CHAPTER 2

EXPERIMENTAL

2.1 Apparatus

1. Polypropylene tubes, NIPRO (Thailand) corp., Ltd., Thailand
2. GF/A filter papers, Whatman International Ltd., England
3. Polypropylene boxes, Raaco, Denmark
4. Syringe filter, 0.45 µm cellulose acetate, Chrom Tech, Inc., England
5. Ultrasonic bath, Transsonic Digital S, Elma, USA
6. Ultrasonic bath, model 8891, Cole-Parmar Instrument Co., USA
7. Analytical balance, Sartorius Basic BA 210s, Germany
8. Oven, memmert model 100-800

2.2 Chemicals

1. Triethanolamine ($C_6H_{15}NO_3$,149.19), 99 %, BDH Chemicals Ltd., England
2. Sodium nitrite ($NaNO_2$, 69.00), 97%, AJAX, Australia
3. N-(1-Napthyl) ethylenediamine dihydrochloride ($C_{12}H_{16}C_{l2}N_2$, 259.18), 90%, Merck, Germany
4. Sulfanilamide ($C_6H_8N_2O_2S$, 172.21), 99%, Merck, Germany
5. Phosphoric acid (H_3PO_4, 98.00), 88%, S.D. Fine Chem. Ltd., India

2.3 Instruments

1. Spectrophotometer, Jasco UV 530, Japan

2. Nitrogen dioxide (NO_2) Test Kit , Environmental Chemistry Laboratory, Chemistry Department, Faculty of Science, Chiang Mai University

2.4 Preparation of solutions

2.4.1 Absorbing solution (20% v/v of Triethanolamine, TEA)

20 ml of TEA was accurately pipetted into 100 ml volumetric flask and adjusted to volume with de-ionized water.

2.4.2 Sulfanilamide solution

10.75 g of sulfanilamide was weighed and dissolved in 28 ml of phosphoric acid and adjusted volume to 500 ml with de-ionized water in volumetric flask.

2.4.3 N-(1Napthyl) ethylenediamine dihydrochloride (NEDA) solution

0.1520 g of N-(1-Napthyl) ethylenediamine dihydrochloride was dissolved in de-ionized water and adjusted to 100 ml in a volumetric flask.

2.4.4 Saltzmann reagent

Sulfanilamide solution and NEDA solution were mixed in a ratio of 10:1. They must be refrigerated and protected from light.

2.4.5 Nitrite standard stock solution (1,000 mg/L)

0.150 g of sodium nitrite ($NaNO_2$) was dissolved in de-ionized water and adjusted to 100 ml in a volumetric flask.

2.5 Analytical characteristics

2.5.1 Linear dynamic range

The linear dynamic range was investigated by varying concentrations of nitrite (NO_2^-) in a range from 0.02 - 10 mg/L. It was then determined by plotting absorbance versus concentrations of nitrite standard solution.

2.5.2 Calibration curve

A calibration curve was constructed by plotting concentrations of nitrite standard solution versus absorbance obtained from spectrophotometry. Nitrite solutions were prepared from 1,000 mg/L stock standard solution and diluted in a range from 0.01-1.0 mg/L. After that 2 ml of Saltzmann reagent were added. Standard solutions were measured by spectrophotometry by using reagent as blank.

2.5.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

In analytical chemistry, the LOD is the lowest concentration of the analyte that can be detected with a given degree of confidence. LOQ is a parameter for quantitative assays for low levels of compounds in the sample matrices and used particularly for determination products or low levels of active constituent in a product (Christian, 2001). The limits of detection and quantitative were calculated as follows:

$$LOD = 3 \times SD \quad (2.1)$$

$$LOQ = 10 \times SD \quad (2.2)$$

Where: SD is a standard deviation from 10 times measurement of the lowest concentration of nitrite standard solution used in calibration curve.

2.5.4 Repeatability and reproducibility

Repeatability is the results of standard deviation of measurements repeated by the same analyst on the same instrument within a short time period (IUPAC Compendium of Chemical Terminology, 1997). The repeatability in this work was checked by 10 times continuously measurement of absorbance of the 0.2 mg/L nitrite standard solution by spectrophotometer at 540 nm.

Reproducibility is the closeness of agreement between independent results obtained with the same method on identical test material but under different conditions (different operators, different apparatus, different laboratories and after different intervals of time). IUPAC Compendium of Chemical Terminology (1997), reproducibility of the system in this work was pursued by preparing 10 flasks of 0.2 mg/L nitrite standard solutions followed by analysis in the same manner. The results were estimated by standard deviation (SD) and the percent relative standard deviation (%RSD).

2.6 NO_2 test kit and its user instruction (Chalermrom, 2008)

The NO_2 test kit composes of a polypropylene diffusion tube, a plastic shelter, specific chemical reagents, a plastic dropper and a disposable syringe and a standard

color chart (Figure 2.1). A test procedure is illustrated in Figure 2.2. Details are described as following.

