Chapter 4

MATERIALS AND METHOD

4.1 SOIL SAMPLING

4.1.1 Equipment

- Soil sampler: 30 mm diameter stainless soil sampler (or soil auger) (Figure 4.1).

- Big hammer to knock the soil auger into the soil;

- Plastic bags;

- Marker, tape, labels;

Figure 4.1 The view of soil sampler.

4.1.2 Sampling method and design

At each sampling site, the soil samples were collected from 3 pits about 1 m from each other. Care was taken to avoid shaking and vibrating the soil sampler when hammering it into the soil in order to ensure the accuracy of sampling depth. Three samples with depths of 0 - 10 cm; 20 - 30 cm; 50 - 60 cm were collected from each pit. Portions from a given depth in the three pits were combined into a single sample. Samples were mixed, labeled and kept in plastic bags and transferred to the laboratory. The distribution of soil sampling sites is shown in Figure 4.2.

4.2 PLANT SAMPLING

Five plant species were chosen for investigation of Pb and Zn level in plant tissues. These species were chosen with regard to results of thesis work of Le Quang Bao, an ERA student who was working at the same time in the same study area as the author of this thesis. They are:

- *Apluda mutica* L. (Gramineae)
- *Anisocampium cumingianum* Presl (Athyriaceae)
- *Ligodium flexuosum* (L.) Sw. (Schidaceae)
- *Microstegium vagans* (Nees ex Steud.) A. Camus (Gramineae)
- *Selaginella repanda* (Desv.) Spr. (Selaginellaceae)

Plant samples were collected in area chosen through having been identified as most severely affected by heavy metals in soil.

About 300 g of whole plant of each species were collected at each sampling point. Care was taken to avoid damaging plant samples during collection and transportation to laboratory.
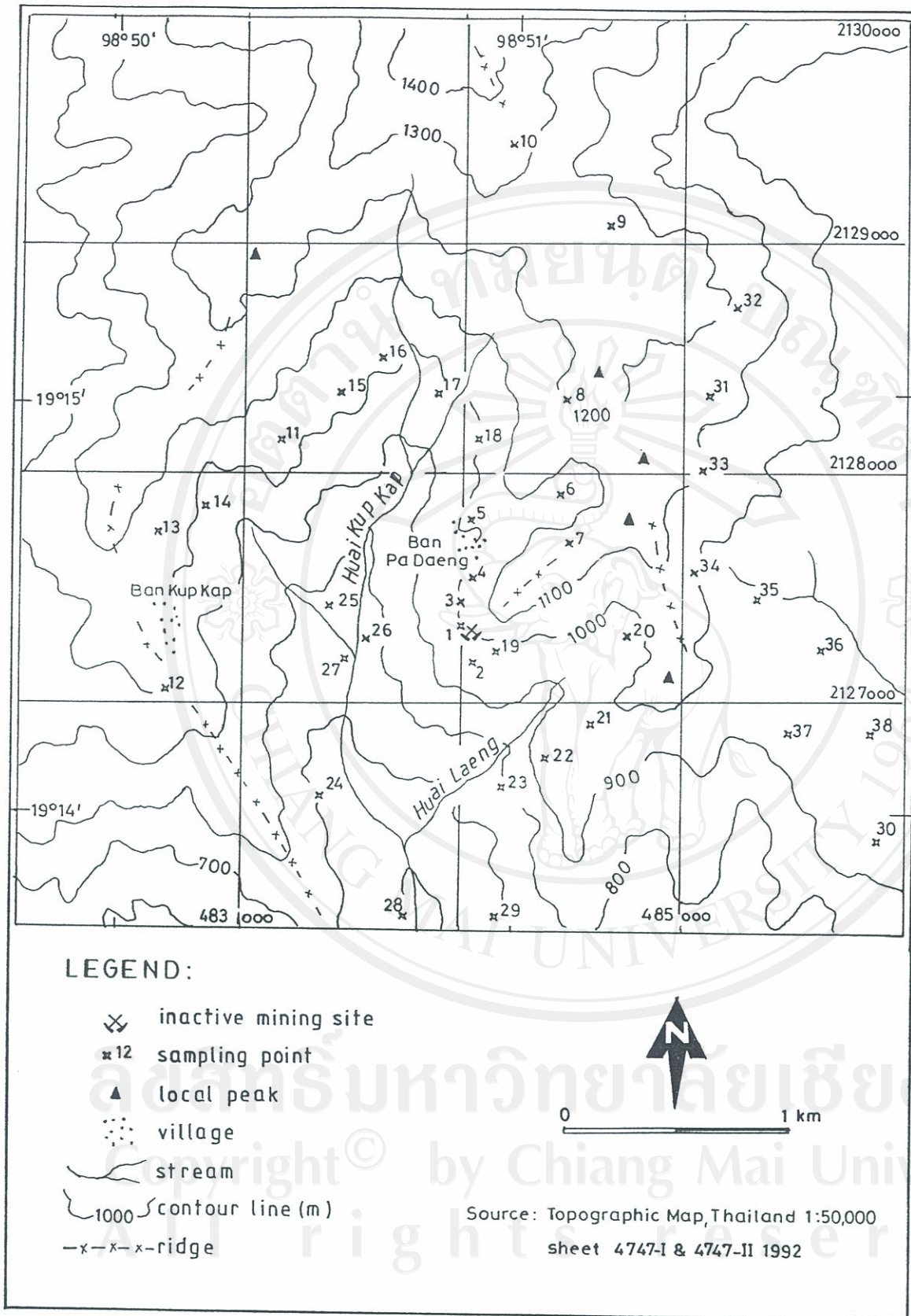
Figure 4.2. Distribution of sampling sites for soil in study area

