

METHODS OF LABORATORY ANALYSES

1. Physical Analysis

1.1 Particle Size Analysis

Particle size analysis was carried out to evaluate soil texture. Ten grams of air dried soil sample was pretreated with 5% hydrogen peroxide to remove organic matter. For dispersion of soil, the suspension was placed in a milk shake container and 10 mL of 5% sodium hexametaphosphate, a dispersing agent, was added. The volume of the contents was made up to about 200 mL with deionized water.

The contents were stirred for 15 minutes on the milk shake mixer. The contents were then sieved through a 300-mesh (0.047 mm) sieve into a one litre cylinder and volume was made up to about 200 mL with deionized water. The sand grains that remained in the sieve were dried at 105°C for overnight and were weighed. The suspension in the cylinder was stirred well with an agitator in an up-down motion for 30 s. The pipette method was used as a direct sampling procedure. Twenty-five millilitres of suspension was pipetted out from a depth of 10 cm for clay at appropriate times based on Stoke's Law (i.e. at 28°C for <0.002 mm sized fraction sampling time at 10 cm depth is 6.5 h). Suspensions were dried at 105°C and weighed (Gee and Bauder, 1986). The amount of sand, silt and clay were calculated. The percentage of clay (<2 µm), silt (0.002 to 0.05 mm) and sand (0.05 to < 2 mm) were plotted on ternary plots and soils were classified using soil textural triangle classes (Soil Survey Staff, 1999).

The clay fraction for mineralogical analysis was separated using the above procedure to obtain 10 g of clay fraction. The clay suspension was transferred from the measuring cylinder to a plastic container, by repeated suspension and decantation until little clay was left in suspension. The clay suspension was next

flocculated by adding excess solid NaCl and the supernatant was then decanted. The flocculated clay was transferred to a centrifuged tube to wash and remove excess salt. The procedure was repeated several times until the conductivity of suspension was equal to that of the deionized water. The washed clay fraction was dried in an oven at 60°C for further analysis.

1.2 Bulk Density (BD)

Bulk density is the mass of dry solid per unit bulk volume of the soil. The bulk volume includes the volume of both solid and pore space. Bulk density varies with structural condition of the soil. It is often used as a measure of soil structure. The undisturbed clod sample (size of about 40 g oven-dry weight) was oven dried at 105°C. The clod and attached thread were weighed in air the clod was then dipped into paraffin wax. The paraffin wax-coated clod was weighed in air and in water. The difference in these weights provides the weight of water that has same volume as the bulk volume of the paraffin wax-coated clod. The density of water and paraffin, weights of oven-dry clod, in air, clod plus paraffin coating in air and water were used to calculate the bulk density which is reported in units of mg m^{-3} (Blake and Hartge, 1986).

2. Chemical Analyses

2.1 Soil Reaction (pH)

Soil pH was determined in water and 1N KCl at a solid to solution ratio of 1:1. The contents were stirred with a glass rod for 30 minutes before measuring the pH by a standardized pH meter (National Soil Survey Centre, 1996).

2.2 Organic Matter (OM)

The organic matter content of soil was indirectly estimated through multiplication of the organic carbon concentration by 1.724. The organic carbon was determined according to the Walkey and Black wet oxidation procedure. This involved wet combustion of organic carbon with a mixture of potassium dichromate and sulfuric acid. After reaction the residual dichromate was titrated against ferrous sulphate (Nelson and Sommer, 1996). A weight of 0.5 g of soil (<0.5 mm) was placed in a 250 mL Erlenmeyer flask. Five mL of 1 N $K_2Cr_2O_7$ was added and the flask was swirled gently to disperse the soil into suspension. Then 10 mL of concentrated H_2SO_4 was added to the flask, swirled gently until the soil and reagents were mixed. The solution takes on a greenish cast and then changes to dark green. The flask was allowed to stand with occasional swirling for 30 minutes. Then 30 mL of deionized water was added to the flask, swirled gently then 3-4 drops of o-phenanthroline indicator were added and the solution was titrated with 1 N $FeSO_4$ until the colour changed to a red end point.