1) Remove the cap of the diffusion tube out and put GF/A filter paper in a diffusion tube. Add 2 drops of 20% triethanolamine (absorbing solution).

2) Fix the sampling tube in a protective shelter and hang at the sampling site above ground at least 1.5-2.0 meters. A recommended sampling duration is 3 days. However, an exact exposure duration has to be recorded.

3) At the end of sampling time, extract by adding 2 ml of de-ionized water using a syringe, shake and hold for at least 10 minutes.

4) Transfer 1 ml of solution in to small glass tube. Add 2 ml Saltzmann reagent close the cap and mix well. Wait 10 minutes until color development was completed. After the specified time, compare color of the sample solution with color of the nitrogen dioxide standard color chart to find out the sample concentration. When the developed color lies between 2 standard colors, read out value between the 2 standard values e.g. 0.1-0.4 mg/L or 10.6-42.5 ppbv.

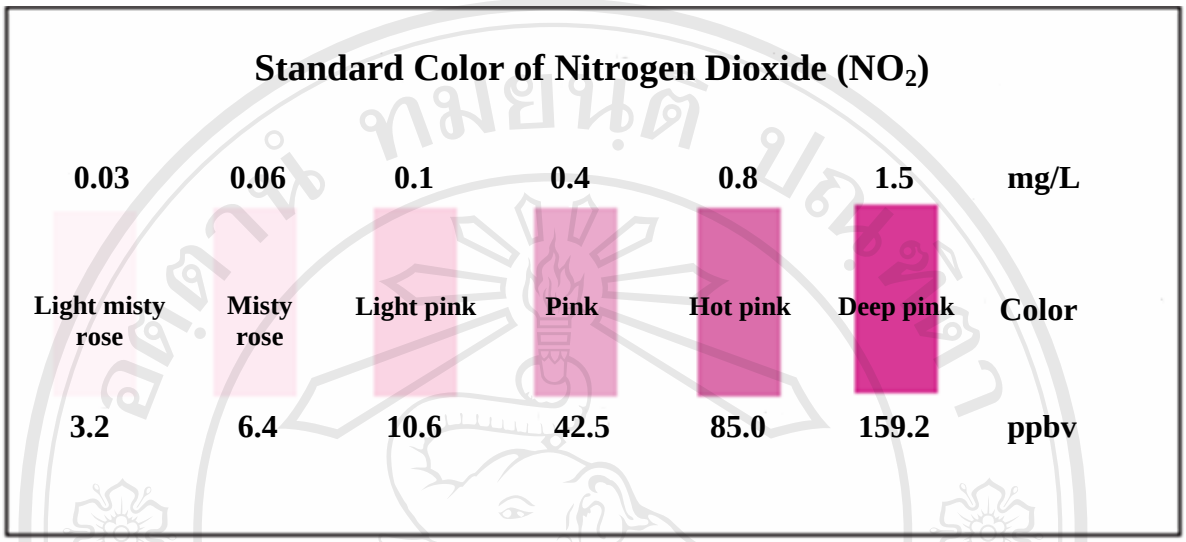


Figure 2.1 Standard color chart of nitrogen dioxide in mg/L and ppbv (based on 3 days exposure), adapted from Chalermrom (2008)

