4. MATERIALS AND METHOD

4.1. Apparatus

1. Atomic Absorption Spectrometer (AAS) and hollow cathode or electrodeless discharge lamps for all analyzing elements.

AAS is the most widely used technique for the determination of trace metals. A Perkin-Elmer 2380 is available in the Department of Geological Sciences, Chiang Mai University and is used for analysis of soil elements concentration. Atomic absorption detection limits depend on the type of atomizer and the sample matrix. Generally these are in the order of micrograms per milliliter to below nanograms per milliliter. These detection limits are usually adequate for determining trace metals in a wide variety of samples such as soils, sediments, sludges and rocks.

A block diagram of an atomic absorption unit is given in Figure 4.1. Atoms absorb radiation only at discrete wavelengths characteristic of the absorbing species. Thus radiation from the source, produced from a vapor of the metal of interest, is absorbed at a discrete wavelength(s) by atoms of that element in the atomizer. As a result, the radiation beam intensity is attenuated by an amount that is atomized. The source radiation is modulated by an amount that is proportional to the concentration of the element under consideration in the atomizer. The source radiation is modulated (chopped or electronically modulated) so that selective amplification of the lamp signal is possible. This effectively eliminates amplification of flame emission. The latter, however, is a source of noise. The function of the atomizer is to produce free atoms from the introduced sample. In atomic absorption the atomizer is usually a flame or furnace type device. Radiation of a characteristic wavelength is most frequently produced by a hollow-cathode or electrodeless discharge lamp. The desired wavelength is isolated from other absorbing or nonabsorbent lines by a monochromatic.

The transducer (detector) is commonly a photomultiplier tube. Absorbance, the quantity usually measured in AAS, can be expressed as follows:

$$A = \log I_0/I = a\ b\ c$$

where I_0 is the intensity of the incident beam,

I is the intensity of the transmitted beam,

a is a constant (characteristic of the particular system),

b is the path length of the optical beam (which can be kept constant)

and *c* is the concentration of the element of interest I atomizer.

This equation predicts a linear relationship between the absorbance and the concentration of the considered element. Best precision is obtained in the middle straight line portion of the curve (Van Loon , 1985).

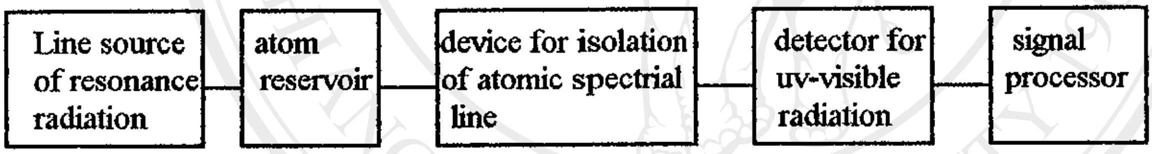

Figure 4.1. Block diagram of an atomic absorption spectrometer (AAS)

2. pH-meter Vibret model 46 A
3. Rockmill
4. Analytical balance (Sartorius 2462)
5. Hot plate
6. Oven
7. Muffle furnace (Galankamp)

8. Fume cupboard
9. Platinum crucible with lids
10. Air compressor
11. Volumetric flasks 1000 ml, 500 ml, 250 ml, 200 ml, 100 ml and 50 ml
12. Elynmayer flasks, 250 ml
13. Beakers
14. Funnels and funnel stand
15. Ashless filter papers Whatmann, no.42
16. Glass filter papers
17. Plastic bottles, 250 ml and 150 ml
18. Acetylene and nitrous oxide gas
19. Pipette fillers
20. Pipette (both calibrated and bulb)
21. Watch and clock glasses
22. Glass and Teflon coated rods
23. Sa-paper

