CHAPTER 2

Methodology

2.1 Apparatus, Chemicals, Equipment and Instrument

2.1.1 Apparatus

1) 5, 10 mL graduate pipette, Witeg, Germany
2) 10, 20, 25 mL transfer pipette, Witeg, Germany
3) 100 to 1000 µl micropipette, Brand, Germany
4) 2000 mL volumetric flask, Technico, England
5) 1000 mL volumetric flask, Duran, Germany
6) 50, 100, 250 mL beaker, Duran, Germany
7) 100 mL sylinder, Duran, Germany
8) 0.45 µm, Ø 13 mm cellulose acetate syringe filter, Vertical, Thailand
9) Four-Stage filter holder, Nilu, Norway
10) Filter membrane (F0), 1.0 µm, Ø 47 mm polytetrafluoro-ethylene (PTFE), Whatman 41, UK
11) Filter membrane (F1), 1.0 µm, Ø 47 mm polyamide, Pall, USA
12) Filter membrane (F2, F3), No. 41, Ø 47 mm cellulose, Whatman, UK
13) Filter paper chromatography, No.590, 400 x 400 mm cellulose, ADVANTEC, Japan
14) Polypropylene tube
15) Plastic gloves
16) Plastic zip bag
17) 200 mL spray bottle
18) 30, 100 mL polyethylene bottle
19) Funnel

2.1.2 Chemicals

1) Sodium (Na^+) standard solution 1000 mg/L, Merck, Germany
2) Ammonium (NH_4^+) standard solution 1000 mg/L, Merck, Germany
3) Potassium (K^+) standard solution 1000 mg/L, Merck, Germany
4) Calcium (Ca^{2+}) standard solution 1000 mg/L, Merck, Germany
5) Magnesium (Mg^{2+}) standard solution 1000 mg/L, Merck, Germany
6) Chloride (Cl^-) standard solution 1000 mg/L, Merck, Germany
7) Nitrate (NO_3^-) standard solution 1000 mg/L, Merck, Germany
8) Sulfate (SO_4^{2-}) standard solution 1000 mg/L, Merck, Germany
9) Acetonitrile (CH_3CN), 99.8%, Merck, Germany
10) Sodium carbonate anhydrous (Na_2CO_3), 99.9%, Scharlau, Spain
11) Sodium hydrogen carbonate ($NaHCO_3$), 99.7%, Scharlau, Spain
12) Sulfuric acid (H_2SO_4), 95-97%, Merck, Germany
13) Nitric acid (HNO_3), 65%, Merck, Germany
14) 2,6-Pyridinedicarboxylic acid, 99%, Sigma-Aldrich, India
15) Potassium carbonate (K_2CO_3), 99%, QRëC, New Zealand
16) Ortho-Phosphoric acid (H_3PO_4), 85%, Labscan, Thailand
17) Hydrogen Peroxide (H_2O_2), 40%, Carlo Erba, Italy
18) Thymol (2-isopropyl-5-methylphenol), 99.5%, Labchem , Australia
19) Powdered Precision Cleaner, Alconox, USA
20) Deionized water, Chemistry Department, Chiang Mai University

2.1.3 Equipment

1) Analytical balance, AB304-S, Mettler Toledo, Switzerland
2) Ultrasonicator, P 300 H, Elma, Germany
3) Ultrasonicator, T 490DH, Elma, Germany
4) Oven, UE 400, Memmert, Germany
5) Vacuum pump, Rocker 300, Keika Ventures, USA
6) pH meter, 744, Metrohm, Switzerland
7) Conductivity meter, CyberScan CON 1500, Eutech, Singapore

2.1.4 Instrument

Ion Chromatograph, 882 Compact IC plus, Metrohm, Switzerland, consisting of

a) 838 Advanced Sample Processor
b) Anion guard column, Metrosep A Supp 4/5 Guard
c) Anion separation column, Metrosep A Supp5 250/4.0 mm
d) Anion self-generating suppressor
e) Cation guard column, Metrosep RP 2 Guard/3.5
f) Cation separation column, Metrosep C4-100/4.0 mm
g) Conductivity detector
h) Output, MagIC Net 2.6 program

2.2 Monitoring of atmospheric particulate deposition by four-stage filter pack

2.2.1 Sampling site

The sampling site is one of the sites belongs to the Acid deposition monitoring station in Thailand. It was located at the meteorological station in the area of Mae Hia Research Center, Chiang Mai University, Muang District, Chiang Mai Province. This station was located at longitude 98^{o} 55' 54.3" E and latitude 18^{o} 45' 40.3" N. Its elevation is 334 meters above sea level. It is situated in a large open meadow closed to the reservoir. The sampling site has been classified as a sub-urban area (Chantara et al., 2012). The major pollutant emission sources around the sampling sites are Chiang Mai International Airport (3 km east), tourist spots (the Night Safari and the Royal Garden) (2.5 km southwest) and Chiang Mai City (6 km northeast). The land use profile of the sampling site is shown in Figure 2.1 and the surrounding detail of sampling site is shown in Table 2.1and Figure 2.2

