

### **3. EXPERIMENTAL**

#### **3.1 Study Sites and Species Selection**

##### **3.1.1 Study Area and Climate**

Chiang Mai, the second largest city in Thailand, is a large economic and cultural center. The area is located in a monsoon zone which is influenced mainly by two monsoons, southwest and northeast and secondarily by cyclonic storms and intertropical fronts, which are typical of the climate of northern Thailand.

The rainy season lasts from May to October and approximately 85 to 90 percent of the annual rainfall occurs during the southwest monsoon. In general, rainfall occurs through three causes, namely orographic, convective, and cyclonic. The average rainfall varies from 0.6 to 251.2 mm per month with a minimum during February due to dry cool air mass movement from the arctic. The maximum rainfall occurs in August as a result of storms from the Indian Ocean.

##### **3.1.2 Plant Species Selection**

Five plant species were selected to investigate changes in lead content of their leaves. The aim of the collection was to consider their availability both in terms of abundance and distribution so that unbiased sampling was done. The plant species were examined for their capacity to accumulate lead in relation to the species, the site

of origin, and the season. Sampling was done in July (rainy season) and December (cool, dry season).

The five species are:

1. *Dimocarpus longan* Lour. *spp. longan var. longan* (Sapindaceae), a native and widely cultivated fruit tree;
2. *Mangifera indica* L. (Anacardiaceae), an introduced and very commonly cultivated fruit tree;
3. *Bougainvillea spectabilis* Willd. (Nyctaginaceae), a very popular introduced ornamental scandent species;
4. *Amaranthus gracilis* Desf. (Amaranthaceae), a widespread herbaceous dicot weed; and
5. *Eleusine indica* (L.) Gaertn. (Gramineae), a very common herbaceous, monocot grass weed.

Voucher specimens for all five species are deposited in the CMU Herbarium (Appendix A. Photos 1-5).

### 3.1.3 Description of the Study Sites

Thirteen study sites were chosen in Chiang Mai, of which 12 are located in the main traffic road junction areas, and the other one, the control site, off to the west of the road sites. Figure 3.1 shows a map of the locations of the study sites.

