO_3 1:3 in order to clean SiO_2 from the samples The process was repeated until the sample was free from SiO_2. The samples were dissolved with 5 ml 1:3 HCl, then transferred to 50 ml volumetric flask rinsed the crucible with some hot water. The solution was boiled for 2 minutes (with boiling chips) to change meta- and pyrophosphates (formed during evaporation with HNO_3) back to orthophosphates. After solution was cool, the solution was filled up with H_2O to final volume of 50 ml, then it was shaken thoroughly and filtered through blue band filter paper.

For Phosphorus analysis, a certain amount 7 ml of ash solutions were mixed with 3 ml Molybdate - Vanadate solution and brought to a volume of 10 ml with 1:30 diluted HCl. The color intensity of the solution was constant almost after 2 hours.

Measurement of sample

1. Measurement of phosphorus. Solution was measured by using Spectrophotometer wavelength 436 nm. with 1-10 mg/l of calibration.
2. Measurement of potassium and calcium. Solution was measured by using Flame-Photometer ELEX 6361 with 10-100 mg/l of calibration.
3. Measurement of magnesium, manganese, iron and zinc. Solution of those elements was measured by using Atomic absorption spectrophotometer (Unicam 939): Mg was measured at wavelength 285.2 nm (0.2-1.0 mg/l calibration), Mn was measured at wavelength 279.5 nm (0.2-1.5 mg/l calibration), Fe was measured at wavelength 248.3 nm (0.2-1.0 mg/l calibration) and Zn was measured at wavelength 213.9 nm (0.1-0.4 mg/l calibration).

Calculation

$$\frac{(\text{mg/l sample} - \text{mg/l blank}) \times V_e}{20 \times W_s}$$

wherein: W_s = weight of sample (g)

V_e = volume used in dilution (ml)

2.3. Analysis of Boron

Boron analysis, 0.3 g of dry samples was used and it was put in Quartz crucibles in the oven, then it was heated in 500 ^{0}C for 3 hours, then decreased to 400 ^{0}C for 1 hour, 300 ^{0}C for 1 hour and 200 ^{0}C for 1 hour. The samples were left overnight, in case the ash was not clear as white or gray color, few drops of 3% H_2O_2 was added and samples were reheated at 500 ^{0}C for 2-3 hours. Digestion was done with 3 ml of 1:30 mixed solution (1HNO_3: 30 Beryllium standard) samples were left at room temperature for 1 hour. Samples were then transferred into plastic bottle for further analysis.

The solution was diluted in 1:20 mixture of 1HNO_3: 30 Beryllium standard. Sample of 5 ml was pipetted to automatic feeder using ICP-MS instrument.

Calculation;

$$\frac{(\mu g/l \text{ sample} - \mu g/l \text{ blank}) \times V_e}{200 \times W_s}$$

wherein: W_s = weight of the sample (g)

V_e = volume used in dilution (ml)

4. Analysis of Sulfur

Sulfur analysis was conducted using 1 mg of Tungsten oxide in cup size 5×9 mm. with 4-6 mg of dry sample. Cup was then closed and tighten as small as possible, broken the cup needed to be avoided. Sample was transferred to tray and analysed using the CE – Instrument model CNS 2500. The samples were automatic calculated to percentage of sulfur.

3. Comparison of the nutritional concentration in leaves and fruits of lychee at four cardinal points

The study was conducted on lychee tree growing in farmer's orchard at Maesa Mai village, Maerim district, Chiang Mai province during the fruit season January – April, 2001.

For leaf analysis, samples were collected every three weeks interval from flowering stage to fruit harvesting stage. Five compound leaves were sampled from each cardinal point of lychee tree. Randomized Complete Block Design was used. There were 10 replications (trees) in each four cardinal. Leaf samples were prepared and analysed following the same method described in **2.**

Eight lychee fruits were sampled from four cardinal points of each tree. Peel, seed and aril of fruit samples were separated. They were placed in the oven and dried at 65 ^{0}C for 2 days except for aril which was dried at 72 ^{0}C for three days.