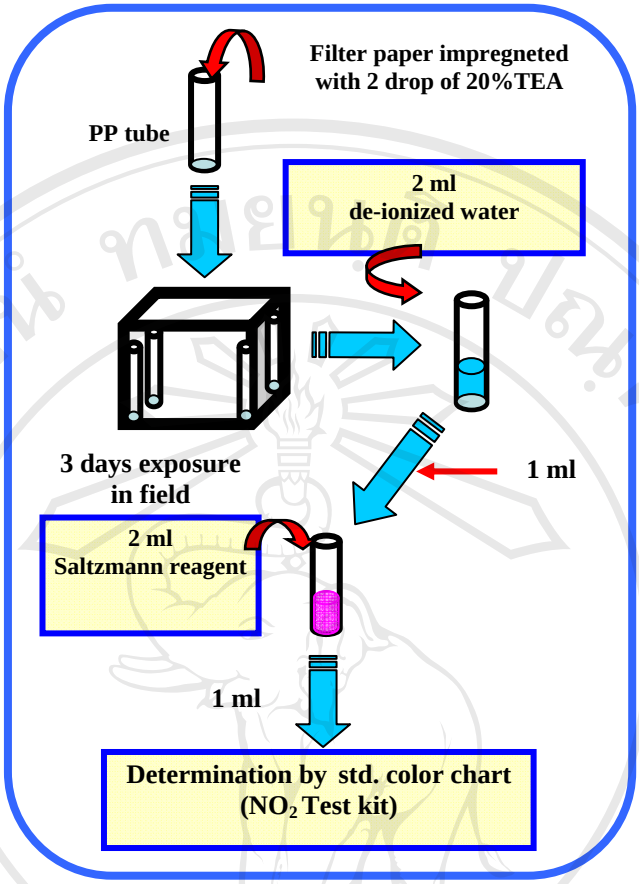


Figure 2.2 Passive sampling of NO_2 and sample analysis by NO_2 test kit

2.7 Passive sampler for NO_2 determination

2.7.1 Preparation of diffusion tube

Figure 2.3 shows polypropylene (PP) passive diffusion tubes with a length of 5.3 cm and an internal diameter of 1.3 cm. They were cleaned and sonicated for one hour, then rinsed with de-ionized water. Filter paper, Whatman GF/A, was cut in a circle with the diameter being equal to inner diameter of the tube. The filter paper was sonicated for 30 minutes for clean and rinsed twice with distilled water before drying

at 60^{o}C for 24 hour. After that, the filter paper was placed at the bottom of the tube and left in a desicator prior to use (Chalermrom, 2008).

Figure 2.3 Polypropylene tube (PP)

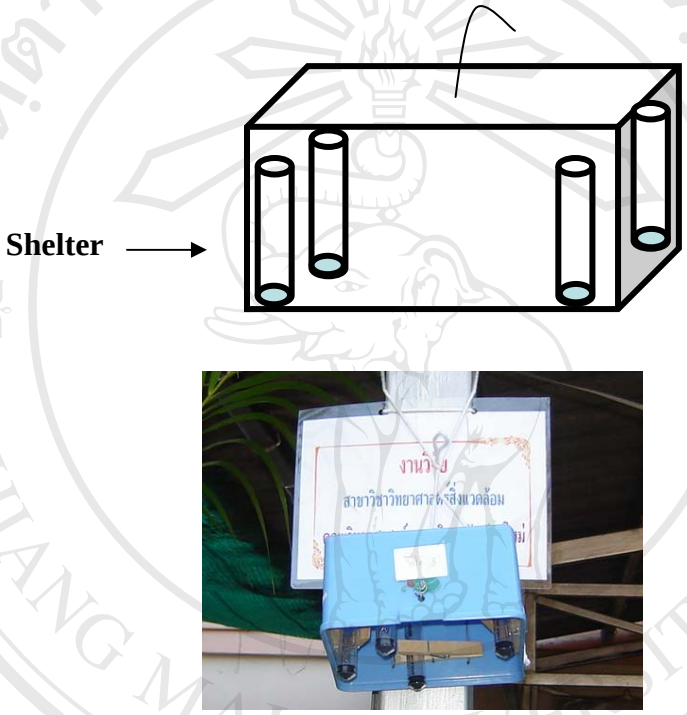
2.7.2 Exposure of diffusion tube

On the exposure day, 50 µl of 20% TEA in de-ionized water was directly added onto the Whatman GF/A filter paper (Ozden and Dogeroglu, 2007; Chalermrom, 2008). The diffusion tubes were vertically fixed, with the open end facing upward, inside the shelter to protect them from meteorological effects i.e. wind, sunlight and rain. Configuration of the passive samplers is illustrated in Figure 2.4. The protective shelter containing three replications of sampler and one blank of tubes was hung at 1.5 – 2.0 m above ground level. The samplers were exposed for 3 days continuously (Sunday to Tuesday) in the 2nd or 3rd week of the month. Exposure of the passive sampling tubes and gas diffusion pathway.

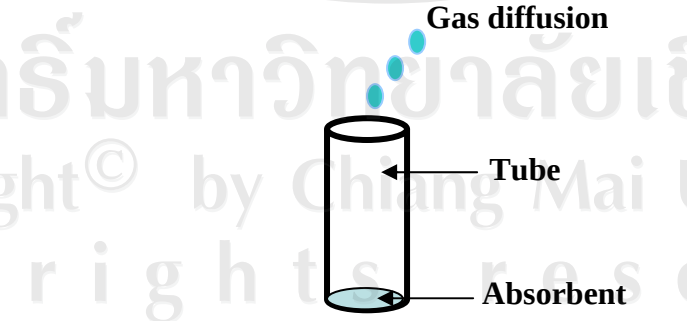
After 3 days of exposure, the tubes were collected, closed with caps and tighten with parafilm. They were placed in plastic zip lock bags, transferred to the laboratory

and stored in a refrigerator until the time of analysis. The exact times of installation and collection were noted for calculation of the NO_2 amount as shown in Figure 2.4.

In the field, the diffusion tubes were prepared using the same procedure. Three replications of sampler and one blank tube were fixed inside the protective shelter at the field and the caps of the sampling tube were removed.



