

**CHEMICAL ANALYSES OF PICA SOIL EATEN  
BY THE VILLAGERS IN SISAKET PROVINCE**

**Mr. Songkot Utara**

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นายทรงกฎ อุดรา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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Suranaree University of Technology has approved this thesis submitted in  
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Thesis Examining Committee

.....  
(Asst. Prof. Dr. Malee Tangsathitkulchai)  
Chairperson

.....  
(Asst. Prof. Dr. Tritaporn Choosri)  
Member (Thesis Advisor)

.....  
(Assoc. Prof. Dr. Saowanee Rattanaphani)  
Member

.....  
(Assoc. Prof. Dr. Vichitr Rattanaphani )  
Member

.....  
(Assoc. Prof. Dr. Tawit Chitsomboon)  
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.....  
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Dean of the Institute of Science

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**(CHEMICAL ANALYSES OF PICA SOIL EATEN BY THE VILLAGERS IN SISAKET PROVINCE)**

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นำดินตัวอย่างที่ชาวบ้านกิน ในเขตจังหวัดศรีสะเกษ 4 ตัวอย่าง มาวิเคราะห์สมบัติทางกายภาพและทางเคมี ได้แก่ สีดิน ความชื้น ปริมาณสารที่หายไปหลังการเผา ความเป็นกรด-เบส ปริมาณสารอินทรีย์ ความสามารถในการแลกเปลี่ยนประจุบวก ความเข้มข้นของธาตุบางชนิด ความเข้มข้นธาตุเหล็กและธาตุสังกะสีที่ร่างกายสามารถดูดซึมได้ ผลของการทดลองมีดังนี้สีของดินมีค่าสี 7.5YR8/3 - 10YR6/1 ความชื้นในดินมีค่า 1.42 - 6.35 เปอร์เซ็นต์ ปริมาณสารที่หายไปหลังการเผามีค่า 7.26 - 9.98 เปอร์เซ็นต์ ความเป็นกรด-เบส มีค่า 4.30 - 4.95 ปริมาณสารอินทรีย์ในดินมีค่า 0.28 - 2.73 เปอร์เซ็นต์ ความสามารถในการแลกเปลี่ยนประจุบวกมีค่า 2.14 - 12.65 เซนติโมลต่อกิโลกรัม ความเข้มข้นของธาตุบางชนิดในดินมีดังนี้สังกะสี 48 - 59 มิลลิกรัมต่อกิโลกรัม เหล็ก 7.28 - 19.08 กรัมต่อกิโลกรัม แมงกานีส 51 - 88 มิลลิกรัมต่อกิโลกรัม ไทเทเนียม 8.33 - 9.71 กรัมต่อกิโลกรัม แบริียม 2.79 - 3.22 กรัมต่อกิโลกรัม โพแทสเซียม 623 - 2055 มิลลิกรัมต่อกิโลกรัม ซีลีคอน 254.85 - 316.47 กรัมต่อกิโลกรัม และ อะลูมิเนียม 50.29 - 108.91 กรัมต่อกิโลกรัม แมกนีเซียม พบเฉพาะในตัวอย่างที่ 4 โดยมีความเข้มข้น 405 มิลลิกรัมต่อกิโลกรัม ปริมาณธาตุเหล็กที่ร่างกายสามารถดูดซึมได้มีความเข้มข้น 22.35 - 79.91 มิลลิกรัมต่อกิโลกรัม ปริมาณธาตุสังกะสีที่ร่างกายสามารถดูดซึมได้มีความเข้มข้น 0.52 - 0.86 มิลลิกรัมต่อกิโลกรัม

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**SONGKOT UTARA: CHEMICAL ANALYSES OF PICA SOIL  
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**THESIS ADVISOR: ASST. PROF. TRITAPORN CHOOSRI, Ph.D.**

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Four pica soil samples from Sisaket province were analyzed for their physical and chemical properties, which were; soil color, moisture content, loss on ignition, pH, organic matter, cation exchange capacity, concentration of certain elements, bioavailable iron, and bioavailable zinc. The experimental results were as follows: color of soil samples ranged from 7.5YR8/3 - 10YR6/1. Moisture content ranged from 1.42 - 6.35 %. Loss on ignition ranged from 7.26 - 9.98 %. The soil pH ranged from 4.30 - 4.95. Organic matter contents ranged from 0.28 - 2.73 %. The cation exchange capacity ranged from 2.14 - 12.65 cmol/kg. The concentration of certain elements were 48 - 59 mg/kg for zinc, 7.28 - 19.08 g/kg for iron, 51 - 88 mg/kg for manganese, 8.33 - 9.71 g/kg for titanium, 2.79 - 3.22 g/kg for barium, 623 - 2055 mg/kg for potassium, 254.85 - 316.46 g/kg for silicon, and 50.29 - 108.91 g/kg for aluminium. Magnesium was found only in sample 4 with the concentration of 405 mg/kg. Bioavailable iron ranged from 22.35 - 79.91 mg/kg. Bioavailable zinc ranged from 0.52 - 0.86 mg/kg.

School of Chemistry  
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Signature of Student.....  
Signature of Advisor.....  
Signature of Co-Advisor.....  
Signature of Co-Advisor Jatuporn Wittayakun

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## List of abbreviations

WDXRF	wavelength dispersive x-ray fluorescence
AAS	atomic absorption spectroscopy
cm	centimeter
nm	nanometer
g	gram
kg	kilogram
ml	milliliter
mm	millimeter
mg	milligram
h	hour
min	minute
mA	milliampere
cps	count per second
rpm	revolution per minute
abs	absorbance
$^{\circ}\text{C}$	degree celcius
kv	kilovolt
ppm	part per million
M	molarity
N	normality
% w/w	percent weight by weight
% w/v	percent weight by volume
RDA	Recommended Daily Dietary Allowances
RNI	Recommended Nutrient Intake
RDI	Recommended Daily Intake

# **Chapter I**

## **Introduction**

Pica originates from the Latin word for magpie, a species of bird that has a habit of eating or carrying away all manner of extraneous objects (Halsted, 1968; Solyom, et al., 1991; Moore and Sear, 1994; Eastwood, 1997; Rose et al., 2000). Generally, pica is an ingestion of nonfood substances or a craving for nonfood substances (Moore and Sear, 1994; Ziegler, 1997).

Scientists have been using Greek word "phagein" meaning, "to eat" as a suffix for calling pica behavior (Moore et al., 1994). There are many specific forms of pica whose names have been derived from the Greek words for the ingested of various substances as listed in Table 1.1. Several hypotheses have been advanced to explain the reasons for pica behavior, including the body needs to acquire certain missing nutrients, hunger, cultural tradition, prevention of nausea, and attention seeking. Rees (1983) suggested that, "When the body is zinc deficient, the brain apparently senses this, and directs the organism to seek out in its environment sources of food or even non food, that will satisfy this need". In addition, there is also a strong link between pica and iron deficiency. However, the exact relationship is not clear (Boyle and Mackey, 1999).

Geophagia or geophagy is defined as the habit of eating clay and other types of soil (Moore and Sears, 1994). Historically, geophagy was known and recorded in 13<sup>th</sup> century in Greek and Roman ages (Rose et al., 2000).

The term geophagy was first mentioned by the great philosopher, Aristotle (Mahaney et al., 2000). During 1799 to 1804, Von Humboldt reported from his travel in South America that clay was eaten to some extent at all times by the Otomac tribe along the Orinoco River (Halsted, 1968; Abrahams, 1996). Geophagy has been recorded in every region of the world both as idiosyncratic behavior of isolated individuals and as culturally prescribed behavior of particular societies (Reid, 1992). Geophagy has been found in many continents such as Africa (Mahaney et al., 2000; Halsted, 1968; Abrahams and Parsons, 1996; Abrahams, 1997), Asia (Boyle and Mackey, 1999; Mahaney et al., 2000), America (Ziegler, 1997; Boyle and Mackey, 1999), Europe (Ziegler, 1997), and Australia (Callahan, 2000). In addition, ingestion of kaolin, also known as white dirt chalk or white clay, is a relative common type of pica found in the central Georgia Piedmont area (Grigsby et al., 1999). Generally, geophagy is most common in underdeveloped countries where many people are poor and suffer from malnutrition, but it is not confined to them (Oliver, 1997). More details about geophagy are discussed in Chapter II.

### **1.1 Background of the problem and the significance of the study**

Researches pertaining to the soil consumption or geophagy can be traced back more than 10 years. Several researchers found geophagy in various species of living things, including mammals, birds, reptiles, butterflies and isopods (Diamond, 1999). Scientists are interested in geophagy in human because its effects and results are still unclear. Moreover, there is still controversy over whether minerals deficiencies of iron, calcium, and zinc cause the pica or a consequence of its practice (Solyom et al., 1991; Boyle and Mackey, 1999). In addition, geophagy may cause several medical problems in human health, for example, helminthiasis (Abrahams, 1997),

malnutrition, dental injury (Federman et al., 1997), abdominal problem, and toxicity from minerals in soil such as lead, arsenic (Ziegler, 1997), mercury, and phosphorus (Federman et al., 1997). Furthermore, some bacteria, pesticides, and some radionuclides in soil may affect human body (Simon, 1997). Besides, geophagy is an important risk factor for orally acquired nematode infections in African children (Glickman et al., 1999).

