CHAPTER 2

Experimental

2.1 Chemicals, Equipment and Instruments

2.1.1 Chemicals

1. Individual standard solution (1000 µg/ml), BDH, England
    1.1 Aluminum (Al)
    1.2 Arsenic (As)
    1.3 Calcium (Ca)
    1.4 Cadmium (Cd)
    1.5 Cobalt (Co)
    1.6 Chromium (Cr)
    1.7 Copper (Cu)
    1.8 Iron (Fe)
    1.9 Lead (Pb)
    1.10 Magnesium (Mg)
    1.11 Manganese (Mn)
    1.12 Sodium (Na)
    1.13 Nickel (Ni)
    1.14 Potassium (K)
    1.15 Selenium (Se)
    1.16 Silicon (Si)
    1.17 Antimony (Sb)
    1.18 Tin (Sn)
    1.19 Vanadium (V)
    1.20 Zinc (Zn)
2. Acid solutions
    2.1 Nitric acid, Extra Pure grade, 65% Merck, Germany
    2.2 Nitric acid, ISO grade, 65%, Merck, Germany

    2.3 Hydrochloric Acid, PH EUR grade, 37%, Merck, Germany
    2.4 Hydrofluoric Acid, (HF), AR grade, 40- 45%, Sigma-Aldrich, USA

3. Certified Reference Material (CRM)
    3.1 Pond sediment (300.21, Japan)
    3.2 Apple leaf (1515, NIES, USA)
4. Hydrogen peroxide, (H_2O_2), 40% m/v in water, Carlo Erba, Italy
5. Distilled water (conductivity < 0.15 mS/m)

2.1.2 Equipment/Apparatus

1. Quart fiber filter paper QMA grade (Ø 47 mm), Whatman, England
2. Open-system combustion chamber, equipped with:
    2.1 PM$_{2.5}$ and PM$_{10}$ impactors, Mini Vol^{TM}, Tactical
    2.2 Gas analyzer, 350-XL, Testo
    2.3 Temperature sensor,
    2.4 Flow recorder
3. Water purification system,
4. Thermometer
5. Double layer digestion Teflon vessels (50 mm diameter)
6. Multiple size graduate pipette, Witeg, Germany
7. Multiple size beaker, Duran, Germany
8. Multiple size volumetric flask, Duran, Germany
9. Plastic Bottle for sample storage 30, 60 ml
10. Hot air oven, UE 400, Memmert, Germany
11. Desiccators
12. Ultra-Sonicator, True-Sweep 690T, Crest, USA.
13. Ultra-sonicator, P300H, Elma, Germany
14. Analytical Balance, AB-304S, Mettler Toledo, Switzerland
15. Microbalance, MX5, Mettler Toledo, Switzerland
16. Hotplate stirrer HTS-3001, LM

2.1.3 Instruments

1. Inductively Coupled Plasma-Optical Emission Spectrophotometry, Prodigy 7, Teledyne Leeman, USA (Chiang Mai Rajabhat University)

2.2 Research Framework

Problem identification was the outcome of previous studies verifying relation of pollution during the dry season with open burning (Chantara et al., 2012; Khamkaew et al., 2016). Sampling contained field sampling (biomass sampling) and laboratory sampling (open burning simulation and $PM_{2.5}$ and ash sampling). Overall outlines of this research, from identification of problem to sampling and extraction of samples, testing of methodology and elemental analysis are shown schematically in the Figure 2.1.

