

CHAPTER III

METHODOLOGY

3.1 Research Plan and Organization of the Thesis

This research aimed to determine the distributions of cadmium and zinc and their relationship in soil along Mae Ku floodplain. Thus, total concentration and bioavailability fraction concentration of the two elements in soil samples were analyzed. In this research, there were 4 steps of study as follow (see also Figure 3.1):

- (1) Sample collection: soil samples in Mae Ku floodplain area were collected by using grid system.
- (2) Sample preparation: soil samples were prepared for analyzing.
- (3) Laboratory analysis: soil samples were extracted for determination of cadmium and zinc for both total concentration and bioavailability fraction concentrations. The total Cd and Zn concentrations were analyzed following the EPA standard method 3051. The bioavailability Cd and Zn for this study define as the first fraction of BCR method (BCR1), thus the analysis was carried following the method of the Standards, Measurements, and Testing (SM&T-Formerly BCR). The details of method analysis are in the Topic 3.5 Laboratory Analysis. Then the solution from digestion and extraction for total and BCR1 were examined for Cd and Zn by ICP-OES and GFAA
- (4) Data interpretation: Histogram and linear regression statistics were used in this step for analyzing the distribution of Cd and Zn and the correlation among the two elements and its total and BCR1 fractions. In addition, the study results were compared with previous study of NRC-EHWM, 2005.