**Table 1.1** Greek word for some specific forms of pica <sup>(1.1)</sup>

Greek word	Substance Ingested
Amylophagia	starch
Cautopyreiophagia	matches
Coniophagia	dust from venetian blinds
Geomelophagia	potatoes
Geophagia	clay, dirt
Gooberphagia	peanuts
Lectophagia	lettuce
Lithophagia	stones, pebbles, rock
Pagophagia	ice
Stachtophagia	ashes from cigarettes
Trichophagia	hair
Xylophagia	wood toothpicks

<sup>(1.1)</sup> Note from "Pica, Iron Deficiency, and the Medical History" Dennis F. Moore and David A. Sears, 1994, The American Journal of Medicine, 390-393.

It is worthwhile noting that there are two different opinions in the explanation the cause of pica. One group believed that minerals deficiencies might cause pica. This group included Abrahams and Parsons (1997), Moore and Sears (1994), Lanzkowsky (quoted in Abrahams and Parsons, 1996; Simon, 1997), Feldman (quoted in Abrahams and Parsons, 1997), Munoz et al. (1998) and Shisslak et al. (1999). These researchers supported the idea that deficiencies in nutrients such as iron and zinc were associated with pica. Moore and Sears (1994) noted that iron deficiency was usually the primary event and pica a consequence. For example, ice eating (pagophagia), which neither replaces calories nor impairs absorption, is a very common form of pica. The mechanisms whereby iron deficiency produces pica are not known, but iron supplementation relieves cravings rapidly, even before correction of the anemia. Munoz et al. (1998) also suggested that pica seemed to be a consequence of iron deficiency rather than its cause.

The other group considered mineral deficiencies as the consequence of pica. Eating soil will cause mineral deficiencies especially iron due to the interaction between soil minerals (Boyle and Mackey, 1999). Pica is thought by some researchers to cause anemia by binding dietary iron, making it useless to the body. The binding ability of clay comes from the cation exchange capacity (CEC). In general, the surfaces of clay particles in soil are negatively charged and are neutralized by electrostatic attraction of ions having positive charge. The mechanism of replacement between the surfaces of clay and cations is called cation exchange. For this reason, it is believed that soil eaten by human can adsorb some minerals, for example iron and zinc.



Because of the cation exchange processes in clay, many researchers supported the idea that geophagy led to mineral deficiencies. There was suggestive evidence from single case reported that the CEC of clay might also prevent the body from absorption of potassium and mercury. Therefore, it was speculated that the same mechanism might result in the inhibition of absorption of zinc deficiency (Halsted, 1968). In addition, humus, organic matter in soil, can inhibit iron absorption in human body, therefore, eating soil that contains humus can lead to iron deficiency in human. Generally, iron deficiency would cause anemia in human (Moynahan, 1979 quoted in Oliver, 1997).

Many studies had found that iron and zinc deficiencies were associated with geophagy in human. However, the exact relationship between minerals deficiencies and geophagy are not known. It is well established that trace elements, especially iron and zinc have many importance roles on human's health. Iron is the most abundant trace element in human body. Most of iron is found in red blood cells as a component of hemoglobin. Iron is also present in several enzymes responsible for electron transport, e.g., cytochromes, and for activation of oxygen, e.g., oxidases and oxygenases. The principle functions of iron in the body involve oxygen transport within blood and muscle, electron transfer in relation to energy metabolism such as transferring energy within cells and mitochondria. It is also intimately involved in cell proliferation, the production and disposal of oxygen radicals (and peroxide), systematic hormone action, and in some aspects of immune defense.

Zinc is a ubiquitous trace element in human and animal tissues and is connected with the function of numerous enzymes in all areas of metabolism. Zinc is necessary for the activity of more than 100 enzymes associated with carbohydrate and

energy metabolism, protein degradation and synthesis, nucleic acid synthesis, heme biosynthesis, carbon dioxide transport, and many other reactions. Based on these knowledges, many researchers are considering more on the hypothesis that mineral deficiency is really the cause of pica.

In Thailand, geophagy is commonly found in many provinces such as Phetchabun Province, Phitsanulok Province, Nakhon Ratchasima Province, Chaiyaphum Province (Sriood et al., 1999) and Sisaket Province (Komchadluek, 2001). However, the well known case is geophagy in Sisaket Province. Geophagy in Sisaket has been on the news for sometimes because Sisaket is the poorest province in Thailand. Many people are very poor and suffered from malnutrition. Every time the media persons find out some interesting things about this issue, they always make it a big headline. The villagers in Sisaket eat soil for variety of reasons for example eating soil as drug, practicing geophagy because of culture, and consuming soil as food when they do not have enough food to eat. Most villagers believe that soil can be used as traditional medicine for curing some diseases and providing them good health. This belief has passed on from generation to generation.

Since there is very little information concerning geophagy in Thailand so it is interesting to do research on this subject. In this study, the soil eaten by the villagers in Sisaket will be investigated chemically and physically. The chemical parameters to be studied will include the content of organic matter, CEC, soil pH, bioavailable iron, bioavailable zinc and elemental composition. The elemental compositions of pica soil are determined semiquantitatively by x-ray fluorescence spectroscopy technique. The bioavailable iron and zinc, the amount that can be absorbed by our body, will be analyzed quantitatively by atomic absorption spectroscopy technique. Certain

physical characteristics of pica soil such as color, moisture content, and loss on ignition will be investigated as well. The results of this study may help to support the hypothesis that pica soil could be a source of minerals, especially iron and zinc for human body. In addition, the results will provide more data concerning geophagy in Thailand.

## **1.2 Research objectives**

1. Attempts will be made to investigate the chemical composition of the soil samples, collected from the sites in the villages and from the market.
2. Attempts will be made to investigate selected physical characteristics of the soil samples from both sources.
3. To compare the characteristic parameters between the field samples and the samples bought from the market.

## **1.3 Research hypothesis**

Edible soil or pica might contain certain amount of essential metals for human body, especially iron and zinc.

## **1.4 Scope and limitation of the study**

The studies will be conducted on edible soil samples collecting from the sites specified by the villagers of Sisaket that it is the place where they used to get their edible soil and the soil samples bought from the market in Sisaket Province only.

## **1.5 Expected results**

1. The results will provide chemical properties, including pH, cation exchange capacity, elemental composition, bioavailable iron and zinc, and the percentage of organic matter of Sisaket edible soil.

2. The results will provide the physical properties of edible soil such as soil color and loss on ignition.
3. The results might support the assumption that soil could be the source of minerals, especially iron and zinc for human.
4. The results obtained from this thesis will be useful for the relevant organizations such as the Department of Public Health and the administrative personnels of Sisaket Province.

## **Chapter II**

### **Literature reviews**

Geophagy is a very complex behavior. It involves a large number of academic disciplines such as soil science, anthropology, medicine, and environmental geochemistry. Consequently, there are many journals and sources that relate to this topic such as journal in the field of anthropology, soil science, geology, geochemistry, medicine, environment, and zoology. A review of these materials will provide the background to understand more about geophagy.

Geophagy in human is commonly found worldwide. Not only in the under developing countries but also in some areas of developed countries. Many studies had been done about geophagy in various aspects, for example, geophagy as nutritional purposes (Abrahams, 1996), as medicinal purposes (Abrahams and Parsons, 1996; Abrahams, 1997), even the geophagy related to cultural or traditional believe also had been studied (Vermeer and Frate, 1979 quoted Simon, 1998). Some of the researchers also studied the chemical and physical aspects of pica soil (Abrahams, 1997; Abrahams and Parsons, 1997; Aufreiter et al., 1997; Mahaney et al., 2000).

Halsted (1968) suggested that it was an attractive theory that the earth eater was instinctively seeking some nutrient, such as a mineral, that was lacking in his diet. For example, the substance that many geophagists consumed was clay which had a high calcium content. This had been invoked to explain why pregnant

women might crave for clay. However, no objective or controlled data were available to indicate that geophagia occurred because certain minerals were lacking in the diet. He also commented that a plausible explanation of geophagia might be a sequence of events that were as follows: Every infant put things into his mouth. Exploration of the environment was normally via the oral cavity up to the age of about 18 months. Among primitive people the "floor" was usually the bare ground so that geophagia would practically be universal. At that age, the mother would normally be able to stop the child's activity in eating foreign substances. However, if she was an earth eater herself she might not feel this admonition to be important. In fact, she might even encourage it as beneficial. On the other hand, if she worked, or was otherwise unable to observe and control her child, geophagia was likely to continue and to become a habit.