4. 3 DETERMINATION OF Pb AND Zn IN SOIL AND PLANTS BY AAS

4.3.1 Apparatus, equipment and glassware

- Atomic absorption spectrophotometer : Perkin Elmer 2380, USA; (Figure 4.3)

- Rocklabs ring mill, New Zealand;

- Analytical balance: Mettler AE 200, Switzerland;

- Blender: Moulinex, France;

- Hot plate: Barnstead Thermolyne HP 47130-26, England;

- Oven: Stuart Scientific 252, United Kingdom;

- Air compressor: Hitachi 04OP- 7S, Tokico, Japan;

- Volumetric flasks 50, 100, 1 000 ml;

- Sieve 2 mm.

4.3.2 Chemicals

- Concentrated hydrochloric acid (HCl), GR., 37 %, Riedel de Haen, Seelze, Germany;

- Concentrated nitric acid (HNO_3), GR., 70 %, BDH Chemicals, Poole, England;

- Lead nitrate ($Pb(NO_3)_2$): 99.9% purity; Hopkin and Williams, Chadwell Heath, England;.

- Zinc acetate $Zn(CH_3CO_2)_2 . 2H_2O$, GR., 99% purity, Merck, Darmstadt, Germany;.

- Vanadium pentoxide (V_2O_5), 99%, Fluka chemie AG, Buchs, Switzerland;

- Distilled water.

4.3.3 Soil sample preparation and digestion

Sample preparation

All the collected soil samples were dried in an oven at 105° C for 24 hours. The dried samples were sieved through a 2 mm aperture sieve to remove the plant residues, rocks and other oversize particles. Samples were ground in the vibrating machine rockmill for 2 minutes.

Sample digestion (Aqua regia method)

3 g of prepared soil sample ($\pm$ 0.1 mg) were accurately weighed into an Erlenmeyer flask. 10 ml concentrated HNO_3 and 30 ml of conc. HCl were added. The flask was covered by a small beaker. The sample was left to digest overnight. The sample was boiled on a hot plate for at least one hour and, in any case, until brown fumes ceased. The sample was allowed to cool. The sample solution was filtered off into a 50 ml volumetric flask, which was filled to the mark with distilled water. The obtained solution was ready for measuring by AAS analysis.

4.3.4 Plant sample preparation and digestion

Sample preparation

Leaves, stems and root parts of plant samples were separated from another on arrival at the laboratory. The samples were placed in paper bags and put into an oven for drying at 90° C until crisp.

The dried samples were broken up by hand, ground in a blender and then subjected to acid digestion.

Plant sample digestion

1 g ($\pm$ 0.1 mg) of plant sample was accurately weighed. Put it into a Teflon dish. 13 mg of vanadium pentoxide and 20 ml of concentrated nitric acid were added. The sample was left to digest overnight. The sample was heated on a hot plate at a temperature in the range of 75 - 85 $^{\circ}$C until the mixture had evaporated to incipient dryness . The sample was allowed to cool. About 5 ml of distilled water was added to the dish. The solution in dish was quantitatively transferred into a 10 ml volumetric flask with distilled water. At this stage the samples now are ready for measuring by AAS.

4.3.5 Preparation of calibration curves

All the quantitative determinations were carried out by using a calibration curve derived from appropriate standards for Pb and Zn. The standard solutions used for making the calibration curve were aspirated at the same time as the sample solutions. The calibration method is implicitly simple and can provide precise results provided that the concentration of the analytes is within a linear range.