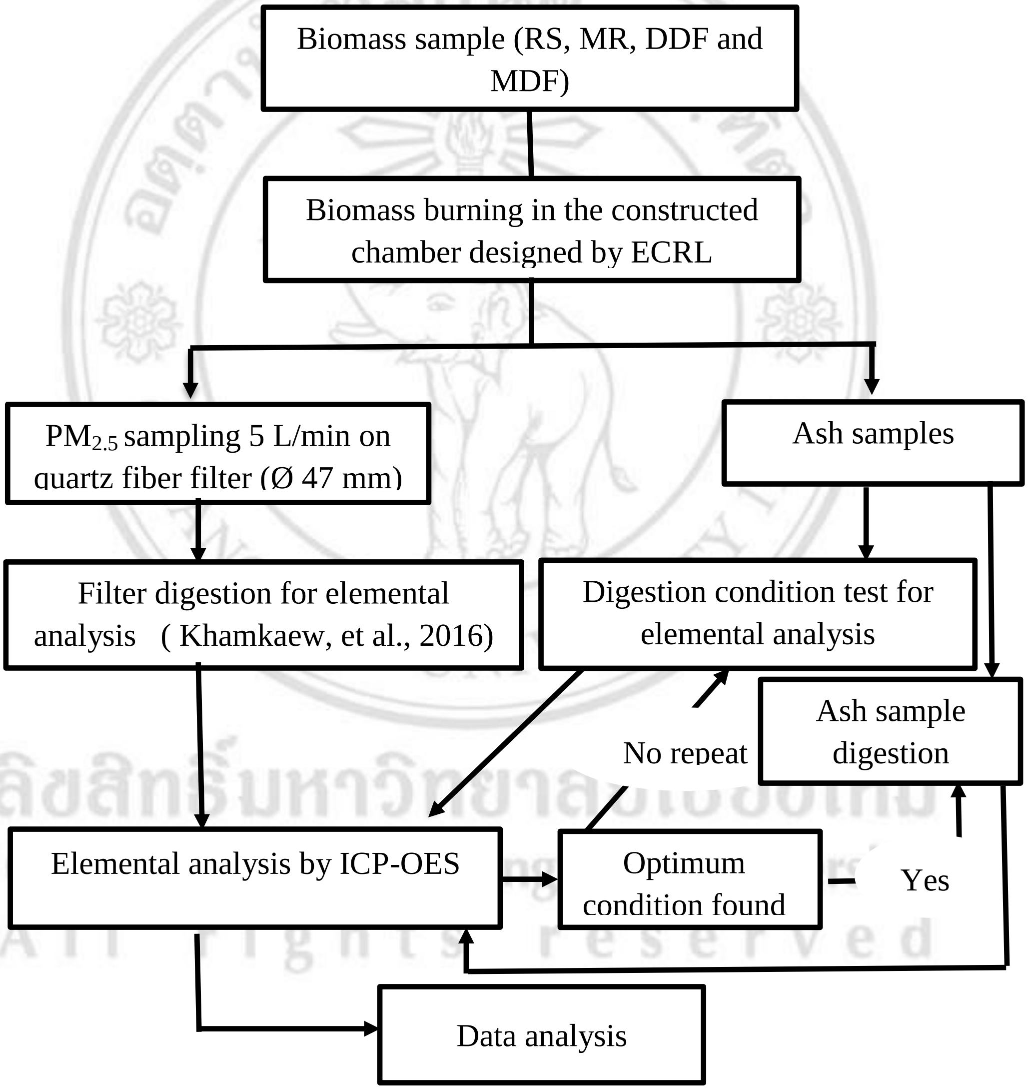


Figure 2.1Diagram of the research plan and processes

2.3 Biomass Sampling and Biomass Burning Experiment

Four types of biomass namely rice straw (RS), maize residues (MR), dry dipterocarps (DDF) and mixed deciduous (MDF) forests leaf litter were selected for this research. Rice and maize are the dominant agricultural crops over UNT. Dry dipterocarps and deciduous forests are main of types of the forest in this area, so they contribute significantly to biomass open burning and pollutant emission in the UNT. Therefore, they were selected and collected from nine provinces of UNT. Information about sampling location and samples code in this research are detailed in Table 2.1. For biomass sampling