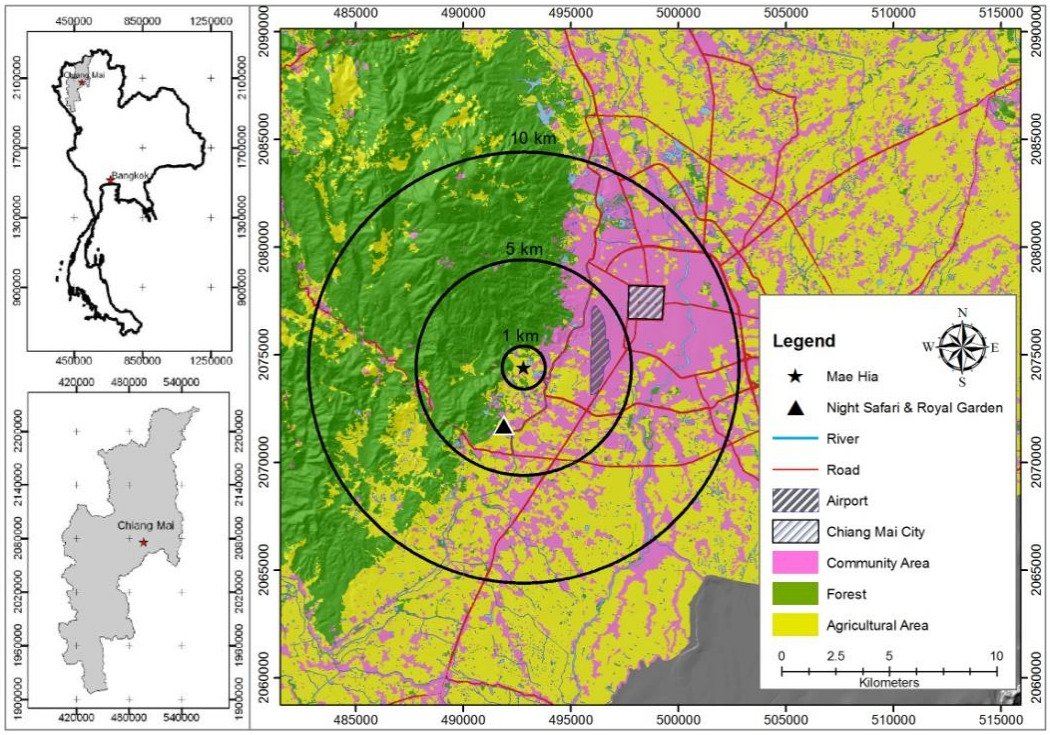


Figure 2.1 Land use profile of Mae Hia Research Center

Table 2.1 Surrounding details of sampling site

Scale Type	Radian	Details
Onsite Scale	0 - 150 m	Area of Mae Hia Research Center are used for research purposes for students of Agriculture and Veterinary Faculties. The rest of the areas are used for the office building, parking lot, housing, etc.
Local Scale	150 m - 10 km	Most of the area are forest, communities and agricultural areas. Other land use is airport, tourist attractions (night safari and Royal garden), Chiang Mai City, golf course, etc.
Regional Scale	10 – 50 km	The radian of this scale cover forest, community, agricultural areas, transportation station, industrial estate, etc.