4.2. Chemicals and materials

1. Concentrated hydrochloric acid (HCl - 37 %) Baker analysed ACS reagent
2. Concentrated nitric acid (HNO_3 -70%) BDH chemical, Spectrosol, for Atomic Absorption Spectroscopy.
3. Lithium tetraborate ($LiBO_3$), Sigma, p. a. grade
4. Lanthanum oxide (La_2O_3) BDH Spectrosol, p. a. grade
5. Caesium chloride ($CsCl_2$) BDH, p. a. grade
6. Potassium iodide (KI), p. A. Grade, May & Baker Ltd., Dagenham, England
7. Sodium borohydride ($NaHB_4$), p. a. grade, Fluka Chemia
8. Sodium hydroxide (NaOH), p. A. Grade, Riedel-de Haen

9. Standard for all analyzed elements: Fe, Mn, Al, Ca, Cu, Cr, Ni, Pb, As, Zn, Mg, Titrosols, Mecrk

10. Dieonized water

11. Distillated water

4.3. Sampling collection

4.3.1. Soil samples

Surface soil were collected from 10 points chosen randomly in around 100 m^2 in each site and mixed. Two samples with depths of 0-2 cm and 2-5 cm were collected. The collected samples were kept in separated labeled plastic bags before drying.

4.3.2. Leaves samples

Leaves of Teak (*Tectona grandis*) were collected at 1.5 m height from different sides of the tree. Teak is found in all the study sites and its leaves have hairs and a large surface area that can keep dust deposited on. Teak leaves were kept in sa-paper to dry out for further scanning under an electron microscope.

4.3.3. Dust samples

Dust samples were collected from bamboo leaves. Bamboo (*Thyrsostachys siamensis*) leaves were taken at a height of 1.5 m . Bamboo is found at all the sites and has glabroous leaves which are easy to wash and remove particles from. The leaves collected were the outermost and youngest. Leaves were kept in plastic bags before washing to retain dust deposited on the leaf surfaces.

Washing of the leaves and measurement of weight of dust samples was done by the following procedure:

500 leaves were taken from each site. Wash the leaves in a 500 ml beaker with 200 ml of distillated water. Dust was filtered through glassfilter paper. Filter papers were dried out at 105^{0}C for 24 hours. The weight of dust samples were calculated by the difference between weight of the filter paper with dust and the weight of the filter paper without dust. Dust samples can be analyzed for heavy metals using a graphite furnace AAS or by a flame AAS if amount of samples is enough for analyze (about 1-2 g). Unfortunately, the long rainy season of 1994 affects on amount of dust deposited on the surface of bamboo leaves. Rain washed away most of deposits. Also, furnace graphite is not available at Chiang Mai University, so heavy metals in dust samples couldn't be analyzed.

4. 4. Vegetation survey:

A vegetation survey was carried out to identify trees species, ground flora, forest type, and their condition and to find out if there is any visible impact from pollution from the mine and power plant on the vegetation forests. This part was completed with help from Mr. James Maxwell, taxonomist in Biology Department, Chiang Mai University.

4.5. Chemical analysis

4.5.1. Sample preparation

All the soil samples collected were dried in a oven at 105^{0}C for 24 hours. The dried samples were sieved with 2 mm diameter sieve. Then samples were again ground in the vibrating machine (Rockmill). The samples were decomposed by two methods of digestion, viz. a aqua regea or tetraborate fusion.

4.5.1.1. Aqua regea

Reagent:

HCl (37%); HNO_3 (70%)

Exactly 3 g of soil samples was put in a conical flask and mixed with 10 ml HNO_3. (70%) and 30 ml HCl (37%) for digestion, i. e. aqua regea. The mixed samples were heated for 1 hour on a hot plate under the fume cupboard. During the heating, condense the vapor of the samples and after 1 hour heating, the samples were allow to cool. The cool samples were filtered into a 50 ml volumetric flask. The filtered sample was diluted with distillated water upto the 50 ml mark.