(a)



(b)

Figure 2.4 The configuration of passive samplers (a) and a gas diffusion pathway (b)

2.7.3 Extraction of sample

After the sampling was completed, 2 ml of de-ionized water were added into the sampling tube. The tube was capped and shaked for 15 minutes. The sample solution was then filtered through 0.45 µm cellulose acetate membrane by the help of syringe.

2.7.4 Analysis of sample

One ml of the extracted sample solution was mixed with 2 ml of Saltzmann reagent in a test tube and standed for 10 minutes until color development was completed. After that, the color of the solution was compared to standard color of the nitrogen dioxide standard color chart to find out its concentration (Chalermrom, 2008). After that, the same solution was measured for its absorbance by spectrophotometry at 540 nm (Bhugwant and Hoareau, 2003; Delgado-Saborit and Esteve-Cano, 2006; Madsen *et al.*, 2007) by using reagent as blank. Comparison of NO_2 concentrations obtained from two measurement methods were analyzed by SPSS version 14.

2.7.5 NO_2 determination from incense burning

Contribution of NO_2 in ambient air could be various. One of the potential source is incense burning (Lee and Wang, 2004). In order to find out how much contribution from burning of incense could possibly influence to NO_2 concentrations in the ambient air especially at the sampling site located near the shrine, therefore the experiment was set up.

The passive diffusion tubes were prepared as described in the section 2.7.1. The incenses sticks used in this experiment are made of a slender piece of wood or

bamboo and to which incense compound are attached. It is 28.5 cm long and has frankincense scent (Fo Ju Dian brand). The shelter containing three replications and one blank of tube was hung at 1.5 meters above the ground both in and outside the room ($3\times3\times3$ m^3) that incenses were burned. The passive sampling tubes were exposed for 1 hour and 8 hours continuously, while nine incenses were burnt until the sampling period was over. After that exposed, the samples were extracted (section 2.7.3) and measured by spectrophotometry.

The experiment was also carried out at the U3 site (Waroros market), which is located in front of the shrine. Shelters containing sampling tubes were hung both inside and outside the shrine for 8, 24 hours and 3 days to find out NO_2 concentrations collected from different position.

2.8 Sampling site

2.8.1 Land – use pattern

Figure 2.5 presents a map of land-use of Chiang Mai Province. The urban area (inner circle) covers 78.55 km^2 (100%) including 5.70 km^2 (7.25 %) of agricultural area, 2.59 km^2 (3.30%) of Chiang Mai International Airport, 60.88 km^2 (77.50 %) of community area, 7.94 km^2 (10.11 %) of forest, park and grassland, 0.07 km^2 (0.09 %) of industry, 0.04 km^2 (0.05 %) of road area and 1.33 km^2 (1.69 %) of water resources. The sub-urban area (outer circle) covers 235.92 km^2 (100%) including 80.00 km^2 (33.91 %) of agricultural area, 77.83 km^2 (32.99 %) of forest, park and grassland, 73.85 km^2 (31.30 %) of community, 0.23 km^2 (0.10 %) of industry, 0.76 km^2 (0.32 %) of road area and 3.25 km^2 (1.38 %) water resources. In rural area (out of the circle within 15 km radius) covers 438.57 km^2 (100%) including 179.93 km^2 (41.03%) of

agricultural area, 137.16 km^2 (31.28%) of community area, 115.59 km^2 (26.36%) of forest, park and grassland, 0.59 km^2 (0.13%) of industry, 0.48 km^2 (0.11%) of road area and 4.82 km^2 (1.10%) of water resources as shown in Table 2.1 (Land development department, 2006).

Table 2.1 Land-use pattern of Chiang Mai Province in 2006

Land-use pattern	Urban		Sub-urban		Rural	
	km^2	**%**	**km^2**	**%**	**km^2**	**%**
1. Agricultural area	5.70	7.25	80.00	33.91	179.93	41.03
2. CM International Airport	2.59	3.30	-	-	-	-
3. Community area	60.88	77.50	73.85	31.30	137.16	31.28
4. Forest, park and grassland	7.94	10.11	77.83	32.99	115.59	26.36
5. Industry	0.07	0.09	0.23	0.10	0.59	0.13
6. Road area	0.04	0.05	0.76	0.32	0.48	0.11
7. Water resources	1.33	1.69	3.25	1.38	4.82	1.10
Total	**78.55**	**100**	**235.92**	**100**	**438.57**	**100**