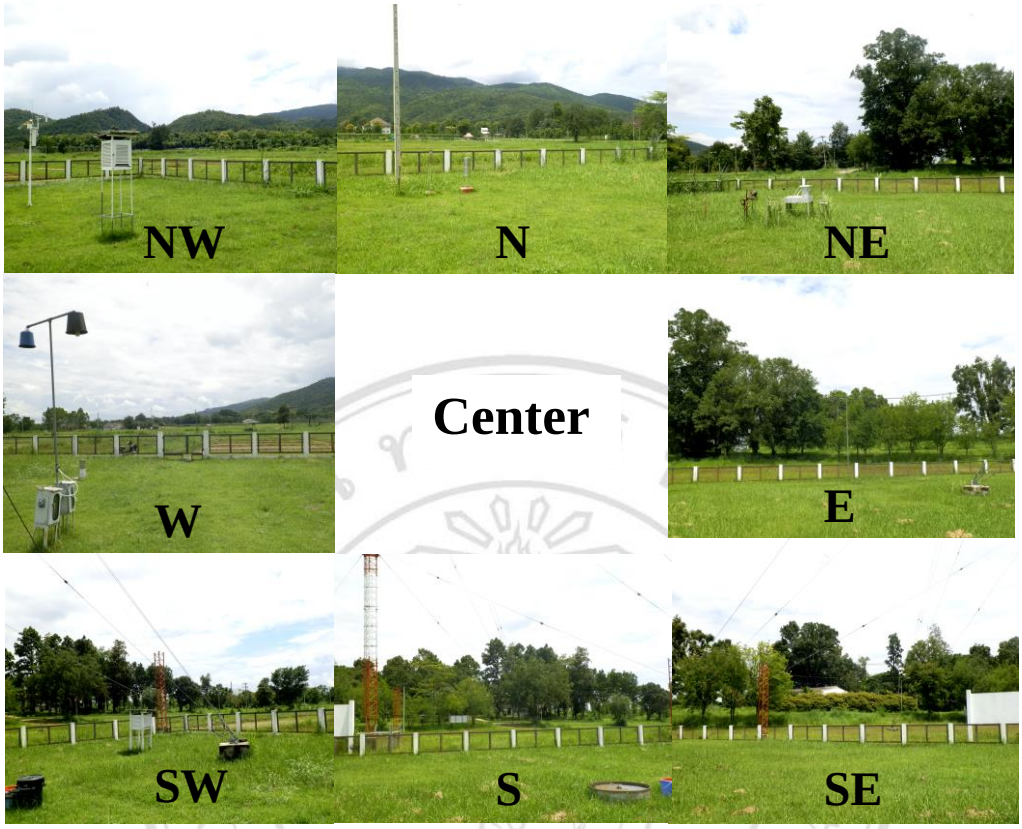


Figure 2.2 Surrounding of the sampling site

2.2.2 Sampling method and sample preparation

1) Filter pack sampling system

Dry deposition sample is proposed to measure the level of air pollution in atmosphere. The main pollutants such as SO_2, HNO_3, HCl, NH_3, and aerosols were collected by four-stage filter pack (Nilu, Norway), which it was controlled an inlet height and flow rate. The inlet of air sampling holder was set up at the height of about 3 meters above the ground. During sampling period, the diaphragm pump was set up for stable flow rate (1 L/min). The filter pack sampling system is shown in Figures 2.3 and 2.4. Four-stage filter pack composes of four filters in line with the air stream Figure 2.3.

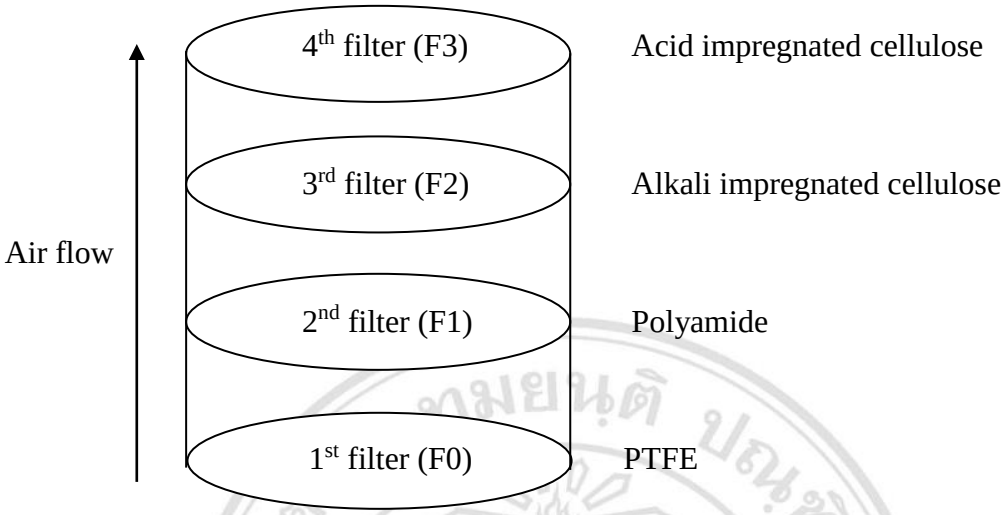
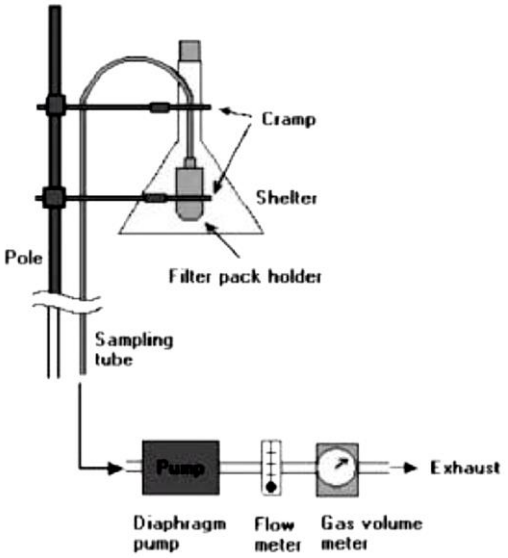