Stock solutions which contained 1 g of Pb or Zn element in 1 l of distilled water were prepared. All working solutions were prepared by proper dilution of the stock solution with distilled water.

Calibration curves were established as a linear regression of plotting points of the absorbance versus concentration.

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

where: - Y : absorbance;

        - X: analyte concentration;

The AF calibration program was written in Assembly programming language. It automatically produces the calibration curve (based on third order regressions between the optimized absorbance and concentration) after input of calibration and enables the users to determine analyte concentration from input absorbance. Figure 4.4 shows the calibration curves for Pb and Zn produced by AF program.

4.3.6 Working conditions of AAS for Pb and Zn

Pb and Zn were analyzed by Perkin Elmer spectrophotometer model 2380, USA under conditions described in Table 4.1.

Table 4.1 Working conditions for AAS analysis of Pb and Zn

Element	Wavelength (nm)	Flame	Concentration for Sensitivity check
Pb	283.3	Air - acetylene	20 ppm
Zn	324.8	Air - acetylene	1 ppm

4.3.7 Calculation of Pb and Zn concentration in samples

Using a regression equation $Y = a + bX$, the concentrations of analytes were calculated by the help of AF program. The final concentration C (ppm) of Pb and Zn in soil is calculated as follows:

$$C = (C_{analyte} \times k_d)/ m$$

Where:- $C_{analyte}$ is concentration (ppm) of analyte computed from equation $Y = a + bX$

- k_d is dilution coefficient.

- m is dry weight of sample (g).

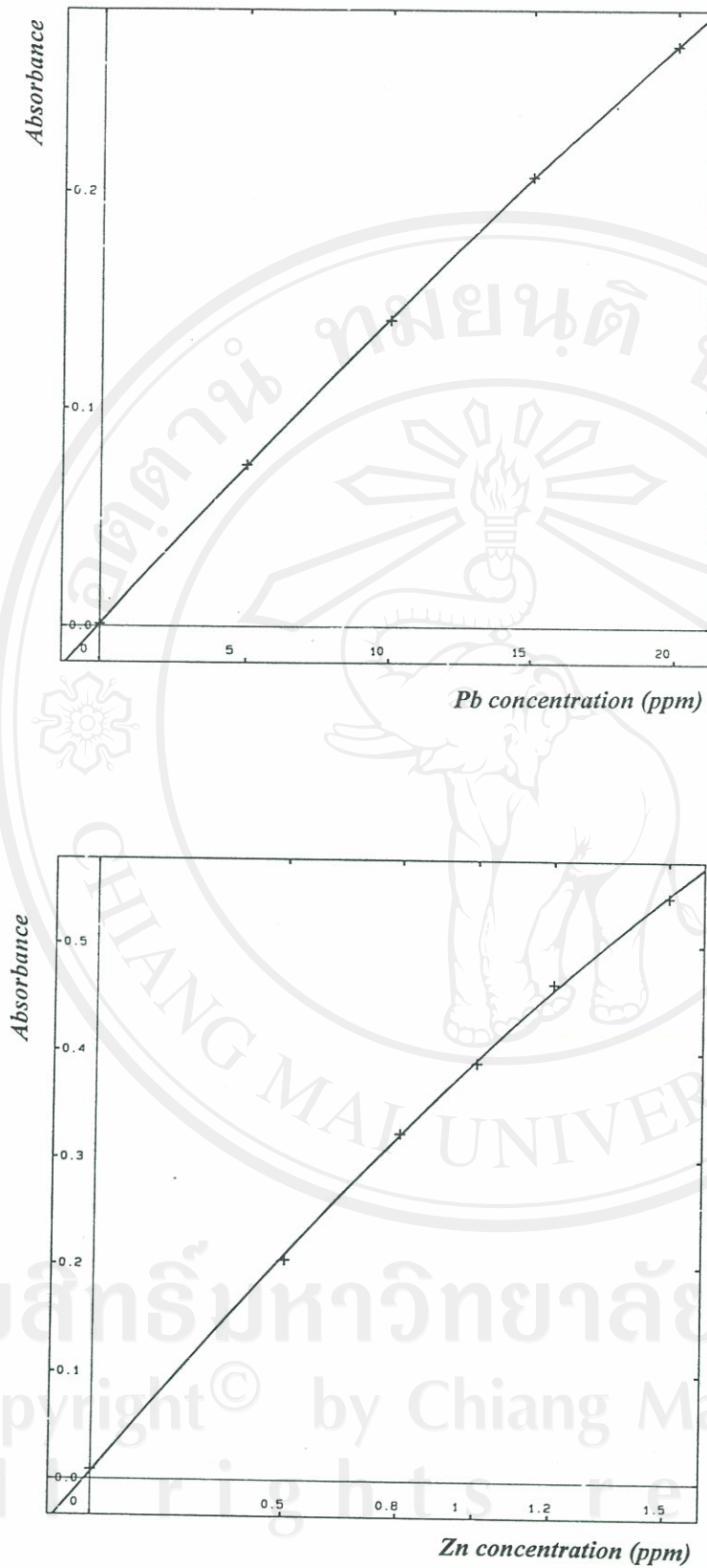Figure 4.3. The flame AAS Perkin Elmer 2380 used in the analysis.