Table 2.1 Information about biomass samples locations and code

Biomass types	site	Location		sample code
		District	Province	
Rice straw (RS)	1	San Pong, Mae Rim	Chiang Mai	1100
	2	Thung Ruang Thong, Chun	Phayao	1201
	3	Huai So, Chiang Khong	Chiang Rai	1300
	4	Maekhammi, Nong Muang khai	Phrae	1400
	5	Moung Kham, Phan	Chiang Rai	1700
Maize Residues (MR)	1	Kong Khaek, Mae Chame	Chiang Mai	2101
	2	Mae Sanean, Muang Nan	Nan	2401
	3	Bua Yai, Na Noi	Nan	2402
	4	Hua Suea, Mae Tha	Lampang	2600
	5	Wiang, Wiang Pa Pao	Chiang Rai	2700
Dry Dipterocarps Forest (DDF)	1	Dan Mae Lamao, Mae Sot	Tak	3103
	2	Mae Chame	Chiang Mai	3106
	3	Tambon Mae La Luang, Mae La Noi	Mae Hong Son	3107
	4	Mae Phrik, Mae Phrik	Lampang	3108
	5	Tambon Wang Nuea, Wang Nuea	Lampang	3110
Mixed Deciduous Forest (MDF)	1	Sa Iap, Song	Phrae	3202
	2	Ban Pong, Hang Dong	Chiang Mai	3203
	3	Tambon Ko, Li	Lamphun	3207
	4	Ban Wiang, Rong Kwang	Phrae	3208
	5	Ping Khong, Chiang Dao	Chiang Mai	3209

2.3.1 Biomass samples detail

Four types of biomass: rice straw (RS), maize residues (MR) and leaf litter from dry dipterocarps (DDF) and mixed deciduous (MDR) forests were selected for this study. Biomass samples collected from nine provinces over northern Thailand. Samples for each biomass type were collected from 5 different locations (Chantara et al., 2017) (Table 2.1).

2.3.2 Biomass sampling

Biomass samples including rice straw, maize residues and forest leaf litters were collected from different areas in Northern Thailand using 2 m x 2 m quadrats (Chantara et al., 2017) (Figure 2.2).

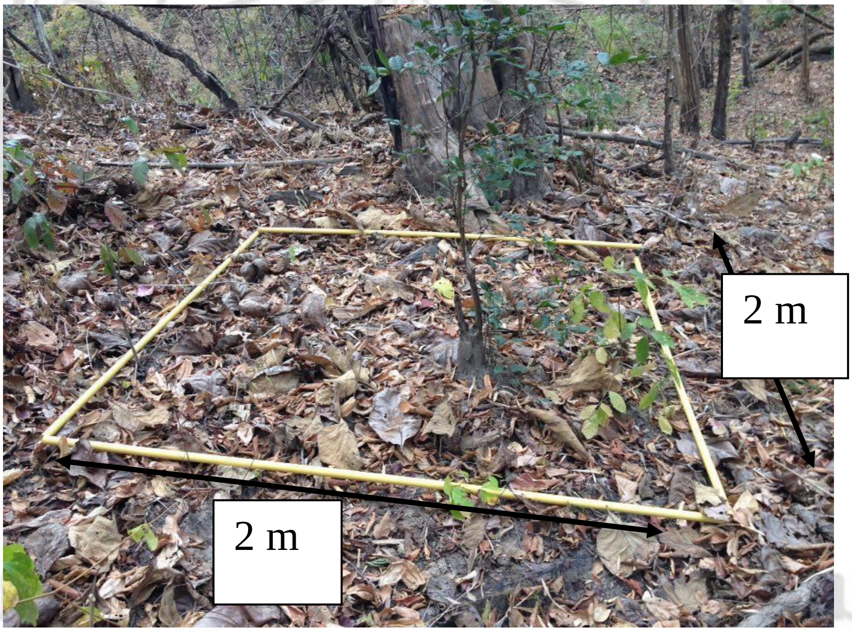


Figure 2.2 Biomass sampling grids (2m x 2m) (Chantara et al., 2017)

Agricultural residues such as rice straw and maize residues were collected from crops planting areas after the harvest period, while forest leaf litters were gathered in dry season. Each type of biomass was collected from five various locations in triple replication from UNT and prior to burning experiment all sampled biomasses were air dried and homogenized (Figure 2.3).

(a) (b)

Figure 2.3 Sampling process of agricultural residues, (a) 2 m x 2 m grid for biomass sampling, and (b) rice straw sample (Chantara et al., 2017)

2.3.3 Open burning simulation

Collected, dried and homogenized biomass samples were prepared. Burning experiment was carried out in the constructed open-system combustion chamber. Each biomass sample from each location was burned in triplicate. $PM_{2.5}$ and ashes were collected during and after open burning simulation as detailed in the following.

