MATERIALS AND METHODS

Materials

1. Soil maps of provinces in Thailand (scale 1:100,000) published by Department of Land Development, Ministry of Agriculture and Cooperatives.

2. Geology maps of Thailand (scale 1:250,000).

3. Topographic maps of Thailand (scale 1:50,000) published by Royal Thai Survey Department.

4. Soil maps of salt affected soil distribution in Thailand (scale 1:100,000) published by Department of Land Development.

5. Standard soil survey and field sampling kits (Kheoruenromne, 1999; Soil Survey Division Staff, 1993).

6. Laboratory instruments for physical, chemical, mineralogical and micromorphological analyses of soils.

Sampling Sites

The 5 study areas are located in Northeast Thailand between coordinates (UTM) 1686032 and 1805233 N and 48 0226080 and 48 0372287 E (Figure 3). Location 1 is in amphoe Phra Yuen, Khon Kaen province, location 2 in amphoe Phimai, Nakhon Ratchasima province, location 3 in amphoe Suwanaphume, Roi Et province, location 4 in amphoe Ban Phai, Khon Kaen province and location 5 in amphoe Bua Yai, Nakhon Ratchasima, province. These soils have developed from diverse sediments on erosional terraces where there are various depths of alluvium and wash deposits.

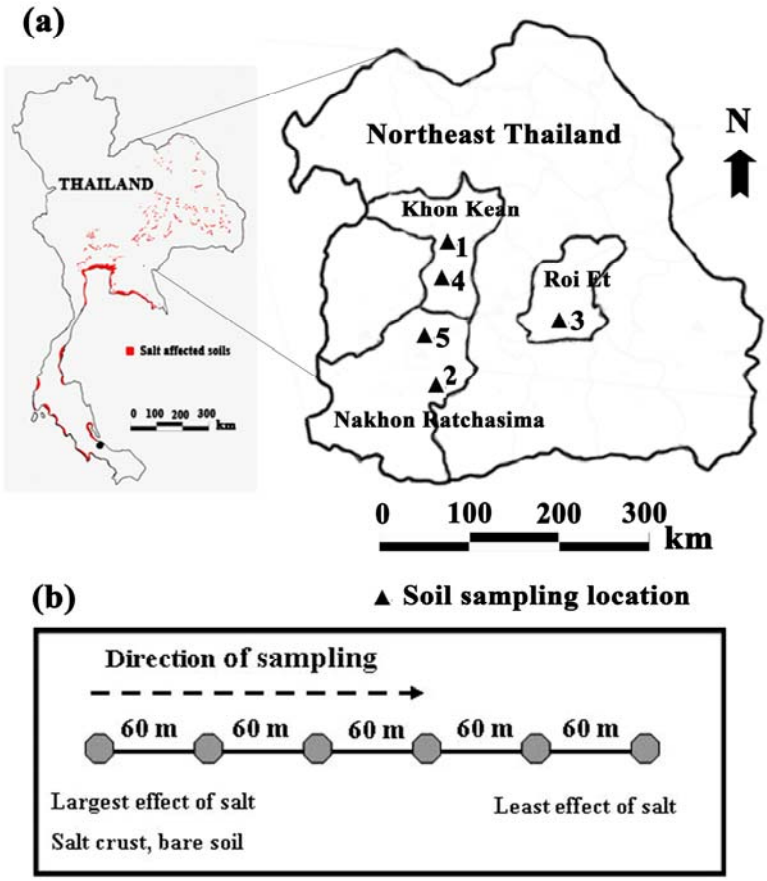

Figure 3 Soil sampling sites, locations of soil sampling sites and soil sampling strategy; (a) distribution areas of salt affected soils in Thailand and locations of soil sampling, (b) direction line illustrating soil sampling strategy at each location.

In each of the study areas some of the soils are salt affected due to saline parent rocks and associated saline groundwater (Department of Land Development, 1991) and exhibit salt crusts, bare areas and halophytic plants. Five or six pedons were collected from linear traverses that included the highest and lowest levels of salt accumulation. Pedons were separated by about 60 m on each transverse and 28 pedons were sampled for this study. Soil profiles were described and sampled by genetic horizon according to standard field study methods (Soil Survey Division Staff, 1993; Kheoruenromne, 1999). Two study locations had sandy soils (locations 1 and 5), two had clayey soils (locations 2 and 4) and at one (location 3) the soils had sandy topsoils and clayey subsoils.

Field Analyses

Pedon analysis in soil pits was carried out at each site including detailed profile description and sampling of soil from each genetic horizon by standard field study methods (Soil Survey Division Staff, 1993; Kheoruenromne, 1999). Soil samples included bulk samples, clod samples, core samples and Kubiena samples.