Figure 2.3 Schematic diagram of four-stage filter pack



(a)

(b)

(c)

(d)

Figure 2.4 Filter pack sampling system

(a) Schematic diagram of filter pack sampling system (EANET, 2003)

(b) On-site for filter pack sampling set

(c) Connection of filter pack holder

(d) Pump

The aerosols are collected on the first filter (F0: made of polytetrafluoro-ethylene; PTFE) mounted in front of the other filters (F1, F2 and F3) collecting gases, while gaseous substances such as SO_2, HNO_3, HCl and NH_3 will pass though this filter. The second filter (F1: made of polyamide) collects all HNO_3 and partial SO_2, HCl and NH_3 from the sampling air. The remaining SO_2 and HCl react with alkali substance on the third filter (F2: made of cellulose). The remaining NH_3 reacts with acid substance on the fourth filter (F3: made of cellulose) after passing through the first, second and third filter. The specifications of collected species in each filter are summarized in Table 2.2.

In this work only the first filter (F0) was analyzed for ion concentrations.

Table 2.2 Specifications of filters and collected species

Stage	Filter type	Collected species
1st(F0)	Polytetrafluoro-ethylene (PTFE) or Teflon	Aerosol
2nd(F1)	Polyamide or Nylon	HNO_3, Partial SO_2, HCl, NH_3
3rd(F2)	Cellulose impregnated by K_2CO_3	SO_2, HCl
4th(F3)	Cellulose impregnated by H_3PO_4	NH_3

Note; Only F0 was used and analyzed in this work for comparison of leaf-washing method

2) Preparation of first filter (F0)

Filters were handled in the laboratory only under the clean conditions. Moreover, the plastic gloves and tweezers should always be used when handling filters. Only a clean filter holder should be used to prevent contamination. However, filters were always sealed by plastic zip bag to avoid the contamination by ambient pollutants before and after the sampling time. The glassware were washed by deionized water and dried in an oven at 60°C. After dried, cleaned glassware were prevented from contamination by kept in plastic zip bag.

3) Four-stage filter pack installation

The four-stage filters pack was connected with an air pump, which flow rate of air input was 1 L/min. It was set up at the height of about 3 meter above ground. All of samples were collected for every 10 days. Therefore, 3 samples per month were collected. Four-stage filter pack holder set was closed with plastic cap and stored in clean plastic zip bag after stop sampling. The collected filter pack was transferred to laboratory for extraction and ion analysis.

4) Sample extraction

As mentioned before, only the F0 was used for thesis work. Samples and blanks were extracted by 20 mL of deionized water in polypropylene tubes with caps and then sonicated in the ultrasonicator (T 490DH, Elma, Germany) for 30 minutes. After extraction, sloution were filtered by cellulose acetate membrane (pore size 0.45 μm, Ø13 mm) and stored in polyethylene bottles and kept in refrigerator at 4°C.

2.3 Monitoring of atmospheric particulate deposition by leaf-washing method

2.3.1 Study area

The sampling tree for leaf-washing method (Figure 2.5) is about 150 meters in NE direction for from the acid deposition monitoring station as shown in Figure 2.1 with an elevation of 337 meter above sea level.

The sampling tree is surrounded by small paved and dirt roads. It is about 50 meters far from the paved roads in north, northeast and east directions. The dirt roads are ~ 100 meters far in the south and southwest directions.