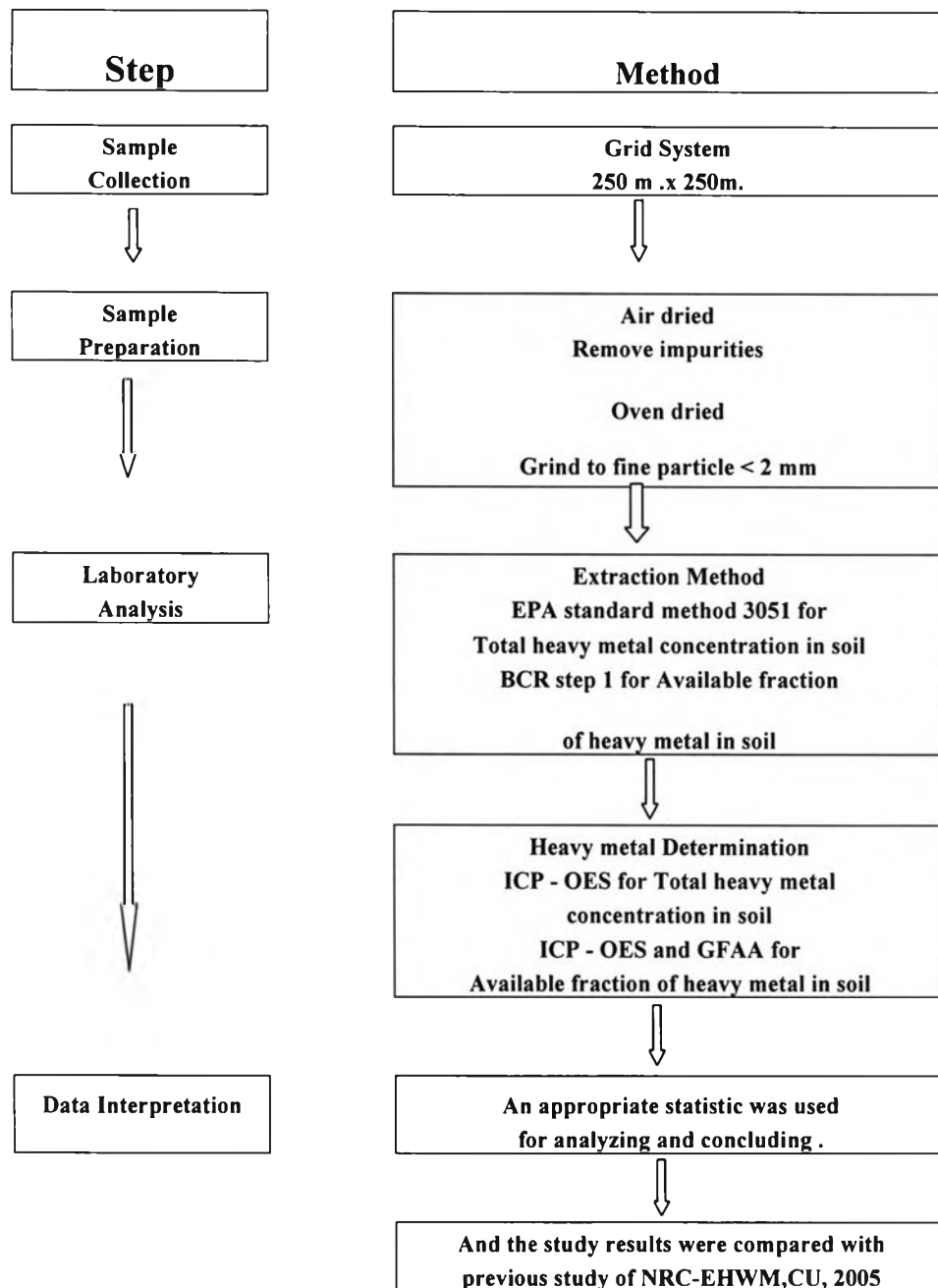


Figure 3.1 Flow Chart of the Study.

3.2 Location and Land Use of the Study Area

The study area is the floodplain area located in the Mae Ku Watershed, Mae Sot district, Tak province in northern Thailand. The study area is about 2.5 Km. x 2.25 Km. (Figure 3.2) which covers three villages that are a part of the Mae Ku sub-district, namely Ban Mae Ku Noi, Ban Mae Ku Nua, and Ban Mae Ku Tai. This area has directly received the run-off from the highlands where some parts of the zinc mine premise is located. Huai Mae Ku is the creek in this area which is used for agriculture purposes. Almost of this area was used for rice cultivation. Now, this area and the others area have been prohibit for rice cultivation because of the problem of cadmium contaminated in those areas. Figure 3.3 and Figure 3.4 show the existing land use.

3.3 Sampling Plan

In this study, the topographic map scale 1:50,000 (series L7017, map sheet 4742 III, Mae Sot district) is the tool which was used in order to design the boundary area and specify the positions of the sampling. A grid system was applied to prepare for the soil collection (250 m. x 250 m. / sample.). There were 10 gridlines which started from line A to line J. And each line had 11 points of soil samples (A1-A11 to J1-J11). The position of each point was indicated in the document for the sample collection.

