Chapter 4

MATERIAL AND METHODS

4.1. Materials

4.1.1 Apparatus

1. Atomic Absorption Spectrophotometer (AAS) : Perkin Elmer 2380, USA with Hollow Cathode Lamps (HCL) or Electrodeless Discharge Lamps (EDL) for analyzing of heavy metals (As, Cr, Ni, Co).
2. Rocklabs ring mill, New Zealand for grounding of the oven-dried soil samples.
3. Analytical balance : Mettler AE 200, Switzerland
4. Hot plate : Barustead Thermolyne HP 47130-26, England
5. Oven
6. Muffle furnace : Galankamp
7. Fume cupboard
8. Silica crucibles
9. Air compressor : Hitachi 04OP-7S, Japan
10. Volumetric flasks (1000 ml, 500 ml, 250 ml, 200 ml, 100 ml and 50 ml)
11. Beakers
12. Ashless filter papers (Whatman 589, diameter 110 mm)
13. Pipette (calibrated pipette and bulb pipette)
14. Tullgren funnel
15. Stereo Microscope (Olympus)
16. Soil thermometer
17. Hygrometer
18. Quadrate (20 x 20 cm^2)

19. Plastic bags

20. Spade

4.1.2. Chemicals

1. Concentrated hydrochloric acid (HCl-37%) Baker analysed reagent
2. Concentrated nitric acid (HNO_3-70%) BDH Chemical, Spectrosol for AAS
3. Potassium iodide (KI), p.A. Grade, May & Baker Ltd., Dagenham, England
4. Sodium borohydride ($NaBH_4$), p.A. Grade, Fluka Chemia
5. Sodium hydroxide (NaOH), p.A. grade, Riedel-de Haen
6. Standard solutions for measured elements (As, Cr, Ni, Co)
7. Ethyl alcohol (70%)
8. EDTA
9. Distilled water

4.2. Methods

The study was carried out during the rainy season, 1998. The soil physicochemical parameters and biological parameters were sampled and measured monthly from July to October.

4.2.1. Soil physicochemical parameters

Soil physicochemical parameters, *viz.* temperature, pH, organic matter, soil moisture content, soil field capacity, and heavy metal concentrations (*e.g.* As, Co, Cr, Ni) were measured in the field and analyzed in the laboratory of the Biology Department and laboratory of the Geological Sciences Department, Chiang Mai

University. The physicochemical data were needed to evaluate the current status of heavy metal contamination at every study site. For the laboratory measurements, soil samples were collected from five points chosen randomly in a 100 m^2 area in each study site and mixed. Four soil samples were collected monthly from each study site, placed in plastic bags and taken to laboratories for measurements of various soil physiochemical parameters.

Soil temperature

Soil temperature was measured in situ by pegging a soil thermometer into the soil. The reading was taken after 10 minutes.

Soil pH

Twenty grams of air-dried soil sample were put in small beakers and 40 ml of distilled water was added. The solution was stirred continuously and allowed to settle for 1 hour. The pH was then measured by a pH-meter.

Soil organic matter (SOM)

Soil samples were dried in an oven at 110 °C for 24 hours. About 3 g of oven-dried soil was weighed accurately on an analytical balance in a silica crucible and heated in a muffle furnace at 550 °C for 2 hours. The sample was then allowed to cool in a desiccator and reweighed. The soil organic matter was calculated with the formula :

$$\text{Soil organic matter (\%)} = \frac{\text{(initial weight - final weight) of sample}}{\text{initial weight of oven-dried sample}} \times 100\%$$

Soil moisture content (SMC)

Sixty grams of fresh soil were put in a previously weighed paper bag, weighed and dried in oven at 110 °C for 24 hours. After cooling, the sample was reweighed and soil moisture content was calculated as follows :

Soil moisture content (% H_2O) = [(Wf-Wd)/(Wd-Wb) x 100%

Where Wf : the weight of fresh soil and paper bag

Wd : the weight of dried soil and paper bag

Wb : the weight of paper bag

Soil field capacity (SFC)

Soil sample was dried in an oven at 110 °C for 24 hours. Twenty five grams of the oven-dried soil were put in a funnel above a flask lined with a filter paper and the water was poured into the soil up to the brim of the filter paper. The water was allowed to drop into the flask until no drops were observed. The soaked soil was then weighed and soil field capacity was calculated with the formula :

Soil field capacity (g) = weight of soaked soil/25 g of oven-dried soil

4.2.2. Heavy metal analysis

Heavy metals *viz.* Arsenic (As), Cobalt (Co), Chromium (Cr) and Nickel (Ni) in soils were measured by using Atomic Absorption Spectrophotometer (AAS) in the laboratory of Geological Sciences Department, Faculty of Science, Chiang Mai University.

Sample preparation

Soil samples were dried in an oven at 105 °C for 24 hours. The dried soil samples were then crushed by using a mortar and sifted through a two millimeter aperture sieve to remove plant debris, rocks and other oversize materials. About 80 g of each sample was ground in the electric Rocklabs ring mill for 5 minutes.

Sample digestion (Aqua regia method)

Approximately 3 g of ground soil sample was accurately weighed using an analytical balance and transferred to an Erlenmeyer flask where 10 ml of concentrated HNO_3 and 30 ml of concentrated HCl were added. The mouth of the Erlenmeyer flask was covered with a small beaker and the sample was left to digest overnight. The sample was then heated up to 75-85 °C on a hotplate inside a fume board for 1 hour, until no brownish fume was observed. The heated solution was allowed to cool and filtered through Whatman ashless filter paper and made up to a volume with distilled water in 50 ml volumetric flask. The obtained solution was ready for heavy metal measurement by AAS.