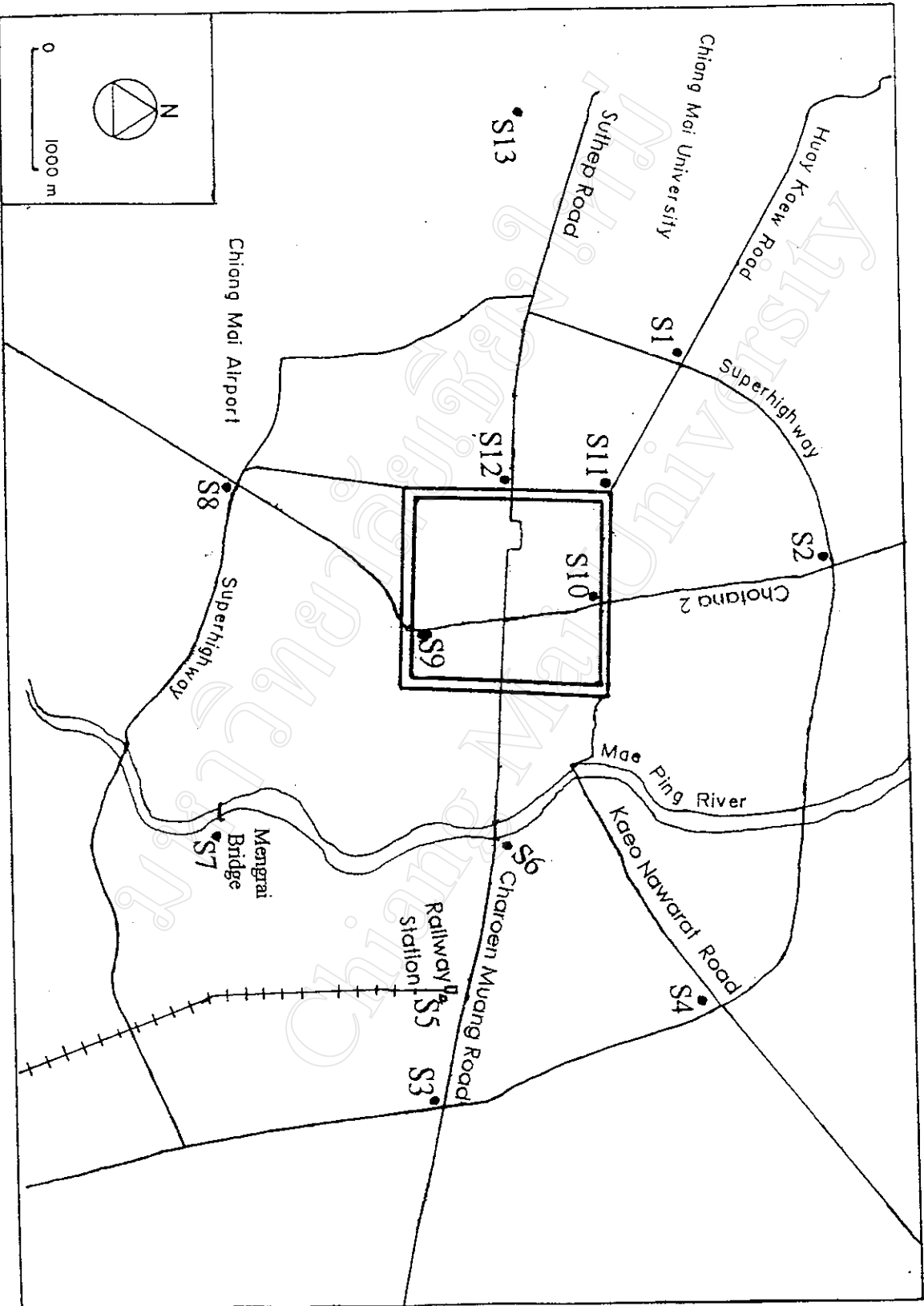


Figure 3.1 Map showing the locations of the study sites

**Super Highway: Sites 1, 2, 3, 4**

Super highway is a major traffic road in Chiang Mai City. Four study sites 1, 2, 3, and 4 are located at road junctions along this highway.

At sampling site 1 and site 2, *Bougainvillea* was collected from the plant block in the middle of road, *Amaranthus* and *Eleusine* were collected from just beside the busy road, while *Dimocarpus* and *Mangifera* were taken about 15 m from the busy road.

Both *Dimocarpus* and *Mangifera* were collected about 15 m away from the road at site 3, and they were collected about 10 m away from the main road at site 4. For both sites 3 and 4 *Bougainvillea*, *Amaranthus* and *Eleusine* were collected just beside the road.

**Chiang Mai Moat: Sites 9, 10, 11, 12**

The traffic beside the Chiang Mai Moat is busy and congested. Four study sites are distributed along this Moat, which are sites 9, 10, 11, and 12.

Site 9 is located at Chiang Mai gate. All species plants were taken from just beside the road at this sampling site.

Site 10 is located at Chang Phuak gate. *Bougainvillea*, *Amaranthus* and *Eleusine* were taken from roadsides, while *Dimocarpus* and *Mangifera* were taken about 10 m away from the road.

Site 11 is situated to the southwest of the intersection of Huay Kaew road with Moat-side road. Site 12 is southwest of Suan Dok gate. All species were collected from just beside the road at these two sampling sites.

**Along Mae Ping River: Sites 6, 7**

Mae Ping River is a major river in Thailand. Traffic beside this river is also busy, especially near bridges over the river.

At site 6, located to the northeast of Nawarat bridge, *Bougainvillea*, *Amaranthus* and *Eleusine* were collected from roadsides, while *Dimocarpus* and *Mangifera* were taken about 15 m from the road. Site 7 is to the east of Mengrai bridge. *Bougainvillea*, *Amaranthus* and *Eleusine* were collected from roadsides, *Dimocarpus* 40 m from the road and *Mangifera* about 15 m from the busy road.

**Railway Station: Site 5**

In sampling site 5, located at the Railway Station, *Bougainvillea*, *Amaranthus* and *Eleusine* were collected from roadsides, while *Dimocarpus* and *Mangifera* about 5 m away from the road.

**Airport Plaza: Site 8**

Site 8 is located at the southwest intersection of Hangdong road and Super Highway. *Bougainvillea*, *Amaranthus* and *Eleusine* were collected from the roadside, *Dimocarpus* and *Mangifera* about 10 m away from busy road.