(a) (b)

Figure 2.4 Open burning simulation, (a) dried biomass in burning tray, and (b) burning process.

a) Combustion chamber: an open-system combustion chamber designed by environmental chemistry research laboratory (ECRL) was constructed. The combustion chamber consists 1 m^2 burning tray, located under 4m^2 hood (shell) which is connected to a 4m horizontal smoke stack with 30 cm in diameter. The stack is equipped with PM samplers (Intra et al., 2012) , a gas analyzer (350-XL, Testo), flow meter (TSI, 4000) to determine flow rate of air in chimney and temperatures sensor (Figure 3.5).

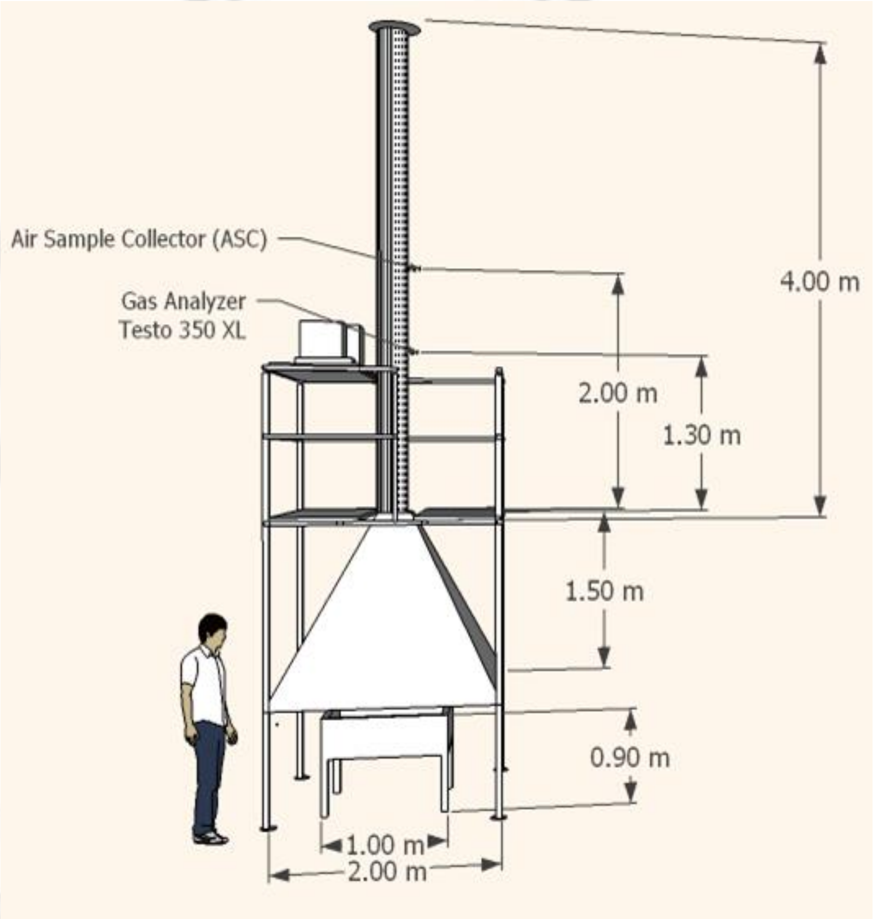
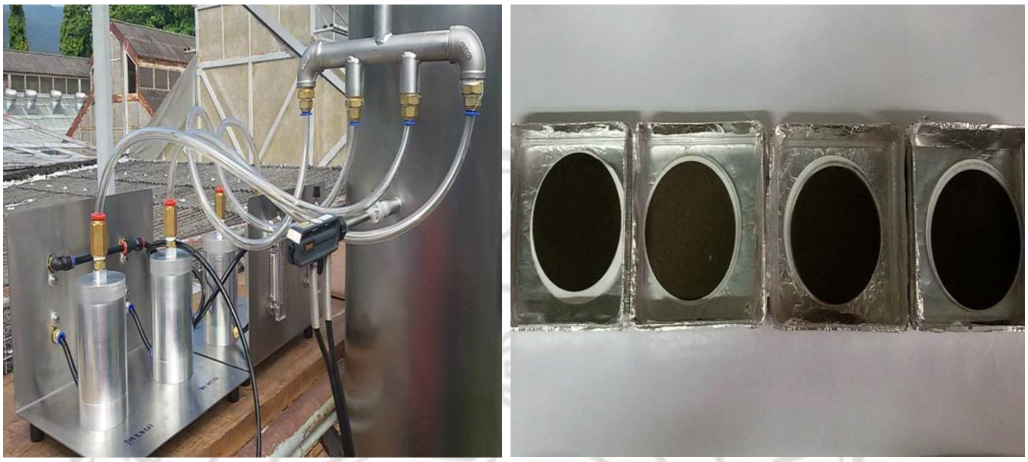