Hunter and Kleine (1984) had done the analyses of pica soil eaten by pregnant women in the central of America. They concluded that geophagy in pregnancy provided a valuable supply of minerals and trace elements on an incremental, supplemental basis. The average rate of consumption of the holy clay (pica soil) tablets, supplied helpful amounts of calcium, potassium, magnesium, iron, zinc, copper, nickel, manganese, cobalt, and selenium, all of which were vital to the healthy growth and development of human fetus. They also commented that a tablet of pregnancy clay as a form of folk medicine compared favorably on an empirical basis with western pharmaceutical supplements, in terms of the limitations of poverty and opportunity in the developing world.

In the study of the detoxification and mineral supplementation as function of geophagy, Johns and Duquette (1991) used clay recovered from an archaeological site

occupied by *Homo erectus* and early *H. sapiens* and seven edible clay samples from Africa. They found out that two sample groups were indistinguishable mineralogically, in detoxification capacity, and in available minerals. African clay released calcium, copper, iron, magnesium, manganese, or zinc in amounts of nutritional significance from some clay samples but not from others. They used x-ray fluorescence technique for the analysis of total elements in pica soil. Identification of minerals in pica soil samples was carried out by x-ray diffraction technique whereas mineral elements released were measured by atomic absorption spectroscopy.

Recently, nutritional properties of pica soil were discussed by Mills in 1996. He stated that, " The eating of clay with high cation exchange capacity can release many elements under acid digestive conditions that can significantly supplement the intakes of available copper, iron, manganese, and zinc for some subsistence communities. They may also release macrominerals, the need for which may be the underlying stimulus to geophagia". (Mills, 1996 quoted in Ziegler, 1997).

Geissler et al. (1997) studied geophagy among school children in Western Kenya. They found out that 73% of the children practiced geophagy, eating soil from the surface of termitaria, the edges of paths and gullies, material from the wall of huts, and a chalk-like, soft stone, commonly found in the area. The median amount eaten reported was 28 g daily, ranging from 8 - 108 g.

Abrahams (1997) studied the geophagy and iron supplementation in Uganda. Pica soil samples were analyzed for soil color, mineral composition, pH, carbonate content, organic carbon, cation exchange capacity, total and bioavailable concentrations of 9 selected elements (calcium, sodium, manganese, potassium, lead, zinc, copper, and iron) using standard methodologies. He suggested that in the

country like Uganda where modern pharmaceuticals were either unobtainable or prohibitively expensive, ingested soil might therefore be very important as a mineral supplement for the health of individuals providing they were consumed sensibly.

Abrahams and Parsons (1997) studied the appraisal of three geophagical materials from the tropic countries: Thailand, Uganda, and Zaire. Soil samples from Thailand were consumed by the female of Akha tribe in the north during pregnancy, menstruation, and after child bearing. The samples were obtained from a village close to the Thai-Burmese border. Soil samples from Uganda and Zaire were purchased from a roadside in Kampala and a market at Mudake respectively. In Uganda, soil was wrapped in a document advertising its medicinal properties, for example, they could be used in the treatment for a variety of ailments, including coughs, stomach troubles, skin diseases, poisoning, and paralysis. In Zaire, soil was sold in a baked state and needed to be ground up before consumption. Pica soil could be supposedly alleviate a variety of ailments, for example, one - half of one teaspoonful of soil each day for 4 days was the recommended prescription for the treatment of smallpox. They had conducted a study on selected physical and chemical characteristics of the three soil samples. Physical characteristics were soil color and soil texture. Chemical characteristics were pH, organic matter content, cation exchange capacity. Available nutrients; calcium, copper, iron, magnesium, manganese, and zinc, were measured by atomic absorption spectrophotometry technique. Potassium and sodium were measured by flame photometry technique, whereas phosphorus was measured by visible spectrophotometry technique. They concluded that iron was a mineral nutrient, which could be supplied to humans in significant amounts via ingested soil.



They also commented that geophagy provided a direct link between the geochemistry of soil and human health.

Aufreiter et al. (1997) studied the geochemistry and mineralogy of pica soil eaten by humans on three continents. Soil samples were from China, United States, and Zimbabwe. China soil samples were used as famine food; United States soil was consumed for general health benefits; soil samples from Zimbabwe were used as medicine for digestive problems (diarrhea). Soil samples were analyzed for particle size, soil color, mineral composition, and total elements (macro, trace and rare earth elements). The technique used for analysis of total elements in soil was instrumental neutron activation analysis. Identification of minerals in soil samples was carried out by x-ray diffraction technique. Their studies revealed patterns of element levels and clay minerals relatively consistent with the effects desired or reported by consumers.

The review paper of soil ingestion by Simon in 1998 suggested that psychological and cultural associated with geophagy. Example of psychological factor that had been implicated with geophagia included mental retardation (Kanner, 1984 quoted in Simon, 1998), habit (Halsted, 1968), emotional neglect (Cooper, 1957 quoted in Simon, 1998), persistence of an infantile hand-mouth behavior (Robischon, 1971 quoted in Simon, 1998) and cultural tradition (Vermeer and Frate, 1979 quoted in Simon, 1998). Geophagia was sometimes associated with more primitive societies. For example, geophagy among Australian aboriginals was discussed by Bateson and Lebroy (Bateson and Lebroy, 1987 quoted in Simon, 1998). The reported on 11 cases of geophagia among the Australian aborigine which were examples of extreme consumption of white clay, often resulting in colon blockage or sometimes perforation. The purposes of clay ingestion were believed to cure

stomachaches and diarrhea or to prevent poisoning of some fishes. Clay was also eaten by the aborigines to allay their hunger or to treat hookworm infestation. In addition, they ate clay sometimes during celebrations when clay was used as a pigment for body painting.

Geissler et al. (1998) studied geophagy on iron status and anemia among primary school children in Western Kenya. The technique used for analysis of iron, zinc and aluminum was atomic absorption spectrophotometry. They found out that iron depletion and anemia were associated with geophagy, but only serum ferritin concentrations was shown to be dependent upon geophagy in the regression model studied. The results suggested that soil could provide an average 4.7 mg iron to a geophageous child which was equivalent to 32 percent of the Recommended Nutrient Intake (RNI) for girls or 42 percent of the RNI for boys.

Geissler et al. (1999) found that most of the pregnant women (73 %) in Kenyan coast ate soil regularly. Women mainly ate soil from the walls of houses and their estimated median daily ingestion was 41.5g. In addition, they found that soil eating was more than just a physiologically induced behavior; it was a rich cultural practice.

Smith et al. (2000) studied the bioaccessibility of essential and potentially toxic trace elements in tropical soil samples from Mukono District, Uganda. Chemical analyses of the major and trace elements were undertaken by wavelength dispersive x-ray fluorescent technique. Where the concentration of a particular element was below the detection limit, the analyte was determined by inductively couple plasma technique. They found that the median percent bioaccessibility values for cerium (1 % - 15 %), magnesium (7 % - 33 %), iron (0.1 % - 2.9 %), and other trace elements varied considerably, but were generally higher in geophagic soil (herbal-soil remedies

and termite nest soil) than common (ground) soil samples. However, only in the case of iron, did the ingestion of soil accounted for a major proportion of recommended daily intake.

Mahaney et al. (2000) had conducted a research on the mineral and chemical analyses of pica soil eaten by humans in Indonesia along with the control samples. Pica soil samples were analyzed for particle size, soil color, mineral composition, alkaloid adsorptive capacity and total elements (macro, trace and rare earth elements). The technique used in the analysis of total elements in soil samples was instrumental neutron activation analysis. Mineral composition in soil samples was identified by x-ray diffraction technique. The findings were as follows: (1) the eaten soil had a high content of hydrated halloysite and kaolinite, the clay minerals, that were pseudofoms of the pharmaceutical Kaopectate<sup>TM</sup>, (2) smectite was also present in much greater quantity than was usually found in Kaopectate<sup>TM</sup>, (3) sodium, manganese, potassium and sulfur were possible candidates driving this geophagy behavior, (4) iron, which was often higher in eaten soil, was relatively high, but did not have a higher concentration relative to the control samples, (5) cobalt and chromium, two important trace elements in human nutrition and diet, were marginally but not markedly higher in the eaten soil. In addition, they found that some soil samples could adsorb dietary toxins, presented in the plant diet or those produced by microorganisms.

As mentioned earlier, nutritional hypotheses were most commonly cited, which attributed geophagy to specific deficiencies of minerals such as iron and zinc (Rose et al., 2000). Many studies described patients with low iron or zinc level whose geophagy behavior diminished with iron and zinc replacement, although the empiric evidence for zinc was less convincing than it was for iron.

## **Chapter III**

### **Materials and methods**

#### **3.1 Sample collection and storage**

Sources of samples were as follows: sample 1 and 2 were purchased from local market in Muang District, Sisaket Province. Sample 3 was collected from Ban Yang Gud, Tambol Mak Kieb, Muang District, Sisaket Province. Sample 4 was collected from Ban Bo, Tambol Nong Chiang Toon, Prank Goo District, Sisaket Province. It seemed that sample 1 and 2 came from Ban Yang Gud site. All samples were stored in sealed plastic bags at 4 °C in a refrigerator until analysis.