Bulk samples were air-dried, gently crushed and then passed through a 2-mm sieve. The resultant <2 mm samples were used for general laboratory analysis. The clod samples were used for bulk density measurement. The core samples from topsoil were used for hydraulic conductivity measurements. Kubiena samples were used for optical microscopy and SEM analysis.

Laboratory Analyses

Physical Analyses

Particle Size Analysis

Particle size analysis was carried out to evaluate soil texture. A mass of 10 g air dried soil sample was pretreated to remove free soluble salt by washing in distilled water, centrifuging and decanting. For dispersion of soil, the soil 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 deionised 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^{o}C 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 from a depth of 10 cm for clay at appropriate times based on Stoke's Law (i.e. at 25^{o}C for <0.002 mm sized fraction sampling time at 10 cm depth

is 6 hr). Suspensions were dried at 105^{o}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, 1998).

Hydraulic Conductivity (Ksat)

A saturated undisturbed soil (core sample) was installed in a cylinder and supplied with water to the bottom of the sample. During the water flow in the standpipe, measured the time for the water level to fall from H_1 to H_2. Calculation of the hydraulic conductivity was from the equation:

$$Ksat = (aL/At) \log_e (H_1/H_2)$$

where Ksat= hydraulic conductivity value, a = the cross sectional area of the standpipe, L= the soil sample length, A= cross section area of the sample, t= time, H_1 = height of initial water and H_2= height of final water (Klute and Dirksen, 1986)

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 2-3 cm, diameter) was oven dried at 105 ^{o}C. The clod and attached thread were weighed in and 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 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 in water were used to calculate the bulk density which is reported in units of Mg m^{-3} (Blake and Hartge, 1986).

Chemical Analyses

Soil Reaction (pH)

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

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 Walkley 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 sulfate (Nelson and Sommers, 1996). A weight of 1.0 g of soil (< 0.5 mm) was placed in a 250 mL Erlenmeyer flask. Five mL of 1 N K_2Cr_2O7 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 took on a greenish cast and then changed 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 color changed to a red end point.

Total Nitrogen

A ground soil weighing 1.0 g for clayey and 3.0 for sandy soil was placed in micro kjeldahl flask and digestion mixture solution 5 mL were added, swirled vigorously and digested, rotating the flask frequently until fumes were emitted. Continued digestion for at least 1 hour after mixture became white. Cooled to room temperature and added

water made up to about 50 mL. Shake until the contents of the flask were 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. Distilled for 7 minutes, added 5 mL H_3BO_3 acid indicator for containing NH_3. And, titrated the absorbed ammonia with 0.01 N H_2SO_4 until color changed from green to an end point of pink color (Jackson, 1965).

Available Phosphorus

A soil sample weighing 3 g was placed in the 250 mL flask and added Bray II extracting solution 30 mL, shaken for 40 seconds. The contents were filtered with No. 42 Whatman filter paper. Aliquot 1-10 mL was pipetted in volumetric flask 25 mL and adjusted by distilled water. After 10 minutes, solution was transferred to tubes for determining by spectrophotometer at wavelength 882 mili-micron. A standard curve for 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg P kg^{-1} was prepared (Bray and Kurtz, 1945).

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 for clayey and 25 g for sandy soil was placed in an Erlenmeyer flask and approximately 50 mL 1 N NH_4OAc, at pH 7.0, were 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. Calcium, Mg, K and Na contents in the leachate were determined by atomic absorption spectrophotometry.

Extractable Acidity

Extractable acidity is the acidity released from the soil by barium chloride-triethanolamine solution buffer ($BaCl_2$-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 4 g was placed in an Erlenmeyer flask and 100 mL of buffer solution at 8.2 (0.5 N $BaCl_2$ H_2O and 2 N triethanolamine) were added. The contents were topped by rubber bung, shaken for 30 min, left to stand overnight and shaken 30 min then left for 2 hours. Taken 50 mL of the content by using the pipette controller, placed to new Erlenmeyer flask. The extracts were added 3 drops of mixed indicator (bromocresol green and methyl red in 95% ethyl alcohol). The extract was titrated with 0.2 N HCl. The acid was added drop by drop until the color changed from green to an end point of purplish red color. The buffer solution was titrated with 0.2 N HCl as the blank in the same condition as extract samples. The amount of HCl consumed was used to calculate the extractable acidity expressed as cmol H^+ kg^{-1}.

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 Center, 1996):

The CEC by NH_4OAc at pH 7.0 was determined by saturating the exchange sites with an index cation (NH_4^+), washing the soil free of entrained index cation, displacing the index cation (NH_4^+) adsorbed by soil and measuring the index cation. A soil sample weighing 10 g for clayey and 25 g for sandy soils was placed in an Erlenmeyer flask, to which 50 mL of 1N NH_4OAc, 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 1N 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 washings with 25 mL of 10% acidified NaCl, and filtrates were collected in filtering flasks. The filtrates were transferred to a Kjeldahl flask to which 25 mL of 50% NaOH were added. 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 color changed from green to the pink end point. The volume of H_2SO_4 was 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).