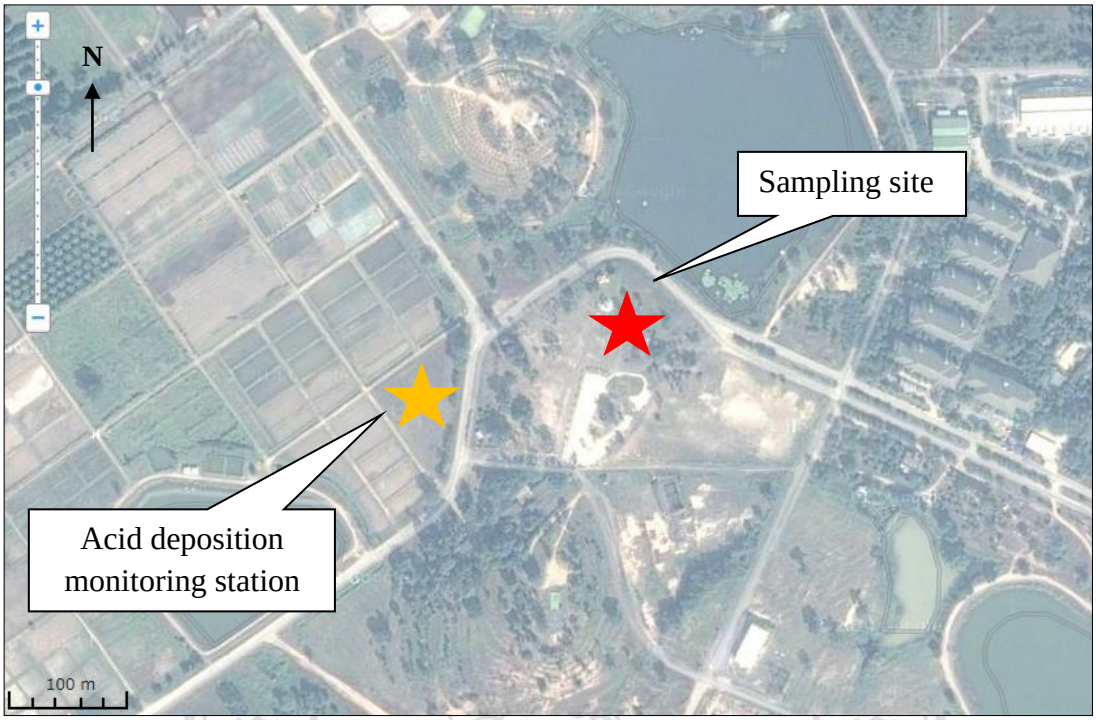


Figure 2.5 Map of sampling site **(**http://map.longdo.com/?gmap=1&hybrid=1**)**

2.3.2 Selection of sampling tree

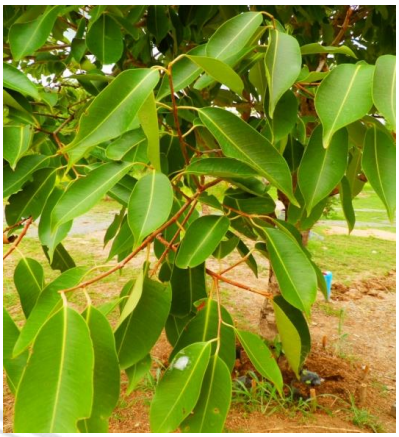
The criteria of sampling tree selection was mainly considered based on leave falling cycle. The selected tree was an evergreen tree with appropriate leaf size and leaf surface. Its canopy was higher than 3 meters according to the height of four-stage filter pack. I should be located near the sampling station for comparison study.

A jambolan plum tree (*Syzygium cumini*) was selected as the sampling tree. The characteristics of the tree are shown in Figure 2.6. Jambolan plum tree is native for tropical region. It is an evergreen tree, with horizontal leaf and smooth surface. The selected tree was about 6 meters high and its canopy was about 4 meters in diameter. It is located near the monitoring station of the EANET site. The scientific classifications of jambolan plum tree are shown in Table 2.3.

Table 2.3 Scientific classifications of jambolan plum tree (USDA, 2014)

Scientific classifications	**Jambolan plum tree**
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	*Syzygium*
Species	*Syzygium cumini* (L.) Skeels

(a)



(b)

(c)

(d)

Figure 2.6 Photos of jambolan plum tree (*Syzygium cumini*)

(a) Tree

(b) Leaves

(c) Flowers

(d) Fruits

2.3.3 Selection of sampling leaves

Leaf samples were randomly selected from 8 directions, while blank samples were randomly selected around the tree canopy. All of the selected leaves were at the outer part of the tree canopy. All of them laid in horizontal position or a little turning upward, in consideration of particulates deposition on the leaf surface. Moreover, size of the selected leaves was almost the same and the leaf colour was greenish showing leaf maturity. If the leaf was too old it might be fallen off easily, but if the leaf was too young, it might be too small and was also easily destroyed by insects, etc.

2.3.4 Sampling method

1) Sampling duration

Atmospheric particulate deposition sampling was conducted from July 2012 to March 2013; 4 months in wet season (July 2012 – October 2012) and 5 months in dry season (November 2012 – March 2013) . The samples were collected once a month (~ 30 days period) in wet season and every 10 days (3 times a month) in dry season.

2) Particulate sample collection from leaves

The sampling tree canopy was divided into 8 directions (N, NE, E, SE, S, SW, W and NW) at about 3 meters above ground level (Figure 2.7), in which 3 leaves were randomly selected from each direction. In total 24 leaves were collected each time from around the canopy and counted as leaf samples. Five more leaves around the tree canopy were randomly selected at the same level of sample leaves and used as blanks. In total, 29 leaves were selected. The petioles of all selected leaves were tided by plastic rope for recognition in the next sampling (Figure 2.8(b)). In conclusion, 24 samples and 5 blank samples were collected each month in wet season and 72 samples (24 x 3) and 15 blank samples (5 x 3) were collected each month in dry season.