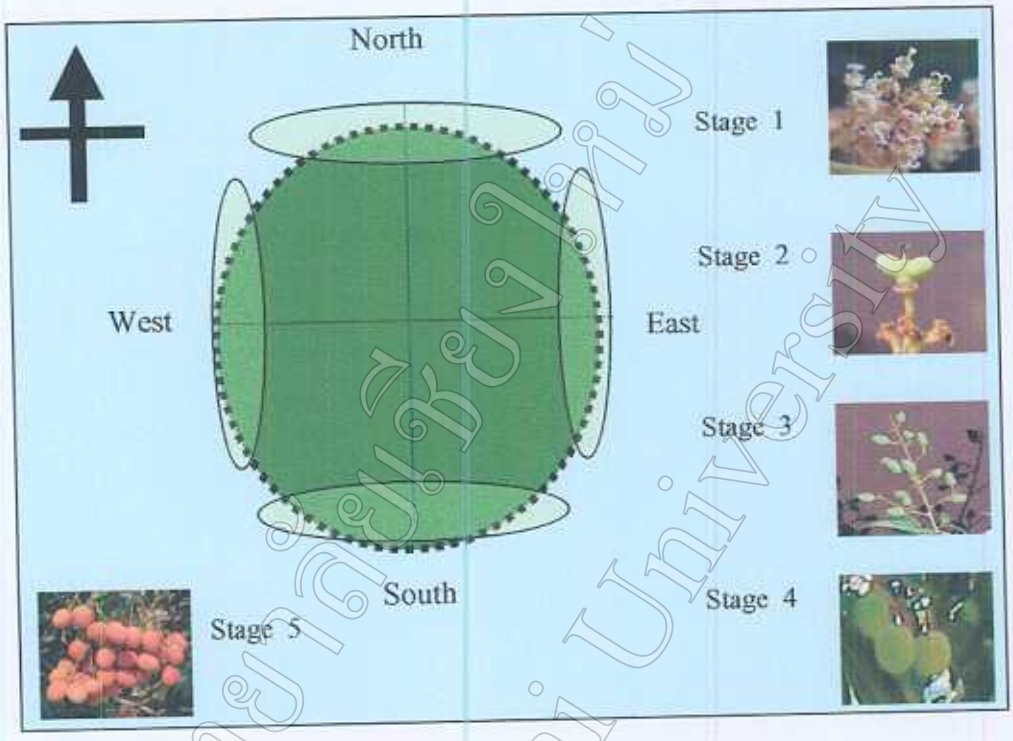

Figure 3.2 Locations where leaf and fruit were sampling at four cardinal points

Data collection;

1. Analysis of nutrient concentration of lychee leaves. The extraction of the nutritional concentration in leaves were analysed for N, P, K, Ca, Mg, S, Mn, Zn, Fe and B using the same methods described for leaf sample analysis.

2. Analysis of mineral concentration of lychee fruit parts. The extraction of the nutritional concentration in lychee fruits were analysed for N, P, K, Ca, Mg, S, Mn, Zn, Fe and B by using the same methods described for leaf sample analysis.

4. Comparison of the quality of lychee fruit at four cardinal points

The experiment was conducted on trees closed to area of fruit sampling for nutrient analysis (experiment 3). Each five fruits of lychee were randomly sampling at four cardinal points of each tree at harvesting stage. Those samples were used for fruit quality analysis.

Data collection

1. Analysis of lychee fruit color. Fruit color was measured by colorimeter instrument model Minolta CR-300

   The measurement was based on the color system be presented as values L* a* and b* (Figure 3.3)

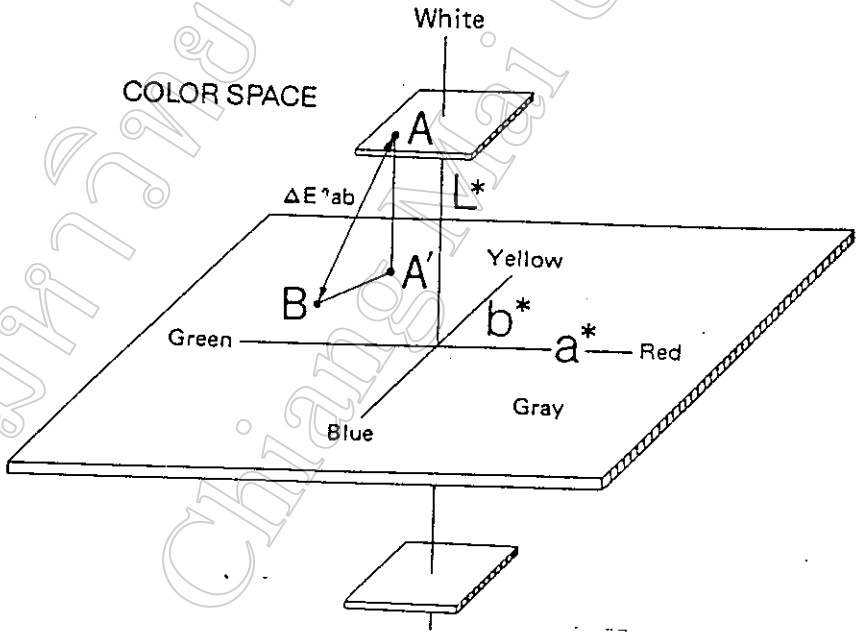

Figure 3.3 L* a* and b* color system

The centre was achromatic and as the point moved out from the centre, the saturation of the color increased. The L* value gave a measurement of the brightness or darkness of the sample. The a* value gave the red-green characteristics of the sample, while the b* value showed the yellow-blue (Minolta Co.Ltd., 1994)

3. The weight of fresh fruit and dried fruits. Samples were measured for width and length of fruits in centimeter and weight.
4. The amount of titratable acid (TA): 5 ml lychee juice was titrated with 0.1 N NaOH and 1% Phenopthalene was used as indicator (Rujjanakrikant and Rattanapanont, 1990). TA as citric acid equivalent was calculated using the empirical formula;

$$TA = \frac{\text{volume of NaOH (ml)} \times \text{conc. of NaOH} \times \text{mol. Wt. of citric acid} \times 100\%}{\text{Volume (ml) of fruit juice}}$$

5. Total soluble solid (TSS): Samples were measured by digital refractometer in ^{0}Brix.

5. The Relationship of nutrient concentration in soil, leaves and fruits of lychee

Regression method was used for analysis the relationship of nutritional concentration in soil, leaves and fruits from each soil characteristic and orchard.