2.3 Total N

A ground soil weighing 1.0 g was placed in micro kjeldahl flask and digestion mixture solution 5 mL was added. Swirl vigorously and digest, rotating the flask frequently until fumes are emitted. Continue digestion for at least 1 hour after mixture becomes white. Cool to room temperature and added 15 mL water. Shake until the contents of the flask are thoroughly mixed. The contents were next filtered using No. 5 Whatman filter paper. The 10 mL of aliquot was placed in distillation flask and 5 mL of 10 N NaOH was added. Distill for 7 minutes, add 5 mL H_3BO_3 acid indicator for containing NH_3 . Titrate the absorbed ammonia with 0.01 N H_2SO_4 until colour changed from green to an end point of pink colour.

2.4 Available Phosphorous

A soil sample weighing 3 g was placed in the 250 mL flask and added BrayII extracting solution 30 mL, shake 40 second. The contents were filtered with No. 42 Whatman filter paper. Aliquot 1-10 mL was pipette in volumetric flask 25 mL and adjusted by distilled water. After 10 minutes, solution was transferred to tubes for determining by spectrophotometer at wave length 882 mili-micron. Standard solution at different concentrations also was determined.

2.5 Available Potassium

A soil sample weighing 10 g was placed in an Erlenmeyer flask and approximately 50 mL 1 N NH_4OAc , at pH 7.0, was added, swirled and allowed to stand overnight. The contents were next filtered using a Buchner funnel with No. 42 Whatman filter paper and a 250 mL suction flask. The volume of the extract was made up to 100 mL. K contents in the leachate were determined by atomic absorption spectrophotometry.

2.6 Extractable Bases

The bases (Ca^{2+} , Mg^{2+} , Na^+ and K^+) that are extracted by NH_4OAc extraction are generally exchangeable bases located on the cation exchange sites of the soil (Chapman, 1965). A soil sample weighing 10 g was placed in an Erlenmeyer flask and approximately 50 mL 1 N NH_4OAc , at pH 7.0, was added, swirled and allowed to stand overnight. The contents were next filtered using a Buchner funnel with No. 42 Whatman filter paper and a 250 mL suction flask. The volume of the extract was made up to 100 mL. Ca, Mg, K and Na contents in the leachate were determined by atomic adsorption spectrophotometry.

2.7 Extractable Acidity

Extractable acidity is the acidity released from the soil by barium chloride-triethanolamine solution buffer (BaCl₂-TEA) at pH 8.2 (Thomas, 1987b). It includes all acidity generated by replacement of the hydrogen and aluminum ions from permanent and pH-dependent exchange sites. A soil sample weighing 5 g was placed in an Erlenmeyer flask and 15 mL of buffer solution at 8.2 (0.5 N BaCl₂.H₂O and 0.2 N Triethanolamine) were added. The contents were stirred and allowed to equilibrate for 30 min before filtering using the Buchner funnel procedure. The contents were given 3 additional washing with 20 mL buffer solution and 5 washing with 20 mL of the replacing solution (0.5 N BaCl₂.H₂O and 0.4 mL of buffer solution in 1 L). The volume of the extracts was made up to 100 mL and 5 drops of mixed indicator (bromocresol green and methyl red in 95% ethyl alcohol) were added. The extract was titrated with 0.2 N HCl. The acid was added drop by drop until the colour changed from green to an end point of purplish red colour. The amount of HCl consumed was used to calculate the extractable acidity expressed as cmol H⁺ kg⁻¹.