Figure 2.5 Diagram of Open-system combustion chamber (Chantara et al., 2017).

b) $PM_{2.5}$ sampling from biomass burning: approximately one kilogram of dried biomass for each replications of each biomass sample type was burned in the open-system combustion chamber. $PM_{2.5}$ samples were collected by PM sampler (Intra et al., 2012.) (Figure 2.6, (a)) with a flow rate of 5 L/min on quartz fiber filter (Ø 47 mm, Whatman) (Figure 2.6, (b)) during the BB in the open-system combustion chamber. Quartz fiber filters were desiccated for at least 24 hours and weighed to determine the net weight of the filter paper before simulation of open burning and similar process was applied post burning to specify amount of collected $PM_{2.5}$ during the biomass burning.



(a) (b)

Figure 2.6 $PM_{2.5}$ sampling process during open burning simulation, (a) $PM_{2.5}$ samplers, and (b) Quarts fiber filter after sampling.

c) Ash sampling: after completion of open burning simulation in open-system combustion chamber, the ashes left over were collected, weighed, sieved and packed (Figure 2.7 (a)&(b)) after cooling down in the open area to determine the ratio of burned and unburned biomass and further elemental analysis.

(a) (b)

Figure 2.7 Ash sampling after open burning, (a) ash remained in the burning tray of the chamber, and (b) Sieved and packed ash samples (Chantara et al., 2017).

2.4 Analysis of $PM_{2.5}$ and Ash Samples from BB

2.4.1 $PM_{2.5}$ sample extraction

Before and after sampling, quartz fiber filters were kept in a desiccator for at least 24 hours and weighed by using microbalance (MX5, Mettler Toledo, Switzerland) (Figure 2.8 (a)). After that each filter was cut into halves for ion and metal analysis. Half of each sample (filter paper) was extracted in 4 ml of aqua-regia (HCl and HNO_3 3:1 v/v) solution at 140°C in a double layer Teflon digestive bomb for 4 hours to dissolve all elements (Figure 2.8 (b)). The solutions extracted were cooled down to room temperature, filtered by nylon filter with 0.45 μm pore size using a sterilized syringe and the volume adjusted to 25 ml with 2% (V/V) nitric acid solution (Khamkaew et al. 2016; Yafa and Farmer, 2006).


(a) (b)

Figure 2.8 (a) Microbalance, and (b) double layer Teflon digestive bomb

2.4.2 Ash samples extraction

For extraction of ash samples, 4 acidic extraction conditions including 1) $HCl:HNO_3$ (3:1 v/v) (Kröppl & Lanzerstorfer, 2013; Yafa & Farmer, 2006), 2) $HNO_3:HF$ (3:2 v/v), 3) $HNO_3:HCl:HF$ (3:1:0.5 v/v/v) (Yafa & Farmer, 2006), and 4) $HNO_3:H_2O_2$ (2:1 v/v) (Bakisgan, et al., 2009) were tested (Table 2.2). The optimum method was specified from outcome of percentage recoveries for targeted elements, while other extraction conditions were set similar to $PM_{2.5}$ extraction. All conditions containing hydrofluoric scid (HF) were heated at 70 °C for 20 minutes to remove HF from solutions matrix prior to adjusting the volume to 25 ml with 2% (V/V) nitric acid.