4.5.1.2. Lithium tetraborate fusion procedure

This method describes the determination of silicon, aluminum, iron, calcium, magnesium, and manganese in silicates and carbonates, and may be applicable to other elements and matrices. The samples are decomposed by means of lithium tetrtaborate fusion. The advantages of using a lithium tetraborate fusion instead of acid attack are that it is faster, it needs no special equipment and it dissolves refractory minerals which are particularly resistant to acid mixture (Bettinelli, 1983)

Reagents

HCl 37 %

Lithium tetraborate $LiBO_3$

Lanthanum solution 10 % prepared by dissolving 10g of La_2O_3 (BDH spectrol grade) with concentrated HNO_3 followed by dissolving 20 g of $CsCl_2$ (BDH grade) with distillated water in a 200 ml volumetric flasks. The volume was made up to the 200 ml mark with distilled water after allowing the mixture cool to room temperature.

Standard solutions:

Standard solutions should be prepared by addition of the stock solutions described in the standard conditions set for each element and should contain concentrations of HCl, $LiBO_3$,and La similar to the sample solutions. A reagent blank should also prepared.

Tetraborate fusion:

Place a 0.1g sample in a platinum crucible and add about 0.6 g of $LiBO_3$. Thoroughly mix the sample and $LiBO_3$ with a small stirring rod and fuse the mixture at 1000^0C in a muffle furnace for 15 minutes. When the fusion is complete, quench the crucible in cold, distillated water. Place the crucible in a tall form 100 ml beaker with a small Teflon-coated stirring bar, and add 20 ml of water and operated magnetic stirrer then empty the contents into a beaker. Add hot water and acid again and stir until the solution is completetely dissolved. Transfer the solution quantitatively to a 200 ml volumetric flash and dilute to volume after cooling.

4.5.1.3. Particulate on filters.

Place the filter containing 10-20 mg of suspended matter in a Teflon vessel, add 2 ml concentrated HNO_3 and 0.5 ml concentrated $HClO_4$. Keep in a closed vessel at room temperature for about 1 hour. Heat in an oven at 100^0C for 1 hour or in a pressure cooker for 0.5 hour. Evaporate the sample to white fumes on a hot plate at 250^0C. After cooling, add 1 ml concentrated HF for Nuclepore or Milipore filters and 5 ml for glass fiber filters. Evaporate the sample to near dryness. Dilute the residue with 10 ml of 6% v/v 1:1 mixture of HNO_3 and $HClO_4$ and heat on a hot plate for 5 minutes. If necessary, filter the sample through a Whatman a 540 filter. Transfer the sample to a 10 ml volumetric flask and adjust the volume with the same add mixture. Analyze three blank filters together with the samples (Van Loon, 1985). This analysis was not able to performed because of technical problem and lack of experience.

4.5.2. Analysis

All the quantitative determinations were carried out by preparing calibration curves for appropriate standards for each element and aspirating in the atomic absorption spectrometer at the same time as the samples were aspirated. The calibration method is quite simple and precise if concentrations are between a linear range. This based on the fact that absorbance, the quality usually measured in AAS can be expressed as linear relationship with concentration of the considered element. Best precision is obtained in the middle straight line portion of the curve.

4.5.2.1. Standard solutions

1. Stock solution:

Stock solutions were prepared by diluting commercially available Merck Titrisols ampoule which contain 1 g of element in 1000 ml distillated (deionized) water.

2. Working solutions

Working solution 100 or 50 ppm were prepared by diluting the stock solution with distillated water

3. Standards solutions

Standard solution were prepared by diluting appropriate aliquots of working solutions in 100 ml volumetric flasks. Concentration standards were chosen according the absorbance in the linear range for optimal conditions for each element. Standard solutions should be prepared by addition of the stock solutions described in the standard conditions set for each element, and should contain concentrations of HCl, HNO_3, Lithium metaborate... as the samples. A reagent blank also should be applied. Standard solutions of Ca, Mg and Fe were added with sufficient amounts of lanthanum oxide and caesium chloride solution to provide the final concentration of lanthanum and caesium at 1 percent.