2.8 Cation Exchange Capacity (CEC)

The CEC is defined as the sum total of the exchangeable cations that a soil can adsorb. It is dependent upon the negative charges of soil component. Two main methods of CEC determination were used (Thomas, 1987a; National Soil Survey Centre, 1996)

CEC by NH₄OAc at pH 7.0 was determined by saturating the exchange sites with an index cation (NH₄⁺), washing the soil free of entrained index cation, displacing the index cation (NH₄⁺) adsorbed by soil and measuring the index cation. A soil sample weighing 10 g was placed in an Erlenmeyer flask, to which 50 mL of 1 N NH₄OAc, pH 7.0 were added. The flask was stirred occasionally and allowed to stand overnight. The contents were filtered by the Buchner funnel procedure. The soil was

next given 6 washings with 25 mL of 1 N NH_4OAc , and 5 washings with 25 mL of 95% ethyl alcohol. The aliquots from these washings were discarded. The index cation was next displaced by giving 6 washing with 25 mL of 10% acidified NaCl , and filtrates were collected in filtering flasks. The filtrates were transferred to Kjeldahl flask to which 25 mL of 50% NaOH were added. A fifty mL of 4% boric acid was placed into a 100 mL flask and 5 drops of bromocresol green methyl red indicator were added. The Kjeldahl flask was connected to the distillation unit and the boric solution flask with condenser, and was then distilled for 30 min. The solution was titrated with 0.01 N H_2SO_4 until colour changed from green to the pink end point. The volume of H_2SO_4 recorded and used to calculate the CEC as cmol kg^{-1} .

The cation exchange capacity (CEC) at pH 8.2 was calculated by summing the NH_4OAc extractable bases plus the BaCl_2 -TEA extractable acidity (at pH 8.2) (National Soil Survey Center, 1996).

2.9 Base Saturation

Base saturation percentage by NH_4OAc at pH 7.0 is equal to the sum of bases extracted by NH_4OAc , divided by the CEC by NH_4OAc , and multiplied by 100. Base saturation percentage by sum of cations is equal to the sum of bases extracted by NH_4OAc , divided by the CEC by sum of cations, and multiplied by 100 (National Soil Survey Center, 1996).

2.10 Extractable Fe, Al, Mn

2.10.1 Dithionite-Bicarbonate (DCB) Extractable Fe, Al, Mn

The amounts of iron extracted from soils by various dissolution methods (so called specific reagents) are commonly taken to indicate particular forms of this element in soil. The results are useful in studies of soil classification, soil

genesis and soil behavior. The method extracts virtually no Fe, Al or Mn from most crystalline silicate minerals, and thus provides an estimate of “free oxide” (i.e. non silicate Fe) in soils.

One gram of soil (< 0.5 mm) was weighed into a 100 mL centrifuge tube to which 45 mL of buffer solution (0.3 M Na-citrate + 0.1 M Na-bicarbonate) were added. The tube was then placed in a water bath at 80°C. One gram of Na-dithionite powder was added to the tube, the mixture was stirred constantly for 1 minute and occasionally during next 15 minutes. Ten mL of NaCl saturated solution and 10 mL acetone were added to promote flocculation. The tube contents were centrifuged for 15 minutes at 2000 rpm. Clear supernatant was decanted into a 250 mL volumetric flask. This extraction procedure was repeated twice, then the volume was made up to 250 mL with deionized water and the solution was kept for further analysis. For determination of iron by atomic absorption spectrophotometry (AAS), standard solutions of these elements were prepared in a matrix of extracting solution (Mehra and Jackson, 1960).

2.10.2 Oxalate Extractable Fe, Al, Mn

A subsample of one gram of soil (<0.5 mm) was weighed into a 250 mL centrifuge tube, 50 mL of 0.2 M ammonium oxalate solution at pH 3.0 were added to the tube, The tube was shaken for 4 h in darkness. Next five drops of 0.4% superfloc were added to the tube, which was swirled and then centrifuged, clear supernatant was kept for further analysis by AAS (McKeague and Day, 1966).

2.10.3 Pyrophosphate Extractable Fe, Al, Mn

Crystalline oxides and silicates of Fe, Al and Mn, various organic complexes occur in soils. Pyrophosphate solution has been used to extract organic complexes of iron (McKeague, 1967).

A subsample of 1 g of soil (<0.5 mm) was weighed into a 200 mL shaking tube and 100 mL of 0.1 M sodium pyrophosphate solution were added before shaking overnight. Fifty mL of solution were transferred into a 50 mL centrifuge tube, 3 drops of 0.4% superfloc were added, and mixed thoroughly before centrifuging the tubes. Clear supernatant was kept for measuring iron by AAS.