Table 2.2 Specification of selected acidic conditions for optimizing ash extraction condition

No	Condition	Ratio (v/v)
1	Hydrochloric Acid : Nitric Acid ($HCl:HNO_3$)	3:1
2	Hydrofluoric Acid : Nitric Acid ($HF:HNO_3$)	2:3
3	Hydrofluoric Acid : Hydrochloric Acid : Nitric Acid ($HF:HCl:HNO_3$)	0.5:1:3
4	Nitric Acid : Hydrogen peroxide ($HNO_3:H_2O_2$)	2:1

2.5 Analysis of $PM_{2.5}$ and Ash Samples by ICP-OES

2.5.1 Method validation

Method validation is an important requirement for any package of information and researches. In an analytical measurement, the overall objective is to obtain consistent, reliable and accurate data. Validated analytical methods play a vital role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories. For validation and verification of the analytical method used for this research, accuracy, precision, linearity and stability of instrument (sustainability) were determined in the following instances.

2.5.2 Elemental analytical framework

As mentioned in the extraction method, $PM_{2.5}$ elemental analysis which was tested and certified by Khamkaew et al., (2016) applied to determine elemental composition of $PM_{2.5.}$ For extraction of ash samples four acidic conditions were tested and optimum extraction condition was selected, the overall analytical procedure for determining elemental composition illustrated in Figure 2.9.