#### **3.2 Cleaning of the glasswares and plasticwares**

All glasswares used in the analysis of bioavailable iron and zinc and cation exchange capacity were washed, rinsed, and then soaked in 10 % (v/v) nitric acid for 24 hours. Plasticwares were soaked in 1 % (v/v) nitric acid for 24 hours. Finally, before use all glasswares and plasticwares were rinsed with deionized water and left at room temperature for drying.

#### **3.3 Sample pretreatment and sample preparation**

Samples were pretreated according to Lagen (1996). After that the samples were divided into small fractions suitable for the analysis by the method of coning and quartering as explained by Radojevic and Bashkin (1999).

**Materials and equipment**

1. Plastic trays for drying sample
2. Spatula
3. Beakers: 1500 ml
4. Agate mortar and pestle
5. Standard sieve 2 mm ; Analysensieb, Retsch, USA
6. Analytical balance ; Model 250A, Precisa, Switzerland

**Procedure**

- About 100 g of soil sample were air - dried by spreading the sample evenly on a tray and kept in a dry and dust free place at room temperature for about 3 days. The sample was considered to be dried if the weight of soil sample between the third and fourth day did not change more than 5 % in 24 hours.
- Sieved the dried sample using a standard 2 mm sieve.
- Crushed the sample materials left on the sieve using the agate mortar until the particle size of the sample was less than 2 mm.
- Mixed the sample thoroughly for about 30 minutes in a 1500 ml beaker using a clean spatula.
- Poured the sample onto a clean plastic tray in a cone shape.
- Flatten the cone and formed a circular layer of soil.
- Divided the circular layer of soil into four even parts using a clean spatula and discarded the two opposite quarters.

- Combined the two retained parts and repeated the coning and quartering until obtaining about 10 g of soil sample, the size appropriated for the chemical and physical analyses.

### **3.4 Physical analyses**

Physical parameters are important in describing the soil properties. It is not only closely related to the chemical composition of soil but also related to many physicochemical and biological processes. In this study, the physical parameters measured, were soil color, moisture content, moisture correction factor, and loss on ignition at 900 °C. All parameters were investigated in triplicate, and reported only the average value.

#### **3.4.1 Soil color**

Soil color is one of the most commonly measured parameter for soil physical properties. It can be used to predict or estimate the chemical properties of soil. For example, white color soil contains carbonates of calcium and magnesium. On the other hand, soil becomes red or yellow because of iron oxide or hydroxide (Singer and Munns, 1999). Soil color is usually measured by comparing the soil color with color on a standard color chart. The collection of color chart generally used with soil is a modified version of the collection of chart appearing in the Munsell Book of Color and includes only the portion needed for soil color identification.

The Munsell notation identifies color by using three variables: hue, value, and chroma. Hue is the dominant spectral color, that is, whether the hue is a pure color, or a mixture of pure colors. Mixtures are identified numerically according to the amount of pure colors used to produce the mixture. For example, 5YR is an equal mixture of yellow and red. As the number increases, the amount of the first color (yellow)

increases and the second color (red) decreases. Value and chroma are terms that refer to how the hue is modified by the addition of gray. Value is the degree of lightness or brightness of the hue reflected in the property of the gray color that is being added to the hue with 0 being black and 10 white. Chroma is the amount of gray of a particular value that is mixed with the pure hue to obtain the actual soil color. The lower the chroma, the closer the color is to the pure gray of that value.

#### **Materials and equipment**

1. Munsell color chart
2. Spatula

#### **Procedure**

- Compared the color of air dried soil samples with Munsell color chart and reported in the letter code.
- The intensity of light may affect the results of this observation so the color comparison should be done with enough light.

#### **3.4.2 Soil moisture content and moisture correction factor**

The measurement of water or moisture content is important in the analysis of soil. Water plays many essential roles in soil system. For example, water acts as a solvent and transporting agent for numerous substances. It provides a medium in which chemical reactions can proceed.

The gravimetric method is used for the analysis of moisture content (Lagen, 1996). In this method, moisture content is the mass of water which can be removed from the soil by heating at 105 °C for 12 hours, expressed as a percentage of the dry mass. The moisture content is calculated based on the oven dried sample weight.

### Materials and equipment

1. Analytical balance ; Model 250A, Precisa, Switzerland
2. Oven ; Model 400, Memmert, Germany
3. Porcelain crucibles
4. Tongs
5. Desiccator

### Procedure

- Heat the crucibles in the oven at 105 °C for 2 hours to constant weight.
- Cooled down to room temperature in a desiccator for 1 hour.
- Weighed each empty crucible (A, g).
- Added approximately 2 g of soil sample into the preweighed dry crucible and reweighed the crucible (B, g).
- Heat the crucibles in the oven at 105 °C for 12 hours to constant weight then transferred the crucibles to a desiccator and allowed to cool to room temperature for 1 hour.
- Reweighed the crucibles (C, g).

### Calculation

$$\text{Moisture content (M\%)} = \frac{(B - C) \times 100\%}{(C - A)} \dots\dots\dots(3.1)$$

$$\text{Moisture correction factor (mcf.)} = \frac{(100 + M\%)}{100} \dots\dots\dots(3.2)$$

#### 3.4.3 Loss on ignition (L.O.I)

Loss on ignition is the mass of water, organic matter and other substances which were removed from the soil after heating at 900 °C, expressed as a percentage of the ignite mass. Loss of sample mass on ignition can be served as a rough



indication the amount of organic matter present in soil samples (Radojevic and Bashkin, 1999). Usually, organic matter is estimated by igniting the soil samples at 550 °C. But the carbonate compounds in soil are also decomposed at high temperature, so the result obtained is usually higher than the exact value. Measurement of L.O.I was being done according to Lagen (1996).

### **Materials and equipment**

1. Muffle furnace ; Model A-550, Vulcan<sup>TM</sup>, USA
2. Porcelain crucibles
3. Analytical balance ; Model 250A, Precisa, Switzerland
4. Tongs
5. Desiccator

### **Procedure**

- Heat the crucibles in the oven at 105 °C for 2 hours to constant weight.
- Cooled down to room temperature in a desiccator for 1 hour.
- Weighed each empty crucible (A, g).
- Added 2.0000 g soil sample to the crucible.
- Heat the crucibles in the oven at 105 °C for 12 hours to constant weight then transferred the crucibles to a desiccator and allowed to cool to room temperature for 1 hour.
- Reweighed each crucible (C, g).
- Placed the crucibles with soil sample in the muffle furnace, gradually increased the temperature to 900 °C, and maintained this temperature for 4 hours.

- Turned off the furnace, allowed the crucibles to cool down in the furnace for about 3 hours to the temperature that enable to handle the crucible with tongs.
- Removed the crucibles from the furnace and allowed to cool to room temperature in a desiccator for 1 hour.
- Reweighed each crucible (D, g).

### Calculation

$$\text{L.O.I (\%)} = \frac{(C - D)}{(C - A)} \times 100\% \dots \dots \dots (3.3)$$

## 3.5 Chemical analyses

In this study, the following parameters were being investigated.

- Soil pH
- Percent organic matter content
- Cation exchange capacity
- Elemental composition
- Bioavailable iron and zinc

The parameters were investigated in triplicate for each sample and reported only the average value.

### 3.5.1 Soil pH

Soil pH is the first chemical parameter measured in this study. In general, it can be defined as the pH of water in equilibrium with soil (Singer and Munns, 1999).

The standard method was used for the soil pH measurement (EPA, 1995).

**Materials and equipment**

1. pH meter with combination pH electrode ; Model 50215: HACH, USA
2. Beakers : 50 ml
3. Magnetic stirrer with magnetic bar
4. Analytical balance ; Model 250A, Precisa, Switzerland

**Reagents**

1. Standard buffers solution pH 4, 7, and 10 ; Sigma Chemicals, USA
2. Deionized water

**Procedure**

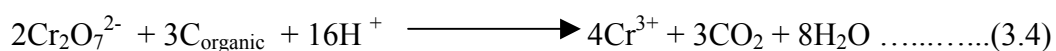
- Calibrated pH meter using standard buffer solutions according to the manufacturer's direction.
- Added 20.0000 g of air dried soil sample into 50 ml beaker.
- Added 20 ml of water and stirred for 5 minutes.
- Let the soil suspension stood for about 1 hour to allow most of the suspended clay to settle out from the suspension.
- Took three pH readings of the clear supernatant solution.
- If the sample temperature differed by more than 2 °C from the buffer solution, the measured pH values should be corrected.
- Reported the results as "soil pH measured in water at the temperature the test was conducted".