The selected leaf attached to the tree was washed by 100 ml of deionized water (DIW) using a sprayer into a polyethylene bottle, which was weighed before used. After sample collection, the bottle containing water sample was weighed again to get the weight of the sample. At the beginning, selected leaves were cleaned by washing with DIW (100 ml/ leaf) using a sprayer and leave them until the next sampling period.

When the sampling period was completed in each circle, the selected leaf was washed with 100 ml of DIW again and kept as a sample in a polyethylene bottle. In case of blank sample, the leaf was washed twice with 100 ml DIW/time. The first fraction was discarded, while the second fraction was collected as a blank sample. All samples were stored in the refrigerator until analysis. Figure 2.8 shows steps of sample collection by the leaf-washing method.

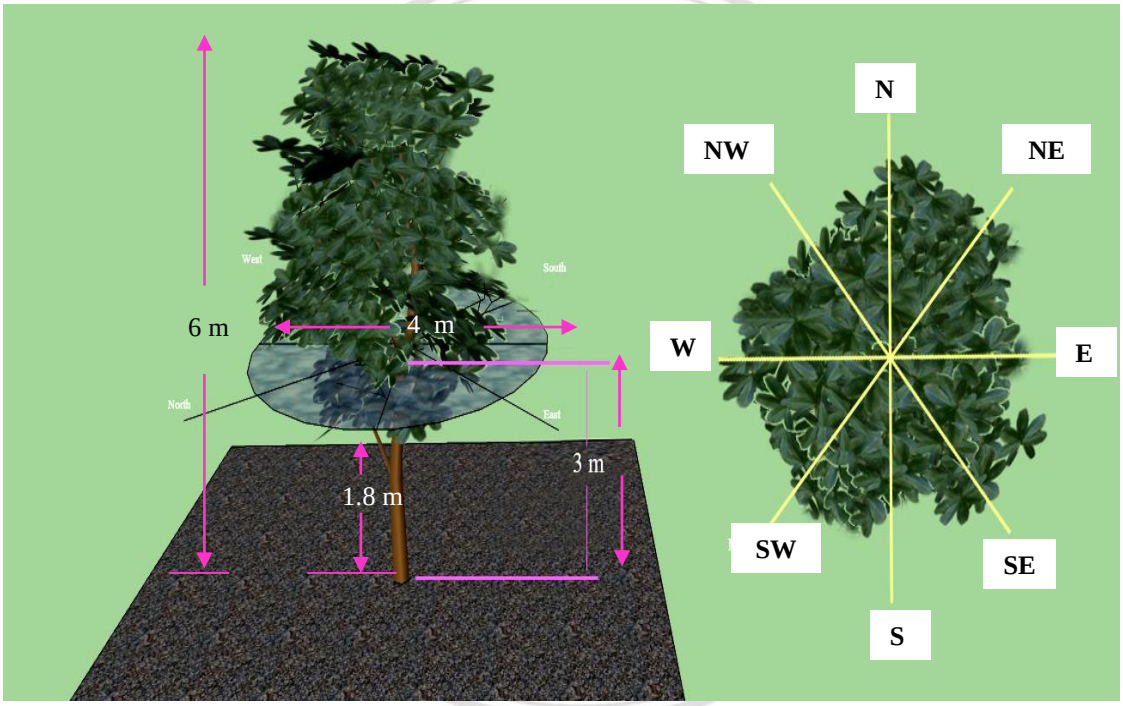


Figure 2.7 Diagram of sampling tree and sampling directions

(a)

(b)

(c)

Figure 2.8 Photos of sample collection by leaf-washing method

(a) Sample collection

(b) Leaf marked by plastic rope

(c) Leaf washing by using a sprayer

2.3.5 Chemical analysis of samples

The bottles used for sample container were pre-and post-weighed for calculation of water volume. Washed solution was divided into two parts. The first part (about 30 mL) was for measurement of electro-conductivity (EC) and pH, while another part was filtered by cellulose acetate syringe filter (pore size 0.45 μm, Ø 13 mm) and analyzed for anions and cations by Ion Chromatograph (IC).

1) Electro-conductivity (EC) measurement

Measurement of electro-conductivity is to determine the total dissolved ions in solution. The samples were measured by CyberScan CON 1500, Eutech, Singapore, in mS/m unit. During the measurement temperature was controlled at 25 °C.