**Control: site 13**

Site 13, a control site, is about 500 m off to the west of the road sites. This sampling site is less contaminated with lead from motor traffic. All five species plants were taken from a house yard at this sampling site.

### 3.2 Chemicals

- (1) Nitric acid ( $\text{HNO}_3$ ), GR, 65%. E. Merck
- (2) Vanadium Pentoxide ( $\text{V}_2\text{O}_5$ ) as catalyst
- (3) Sodium borohydride ( $\text{BH}_4\text{Na}$ ), assay >97.0%, Fluka Chemica
- (4) Potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ), Zur Analyse, E. Merck
- (5) Lactic acid, 90%, DAB, Ph Eur, BP, E270
- (6) Solid NaOH for preparation of 4%  $\text{BH}_4\text{Na}$  solution.
- (7) A 1000mg/L lead (II) stock standard solution was prepared by dissolving 0.3991 g of lead nitrate in 250 ml of 0.2% nitric acid.
- (8) Nitrogen, OFN grade, Thai Industrial Gases Co. Ltd.

### 3.3 Equipment

- (1) Blender Mill 2, Moulinex, made in France
- (2) Atomic absorption spectrophotometer, 2380, Perkin-Elmer
- (3) Hydride generator, model MHS 10, Perkin Elmer

### 3.4 Methods

### 3.4.1 Sampling and frequency

About 100g of leaves of each of the five species were collected at each sampling site and put in polyethylene bags. Leaves of *Dimocarpus* and *Mangifera* were collected at 1.5m above the ground level. Leaves, branches, and flowers of *Bougainvillea* were collected, while entire plants of *Amaranthus* and *Eleusine* were taken.

Samples from 13 sites were collected in July 1995 and December 1995, representing respectively the rainy season and dry season. All samples were taken to the CMU Herbarium, Department of Biology, to dry.

### 3.4.2 Sample treatment

Each plant sample was divided into two parts immediately on arrival at the Herbarium. One part was washed using a sequence of tap water and distilled water. The sample was then placed in paper bag and put into an oven for drying at around 90°C until crisp. The other part was placed directly in a paper bag for drying.

The dried samples were broken up by hand and ground in a blender mill and then underwent acid digestion (wet digestion).

### 3.4.3 Wet digestion

Sample solutions were prepared by the following procedure:

About 1 g of ground dried plant sample was accurately weighed in a p.t.f.e. dish. 13 mg of vanadium pentoxide as catalyst and 20 ml of concentrated nitric acid were added to each dish and were left to pre-digest for several hours. The samples were evaporated to incipient dryness on a hot plate at a temperature of 75-85 °C. The p.t.f.e. dishes were cooled and about 5 ml of distilled water was added to each dish. The resulting solution was then transferred into a 10 ml volumetric flask and made up to the volume with distilled water (Madrid, 1990).

#### **3.4.4 Determination of sample solutions**

Measurements were made on a Perkin-Elmer 2380 Flame AAS machine. The lead hydride generation system was applied to the determination of lead.

Each sample was analyzed by placing 1 ml of prepared sample solution, 1.0 ml of 10% (w/v) lactic acid and 1.0 ml of 5% (w/v) potassium dichromate in a reaction flask. Lead hydride was generated by adding 2.0 ml of 4% (w/v) sodium tetrahydroborate solution and transported to the photo absorption cell by nitrogen. Analytical output was recorded as peak areas.

All the quantitative determinations were carried out by obtaining calibration data at the same time as the samples were determined. Peak area was related to mass of lead by curvilinear regression.

### **3.5 Analytical Quality Control**



### **3.5.1 Recovery experiments**

Several pre-experiments were carried out to determine recoveries for each of the five investigated species. This was done by spiking a known amount of standard solution into the dried ground sample. The spiked samples were measured under the same conditions as the analyzed samples. The percentage recoveries were calculated after measurements had been made by AAS under optimum conditions.

### **3.5.2 Reference materials measurement**

In order to test the reliability of the analysis system and method, two certified reference plant materials were measured in same way as the samples. These two materials are CRM 281 rye grass supplied by the European Community Bureau of Reference, Brussels, and IAEA/V-10, hay supplied by the International Atomic Energy Agency, Austria.