2.11 X-ray Fluorescence (XRF) Analysis for Bulk Soil Samples

Major and minor elements in soil samples were determined on pelleted samples using a Philips PW1480 XRF spectrometer (Jones, 1982). Pelleted samples were produced with a boric acid back and edge designed to fit into Philips sample holders (PW 1480). A teaspoon of boric acid (fine powder of B.P. grade) was poured into the sample holder to encase the sample.

2.12 Inductively Coupled Plasma-Mass Spectrometry

Trace element content of whole soil samples was determined using inductively coupled plasma-mass spectrometry (ICP-MS). For aqua regia digestion, 0.25 g of fine ground whole soils were dissolved in 2 mL of conc. HNO₃ and 5 mL of Conc. HCl at 130°C for 1 h. The volume was made to 20 mL mark with MilliQ water. Supernatant can be poured off into suitable clean and marked vials. An analysis was carried out on Perkin Elmer Elan 6000 ICP-MS. Data were assessed for accuracy and precision using a rigorous control system including reagent blanks and a certified international reference material (STSD-1).

The aqua regia digestion does not completely dissolve some minor and highly resistant sand size minerals but results closely approximate the total concentrations of trace elements, while the XRF values are true totals. Therefore Si, Al, Fe, K, Ca, Mg, Na, Ti and S were determined by XRF using a Philips PW1480 XRF spectrometer. Manganese, Cr, V, P, Zn, Rb, Li, Ni, Sr, Cu, Ga, Pb, Co, As, Be, U, Ag and Cd were determined by ICP-MS on acid digests.

3. Mineralogical Analysis

X-ray diffraction analysis was used to identify and to make semiquantitative measurements of the crystalline mineral components of clay fraction. The clay fraction from sedimentation was pretreated using 4 treatments. The clay after Mg^{2+} and K^+ saturation were placed on the ceramic plates, dropped with $\approx 10\%$ glycerol on the Mg^{2+} saturation plates for the glycerol treatment, and heated to $550^\circ C$ on K^+ saturation slide for heat treatment (Brown and Brindley, 1980). Minerals were determined for all horizons with X-ray diffraction (XRD analysis using a Philips PW-3020 diffractometer with a graphite diffracted beam monochromator ($CuK\alpha$, 50 kV, 20 mA). Clay fractions were scanned respectively from 4 to $45^\circ 2\theta$ and 4 to $65^\circ 2\theta$, using a step size of $0.02^\circ 2\theta$ and a scan speed of $0.04^\circ 2\theta \text{ sec}^{-1}$. Relative proportions of various minerals were calculated by comparing the XRD peak intensity with the intensity for standard minerals (Klug and Alexander, 1974; Brown and Brindley, 1980; Whittig and Allardice, 1986).

3.1 Analysis of Kaolins

3.1.1 Preparing Kaolin Samples

Representative soil kaolins were sampled for fourteen sites in Thai paddy soils where kaolin is the dominant clay mineral. Soil samples were dried and passed through a 2 mm sieve. Organic carbon was removed from < 2 mm soil using H_2O_2 and free iron oxides were removed by dithionite-citrate-bicarbonate treatment following the procedure of Mehra and Jackson (1960) before the clay fraction was collected by dispersion and sedimentation. At this stage the clay was examined by XRD to confirm that it consisted almost entirely of kaolin-group minerals and henceforth these purified soil clay materials are referred to as kaolins.

3.1.2 Cation Exchange Capacity (CEC) for Kaolin Concentrate

Cation exchange capacity (CEC) was measured using 0.01 M silver thiourea solution at pH 4.7 to displace the exchangeable cations (Rayment and Higginson, 1992). This CEC method involves the equilibration of 0.1 g air-dry Kaolin and 0.01 M (AgTU)⁺ for 16 h, with end-over-end shaking, followed by centrifugation and analysis of the residual Ag⁺ by AAS in the presence of La. It should not be used on samples where CEC values above ~30 cmol kg⁻¹ are expected.