**3.5.2 Organic matter content**

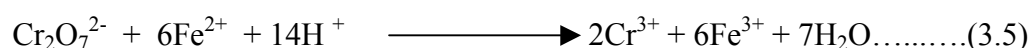
Organic matter is the fraction of plant, organism, and animal residues remained after chemical, biological and physical decomposition of the soil. As a result of decomposition, inorganic nutrients (N, P, K, Ca, Mg, Fe, Cu, Zn and Mn) in plant and

animal tissues are released into the soil. Organic matter affects the physical, chemical, and biological properties of soil. Physically, organic matter increases darker color to soil, increases bulk density and improves aggregation of soil particles. Chemically, organic matter increases cation exchange capacity because soil organic matter has a high specific surface area as great as  $800 - 900 \text{ m}^2\text{g}^{-1}$  (Sparks, 1995) Biologically, organic matter is the main source of food for microorganism. Organic matter in soil is determined by dichromate method or wet oxidation method (Radojevic and Bashkin, 1999), in which the organic carbon will be completely oxidized by excess dichromate in acid condition. The excess dichromate is determined by back titration with ferrous sulfate using ferroin as indicator. Finally, the organic matter content is obtained by multiplying the organic carbon by the factor 1.72. The factor of 1.72 is due to the fact that soil organic matter generally has a carbon content of about 58 % (i.e.  $1/0.58 = 1.72$ )(Radojevic and Bashkin, 1999).

In the determination of organic carbon, a known excess of  $\text{K}_2\text{Cr}_2\text{O}_7$  is added to the soil sample together with  $\text{H}_2\text{SO}_4$ . The organic carbon is oxidized to  $\text{CO}_2$  according to the reaction:



The unreacted excess of dichromate remaining after the reaction is determined by back titration with ferrous sulfate:



The amount of organic carbon is calculated by the difference between the amount of  $\text{Cr}_2\text{O}_7^{2-}$  in equations (3.4) and (3.5).

**Materials and equipment**

1. Reflux apparatus : 500 ml round bottom flask, condenser, and heating mantle (Figure 3.1)
2. Beakers : 50 ml
3. Volumetric flasks : 100 ml, 1 liter

**Reagents**

1. Potassium dichromate ( $K_2Cr_2O_7$ ) ; Sigma Ultra (99.5 %), Sigma Chemicals, St. Louis, USA  
  
0.0830 M  $K_2Cr_2O_7$   
  
Dried  $K_2Cr_2O_7$  in an oven at 105 °C for 2 hours. Dissolved 24.5180 g in water and diluted to 1 liter in a volumetric flask. This solution was stable indefinitely.
2. Ferrous sulfate ( $FeSO_4 \cdot 7H_2O$ ) and 1,10-phenanthroline monohydrate ; A.C.S. reagents, Aldrich Chemicals, Milwaukee, WI, USA  
  
Ferroun indicator  
  
Dissolved 0.7000 g  $FeSO_4 \cdot 7H_2O$  and 1.4850 g 1,10- phenanthroline monohydrate in water then diluted to 100 ml in a volumetric flask.
3. Concentrated  $H_2SO_4$  ; Analytical Reagent , Labscan Asia, Thailand
4. Ferrous ammonium sulfate ( $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ ) ; A.C.S. reagent, Aldrich Chemicals. Milwaukee, WI ,USA  
  
0.2000 M  $Fe(NH_4)_2(SO_4)_2$   
  
Dissolved 78.3900 g  $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$  in water then added 20 ml concentrated  $H_2SO_4$ , cooled and diluted to 1 liter in a volumetric flask.

### Procedure

- Weighed 0.5000 g (W, g) soil sample into a refluxing flask.
- Added 10 ml 0.0830 M  $K_2Cr_2O_7$  to the refluxing flask then carefully added 15 ml concentrated  $H_2SO_4$  and swirled gently to mix.
- Connected a refluxing flask to the condenser then turned on cooling water and covered the open end of condenser with a small beaker.
- Refluxed for 1 hour using the heating mantle.
- Cooled and rinsed down the condenser with deionized water, collected the water in refluxing flask. Disconnected the refluxing flask and added about 100 ml deionized water into the refluxing flask.
- Swirled to mix the solution and added 5 drops of ferroin indicator.
- Titrated the solution in the refluxing flask with ferrous ammonium sulfate to the end point, at which the color changed from blue-green to violet-red, recorded the volume of ferrous ammonium sulfate used ( $V_1$ , ml).
- Repeated the experiment with the blank measurement using the same reagents but without the soil sample then refluxed and titrated the blank, using the same procedure as soil sample.

### Calculation

$$\text{Organic carbon (mg g}^{-1}\text{)} = \frac{0.3 \times C \times (V_2 - V_1) \times \text{mcf}}{W} \dots\dots\dots(3.6)$$

where

0.3 = correction factor

C = concentration of ferrous ammonium sulfate  
(0.2041 M)

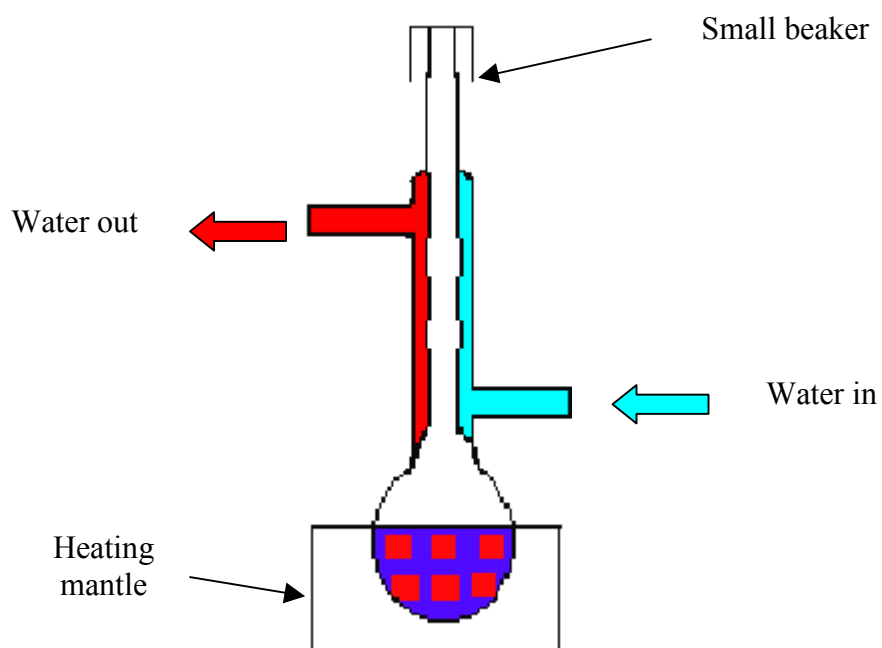
$V_1$  = volume of ferrous ammonium sulfate used  
in the sample determination (ml)

$V_2$  = volume of ferrous ammonium sulfate used  
in the blank determination (ml)

$W$  = weight of sample used (g)

Organic matter (%) =  $1.72 \times$  Organic carbon (%).....(3.7)

The factor of 1.72 is due to the fact that soil organic matter has a carbon content of about 58 % (i.e.  $1/0.58 = 1.72$ ).



**Figure 3.1** Reflux apparatus for the determination of organic matter.

### 3.5.3 Cation exchange capacity

Clay and humus in soil consist of colloidal particles with very large surface areas which have electrical charges associated with their surfaces. Most of the clay particles are negatively charged. The surface charges are usually neutralized by electrostatic attraction of ions having opposite charges. The ions which are bound to clay mineral surfaces by weak electrostatic forces can be replaced by ion exchange processes. Cations which are generally held by electrostatic forces on soil particles are  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ , with increasing  $\text{H}^+$  and  $\text{Al}^{3+}$  in acid soil, and  $\text{Na}^+$  in saline soil. The CEC of soil is defined as its capacity to adsorb and exchange cations. The unit of CEC is expressed in milliequivalent (meq) per 100 g soil. In this study, the CEC was determined according to Lagen (1996). There were three steps in the procedure: (1) removing of the adsorbed cations on the surface of clay particles by treating with excess  $\text{Ba}^{2+}$ , (2) removing the adsorbed  $\text{Ba}^{2+}$  by adding known excess  $\text{Mg}^{2+}$ , (3) measuring  $\text{Mg}^{2+}$  lost from the exchange with  $\text{Ba}^{2+}$  as the CEC using the flame atomic absorption spectroscopic technique.