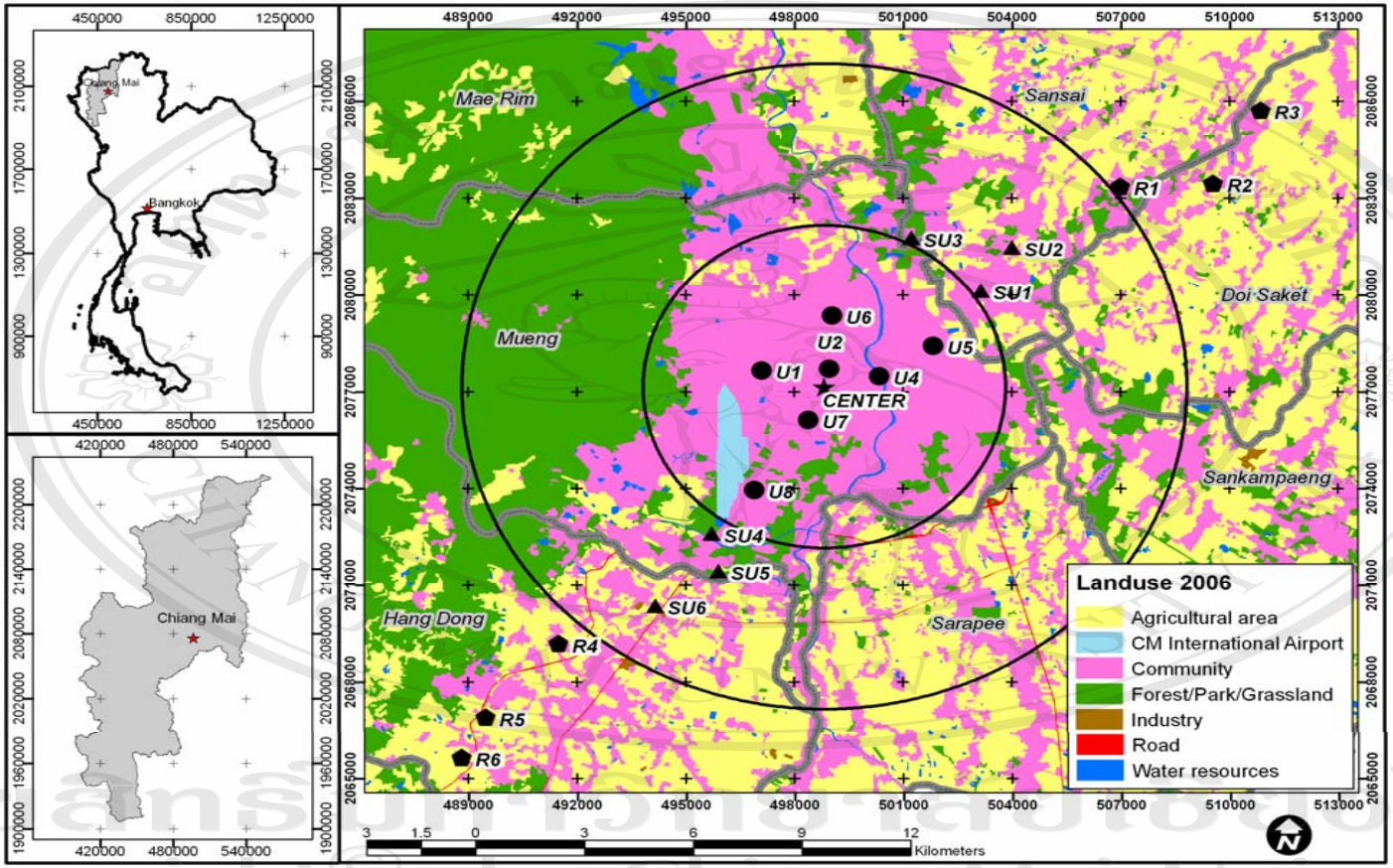


Figure 2.5 Land-use pattern of in Chiang Mai Province in 2006

Source; Land Development Department, Ministry of Agriculture and Cooperative, 2006

2.8.2 Location of sampling site and sampling period

Sampling sites were surveyed and randomly selected within four districts of Chiang Mai Province. There was 20 sites in total and can be divided into urban (8 sites), sub-urban (6 sites) and rural (6 sites) areas based on population density and human activities. The surrounding details of sampling site in different scales are described in Table 2.2. The eight sites of the urban area are located in Muang Chiang Mai District, six sites of the sub-urban area are in Muang Chiang Mai, San Sai and Hang Dong Districts and the last six sites in the rural area are in Doi Saket and Hang Dong District. The sampling duration was 3 days for each exposure. Sampling has been done once a month in the dry season during November 2007 to April 2008. A sampling map using Global Positioning System (GPS) and Geographic Information System (GIS) was constructed (Figure 2.6). Detail is given in the Table 2.3.

Table 2.2 The surrounding of sampling site

Sampling Site	Radian	Details
Urban	0-5 km	The center is intersection of Phapokklao and Rajadamnoen roads in Chiang Mai Moat. Most of areas are communities. The area are covered the resident, office building, Chiang Mai International Airport and transportation network.
Sub-urban	5-10 km	Most of areas are communities and transportation. Other land-use is for agriculture and commerce. Comprehensive to middle ring road and some parts of outer ring road.
Rural	10-15 km	Most of areas are dispersed communities and agricultural. Other land-use is forest. Comprehensive some parts of outer ring road.