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).

Electrical Conductivity (EC)

Electrical conductivity (EC) by the saturation extract method at 25^oC and measured by electrical-conductivity bridge (U.S. Salinity Laboratory Staff, 1954).

Sodium Adsorption Ratio (SAR)

The SAR was computed by dividing the molar concentration of the monovalent cation Na^+ by the square root of the sum of the molar concentration of the divalent cations Ca^{2+} and Mg^{2+} (meq L^{-1}) (U.S. Salinity Laboratory Staff, 1954). The saturaration extract cations Ca^{2+}, Mg^{2+} and Na^+ measured by atomic absorption spectrophotometer (National Soil Survey Center, 1996).

Exchangeable Sodium Percentage (ESP)

The ESP was computed by dividing the exchangeable sodium (ES) by the CEC-7 and multiplying by 100 (U.S. Salinity Laboratory Staff, 1954).

Soluble Salt

A soil sample weighing 10 g was placed in an Erlenmeyer flask, to which 50 mL of DI water were added. The flask was stirred occasionally and allowed to stand overnight. The contents were filtered by the Buchner funnel procedure. Soluble calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) were measured by atomic absorption spectrophotometer. The saturation extracted cations Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} are reported in meq L^{-1} (Rhoades, 1982; National Soil Survey Center, 1996).

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

Major and minor elements in the bulk samples of whole soil samples were determined using a Philips PW1480 X-ray fluorescence (XRF) spectrometer. The samples preparation were prepared by pressed pellets techniques (Karathanasis and Hajek, 1996).

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Lithium, Be, P, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Br, Rb, Sr, Mo, Ag, Cd, I, Cs, Pb and U were determined using by ICP-MS. Soil samples were extracted by aqua regia digestion (HCL and HNO_3) followed by inductively coupled plasma-mass spectrometry (ICP-MS) (Soltanpour *et al.,* 1996; Lynch 1999).

Mineralogical Analysis

X-Ray Diffraction (XRD) for Soil Samples (Random Powder)

Semi-quantitative determination of mineral abundance in whole soil was done on random powders by X-ray diffraction analysis using monochromatised CuKα radiation with a Philips diffractometer at 50 KV and 20 mA. The XRD scans extended from 3^{o} to 70^{o} 2θ with a step size of 0.02^{o} and scan speed of 1.2^{o} per minute. Relative proportions of minerals were calculated by comparing the random powder XRD peak intensity of major

refections with the intensities for a mixture of standard minerals (Brindley and Brown, 1980). Twenty five percent Birch Pit Macon Georgia kaolinite, 25% of Upton – Wyoming montmorilonite, 25% of Morris-Illinois illite and 25% guartz were mixed to provide a mineral standard. Clay minerals percentages were determined from the intensity of their 001 reflections. The total clay mineral abandance (montmorillonite, illite and kaolin) was determined from the intensity of the common reflection at 4.45. The quartz reflection at 3.34 Å d-spacing was used. This procedure provides only semiquantitative mineralogical analyzes but is suitable for identifying major differences and trends in mineralogical composition.

For other mineral the percentages were calculated by comparing peak area intensity with the total peak area intensity of each sample and then normalized data to 100%.

X-Ray Diffraction (XRD) for Clay and Silt Fraction Samples

The clay fraction for mineralogical analysis was separated by using a sedimentation procedure. Pre-treatments of the 2 mm soil to remove organic carbon were by using H_2O_2 (Gee and Bauder, 1986). The clay fraction for mineralogical analysis was separated using the same method with particle size analysis in above procedure to obtain 10 g of clay fraction. 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 free excess salt. The Dithionite-Citrate-Bicarbonate method (DCB) was used to remove iron. The dialysis method was used to remove salt from the clay fraction. The DI water overriding the dialysis tube was replaced several times until the conductivity of the water was equal to that of the DI water.

X-ray diffraction analysis was used to identify and to make semiquantitative measurements of the crystalline mineral components of the clay fraction. The clay fraction from sedimentation was pretreated using 4 treatments. The clay after Mg^{2+} and K^{+} saturation were placed on ceramic plates, washed with ≈10% glycerol on the Mg^{2+}

saturation plates for the glycerol treatment, and heated to 550 ^{o}C for the K^{+} saturation plate for heat treatment (Brindley and Brown, 1980). Minerals were determined for all horizons by X-ray diffraction (XRD) analysis using a monochromatised CuKα radiation with a Philips PW-3020 diffractometer (Cu Kα, 50kV, 20mA). Clay fractions were scanned respectively from 4 to 45° 2θ, using a step size of 0.02° 2θ and a scan speed of 0.04° 2θ sec^{-1}. Silt was prepare as a random powder for some horizons and analyzed using a Philips X'Pert diffractometer (Co Kα, 50kV, 20mA), scanned from 4 to 65° 2θ.