#### Materials and equipment

1. Filter paper ; Whatman No. 1
2. Shaker ; Model GFL 3006, Germany
3. Centrifuge ; Model Universal 30RF, Hettich, Germany
4. Centrifuge tubes : 50 ml with screw caps
5. Micropipet ; Model Pipet Man, Gilson Medical Electronics, France
6. Volumetric pipet
7. Volumetric flasks : 50 ml, 100 ml, 1 liter
8. Graduated cylinder : 100 ml



9. Flame atomic absorption spectrophotometer ; Model Spectra 250 plus,  
Varian, Australia

### Reagents

1. Barium chloride ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ) ; Sigma Chemicals, St. Louis, USA  
0.1000 M  $\text{BaCl}_2$   
Dissolved 24.4000 g  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  in water and transferred to 1 liter volumetric flask. Made up to volume with deionized water.  
0.0025 M  $\text{BaCl}_2$   
Pipetted 25.00 ml of 0.1000 M  $\text{BaCl}_2$  into 1 liter volumetric flask and made up to volume with deionized water.
2. Magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ); Aldrich Chemicals, Milwaukee, USA  
0.0200 M  $\text{MgSO}_4$   
Dissolved 4.9296 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in deionized water and transferred to 1 liter volumetric flask. Made up to volume with deionized water.
3. Lanthanum chloride ( $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ ) ; Sigma Chemicals, St. Louis ,USA  
5.00 % lanthanum solution  
Weighed 133.7000 g of  $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$  into 1 liter beaker. Added about 800 ml water and then carefully added 10 ml HCl 37 %. Allowed to cool and transferred into 1 liter graduated cylinder then made up to volume with deionized water.
4. Hydrochloric acid 37 % ; Analytical Reagent, Labscan Asia. Ltd, Thailand
5. Mg atomic absorption standard solution, 1018 ppm of Mg in 1 wt.%  $\text{HNO}_3$  d 1.010 ; Aldrich Chemicals, Milwaukee, USA  
101.8 ppm Mg

Pipetted 10.00 ml of 1018 ppm Mg and transferred to 100 ml volumetric flask then made up to 100 ml with deionized water.

### **Procedure**

- Weighed 2.5000 g (W, g) soil into 80 ml centrifuge tube with screw cap. Weighed and recorded the weight of centrifuge tube with soil sample (FW, g).
- Added 30 ml of 0.1000 M BaCl<sub>2</sub> to the centrifuge tube, placed centrifuge tube with screw cap on the shaker and shook for 1 hour at 200 /minute.
- After shaking, placed the centrifuge tube with screw cap in the centrifuge and centrifuged for 15 minutes at 9000 rpm.
- Decanted the supernatant (the supernatant was not needed for analysis) then repeated the shaking and centrifugation steps two times, decanted the supernatant each time.
- Added 30 ml of 0.0025 M BaCl<sub>2</sub> to the centrifuge tube and shook for 12 hours at 200 /minute.
- After shaking, centrifuged for 15 minutes at 9000 rpm. Decanted the supernatant. Weighed the centrifuge tube again (SW, g).
- Added 30 ml of 0.0200 M MgSO<sub>4</sub> to the centrifuge tube and shook for 2 hours at 200 /minute.
- After shaking, centrifuged for 15 minutes at 9000 rpm. Decanted the supernatant over a coarse filter paper (Whatman No. 1) into 250 ml erlenmeyer flask.
- Determined the amount of Mg in the filtrated solution using the flame atomic absorption spectroscopic technique with standard addition method.

- Diluted the filtrated solution to appropriated concentration. In this study it was diluted 2500 times to get the Mg concentration in the linear range of the relationship between absorbance and concentration.
- Pipetted 2.00 ml of the filtrated solution into 50 ml volumetric flask and made up to volume with deionized water.
- Pipetted 0.50 ml portions of the diluted filtrated solution into two 50 ml volumetric flasks and added exactly 0, 0.05 ml of 101.8 ppm Mg to each volumetric flask respectively.
- Added 2.0 ml 5 % lanthanum solution into each volumetric flask, made up to volume with deionized water. The standard addition will be 0 ppm Mg and 0.102 ppm Mg respectively.
- Measured the absorbance of the two solutions by flame atomic absorption spectrophotometer.
- Instrument parameters of atomic absorption measurements were as follows:

Wavelength	285.2 nm
Lamp current	4 mA
Slit width	0.5 nm
Air flow	13.0 l/min
Acetylene flow	2.00 l/min
- Calculated the concentration of Mg from the absorbance measured.

### 3.5.4 Elemental composition

Fusion technique was used in the preparation of soil sample for the analysis of elemental composition by XRF technique. Soil samples were mixed with glass-forming lithium tetraborate and fused by a fusion machine. In this technique, the sample was homogeneously dissolved in the borate-flux. After cooling/casting, the borate disk could be analyzed for the elements content by wavelength dispersive x-ray fluorescence (WDXRF) spectrometer.

#### Materials and equipment

1. Bis-crucible Pt/Au alloys, volume 25 ml, net weight 26 g ; Claisse 252, Chemin Saite-Foy, Canada
2. Pt/Au alloys mold, diameter 32 mm, net weight 21 g ; Claisse 252, Chemin Saite-Foy, Canada
3. Fusion machine, the Claisse Fluxer – Bis<sup>TM</sup> ; Claisse 252, Chemin Saite-Foy, Canada
4. Wavelength dispersive x-ray fluorescence spectrometer ; MagiXPro PW 2404, Philips Analytical X - Ray, Almelo, Netherlands
5. Agate mortar and pestle
6. Standard sieve 75  $\mu\text{m}$  ; Analysensieb, Retsch, USA

#### Reagents

1. Lithium tetraborate anhydrous,  $\text{Li}_2\text{B}_4\text{O}_7$  ; Claisse 252, Chemin Saite-Foy, Canada
2. Lithium bromide, LiBr ; Claisse 252, Chemin Saite-Foy, Canada

3. Standard Reference Materials from NIST (National Institute of Standard and Technology, Department of commerce, USA)
  - Standard Reference Material 2709 (San Joaquin Soil)
  - Standard Reference Material 98b (Plastic Clay)
  - Standard Reference Material 600 (Bauxite)
  - Standard Reference Material 679 (Brick Clay)
4. Reference material Kaolin ; MBH Analytical Ltd., UK

#### **Procedure**

- Added 10.0000 g of soil sample into agate mortar and grounded. Sieved the soil sample over 75 $\mu$ m sieve. This fine soil was used in disk preparation.
- Added 1.0000 g soil sample into Bis-crucible.
- Weighed 7.0000 g  $\text{Li}_2\text{B}_4\text{O}_7$  and 0.0300 g of LiBr (used as wetting agent) into the Bis-crucible.
- Carefully mixed the soil sample  $\text{Li}_2\text{B}_4\text{O}_7$  and LiBr in the crucible using a clean spatula.
- Placed the Bis-crucible with mold in the fusion machine (Figure 3.2).
- The fusion machine was pre-programmed for power and fusion time according to the instruction manual.
- After cooling, solidification, and casting, weighed and recorded the weight of the disk which was used for WDXRF analysis.
- Prepared the disk of all the standard reference materials with the same procedure as the soil sample.



**Figure 3.2** Fusion machine with Bis-crucible.



**Figure 3.3** Bis-crucible and mold with disk samples.



**Figure 3.4** Wavelength dispersive x-ray fluorescence spectrometer,  
MagiX Pro PW 2404 Philips Analytical.

### **3.5.5 Bioavailable iron and zinc**

Iron is the second abundant metal (about 5 %) in the earth's crust after aluminum (McDowell, 1992). Iron is a transition metal with an atomic weight of 56. It has two stable oxidation states (+2 and +3) and several unstable oxidation states in aqueous solution, and widely redox potentials depending on the ligands (McDowell, 1992). This property makes iron complexes useful in electron- transfer reactions. Iron concentration in soil ranges from 7,000 to 550,000 mg/kg (Hassett and Banwart, 1992).

Iron is a component of every living organism and it has been said that, "Every living cell whether plant or animal, contains iron" (Sizer and Whitney, 2000). Adult humans (70 kg) are estimated to contain 4 - 5 g iron or 60 - 70 ppm of the whole body (McDowell, 1992). Most body iron exists in complex forms bound to protein, either

as porphyrin or heme compounds, particularly hemoglobin and myoglobin, or as nonheme protein bound complexes such as transferrin, ferritin and hemosiderin. Iron absorption occurs throughout the gastrointestinal tract. The major sites are the duodenum and jejunum. Iron is excreted through the skin, gastrointestinal tract, and the urinary tract.

The 1989 RDA (Recommended Daily Dietary Allowance) of iron for the healthy Thais is 12 mg/day for young boys (10 - 12 years), 15 mg/day for young girls (10 - 12 years), 10 mg/day for men (20 - 29 years), and 15 mg/day for women (20 - 29 years)(The Committee on Recommended Daily Allowances, 1989).

Zinc is the twenty-fourth in abundance of the chemical elements. Zinc is a divalent cation, with an atomic number of 30 and atomic weight of 65.37. Its concentration in the soil depends largely on the parent material. In general, zinc is found associated with sulfide of other metals, including lead, copper, cadmium, and iron (McDowell, 1992).

The common range for total zinc concentration in normal soil is between 10 and 300 mg/kg (Hassett and Banwart, 1992). Zinc plays an important role in human body because it is a component of more than 100 metalloenzymes such as carbonic anhydrase, alcohol dehydrogenase, superoxide dismutase, DNA-polymerase, RNA-polymerase, alkaline phosphatase, and carboxy-peptidase. Because of these, zinc is essential for DNA synthesis, RNA transcription, cell division and cell activation. Moreover, zinc is also necessary for the structural stabilization of organic tissues and membranes. Zinc is found in bone, liver, kidney, muscle and skin.