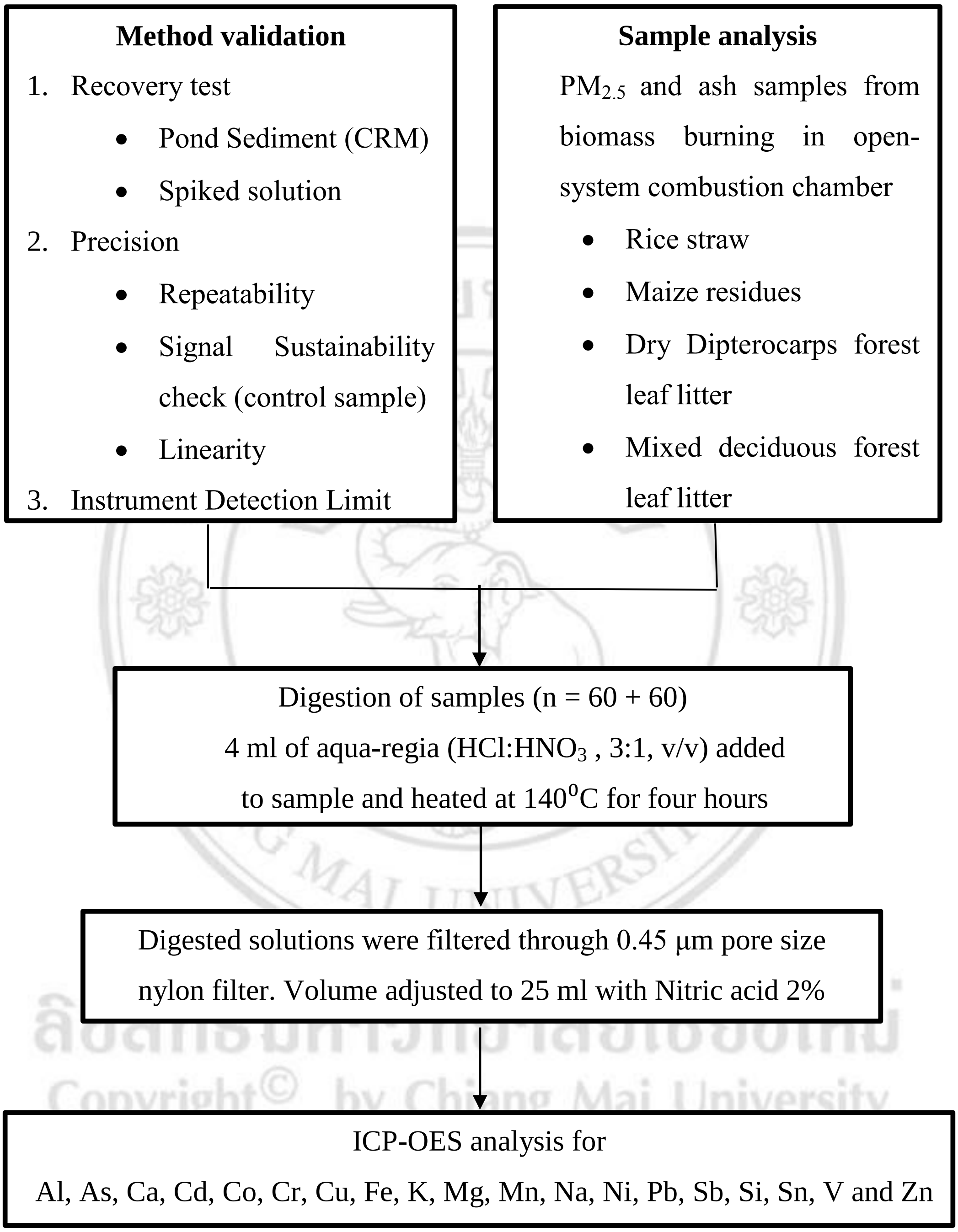


Figure 2.9 Flow chart of analytical method for elemental analysis by ICP-OES

2.5.3 Accuracy of the analysis method

Accuracy of the analysis method was tested by using certified reference material (CRM) including apple leaf (1515, NIES, USA) and pond sediments (No.2, 300.21, Japan). But percentage recovery of some elements such as potassium (K) and sodium (Na) obtained from CRM were very low, therefore a known concentration of standards solution of each element (20 elements) was spiked and extracted under the same condition which was applied for real sample extraction to optimize extraction method for ash sample extraction.

Three sets of 100 mg CRM were extracted using 4 acidic extractions as mentioned in Table 2.2, and elemental analysis was performed by ICP-OES to determine the elemental composition of extracted solutions. Percent recoveries of four methods were compared and the optimum extraction method was selected for digestion of ash samples. The Percent recoveries were calculated from given Certified Reference values (CRV) of CRM for each element applying equation 2.1.

$$\%R = \frac{C_{(calculated)}}{C_{(CRV)}} \times 100 = \frac{CV / m_{(used)}}{C_{(CRV)}} \times 100 \qquad \text{Eq. 2.1}$$

Where $m_{(\mathrm{Used})}$ is 100 mg of CRM, $C_{(\mathrm{calculated})}$ is the obtained concentration (μg/mg), $C_{(\mathrm{CRV})}$ given for each element, C is the concentration extracted and V is volume (25 ml) of extracted solution.

2.5.4 Standard preparation for standard calibration curve

Standard calibration curve is a useful tool to convert the intensity read out from ICP-OES to ppm (μg/ml) concentration of analytes. The standard calibration curve of each element (Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Si, Sn, V and Zn) was developed by plotting range (0.01-10 ppm) of concentrations (0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 2.5, 5.0 and 10.0 ppm) against their emitted light intensity as count per second (CPS).

2.5.5 Performance of the ICP-OES

a) Wavelength selection for ICP-OES

The choice of wavelength is dictated by the need for sensitivity and the need to avoid interferences from the emission lines of other constituents in the sample. Since an analytes atomic emission spectrum has an abundance of emission lines particularly when using a high temperature plasma source, it is inevitable that there will be some overlap between emission lines. ICP-OES conditions set for experiment is given bellow (Table 2.3).

Table 2.3 ICP-OES experimental condition

Operation condition	Setup Value
RF power	1.2 Kw
Plasma flow rate	14 LPM
Auxiliary flow rate	0.3 LPM
Nebulizer gas flow rate	25 PSI
Sample flow rate	1.4 ml/min
Elements	wavelength
Al	396.152
As	189.042
Ca	317.933
Cd	228.802
Co	236.379
Cr	267.716
Cu	324.754
Fe	239.563
K	766.491
Mg	285.213
Mn	257.610
Na	589.592
Ni	231.604
Pb	220.353
Sb	206.833
Se	196.090
Si	288.158
Sn	189.991
V	310.230
Zn	213.850

b) Repeatability

Repeatability of ICP-OES system was verified by running 1 and 5 ppm (µg/ml) of mixed standards solutions, for each concentration 10 repeated measurements in three replications were done under the same condition set for both extracted filter paper and ash samples. The reliability of repeatability measurements was confirmed by calculation of percent relative standard deviation (%RSD) of results from mean, which is calculated from equation.2.2.

$$\%RSD = \frac{SD}{\overline{X}} \times 100 \qquad \text{Eq. 2.2}$$

Where %RSD is percent relative standard deviation, SD is the standard deviation from mean, and $\bar{X}$ is average of the repeated measurements.

c) Detection limit of instrument

The limit of detection (LOD) of ICP-OES for each of the element was determined using standard solution concentration of 0.05 & 0.1 (µg/ml) , for each concentration 10 repeated measurements in three replication were done under the same condition that was set for sample measurement and SD was used to estimate the LOD for each element from equation 2.3.

$$LOD_B = 3SD_B \qquad \text{Eq.2.3}$$

Limit of quantification (LOQ) for each element was calculated similar to LOD from 10 time of SD for each element.