Regression Equations were calculated using Lotus program

Linear Regression equation for absorbance: $Y = a + bX$

Where Y: absorbance , X: concentration

Calibration curves were established by plotting the absorbance versus concentrations.

4.5.3. Working condition of AAS for each element

Working conditions for AAS according the manual are presented in table 4.1.

4.5.4. Calculation of concentration

Using a regression equation ($Y = a + bX$) to calculate the concentration of each element in analyte soil sample

concentration = [(absorbance-a) / b x V x 1000 x q]/ (1000 x w)

Where V: final volume

q: dilution factor;

w: weight of sample

4.5 .5. Hydride generation - a special technique for determination of arsenic

The method is based on the fact that arsenic forms volatile covalent hydride. This can be produced in a solution and volatilized into an atomizer for determination by atomic absorption spectrometry. By using hydride generation virtually all the elements in a sample solution enter the atomizer at one time and thus a large increase in sensitivity in obtained as compared to direct aspiration into a flame.

Reagents: KI 10 %, $NaHB_4$ 3% in 1% sodium hydroxide, HCL 37 %

Prepare standards solutions containing 5 µg. Al/l. Pipette out aliquots of 0, 4, 8,12, 16, and 20 ml containing 0, 20, 40, 60, 80, 100 ng As. Use deionized water to make up each solution to 10 ml. Add 1 ml of 10 % KI solution in each sample and standard to reduce As (v) into As(iii). Add 3ml of 37 % HCL or 6 ml of mixture HCL:H_2O (1:1) and let the reaction perform for 1 hour. Generate hydride of arsenic by adding 3 % the solution of sodium tetrahydroborate(iii) from a semiautomatic hydride generator. Pass analyte gases directly into a hydrogen (nitrogen- diluted) flame for measurement of the transient absorption signals. Peak areas are measured in 20 seconds. The concentration of arsenic in samples were calculated by a calibration curve.

Table 4.1 Working condition for each elements

Element	λ, nm	SBW nm	Flame	Sens. Check (a)
Al	309.3	0.7	NO-A	50.0 (b)
Ca	422.7	0.7	A-A	4.0
Cr	240.7	0.2	A-A	4.0 (c)
Cu	324.8	0.7	A-A	4.0 (e)
Fe	248.3	0.2	A-A	5.0 (c)
Mg	285.2	0.2	A-A	0.3 (c)
Mn	279.5	0.7	A-A	2.5 (c)
Ni	233.0	0.2	A-A	7.0 (c)
Pb	283.3	0.7	A-A	20.0
Zn	213.9	0.7	A-A	

a) Metal concentration (mg/l) in aqueous solution which will give a reading of 0.2 absorbance units

b) Condition of an alkali salt (K, La, Cs as chloride) recommended to control ionization

c) The use of the impact bead will improve sensitivity by about 2x

A-A: Air-Acetylene

NO-A: Nitrogen oxide- Acetylene

4.6. Soil pH measurement

Soil pH was determined after stirring a soil- water solution (1:2.5) after letting the suspension settle overnight.

4.7. Soil moisture and percentage of fine particles

Dry 100 g of soil sample in an oven at 105^0C for 24 hours until thoroughly dry. Soil moisture was calculated by formula:

Soil moisture = (weight of wet soil sample- weight of dried sample)/ weight of dry samples X 100 %

The dried samples were sieved through a 2 mm sieve and weighted. Calculation of the percentage of fine particles was then done.

4.8. Scanning electron microscope

A electron microscope used for scanning fly ash in deposits on teak leaf surfaces. The morphology of the particles was compared with that of fly ash to find out the dispersion pattern of fly ash in the area. By using electron microscope with x-ray florescence can analyze composition of deposits on surface leaves. Unfortunately, the instrument in CMU was not available during study time. I hope I have chance to do it latter.