The main site for zinc absorption in the gastrointestinal tract is the proximal small intestine, especially the jejunum. The excretion of zinc in human body is



through feces, urine and sweat. The major route of excretion is via feces, with smaller amount eliminated by urine. The normal body contains about 2.2 g zinc, with an overall concentration of about 30 ppm (McDowell, 1992). The 1989 RDA of Zn for Thais is 15 mg/day for young boys (10 - 12 years), 15 mg/day for young girls (10 - 12 years), 15 mg/day for men and women (20 - 29 years) (The Committee on Recommended Daily Allowances, 1989).

In this study, bioavailable iron and zinc are defined as the amount of iron and zinc that can be absorbed by human body through the intestinal system. The bioavailable iron and zinc were determined according to Geissler et al. (1998). The condition used for extraction of bioavailable iron and zinc was the simulation of human digestion systems, using the equivalent pH of the stomach and the temperature was maintained at a constant 37 °C, the body temperature.

#### **Materials and equipment**

1. Shaker with thermostated water bath ; Model SWB 5050, National Lanet, Denmark.
2. Atomic absorption spectrophotometer ; Model Spectra 250 plus, Varian, Australia
3. Micro pipet ; Model Pipet Man, Gilson Medical Electronics, France
4. Filter paper ; Whatman No 42
5. Aluminium foil

#### **Reagents**

1. Hydrochloric acid 37 % ; Analytical Reagent , Labscan Asia , Thailand
2. Fe, Zn standard solutions ; Aldrich Chemicals, Milwaukee WI, USA
  - Fe atomic absorption standard solution, contains 1020 ppm of Fe in

1 wt. % HCl,  $d = 1.010$ .

- Zn atomic absorption standard solution, contains 985 ppm of Zn in

1 wt. % HCl,  $d = 1.010$ .

### 3. Preparation of Zn and Fe standard solutions

98.5 ppm Zn

Pipetted 10.00 ml 985 ppm Zn into 100 ml volumetric flask then made up to 100 ml with deionized water.

9.85 ppm Zn

Pipetted 10.00 ml 98.5 ppm Zn into 100 ml volumetric flask then made up to 100 ml with deionized water.

102.0 ppm Fe

Pipetted 10.00 ml 1020 ppm Fe into 100 ml volumetric flask then made up to 100 ml with deionized water.

### **Determination of bioavailable iron**

#### **Procedure**

- Weighed 10.0000 g (W, g) soil sample into 250 ml erlenmeyer flask.
- Added 100 ml 0.10 M HCl and covered the flask with aluminum foil.
- Placed the flask on the shaker with thermostated water bath set at 37 °C and shook for 2 hours at 180 /minute.
- Decanted the solution over a filter paper into 100 ml graduated cylinder, recorded the volume of the solution (V,ml).
- Pipetted 10.00 ml of the solution from the graduated cylinder into four 50 ml beakers

- Measured the amount of Fe in the solution using the atomic absorption spectroscopic technique with standard addition method.
- Added exactly 0, 0.50, 1.00, and 1.50 ml of standard 102.0 ppm Fe into each of the four 50 ml beakers.
- Measured the absorbance of the four solutions by flame atomic absorption spectrophotometer.
- Calculated the concentration of Fe from the standard addition curve.
- Instrument parameters for atomic absorption measurements were as follows:

Wave length	372.0 nm
Lamp current	5 mA
Slit width	0.2 nm
Air flow	20.0 l/min
Acetylene flow	2.00 l/min

### Calculation

$$\text{Bioavailable iron (mg/kg)} = \frac{\text{Fe} \times \text{V}}{\text{W}} \dots \dots \dots (3.8)$$

where

Fe = concentration of Fe from standard addition curve (ppm)

V = volume of sample (ml)

W = weight of sample (g)

## **Determination of bioavailable zinc**

### **Procedure**

- Weighed 10.0000 g (W, g) soil sample into 250 ml erlenmeyer flask.
- Added 100 ml 0.10 M HCl and covered the flask with aluminum foil. The pH of the solution was the pH of gastric juice.
- Placed the flasks on the shaker with thermostated water bath set at 37 °C and shook for 2 hours at 180 /minute.
- Decanted the solution in the flask over a filter paper into 100 ml graduated cylinder, recorded the volume of the solution (V, ml).
- Pipetted 10.00 ml of solution from graduated cylinder into four 50 ml beakers.
- Measured the amount of Zn in the solution using the atomic absorption spectroscopic technique with standard addition method.
- Added exactly 0, 0.05, 0.10, and 0.15 ml of standard 9.85 ppm Zn into each of the four 50 ml beakers.
- Measured the absorbance of the four solutions by flame atomic absorption spectrophotometer.
- Calculated the concentration of Zn from the standard addition curve.
- Instrument parameters for atomic absorption measurements were as follows:

Wave length	213.9 nm
Lamp current	5 mA
Slit width	1.0 nm
Air flow	20.0 l/min



## **Chapter IV**

### **Results and Discussions**

The major aim of this study was to investigate the physical and chemical properties of pica soil from different sources in Sisaket Province in the northeast of Thailand. This chapter showed the results and discussions on the physical and chemical properties of pica soil samples under investigation.

#### **4.1 Physical analyses**

##### **4.1.1 Soil color**

Color is one of the most noticeable characteristics of soil. Color is important because it is related to organic matter content, climate, soil drainage, and soil mineralogy. The colors of the soil are strongly influenced by iron compounds and humus or organic matter. Red, yellow and gray color of soil are usually due to the concentration and the form of iron present. For example,  $\text{Fe}_2\text{O}_3$  (ferric oxide) makes the soil red and  $\text{Fe}_2\text{O}_3 \cdot x\text{H}_2\text{O}$  (hydrated ferric oxide) gives the soil yellowish - brown in color while black color may be due to the organic matter content in soil. So soil color is also used to estimate the organic matter present in soil as shown in Table 4.1. The colors of dry soil and other physical parameters are shown in Table 4.2. The color of the soil samples ranged from 7.5YR8/3 (sample 4) to 10YR6/1 (sample 2). The color code is the color based on the Munsell system which describes color in terms of three variables - hue, value/chroma as mentioned previously.

**Table 4.1** Relationship between soil color and organic matter content in soil <sup>(4.1)</sup>

Munsell notation for moist soil color	Percent organic matter	
	Range	Average
10YR2/1	3.5-7.0	5.0
10YR3/1	2.5-4.0	3.5
10RY3/2	2.0-3.0	2.5
10RY4/2	1.5-2.5	2.0
10YR5/3	1.0-2.0	1.5

<sup>(4.1)</sup>Note from Soils & their environment (22), John J. Hassett and Wayne L. Banwart, 1992, New Jersey: Prentice Hall.

High chroma gives an impression of being 'bright' or 'colorful'. Sample 4 with chroma 3 was slightly brighter than samples 1, 2, 3 which had chroma 1. The results also suggested that sample 4 should contain higher concentration of iron compounds than other samples. This was later found out to be true when doing the measurement of elemental concentration of the soil samples (Table 4.4)

**Table 4.2** Physical parameters and site description of pica soil samples from Sisaket

Samples	Collecting site	Color of dry soil	Moisture content (%)	Moisture correction factor (%)	Loss on ignition (%)
1	From local market	10YR5/1	1.42	1.01	7.26
2	From local market	10YR6/1	2.15	1.02	7.90
3	The pit has water through the year even in summer	10YR5/1	6.35	1.06	9.19
4	Near the small water way that cut through the rice field	7.5YR8/3	4.36	1.04	9.98



Figure 4.1 Map of Sisaket Province.





**Figure 4.2** Pica soil sample 1, local market in Muang District, Sisaket Province.



**Figure 4.3** Pica soil sample 2 ,local market in Muang District, Sisaket Province.



**Figure 4.4** The site for collecting pica soil sample 3, Ban Yang Gud, Tambol Mak Kieb, Muang District, Sisaket Province.



**Figure 4.5** The site for collecting pica soil sample 4, Ban Bo, Tambol Nong Chiang Toon, Prank Goo District, Sisaket Province.



**Figure 4.6** The villagers demonstrated how to eat pica soil by roasting it before eating.



**Figure 4.7** The villagers demonstrated eating pica soil.

#### **4.1.2 Moisture content and moisture correction factor**

Soil sampled from the field contains water, the amount depending on soil properties and the preceding weather conditions. Even after being air - dried, some water still remains in the soil, the amount depending on soil texture and the humidity of the air in the drying room. The water content or the moisture content of soil samples is determined by drying at 105 °C for 12 hours in the oven. The moisture content is the mass of water which can be removed from the soil by that heating treatment, expressed as a percentage of the dry mass. The results of moisture contents as listed in Table 4.2 show that samples 1, 2 which were bought from the local market and had been kept for sometime had less water (1.42%, 2.15%) than samples 3, 4 (6.35%, 4.36%) which were collected from the field. Since the collecting site of sample 3 is a pit filled up with water all year round, the moisture content of sample 3 is the highest. Moisture correction factor ranged from 1.01 % (sample 1) - 1.06 % (sample 3).