3.1.3 Specific Surface Area for Kaolin Concentrate

Specific surface area (SSA) of kaolin sample was measured using the N₂-BET method (Aylmore *et al.*, 1970) with a Micrometric Gemini III 2375 surface analyzer. Accurately weighed with 4 decimals empty test tube and also approximately 0.3000 g air-dry kaolin sample into the test tube. Degas the soil samples overnight and reweighed the kaolin sample plus tube. Load the sample tube on the surface area analyzer run and interpret the data using the software.

3.1.4 X-ray Diffraction (XRD) for Kaolin

X-ray diffraction pattern of kaolins were obtained using CuK α radiation with Philips PW-3020 diffractometer equipped with graphite diffracted beam monochromator. Random power patterns were obtained over the range of 3-70° 2 θ and a scan speed of 0.02° per second to determine the degree of structural order of the kaolins expressed as the HB index (Hughes and Brown, 1979). Oriented kaolins were prepared on ceramic plates and XRD patterns 3-30° 2 θ were obtained after various pretreatments to aid identification of minerals (Brown and Brindley, 1980). Coherently scattering domain (CSD) size of kaolins was calculated from the width at half height of XRD reflections using the Scherrer equation (Klug and Alexander, 1974).

3.1.5 Transmission Electron Microscope (TEM) for Kaolin Samples

For analytical transmission electron microscopy (TEM), specimens were prepared from dispersed samples. A highly diluted suspension of the iron concentrated sample was prepared in distilled water and kaolin particles were dispersed by ultrasonic treatment. A drop of the suspension was deposited on a carbon-coated supported on a Cu grid and examined using a JEOL 2000 FX II electron microscope operated at 80 kV.

3.1.6 Chemical Composition of Kaolin Samples (EDS)

Well-dispersed samples of kaolins, on carbon film were investigated using a JEOL 3000 transmission electron microscope operated at 300 kV. Energy dispersive spectra (EDS) for well separated single crystals were collect at 500-1500 counts per second for 100 live seconds. Elemental compositions of these crystals were calculated using the thin film method (Jepson and Rowse, 1975). The k-factors used were derived from the spectra of standard minerals and confirmed by reference to the spectra of well characterized kaolinites.

3.2 Preparing Smectite Samples

3.2.1 Mineralogical Characterization

The clay fraction ($<2 \mu\text{m}$) was obtained by sedimentation. Organic matter was removed from the clay using the H_2O_2 oxidation method (Kunze, 1965). Free sesquioxides were removed with a solution of sodium dithionite and sodium citrate in a sodium bicarbonate-buffered system (Mehra and Jackson, 1960). The basally oriented Li-saturated clay was prepared on a ceramic plate, heated at 300°C for 2 h, cooled in a dessicator and then glycerol solvated prior to XRD examination (Greene-Kelly, 1955).

3.2.2 Cation Exchange Capacity (CEC) for Smectite

Cation exchange capacity (CEC) was measured using 0.01 M silver thiourea solution at pH 4.7 to displace the exchangeable cations (Rayment and Higginson, 1992). This CEC method involves the equilibration of 0.1 g air-dry kaolin and 0.01 M (AgTU)⁺ for 16 h, with end-over-end shaking, followed by centrifugation and analysis of the residual Ag⁺ by AAS in the presence of La. It should not be used on samples where CEC values above ~30 cmol(+) kg⁻¹ are expected.

3.2.3 Chemical Composition of Smectite

The chemical compositions of individual smectite crystals were determined using transmission electron microscopy (JEOL 3000F Field Emission Gun TEM) with the energy dispersive X-ray (EDX) analysis.