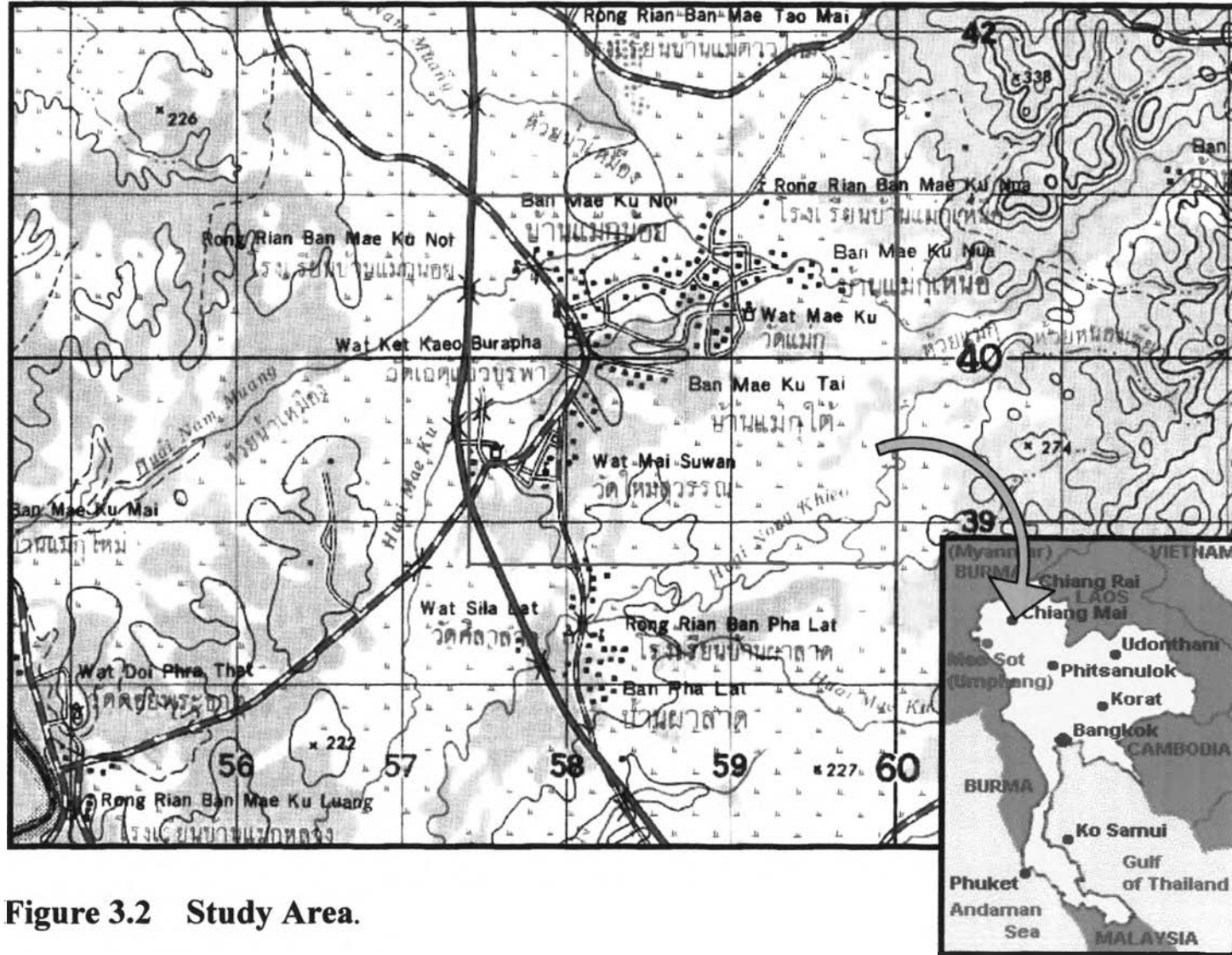


Figure 3.2 Study Area.



Figure 3.3 Existing Land Use (a).



Figure 3.4 Existing Land Use (b).

3.4 Soil Collection

Soil samples collection was carried out on June, 2005. The soil samples were collected at the location indicated in the sampling plan document (250 m. x 250 m. per sample). The GPS was the instrument used in order to express the position of sampling (see Figure 3.5 for soil collection).

If the location in the map was not suitable for sampling, the nearest point was collected instead. However, there were some positions which could not be sampled such as roads, temples, and animal bents. Therefore, the total amount of soil samples was 9. The sampling stations are shown in Figure 3.6.

Soil samples were collected by hoe from the ground level to a depth of around 30 cm. About 1-2 kg samples was collected at each site plastic bags and then sealed in order to reduce oxidizing reactions of the heavy metals in the soil with atmospheric oxygen. Each sample was labeled in order to identify in station of sampling.



Figure 3.5 Sample Collection.