Table 2.3 The Location of study sites in Chiang Mai Province.

Sites	Code	Location	Latitude - longitude
1	U1	Maharaj Nakorn Chiang Mai Hospital (รพ.มหาราชนคร เชียงใหม่)	18º 47´ 26.5˝ N 98º 58´ 20.6˝ E
2	U2	Yupparaj Wittayalai School (ร.ร.ยุพราชวิทยาลัย)	18º 47´ 28.4˝ N 98º 59´ 24.2˝ E
3	U3	Waroros Market (ตลาดวโรรส)	18º 47´ 27.1˝N 99º 00´ 4.1˝E
4	U4	Chiang Mai Regional Police Traffic Center (ศูนย์การจราจรตำรวจภูธร จังหวัดเชียงใหม่)	18º 47´ 21.0˝N 99º 00´ 11.2˝E
5	U5	Pee Nong Dormitory (หอพักพี่น้อง)	18º 48´51.4˝N 99º 01´ 2.0˝E
6	U6	Soi 7 Muen Dam Phra Khot Road (ซอย 7 หมื่นด้ามพร้าคต)	18º 48´ 21.9˝N 98º 59´26.8˝E
7	U7	Muen San temple (วัดหมื่นสาร)	18º 47´ 36.8˝N 98º 59´ 4.3˝E
8	U8	Yu Pin house (small real-estate) (โครงการบ้านยุพิน)	18º 45´ 25.7˝N 98º 58´ 14.0˝E
9	SU1	San Sai Market (ตลาดสันทราย)	18º 49´ 41.4˝N 99º 02´ 37.5˝E
10	SU2	Ban San Sai Noi (บ้านสันทรายน้อย)	18º 50´ 30.5˝N 99º 02´ 16.2˝E

Table 2.3 The Location of study sites in Chiang Mai Province. (continuted)

Sites	Code	Location	Latitude -longitude
11	SU3	Thanawan home-estate (โครงการบ้านธนาวัลย์)	18º 50´ 39.7˝N 99º 01´ 41.7˝E
12	SU4	Ban Tam Nak (บ้านตำหนัก)	18º 45´ 40.1˝N 98º 58´ 34.4˝E
13	SU5	Ban Ton Pin (บ้านต้นปิน)	18º 44´ 3.7˝N 98º 58´ 40.2˝E
14	SU6	Ban Muang Kung (บ้านเหมืองกุง)	18º 44´ 33.3˝N 98º 56´ 24.5˝E
15	R1	Ban Rong Bon (บ้านร้องบอน)	18º 51´ 31.3˝N 99º 04´ 58.5˝E
16	R2	Ban Pha Yak Don Kaew (บ้านพยากดอนแก้ว)	18º 51´ 34.2˝N 99º 05´ 26.2˝E
17	R3	Ban Rong Khi Lek (บ้านร้องขี้เหล็ก)	18º 52´48.1˝N 99º 06´11.0˝E
18	R4	Ban Rai (บ้านไร่)	18º 43´ 49.3˝N 98º 55´ 9.6˝E
19	R5	Aeranthawan temple (วัดเอรัณฑวัน)	18º 42´ 34.8˝N 98º 54´ 0.7˝E
20	R6	Ban Num Phrae (บ้านน้ำแพร่)	18º 41´ 55.1˝N 98º 54´ 37.7˝E