Relative proportions of various minerals were calculated by comparing the XRD peak intensity with the intensity for standard minerals (Brindley and Brown, 1980).

X-Ray Diffraction (XRD) for the Identification of Smectite Mineral group

Generally, smectite group minerals in soils consist of montmorillonite, beidellite or nontronite. To distinguish montmorillonite from beidellite and nontronite one normally uses Greene-Kelley method, where Li saturated clay is heated at 200-300 ^{o}C (Greene-Kelley, 1953). Fifty milligrams of clay were transferred into 30-mL test tube and washed 3 times with aqueous 3M LiCl and 2 times with 0.01M LiCl in 90% methanol. The Li-saturated clay suspension was deposited on a porous ceramic plate under a suction. The clay on the ceramic plate was allowed to dry slowly at 25 ^{o}C and then heated overnight at 250 ^{o}C in a muffle furnace. The sample was then saturated with glycerol and analyzed by XRD using a monochromatised CuKα radiation with a Philips PW-3020 diffractometer at 50 KV and 20 mA. The clay fraction was scanned from 4 to 45° 2θ, using a step size of 0.02° 2θ and a scan speed of 0.04° 2θ sec^{-1}. A 001 spacing of about 0.95 nm after they pretreatment indicates monmorillonite, and a spacing of 1.78 nm indicates beidellite (Greene-Kelley, 1953; Brindley and Brown, 1980; Singh and Gilkes, 1991).

Clay Suspension under the Transmission Electron Microscope (TEM)

For analytical transmission electron microscopy (TEM), specimens were prepared as dispersed samples. One milliliter of sample in the clay suspension was transferred into the 10-mL test tube, mixed well with 9 mL of deionized water. A drop of the suspension was deposited on a carbon-coated Cu grid and examined using a Jeol 2000 FX II electron microscope operated at 80 kV.

Micromorphological Analysis

Preparation

Kubiena samples were transferred to impregnation mould containers, oven dried at 60-70 ^{o}C for approximately 2-4 week. Before oven drying acetone was used to replace water in micro-pore as it is easily volatized from the samples. This is special consideration for salt affected soils and clay texture soil for reducing the impregnation problem due to soil moisture. The soil samples were impregnated with a 40:60 resin: monostryrene mixture and 5 g of the catalyst benzoyl peroxide ($C_{14}H_{10}O_4$) per liter. The resin mixture was poured onto samples at atmospheric pressure, followed by a period in a vacuum oven while the pressure was slowly increased from 10 to 65 cm Hg, and then the samples were left for 4 hours. The dried impregnated samples were cut to the size of glass slides, polished on one face to smooth and then cleaned with acetone. Blocks were mounted on glass slides with resin and polished to 0.03 mm thickness.

Optical Analysis

The thin sections were analyzed under a polarizing microscope using standard micromorphological techniques as described by Bullock *et al.* (1985).

SEM Analysis of Soil Thin Section

The polished thin section samples were first analyzed by optical microscopy. The areas of interest were selected and coated with carbon for examination using a backscattered electron image, secondary electron image and elemental mapping on a VPSEM ZEISS 1555 scanning electron microscope operated at 15 kV electron beam accelerating voltage. Energy dispersive spectrometer (EDS) quantitative microanalysis was used to determine the chemical composition of distinct micromorphological features such as soil plasma matrix, nodules and salt crystals (White and Dixon, 1995). Only fine fraction (eg. clay coat, clay matrix, nodules and salt crystal) were pointed for EDS analysis. The EDS detector used was a Be-window Oxford Instruments Link Analytical detector running ISIS software and the analysis results were standardized based on standard natural minerals, metals and synthetic compounds.

Statistical Analysis

Correlation Matrix

The Statistica program (version 6.1) was applied to analyze relationships between the chemical properties, and identified relationships significant at $p < 0.05$.

Factor Analysis

The concentrations of elements in whole soil were statistically analyzed using factor analysis and principal component analysis with the Statistica Program (Version 6.1). Factor analysis, a widely used multivariate statistical method, was employed to interpret data. This technique reveals the correlation structure of the geochemical variables allowing the identification of affinity groups of elements. Also, this method was applied to identify the affinity groups of soil samples (Evans *et al.,* 1996; Kumru and Bakaç, 2003).