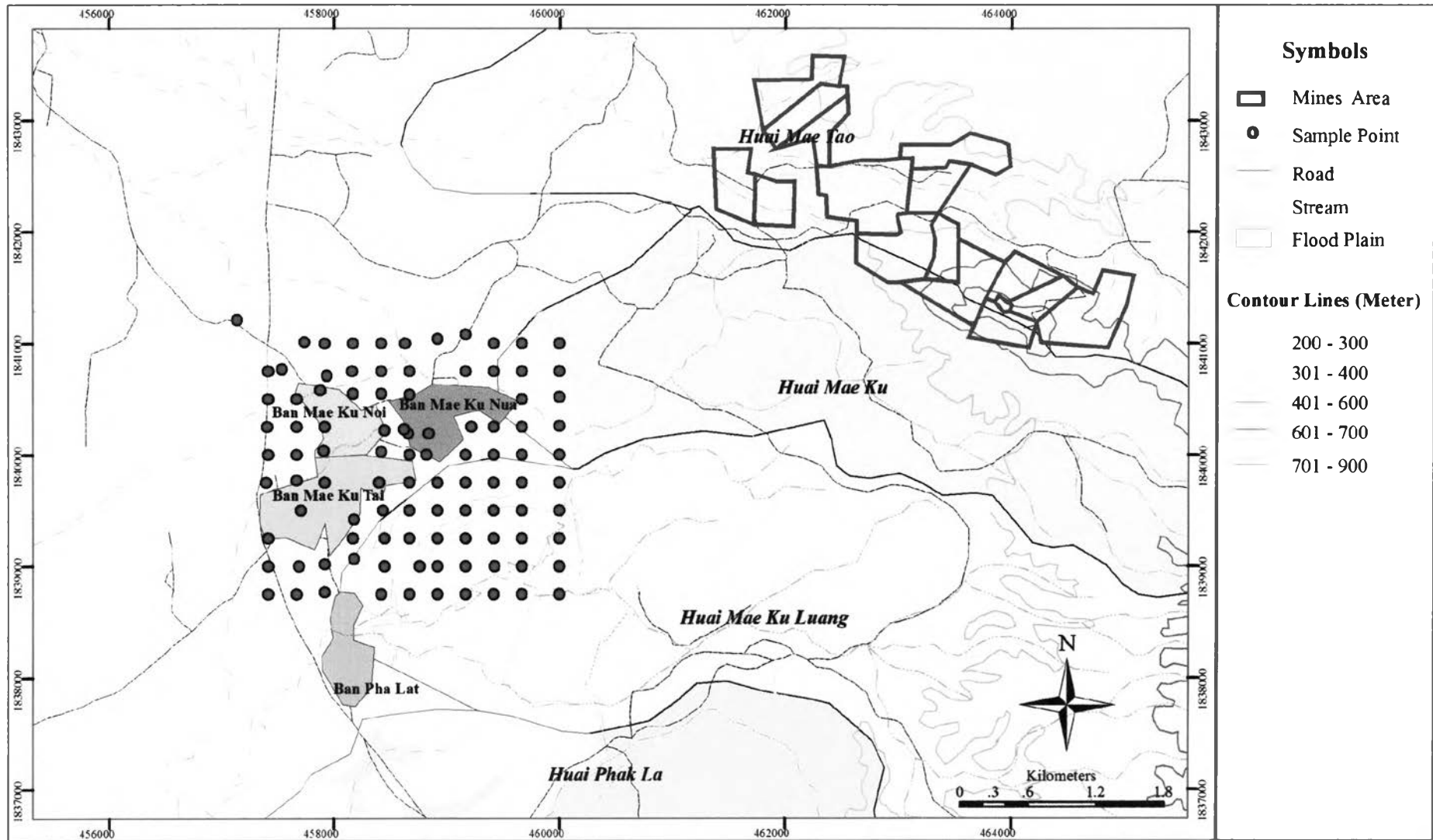


Figure 3.6 Sampling Stations

3.5 Laboratory Analysis

3.5.1 Material and Instruments

- Glassware: all glassware was soaked in 10 % of nitric acid and rinsed with de-ionized water before use.

- Chemical substances: analytical grades of chemicals were used in this step.