Note; U = Urban area, SU= Sub-urban area and R = Rural area

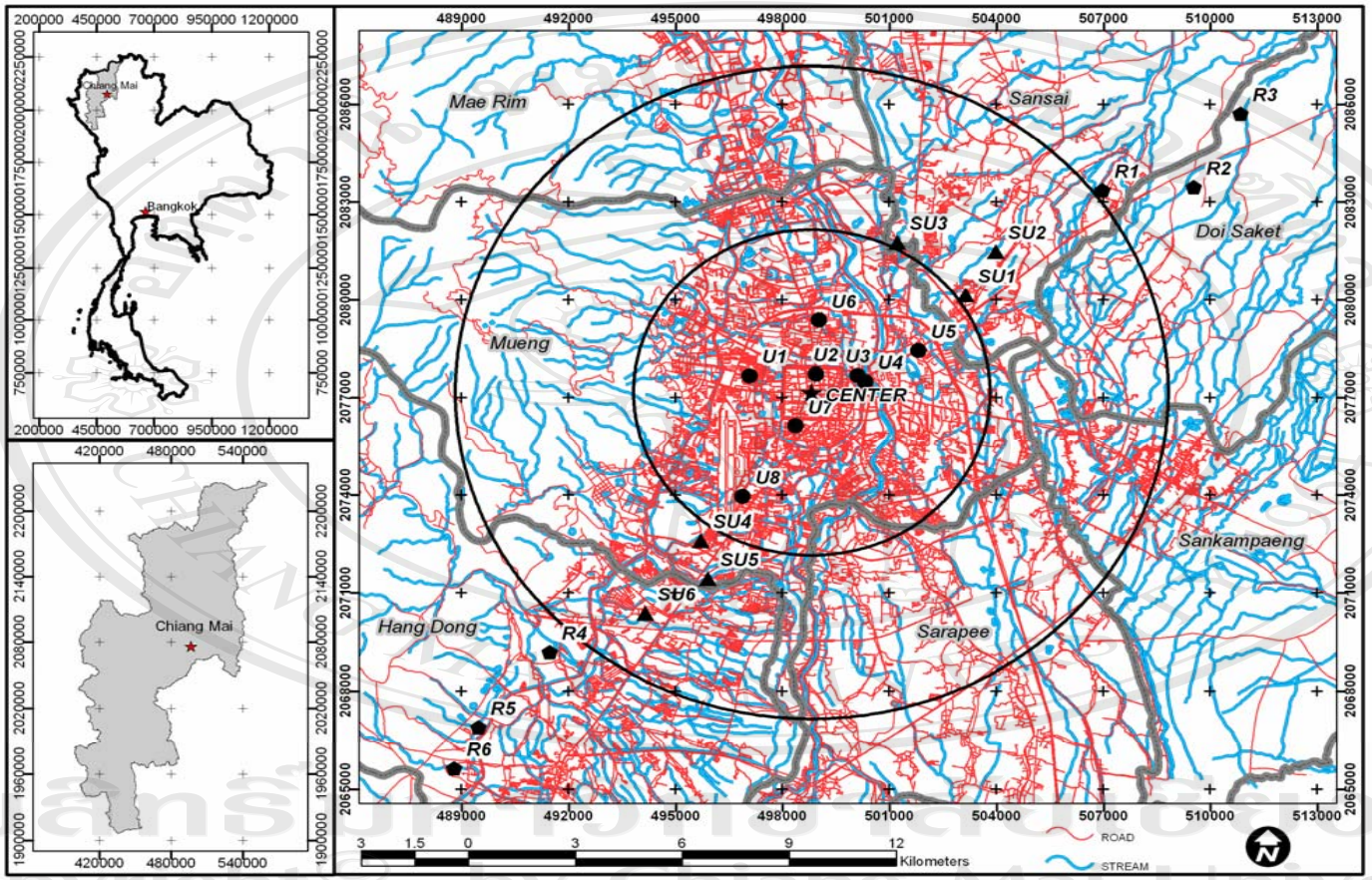


Figure 2.6 Location of the study sites in Chiang Mai Province

2.8.3 Sampling site description

a. Urban area

- U1: This sampling site is situated inside the Maharaj Nakorn Chiang Mai Hospital, Intawaroros road, Suthep Sub-district, Muang District. It is in a small park near the parking lot (Figure 2.7). The hospital is approximately 200 m distance from Intawaroros road, which has high traffic density especially during rush hour.

- U2: This sampling site is in Yupparaj Wittayalai School, Prapokklao road, Sriphum Sub-district, Muang District. The school was surrounded by small roads (~8 m width) with high traffic density especially during rush hour. The air quality monitoring station of the PCD is also located in this school (Figure 2.8).

- U3: This sampling site is located at Lao Jo lane, Waroros market, Chang Moi road, Chang Moi Sub-district, Muang District. The site is just in front of the shrine, which is surrounded by small (~ 4 m width) roads with high traffic density and human activities all day long. The site is opposite of the motorcycles parking lot (Figure 2.9).

- U4: This sampling site is in the Chiang Mai Regional Police Traffic Center, Prisanee road, Chang Moi Sub-district, Muang District, The center is located at the riverside of the Ping River near Nawarat bridge, Ton Lum Yai market and intersection. This site is approximately 70 m distance from Prisanee road (~ 8 m width), which has guilt a high density every day (Figure 2.10).

- U5: This sampling site is located at Soi 4, Tunghotel road, Wat Kete Sub-district, Muang District. It is in Pee Nong dormitory (Figure 2.11) near the Arkhet bus station. It is approximately 350 m far from super highway.

Figure 2.7 U1; Maharaj Nakorn Chiang Mai Hospital, Muang District.

Figure 2.8 U2; Yupparaj Wittayalai School, Muang District.

Figure 2.9 U3; Waroros Market, Muang District.

- U6: This sampling site is located in Soi 7, Muen Dam Phra Khot Road, Chang Puak Sub-district, Muang District (Figure 2.12). It is in the back of Chiang Mai Rajabhat University and approximately 120 m from Muen Dam Pha Khot road (~ 4 m width). There are dormitory buildings and houses situated along the road.

- U7: This sampling site is inside the Muen San Temple, Haiya Sub-district, Muang District. The site is approximately 110 m from Wua Lai road (~ 8 m width), where the silver handicraft shops are located, with high traffic (Figure 2.13).