Figure 4.4. Calibration curves for determination of Pb and Zn

4.4 QUALITY CONTROL FOR ANALYTICAL METHOD

4.4.1 Precision test

The test was carried out with the aim to investigate the degree to which the analytical method is free of random errors. About 10 % of samples were analyzed twice with the same procedure. The obtained data were used for calculation of standard deviation and coefficient of variation of the analytical method.

4.4.2. Accuracy test

The test was carried out to confirm the accuracy with the aim of investigating the degree to which the analytical method is free of systematic error or bias. Two reference soil samples provided by International Atomic Energy Agency (IAEA), Austria with certified concentration values of trace elements were used for this test. These two reference materials are soil No 5 and soil No 7 distributed by IAEA. Other reference material rye grass which is supplied by IAEA was analyzed to confirm the accuracy of the analytical method applied for plant tissue analysis.

4.5 SOIL PH MEASUREMENT:

4.5.1 Equipment

- Laboratory pH meter: Vibret, United Kingdom;

- Analytical balance: Metter AE 200, Switzerland;

- Bottles with screw caps.

4.5.2 Method

In the laboratory soil pH can be determined by stirring a soil : water mixture (1:2.5) and allowing the suspension settle overnight.

4.6 SOIL ORGANIC MATTER (OM) MEASUREMENT

Methods used to determine the organic matter content of soils include either the percentage loss in weight after pyrolysis in a furnace at $600\ ^{\circ}C$ for 2 h or the oxidation of C by acid dichromate followed by the titration of excess dichromate. During this thesis work the first method was used for determination of organic matter in soil samples.

4.6.1 Equipment

- Analytical balance: Mettler AE 200, Switzerland;

- Furnace: Gallenkamp FR 740, England;

- Vitreous silica crucibles ;

4.6.2 Method

10 g ($\pm$ 0.1 mg) of air dried soil sample was weighed into a crucible. The sample was roasted in the furnace at $600\ ^{\circ}C$ for a period of 2 hours. The sample was allowed to cool in a desiccator. The loss in weight of the sample on roasting was determined. The content of organic matter (%) was calculated as follows:

OM (%) = [(Initial weight - Final weight) x 100]/ initial weight

4.7 APPLICATION METHOD OF GIS (IDRISI SYSTEM) FOR MAPPING AND ASSESSING HEAVY METAL CONTAMINATION IN SOIL.

- The topography map of the 9 km^2 of the study area was scanned.

- The main landmarks such as school, village, streams etc were digitized. The sampling sites were digitized in the map. Thus a vector file was created from the digitized map.

- An image file was created from the vector file of sampling sites.

- A separate value file for the map of soil contamination by Pb and by Zn was created. Before the data were input to the value file, they were classified into different categories which indicated the risk of Pb and Zn level in soil. In this work five categories were used, they are : 1 = study area background level; 2 = slightly high; 3 = medium high; 4 = very high; 5 = extremely high. The numbers given from 1 to 5 also represent the risk value for each element at each site. Site with risk value 1 shows no risk of Pb and Zn contamination, whereas site with risk value 5 shows the highest risk.

- The map of Pb and Zn contamination in soil combined by the image file and value file. Two maps were obtained. They are a lead distribution map and a zinc distribution map for the study area

- The final map was obtained after combining these two maps by achieving the highest risk value for each site among Pb and Zn risk value. Color coding was used on the above defined five categories to relay information of the condition of the area. The final map enables recognition of the risk of Pb and Zn contamination in soil of the study area.

4.8 Pb AND Zn MOVEMENT FROM THE SOURCE OF CONTAMINATION. SOIL-PLANT RELATIONSHIP OF HEAVY METALS

4.8.1 Design of study

- Three transects were set up on the west facing slope below the mine. In each transect 8 squares (2m x 2m) were established. Figure 4.5 shows the design of transects and squares.

- Soil samples and plant samples were collected in each square and were analyzed for Pb and Zn content.