- Certified reference material: natural matrix certified reference material, catalog No. CRM 025-050, Lot No: JG 025 were used for validating the method in this paper

- Microwave digestion and extraction system model ETHOS SEL, Milestone was used for digestion of the total concentration of cadmium and zinc in soil samples.

- Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) model Vista- MPX, Varian was used to analyze the concentration of cadmium and zinc in all samples. The detection limit of this instrument is 0.005 mg/L.

3.5.2 Laboratory Procedures

Sample preparation

Soil samples were dried in an opened air and impurities were removed from samples. Then the samples were then dried at 105 °C for 24 hr in an oven. After that dried soil samples were crushed into fine particle sizes, and then were sieved for selection of the mesh size of less than 125 micron. These fine soil samples were kept in plastic containment before extraction step.

Heavy metal analysis

- Total concentration of cadmium and zinc

The procedure of total digestion was conducted following the EPA standard method 3051 (Microwave assisted acid digestion of sediments, sludge, soils, and oils) as the following details:

Add 0.5g of soil sample from selected mesh size in a digestion vessel, and then 5 ml of 65% HNO₃ was added and the vessel was closed with a Teflon cover. After that, the sample was heated at 170 ± 5°C for approximately 5.5 minutes and remained at 170 - 180°C for another 10 minutes to accelerate the leaching process by microwave digestion system. After cooling, the solution was filtered through a Whatman GC filter. The filtered solution was diluted with de-ionized water to be 50 ml volumetric flask. These solutions were kept in plastic containments for further analysis. The presences of these metals were determined by Inductively Coupled Plasma Optical Emission Spectrometer (ICP - OES).

- Available forms of cadmium and zinc

The SM&T method was proposed by the Standards, Measurements and Test program (SM&T-formerly BCR) of the European Union .This method is the sequential extraction method which was used in this study. This method composes of 3 steps which can extract each form of heavy metal in soil as the following: Step 1, exchangeable fraction: acetic acid, 0.11mol/L is the extractant which use in order to extract the water soluble and weak acid form. This is the form that has the high potential of plant uptake, Step 2, easily reduced fraction: hydroxylamine hydrochloride, 0.5 mol/L (freshly) is the extractant in this step, and Step 3, oxidizable fraction: hydrogen peroxide, 8.8mol/L is the oxidize substance and ammonium acetate; 1.0 mol/L is the extractant.

According to the objective of the study, the BCR step 1 was the procedure which was conducted in this step. The procedure of BCR step 1 can be described as the following:

- (1) Reagents used in the BCR Step 1: acetic acid, 0.11mol/L
- (2) BCR step 1 procedure: after 40mL of acetic acid (0.11mol/L) was added to 1g soil in a 50 mL centrifuge screw cap tube, the sample was extracted by shaking for 16 hrs (end –over-end shaker) at speed of 200 rpm and a room temperature. After that, separate the extract from the residue using a Whatman GC filter. The extractant was kept into polyethylene container for further analysis.

The presence of these metals were be determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

3.5.3 Quality Assurance/Quality Control

For each batch of samples, prepare blank samples by using acidified de-ionized water (no added sample) and follow the same condition as common samples. This blank is useful in determining if samples are being contaminated. For the quality control of soil sample analysis, all samples were done in duplicates both of total concentration and bioavailability fraction of cadmium and zinc. In addition, all soil samples analysis both total concentration and BCR1 were carried out for duplicate analysis.

3.5.4 Data Analysis

The result in $\mu\text{g/g}$ (ppm) was calculated as follows:

$$\text{A metal in soil (Cd or Zn)} = (A \times B / C) \times 1000$$

where

A = Result from analysis in $\mu\text{g/L}$

B = Volume of the digested samples in liter (L)

C = Weight of the digested sample in gram (g)

3.6 Data Interpretation

The correlation between cadmium and zinc concentrations was analyzed by linear regression. The results were compared to the previous studies (NRC-EHWM, 2005) of the relationship between cadmium and zinc concentrations in soil in Mae Toa floodplain.