2) pH measurement

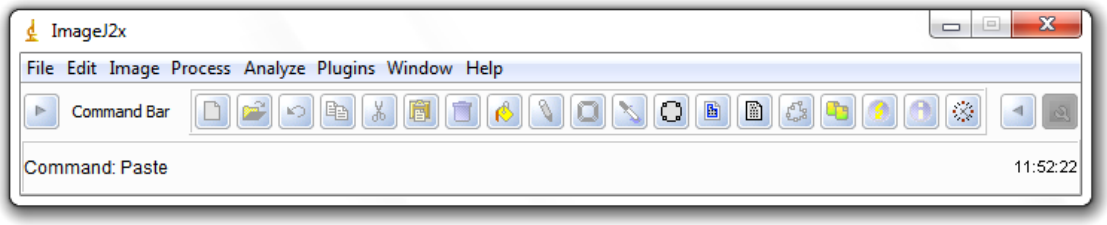
The pH of sample is a measure of the concentration of hydrogen ions in solution. In this study, Metrohm pH meter was used. A temperature of 25°C is recommended for pH measurement.

2.3.6 Measurement of leaf area

The leaf area was determined by using Image J 2X program version 1.43 (Ferreira and Rasband, 2010), which is a Java image processing and analysis program.

All the selected leaves of the sampling tree were photographed at the site for leaf area analysis. The individual leaf was laid on a white paper together with a scale (1cm) for photography. Photos of leaves were measured for their areas by Image J 2X program. This program converted a scanned color image of leaf to grayscale to define region from pixel value. The actual pixel from the picture was then converted to area (mm^2). The program of Image J main window and example for measurement of leaf area are shown in Figure 2.9.

(a)



(b)

(c)

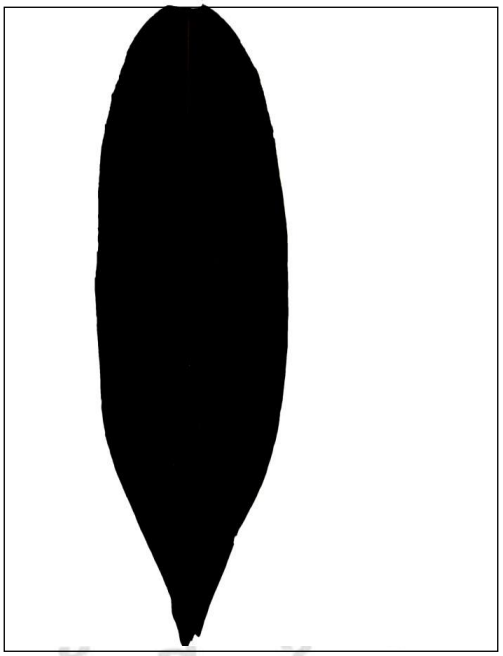


Figure 2.9 Leaf area measurement by Image J program

(a) Image J main window

(b) Photo of leaf with a scale

(c) Converted photo of leaf for leaf area calculation

2.4 Analysis of ion content in dry deposition samples by Ion chromatography

All of samples from four-stage filer pack and leaf-washing method were analyzed for major cations (Ca^{2+}, NH_4^+, Na^+, K^+ and Mg^{2+}) and major anions (Cl^-, NO_3^- and SO_4^{2-}) by Ion Chromatograph (882 Compact IC plus, Metrohm, Switzerland). Ion Chromatograph is shown in Figure 2.10, which the analysis conditions of ion chromatograph for anion and cation are detailed in Table 2.4.

Figure 2.10 Ion Chromatograph (Metrohm, Switzerland).

Table 2.4 Conditions of ion chromatograph for anion and cation analysis

Analysis item	Anions	Cations
Eluent	3.2 mM Na_2CO_3/1.0 mM $NaHCO_3$	1.7 mM HNO_3 and 0.7 mM 2,6-Pyridinedicarboxylic acid
Guard column	Metrosep A Supp 4/5	Metrosep RP 2/3.5
Analysis column	Metrosep A Supp5 250/4.0 mm (250×4.0 mm)	Metrosep C4-100/4.0 mm (100×4.0 mm)
Carrier material	Polyvinyl alcohol with quarternary ammonium groups	Silica gel with carboxyl groups
Suppressor	Anion self-regenerating suppressor with DI water/100mM H_2SO_4	-
Particle size	5 µm	5 µm
Max. pressure	15 MPa	20 MPa
Flow rate	0.70 mL/min	0.90 mL/min
Temperature	20-60 ºC	20-60 ºC
pH range	3-12	2-7
Injection loop	20.0 µL	20.0 µL
Detector	Conductivity	Conductivity

2.4.1 Eluent preparation

1) Eluent preparation for cation analysis

The eluent solution for cation was mixture of 1.7 mM HNO_3 and 0.7 mM 2,6-pyridinedicarboxylic acid (DPA). 237 µL of HNO_3 was pipetted using micropipette, while 0.236 g of 2,6-pyridinedicarboxylic acid was weighed. After that, they were mixed and dissolved in deionized water before adjusted the volume to 2000 mL in a volumetric flask. Eluent was filtered by a filter holder set with nylon membrane filter (pore size 0.45 µm, Ø 47 mm) and degassed by vacuum and ultrasoniccation before analysis.