-U8: This sampling site is in theYu Pin house (small real-estate), Padad Sub-district, Muang District (Figure 2.14). It is located at the back of Hang Dong Tesco Lotus department store. This site is approximately 120 m from Chiang Mai - Hang Dong road (Highway No. 108).

b. Sub-urban area

-SU1: This sampling site is located at the back of San Sai Market, San Sai Luang Sub-district, San Sai District (Figure 2.15). It is near the Y intersection and approximately 100 m from Chiang Mai - Chiang Rai road (Highway No. 118) with high traffic density especially during rush hour.

- SU2: The sampling point is located at Ban San Sai Noi, San Sai Sub-district, San Sai District (Figure 2.16). It is a small village with a number of houses. The site is approximately 150 m from San Sai road (~ 8 m width).

- SU3: This sampling site is located in Thanawan home - estate, Nong Jom Sub-district, San Sai District. It is near by Ruam Chok Market and the middle ring road. It is approximately 70 m from Chiang Mai - Phrao road (Highway No. 1001) with high traffic density especially during rush hour (Figure 2.17).

Figure 2.10 U4; Chiang Mai Regional Police Traffic Center, Muang District.

Figure 2.11 U5; Pee Nong Dormitory, Muang District.

Figure 2.12 U6; Soi 7 Muen Dam Phra Khot Road, Back of Chiang Mai Rajabhat University.

- SU4: This sampling site is situated in Ban Tam Nuk, Mae Hia Sub-district, Muang District (Figure 2.18.). It is opposite the Hang Dong Big C department store and near the middle ring road. It is approximately 40 m from Chiang Mai - Hang Dong road (Highway No. 108).

- SU5: This sampling site is located at Ban Ton Pin, Mae Hia Sub-district, Muang District (Figure 2.19). It is at the back of Kulapunvil home-estate and surrounded by paddy fields. This site is approximately 700 m distance from Chiang Mai - Hang Dong road (Highway No. 108)

- SU 6: This sampling site is located at Ban Muang Kung, Nong Kwai Sub-district, Hang Dong District. It is situated near the outer ring road and approximately 700 m far from Chiang Mai - Hang Dong road. This area is a tourist place for handcrafted earthenware (Figure 2.20).

c. Rural area

- R1: This sampling site at located in Ban Rong Bon, San Na Meng Sub-district, Doi Saket District. It is surrounded by orchard, paddy fields and village's houses. It is approximately 300 m distanced from Chiang Mai - Chiang Rai road (Figure 2.21).

- R2: This sampling site is located at Ban Pha Yak Don Kaew, Talad Khaen Sub-district, Doi Saket District. It is surrounded by paddy fields (Figure 2.22). It is approximately 900 m from Chiang Mai - Chiang Rai road.

Figure 2.13 U7 ; Muen San Temple, Muang District.

Figure 2.14 U8; Yu Pin House (small real-estate), Muang District.

Figure 2.15 SU1; San Sai Market, San Sai District.

Figure 2.16 SU2; Ban San Sai Noi, San Sai District.

Figure 2.17 SU3; Thanawan home-estate, San Sai District.

Figure 2.18 SU4; Ban Tam Nuk, Muang District.

Figure 2.19 SU5; Ban Tan Pin, Muang District.

Figure 2.20 SU6; Ban Muang Kung, Hang Dong District.

Figure 2.21 R1; Ban Rong Bon, Doi Saket District.

- R3: This sampling site is located in Ban Rong Khi Lek, Cheng Doi Sub-district, Doi Saket District. It is surrounded by houses of villagers and paddy fields. There is also a rice mill nearby. This site is approximately 600 m from Chiang Mai - Chiang Rai road (Figure 2.23).

- R4: This sampling site in located at Ban Rai, Ban Wan Sub-district, Hang Dong District (Figure 2.24). It is in the front of Chaiyawoot temple and surrounded by villager's houses. It is approximately 600 m from Irrigation canal road (~ 8 m width).

- R5: This sampling site is located in front of the Aeranthawan temple, Num Phrae Sub-district, Hang Dong district. It is surrounded by paddy fields and orchard. This site is approximately 250 m distanced from Irrigation canal road (Figure 2.25).

- R6: This sampling site is located in Ban Num Phrae, Num Phrae Sub-district, Hang Dong District. It is surrounded by orchard and paddy fields. The site is approximately 500 m from Irrigation canal road (Figure 2.26).

Figure 2.22 R2; Ban Pha Yak Don Kaew, Doi Saket District.

Figure 2.23 R3; Ban Rong Khi Lek, Doi Saket District.

Figure 2.24 R4; Ban Rai, Hang Dong District.

Figure 2.25 R5; Aeranthawan temple, Hang Dong District.

Figure 2.26 R6; Ban Num Phrae, Hang Dong District.