Determination of As, Co, Cr, and Ni

The quantitative determination of these heavy metals was carried out by employing calibration curves derived from appropriate standards for As, Co, Cr, and Ni. The calibration curves were established by aspirating the standard solutions which were prepared by diluting an appropriate stock solution to the working solution in a 1000 ml volumetric flask. The measurements of the standard solutions were made at the same time as the sample solutions on a Perkin Elmer 2380 flame AAS-machine.

As measurement

Concentration of As was measured by applying hydride generation AAS system. A volume of 0.1 ml aliquot of soil sample was diluted to 10 ml with distilled water and 6 ml (1+1) HCl and 1 ml of 10% KI solutions were added. The solution mixture was kept for one hour at room temperature prior to the measurement using a hydride generator model MHS-10 at 193.7 nm wavelength with 3% $NaBH_4$ in 1% NaOH solution as a reducing agent. The analytical output was recorded as peak area.

Co, Cr and Ni measurement

Concentrations of Co, Cr and Ni were determined by direct measurement using AAS.

Calculation of analyte concentration

The concentration of heavy metals was calculated by employing AF-EXE and ABSRPIFT.DAT computer program which can produce the calibration curve automatically based on third order regression between the optimized absorbance and concentration. The result was then mathematically converted into mg/kg dry weight soil.

Quality control

The quality control test was carried out in order to confirm the accuracy of the measurements and the reliability of the methodology applied. By doing this, the degree of systematic errors or bias of the methods can be investigated. A reference soil sample provided by International Atomic Energy Agency (IAEA), Austria with

certified concentration values of trace elements was used. The measurements of the heavy metal concentration in the reference soil were done in the same time as the samples.

4.2.3. Biological parameters

Soil-inhabiting arthropod collection

Soil-inhabiting arthropods were collected monthly from the 12 study sites in area surrounding of the Mae Moh Power Plant. For each study site, two soil samples were collected from a 20 x 20 cm^2 quadrate at 10 cm depth. The collected soil samples were placed separately in plastic bags and brought to the laboratory for extraction of the soil-arthropods using a Tullgren funnels. The extractions were carried out for 48 hours before removing the extracted soil arthropod specimens.

Identification of the specimens collected

The specimens collected were sorted and identified up to family. However, the number of species and number of individuals of each species were counted and recorded to determine ecological properties. The identification of the specimens was based on morphological characteristics using available keys e.g. Borror et al., (1989a), Borror et al., (1989b), Chinery (1976), and Jaques (1947). The specimens collected were kept by the author.

4.3. Data Analysis

4.3.1. Physicochemical parameters

The data of several soil physiochemical parameters including heavy metal concentrations were statistically analyzed using Factorial to compare mean values between study sites regardless of the observation period. Statistical analysis of completely randomized design (CRD) was employed to compare mean values between observation period in every study site. A cluster analysis was also used to group the study sites based on heavy metals concentrations.

4.3.2. Biological parameters

The data for soil arthropods collected were analyzed using a SPDIVERS.BAS of Ecostat Basic Program to determine richness indices, diversity indices, and evenness indices. A Basic Program of SUDIST. BAS was employed to calculate distance coefficients. The similarity between sites were calculated using Sorensen's index. The formula of ecological indices and coefficients were cited from Ludwig and Reynolds (1988).

Species richness indices

An unambiguous and straightforward index of species richness is the total number of species in a community (S or N0). However, since this number depends on the sample size and time spent searching, its use as comparative index is limited. Therefore, two species richness indices were selected in this work which were independent of the sample size :

(1) Margalaef index $R1 = \frac{S-1}{\ln (n)}$

(2) Menhinick index $R2 = \frac{S}{\sqrt{n}}$

where S : total number of species

n : total number of individuals

Species diversity indices

The species diversity indices incorporate both species richness and evenness into a single value. Three species diversity indices were calculated in this work, however, emphasize was put on Hill's diversity indices due to its simplicity for ecological interpretation :

(1) Hill's diversity number $N0 = S$

$N1 = e^{H'}$

$N2 = 1/\lambda$

where N0 : number of all species in the sample

N1 : number of *abundant* species in the sample

N2 : number of *very abundant* species in the sample

(2) Shannon's index $H' = - \sum_{i=1}^{S} (p_i \ln p_i)$

(3) Simpson's index $\lambda = \sum_{i=1}^{S} p_i$

where pi : proportion of individuals belonging to i-th species to the total number of individuals which is computed as :

$$p_i = n_i/N$$

where n_i : number of individuals of the i-th species

N : total number of individuals

S : total number of species

Species evenness indices

Evenness refers to how the species abundances are distributed among the species in a community. In this work, evenness index 5 (E5) was selected which was relatively unaffected by species richness. Evenness index (E5) was known as a modification of Hill's ratio :

$$E5 = \frac{(1/\lambda)-1}{e^{H}-1}$$

where λ : Simpson's index

e^{H} : Hill's (1973) diversity number N1

Similarity coefficient

To compare the similarity of the soil-inhabiting arthropod communities at different sites, Sorensen's index was applied :

Sorensen's index = 2C/A+B

where A : total number of species in community A

B : total number of species in community B

C : total number of species common to both communities

Distance coefficient

Chord distance (CRD) was technically done by projecting the sample units onto a circle of unit radius through the use of direction cosines. The values of CRD express the 'dissimilarity' between the two communities. Chord distance was given by:

$$CRD_{jk} = \sqrt{2(1-ccos_{jk})}$$

ccos is the chord cosine and computed from :

$$ccos = \frac{\sum_{i=1}^{s} (XijXik)}{\sqrt{\sum_{i=1}^{s} X^2_{ij} \sum_{i=1}^{s} X^2_{ik}}}$$

where Xij : number of individuals of the i-th species in sample unit j

Xik : number of individuals of the i-th species in sample unit k

S : number of species