2) Eluent preparation for anion analysis

A mixture of 3.2 mM sodium carbonate (Na_2CO_3) and 1.0 mM sodium hydrogen carbonate ($NaHCO_3$) solution was used as an eluent. The eluent stock solution was prepared by dissolved 3.4046 g Na_2CO_3 and 0.8426 g $NaHCO_3$ in deionized water and into 100 mL volumetric flask. This stock solution was then used to prepare a solution of 3.2 mM Na_2CO_3 and 1.0 mM $NaHCO_3$ by pipetted 20 mL of it into 2000 mL volumetric flask and adjusted volume by deionized water. Eluent was filtered by a filter holder set with nylon membrane filter (pore size 0.45 µm, Ø 47 mm) and degassed by vacuum and ultrasoniccation before analysis. The system was regenerated by a solution of 100 mM H_2SO_4 pumped through a suppressor simultaneously with deionized water. They were filtered by a filter holder set with nylon membrane filter (pore size 0.45 µm, Ø 47 mm)

2.4.2 Preparation of standard solutions and construction of standard calibration curve

The individual ion standard solution of cations (Na^+, NH_4^+, K^+ $Ca^{2+}$and Mg^{2+}) and anions (Cl^-, NO_3^- and SO_4^{2-}) was prepared from its stock solution (1000 µg/mL). Mixed standard solution of anions was prepared and diluted with deionized water to concentration of 10 µg/mL into 25 mL volumetric flask. Concentration of 10 µg/mL standard solution was used for preparation another standard solutions concentration. The same process was done for cations standard solution.

Mixed standard solutions were injected into IC columns under optimum conditions before analysis of ion concentration. The standard solutions were freshly prepared from the mixed stock standard solution every analysis day. Then calibration curve of each ion was plotted between peak areas (y axis) with ion concentrations (x axis).

2.4.3 Determination of Limit of detection (LOD) and limit of quantification (LOQ) of ions analyzed by Ion chromatography

The LOD is the lowest concentration can be detected by instrument. LOQ is a parameter for quantitative assays for low levels of compounds in the sample or the lowest actual concentration of an analysis that can be reliably detected by an analytical procedure.

The LOD and LOQ were checked by injecting 5 times of the lowest concentration (0.05 µg/mL) of mixed anions standard (Cl^-, NO_3^- and SO_4^{2-}) and the lowest concentration (0.05 µg/mL) of mixed cations standard (Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) into ion chromatographic system under the optimum conditions. The LOD was obtained from 3 times, while LOQ was obtained from 10 times, of standard deviation (SD).

2.4.4 Accuracy of ion analysis by Ion chromatography

1) Repeatability

The repeatability is the results of standard deviation of measurements repeated by same method on the same instrument within a short time period (Wiriya, 2008; Sillapapiromsuk, 2013). The repeatability was checked by 5 times continuously injection of a 0.8 µg/mL mixed anion standards (Cl^-, NO_3^- and SO_4^{2-}) and 0.8 µg/mL mixed cation standards (Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) into IC under the optimum conditions.

2) Reproducibility

The reproducibility was checked by injecting a 0.8 µg/mL mixed standard solution of anions (Cl^-, NO_3^- and SO_4^{2-}) and 0.8 µg/mL mixed standard solution of cations (Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) into IC under the optimum conditions once a month for 5 months long.

The results of the repeatability and reproducibility were estimated by standard deviation and the relative standard deviation (RSD) as calculated by using Equation 2.1.

$$\% \text{ RSD} = \frac{\text{SD}}{\bar{x}} \times 100 \qquad \text{Eq. 2.1}$$

Where % RSD is a percentage relative standard deviation

SD is a standard deviation

$\bar{x}$ is average value

2.5 Comparison of data obtained from four-stage filter pack and leaf-washing methods by statistic

Data including dry deposition amount (mg/m^2) of Cl^-, NO_3^-, SO_4^{2-}, Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+} in wet and dry seasons from both methods were analyzed by statistical method. Analysis of variance (MANOVA) was used for comparison of ion composition between leaf-washing and four-stage filter pack methods. In each method, ion composition in wet and dry seasons were compared for seasonal variation. In addition, pearson correlation (*r*) was used to identify relationships between same pair ion from both methods.