



CHAPTER III

Materials and Methods

3.1 Equipments

The equipments which were used in the experiments are listed as follows.

- Analytical balance, Mettler H 54 AR, Switzerland.
- Environ - Shaker, Lab-line Orbit, U.S.A.
- pH meter, Orion Model 601, U.S.A.
- Atomic Absorption Spectrophotometer, Shimadzu, Japan;
and
- Induce Couple Plasma Spectrometer, Perkin Elmer, U.S.A.

3.2 Reagents

All analytical reagent grade chemicals were used throughout. Heavy metal solutions (as chloride) were prepared in 0.01 M calcium chloride (CaCl_2). Deionized water was used for all treatments.

3.3 Procedure

3.3.1 Sample Collections

Four top soil samples (0-30 cm. surface soil) were Ban Mi, Kamphaeng Saen, Pak Chong and Narathiwat series. They were collected during September, 1986 through April, 1987, and kindly supplied by the members of Land Development Department, Ministry of Agriculture and Cooperative, Thailand. Those samples were collected from different parts of Thailand, i.e., Ban Mi series from Amphoe Ban Mi, Lop Buri Province; Kamphaeng Saen series from Amphoe Kamphaeng Saen, Nakhon Pathom Province, Pak

Chong series from Amphoe Pak Chong, Nakhon Ratchasima Province, and finally, Narathiwat series, from Amphoe Yi-ngo, Narathiwat Province. The first three soils are mineral soils and the last one is organic soils.

All soil samples were air-dried, ground and well mixed, sieved 2.0 mm., except Narathiwat series (organic soils) because of irreversible properties. The moist Nw was used and converted to be dry weight on oven dry basis. To avoid irreversible properties of Nw series they were kept at 4-8°C.

In addition, no metallic tool was permitted in order to prevent metal contamination. All samples were stored and sealed in plastic containers that have already been rinsed by diluted strong acid, such as hydrochloric acid (HCl) or nitric acid (HNO₃). After primary treatment each of mineral soil samples was divided into two parts. They were used as natural and as calcium-saturated soils in the experiments. The chemical and physical properties of each soil sample were analysed by ASA-SSSA, 1965 and 1982 (See Table 3.1).

3.3.2 Calcium - saturated Soil Preparation

Thirty grams of each mineral soil samples were added with 750 milliliters (ml) of the neutral solution (pH=7) of 0.5 M calcium acetate [Ca (CH₃COO)₂]. The suspension was thoroughly shaken about 5 minutes and allowed it to stand overnight. After centrifugation, the supernate was obtained and washed out [4 times with 0.5 M calcium chloride (CaCl₂). Then it was washed with water and with acetone performed. When the test for chloride in leachate (use 0.10 N AgNO₃) becomes negligible, the soil was allowed to drain thoroughly. It is noted that the soil should not allow drying and cracking in order to prevent cleavage and broken edge of soil minerals during washing performances. The Ca-saturated soil samples were obtained after air-dried. (Black et al., 1965; Wada and Abd-Elfattah, 1978; Abd-Elfattah and Wada, 1981).

Table 3.1 The Procedure used in soil physical and chemical analysis.

Characteristics	Analytical Methods
Moisture Content (%)	Oven-dry method
Texture	Pipette method
Clay mineral	X-ray Diffraction method
pH	Glass electrode method
Carbonate	Acid-neutralization method
Cation exchange capacity	Ammonium saturation method
Organic carbon	Walkley - Black method
Free iron oxide	SCS, 1972
Heavy metals	AAS, ICP

Remark : Source ; Black et al.(1965) and ASA-SSSA (1965)

SCS = Soil Conservation Service

AAS = Atomic Absorption Spectrophotometry

ICP = Induce Couple Plasma Spectrometry

3.3.3 Adsorption by Natural Soils

Transfer 0.25 g. of sample to polyethylene flask and add 25 ml of the respective chloride solution containing different concentrations of the heavy metal (Cd, Ni and Zn) viz 10^{-4} to 10^{-3} M which 0.01 M CaCl_2 be supporting electrolyte. The suspensions were shaken thoroughly for 24 hrs. at $30^\circ \pm 2^\circ\text{C}$. The filtrates were acidified by a few drops of concentrate nitric acid (HNO_3) and subsequently analyzed the heavy metal ions by atomic absorption spectrophotometry (AAS) or by Induce Couple Plasma Spectrometry (ICP). The amounts of adsorbed metal cations were calculated as the differences between the amounts of metal presented initially in the solution and those remaining after equilibration. In this calculation, the metals adsorbed in soil (microequivalent per gram soil, $\mu\text{eq/g}$) and those equilibrium concentration (microequivalent per litre, $\mu\text{eq/l}$) were assumed to be studied in the Langmuir adsorption approach. All data refer heavy metal cations being divalent and are expressed on the oven-dry soil basis (Wada and Abd-Elfattah, 1978 ; Abd-Elfattah and Wada, 1981).

3.3.4 The Adsorption by Calcium-Saturated Soils.

The calcium-saturated soils (Bm, Ks, Pc) were treated in the same procedure as natural soil adsorption study. The amount of heavy metal and Ca adsorbed ($\mu\text{eq/g}$) were analyzed in order to be involved the exchange reaction equation approach. The extractable metal with the neutral mixture ($\text{pH} = 7$) between 1 M ammonium acetate ($\text{NH}_4\text{CH}_3\text{COO}$) and 10^{-3} M ethylene diamine tetra acetic acid (EDTA) in the air dry soil were assumed to be involved in an adsorption equilibrium (Wada and Abd-Elfattah, 1979; Abd-Elfattah and Wada, 1981).

3.3.5 Effect of Solution pH on Heavy Metal Adsorption

Placed 0.25 g of mineral soils in polyethylene flask, then added 25 ml of metal solution (10^{-3} M) which 0.01 M CaCl_2 as supporting electrolyte. Before adding those solutions,

the initial pH were adjusted to be 4, 5 and 6 by 0.5 M HCl. after 24 - hrs. shaking, the solution was filtered. The filtrate was analyzed and calculated the concentration of metal both adsorbed in soil and equilibrated in solution (Harter, 1983).

3.3.6 The Desorption of Heavy Metal

After filtration in 3.3.5, those soils were washed with deionized water. To be certain that metal was not desorbed by water washing after filtration the decantate spot checking were made. The supernatant liquids were discarded. Finally, the washed soils were extracted by 1 - hour shaking with 0.01 M HCl (10 ml). Again the solution was clarified by centrifugation and metal in solution was determined by AAS or ICP.

3.3.7 Analytical Determinations

The heavy metals and Ca were determined by AAS from the Scientific and Technological Research Equipment Center Chulalongkorn University and by ICP from the Electrical Authority of Thailand at Phra Pradaeng District, Samut Prakan Province.

3.4 Analysis of Data

3.4.1 Correlation

Solving the correlation between the two parameter c/n , and c which are transformed from the Langmuir equation used the linear regression analysis. Quantitatively, an correlation coefficient (r) is determined between -1 and +1 where an absolute value near 1 means near perfect correlation and a value approaching zero means no correlation. Furthermore, the coefficient of determination, R^2 , indicates that approximately percentage of the variation, is accounted for by a linear relationship (Walpole and Myers, 1972). In addition, the case of computer using has led to the correlation and calculated confidence limits of parameters (adsorption maxima, M and affinity parameter, K).

3.4.2 The Hypothesis Testing

The studying of pH effect on the soil adsorption shows amount of heavy metal under pH variation. In testing for significant difference of the population means are to be performed by the analysis of variance one - way classification (see Appendix C).