2.6 Calculation of Emission Factor (EF)

2.6.1 EF of $PM_{2.5}$ from biomass burning

Emission factor (EF) is a representative value that attempt to relate the quantity of a pollutant released to the atmosphere with an activity associated with the release of that pollutant. These factors are usually expressed in terms of the mass of pollutant emitted per unit mass of dry fuel consumed (i.e. mg kg^{-1}) (Sillapapiromsuk *et al*., 2013). EF of PM$_{2.5}$ of each biomass type was measured from the net mass (m) of PM$_{2.5}$ and dilution factor (DF). Dilution factor of each burning experiment determined using equation 2.4.

$$DF = \frac{V_{(air)}}{V_{(sampling)}} \qquad \text{Eq.2.4}$$

Volume of air (V$_{(air)}$) is directly proportional to the height (h) of smoke (equation 2.5) produced during the burning experiment and square of stack's radius, where h is calculated from average flow rate (m/s) of air (F$_{(air)}$) in the chimney and time (s) during the burning process (equation 2.6). While volume of sampling (V$_{(sampling)}$) is measured from flow rate (F) of PM$_{2.5}$ sampler (5 L/min) (Intra et al., 2012.) and sampling time (min) (equation 2.7).

$$V_{(air)} = \pi r^2 h \qquad \text{Eq.2.5}$$

$$h = F_{(air)} \times t(s) \qquad \text{Eq.2.6}$$

$$V_{(sampling)} = F_{(sampling)} \times t_{(sampling)} \qquad \text{Eq.2.7}$$

Therefore the EFs of PM$_{2.5}$ calculated through this research will represent amount of PM$_{2.5}$ in all smoke produced during the open burning simulation in open system combustion chamber which is obtained from equation 2.8.

$$EF_X = \frac{m_{(PM2.5)} \times DF_X}{m_{dry-biomass}} \qquad \text{Eq.2.8}$$

Where m$_{(PM2.5)}$ is mass of PM$_{2.5}$ and calculated from equation 2.9:

$$m_{(PM2.5)} = m_{(postsampling)} - m_{(presampling)} \qquad \text{Eq.2.9}$$

Where m$_{(pre\text{-}sampling)}$ is net weight of quartz filter paper and m$_{(post\text{-}sampling)}$ is weight of filter plus collected PM$_{2.5}$.

2.6.2 EFs of elements in $PM_{2.5}$ and ash emitted from BB

In this study EF of elements (EF_E) from each type of biomass is calculated in order to specify amount of individual elements emitted from open burning. The EF_E of elements from $PM_{2.5}$ is calculated from ratio of mass of elements in the extracted solution, DF and dry biomass.

$$EF_E = \frac{CV \times DF_X}{m_{dry-biomass}} \quad \text{Eq.2.10}$$

In equation 2.9, C is concentration (µg/ml) of element in the extracted solution and V is the volume (25 ml) of extracted solution. And the EF_E in ash samples are calculated from mass of elements in the extracted solution of ash samples, mass of extracted ash and ratio of unburned (ash remained) and dry biomass.

$$EF_E = \left(\frac{CV}{m_{extracted}}\right) \times \left(\frac{m_{total-ash}}{m_{dry-biomass}}\right) \quad \text{Eq.2.11}$$

2.7 Data Analysis

Multivariate analysis of variance (MANOVA) method is performed in this study to test the differences in the four types of biomass burned, while various elements and $PM_{2.5}$ EFs are considered at the same time. Analysis of variance (ANOVA) performed to test for the difference between of four biomass EF means. Correlations (r) between the various emitted elements and $PM_{2.5}$ from biomass burning were calculated in order to identify their relationships.