- The third transect was located along the stream at the foot of slope, which can be the end point of heavy metal contamination in soil by mine wastes of the mine sorting floor.

4.8.2. Purpose of study

Statistical analysis was carried out for investigation of the dispersal trend of Pb and Zn from the pollution source. Mainly it aims to:

- Investigate the differences between heavy metal level in each transect.

- Compare whether the dispersal of Pb is different from that of Zn. Taking the concentration in the first transect as the source, determine how the concentration of Pb and Zn in second and third transect has been changed from the first transect.

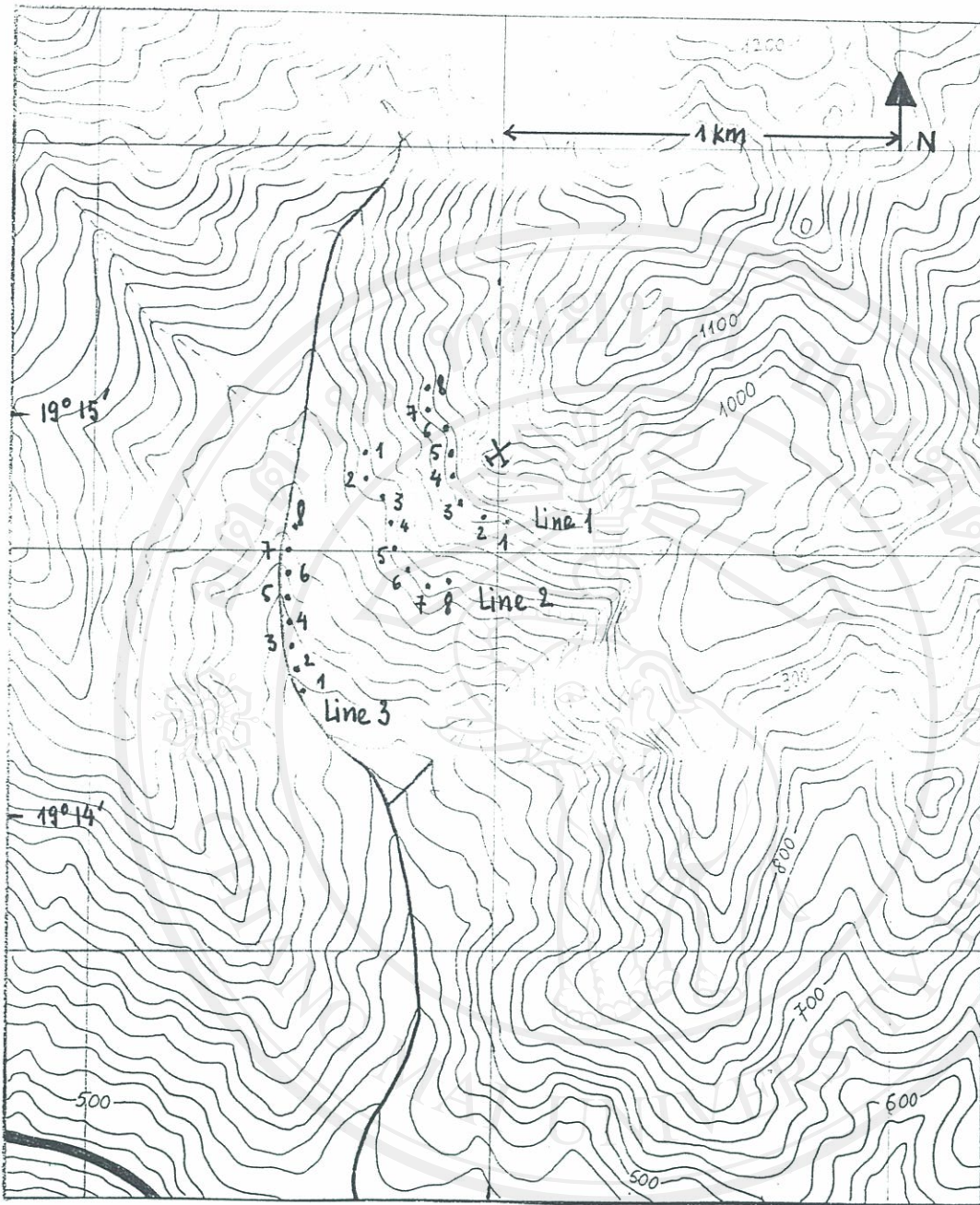- Investigate the relationship between Pb and Zn levels in soil and in plant tissues at the same place.

Figure 4.5. Design of transects and squares under mine sorting floor