XRF analyses of fused discs of the Ca-saturated clay fractions of Ap and Bss3 horizons of Lop Buri soils were obtained using a Philips PW1400 X-ray fluorescence (XRF) spectrometer fitted with a rhodium tube. Zero point seven gram of clay fraction was fused with 7.000 g of lithium meta/tetraborate flux at 1050°C in a platinum crucible. For this analysis, a sample of the clay fraction was Ca²⁺-saturated by four centrifugal washings in 1 M CaCl₂ and then washing with distilled water until free of AgNO₃ detectable chloride. These two clays were chosen for analysis because they consist almost solely of smectite.

3.2.4 Layer Charge Characterization

The layer charge was estimated by XRD measurement of the (001) interplanar spacing of alkylammonium-clay complexes using alkylchains with 6, 7, 10, and 12 C alkyl number (n_c). Alkyl solutions were prepared using a slight

modification of the procedure of Lagaly (1994), which consisted of heating the solution containing the required amount of alkylamine salt, ethanol, water, and HCl at about 60°C for 1 h and adjusting the solution pH to 6.5-7.0 with 0.1 M HCl after cooling. One hundred milligrams of clay was dispersed in a capped tube using a vortex mixer with 1 mL for alkylammonium concentrations of >0.1 M ($n_c < 11$) and 4 mL in the case of 0.05 M solutions ($n_c > 15$), equilibrated overnight in a water bath at 65°C and centrifuged. The treatment was repeated once. The excess alkylammonium salt was removed by washing the samples 8 times with 3 mL of a water-ethanol mixture (2:1) followed by two washings with pure ethanol. Oriented specimens were prepared by thinly spreading the clay-ethanol slurry onto glass slides which were then dried in a desiccator containing P_2O_5 for 24 h prior to XRD analysis.

All XRD traces were obtained on a computer controlled Philips X-ray diffractometer using $CuK\alpha$ radiation and a focusing monochromator. The XRD traces of clay separates were recorded between 2 and 15° 2θ using a step size of 0.02° 2θ . The critical chain length used for the calculation of layer charge is the molecule with the alkyl number n_c (general formula $C_{n_c}H_{2n_c+1}NH_3^+$) where the molecules attain the densest monolayer packing in the interlayer of the smectite. The spacings are mostly between 13 and 14 Å (monolayer) and 17 and 18 Å (bilayer). The procedure for calculating layer charge is given by Lagaly (1994).

4. Thin Sections

4.1 Thin Section Preparation

This is done by putting the soil sample in the box and it is impregnated under vacuum with a polyester resin. When the resin is polymerized, it hardens producing a compact block that encompasses the soil sample, conserving its natural structure with no alterations. The main steps of preparing the samples are as follows: The resins used for fixing the soil samples are hydrophobes, any water must be

extracted first, therefore the soils are oven dried at 60°C and soil water is replaced with acetone. Fixing (impregnation and hardening): Unsaturated polyester resins (40%), which, as they are very viscous, must be reduced by adding styrene monomer (60%). Polymerisation is started by adding a catalyst (Benzyl). To ensure that the resin penetrates well; impregnation of the samples is often carried out at vacuum. After the sample has been impregnated and hardened, cutting and polishing occurs, in which only oil can be used as a lubricant to avoid dissolving soluble minerals (e.g. gypsum). Firstly, the piece of soil included in the plastic block has to be cut with a diamond cut-off saw to obtain a flat surface with the size of the microscopic preparation that is required. After a flat surface is obtained, it is polished to eliminate the traces of cutting and to obtain a flat surface that is as smooth as possible. Mounting: The polished surface is stuck onto a glass microscope slide with a colourless and isotropic cementing agent. After the piece of soil has been stuck to the microscope slide, it is cut to obtain the thinnest slice possible. The sample is trimmed until it has a thickness of about 30 microns.

4.2 Analysis of Thin Sections

The thin section samples were first analyzed by optical microscopy. Areas of interest were selected photographed and coated with carbon of examination using a backscattered electron image and elemental mapping on a JEOL 6400 SEM operated at 15 kV of electron beam accelerating voltage. Electron microprobe analysis (EMPA) and energy dispersive spectroscopy (EDS) quantitative microanalysis were used to determine the chemical composition of distinct micromorphological features (White and Dixon, 1995).