#### **4.1.3 Loss on ignition (L.O.I.)**

Loss on ignition is the mass of water, organic matter and other substances, which can be removed from soil by igniting at 900 °C for 12 hours in the furnace, expressed as a percentage of the ignited mass. Soil which contains appreciable quantities of clay and sesquioxides loses structural water between 105 °C and 500 °C. For example goethite,  $\text{FeOOH}$ , is dehydrated to hematite,  $\text{Fe}_2\text{O}_3$ , at between 280 °C and 400 °C. Most of the organic matter is burnt off at about 325 °C, but losses may continue at higher temperature. Calcium carbonate loses  $\text{CO}_2$  to form calcium oxide from about 770 °C. Thus loss on ignition is an approximate measure of organic matter content in sandy soil but may be up to twice the organic matter content in heavy

textured soils. Loss on ignition of the samples are illustrated in Table 4.2. Loss on ignition ranged from 7.26% (sample1) to 9.98% (sample 4).

## **4.2 Chemical analyses**

### **4.2.1 Soil pH**

Soil pH is a pH of water in equilibrium with soil solution. Soil pH plays important roles in many chemical processes and activities of microorganisms that survive in soil. There are many factors that relate to soil pH, including parent materials, climate, vegetation, fertilizer and lime application (Radojevic and Bashkin, 1999). In general, Soil pH ranges from pH 4 (extremely acidic) to about pH 10 (very strongly alkaline).

The results of soil pH are displayed in Table 4.3. Soil pH ranged from 4.30 (sample 1) to 4.95 (sample 3). These results suggest that all pica soil samples are strongly acidic so the taste of pica soil is sour. Acidic soil which has pH 4 to 5 usually dissolves high, even toxic, concentrations of soluble metal ions. This will provide more available metal ions for the geophagists which may cause health problems if they consumed a lot of soil. However, soil color and pH are important to some geophagists, which have already been noted by a number of research workers (for example, soil pH imparts a sour (acid) or sweet (alkaline) taste to the material consumed) (Laufer, 1930 quoted in Abrahams and Parsons, 1997).

**Table 4.3** Chemical parameters of pica soil samples from Sisaket Province.

Samples	pH	Organic matter (%)	Cation exchange capacity ( cmol (+)/ kg )
1	4.30	1.78	2.14
2	4.41	2.33	12.65
3	4.95	2.73	12.17
4	4.79	0.28	5.54

#### 4.2.2 Organic matter content

Soil organic matter plays important roles in soil forming processes especially with respect to pH. Carbondioxide produced during the decomposition of organic matter results in the building up carbonic acid, which in turn lowers soil pH (Radojevic and Bashkin, 1999). Soil organic matter has a high specific surface area (as great as 800 m<sup>2</sup>/g - 900 m<sup>2</sup>/g) and CEC from 150 cmol/kg - 300 cmol/kg. Thus the majority of a surface soil's CEC is in fact attributable to soil organic matter. Due to high specific surface area and CEC of soil organic matter, it is an important sorbent of ions including toxic metal cations, organic materials such as pesticides which will cause ill effect for the geophagists. The results of pica soil organic matter are shown in Table 4.3. All four samples had low organic matter contents which were between 0.28 % (sample 4) to 2.73 % (sample 3). This finding is similar to the results obtained from pica soil being investigated by Abrahams and Parson (1997). Their samples contained 0.2 % -1.5 % organic matter contents.

#### 4.2.3 Cation exchange capacity

Clay and humus in soil consist of colloidal particles with very large surface areas and these have electrical charges associated with their surfaces. Most of the clay

particles are negatively charged, except in tropical acid soil where they may be charged positively which is the case of the Sisaket pica soil under investigated. The surface charges are usually neutralized by electrostatic attraction of ions having opposite charge. Ions which are held electrostatically on the surface of the soil colloids can be replaced by other ions from the soil solution. The process of replacement is referred to as ion exchange, and it can involve either anion exchange or cation exchange. The cation exchange capacity of a soil is defined as its capacity to adsorb and exchange cations. Cations, which are generally held by electrostatic forces on soil particles, are  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ , with increasing  $\text{H}^+$  and  $\text{Al}^{3+}$  in acid soil, and  $\text{Na}^+$  in saline soil. CEC is a quantitative measure of all the cations adsorbed on the surface of soil particles. The unit of CEC is milliequivalents of charge per 100 g of dry soil (meq/100g) or centimole of charge per kilogram of dry soil (cmol (+) kg). The results on CEC are listed in Table 4.3. The CEC ranged from 2.14 cmol (+)/kg (sample 1) to 12.65 cmol (+)/kg (sample 2).

#### **4.2.4 Elemental composition**

The earth's crust contains a variety of minerals and the elemental composition of soil reflects the elemental composition of the earth's crust. Ten most abundant elements in soil in g/kg are: Si (282), Al (82), Fe (56), Ca (42), Na ~ K (24), Mg (20), Ti (5.7), P (1), Mn (0.95) respectively (Ochiai, Ei-ichiro, 1979). Soil elemental abundance will be further modified by weathering and biological processes. Besides, soil is an open system, materials are constantly added to soil by natural and human activities, and great quantity of materials have been lost from soil due to the constant leaching of precipitation from soil.

As previously mentioned in Chapter II, several researchers suggested that geophagy behavior may closely associated with nutrition or elements requirements. Deficiency in minerals may cause the craving for nonfood substances like soil. So it is interesting to find out the elemental composition of pica soil. In this experiment the elements content in soil samples were determined by WDXRF technique. Due to the limited number of available soil standard reference materials, only nine elements were being determined. The data obtained were analyzed by SuperQ program.

The semiquantitative results of elements content obtained are shown in Table 4.4 and Table 4.5 in the form of oxides and elements respectively. The results showed that total concentrations of iron and zinc in pica soil samples were not higher than the common concentration ranges of iron and zinc in soil; iron 7000 - 550000 mg/kg, zinc 10 - 300 mg/kg (Hassett and Banwart, 1992).

**Table 4.4** Elemental composition of pica soil samples in oxide form.

Oxide form	Concentration (mg/kg)			
	Sample 1	Sample 2	Sample 3	Sample 4
Al <sub>2</sub> O <sub>3</sub>	95021	121449	155648	205769
BaO	3183	3311	3600	3119
Fe <sub>2</sub> O <sub>3</sub>	11033	10409	10516	27285
K <sub>2</sub> O	961	826	750	2475
MgO	ND	ND	ND	0.672
MnO	66	75	85	114
SiO <sub>2</sub>	558280	677078	675753	545251
TiO <sub>2</sub>	14320	14818	16200	13888
ZnO	60	64	64	74



ND = Not detected

**Table 4.5** Elemental composition of pica soil samples in elemental form.

Elemental form	Concentration (mg/kg)			
	Sample1	Sample2	Sample3	Sample4
Al	50295	64282	82384	108914
Ba	2851	2965	3225	2794
Fe	7716	7280	7354	19083
K	797	685	623	2055
Mg	ND	ND	ND	405
Mn	51	58	65	88
Si	260930	316466	315800	254850
Ti	8584	8883	9712	8326
Zn	48	51	51	59

ND = Not detected

Table 4.6 shows the comparison of the ranges of elements content found in pica soil samples from other studies with the results obtained from Sisaket soil samples. Table 4.6 shows the similar elemental composition profiles of pica soil samples, i.e., Al, Fe, and Si are the major elements while Ba, K, Ti, and Zn are the minor ones.

**Table 4.6** Ranges of elemental concentration in selected pica soil samples from other studies compared with the results from Sisaket pica soil.

Researcher/ year	Elements content : concentration in mg/kg								
	Al	Ba	Fe	K	Mg	Mn	Si	Ti	Zn
John and Duquette 1991	61000 - 214100	-	11600 - 60000	-	603 - 8924	155 - 3980	174100 - 363100	2381 - 9684	<10 - 185
Aufreiter et al.,1997	41000 - 97000	340 - 1200	13300 - 71900	3500 - 27000	900 - 6800	133 - 409	300000 - 400000	2400 - 7600	-
Abrahams 1997	-	-	22643 - 81696	1521 - 8322	1058 - 3107	41 - 2247	-	-	35 - 99
Sriood et al., 1999	72700 - 161800	-	-	-	-	45800 - 140100	-	3100 - 7200	-
Mahaney et al.,2000	103000 - 151000	240 - 660	50700 - 75800	10200 - 23000	≤ 6000 - 31000	650 - 2360	-	-	-
Smith et al., 2000	50 - 130000	-	70 - 81600	-	60 - 2820	-	-	-	1 - 79
Sisaket Pica Soil samples 2003	50295 - 108914	2794 - 3225	7280 - 19083	623 - 2055	ND - 405	51 - 88	254850 - 316466	8326 - 9712	48 - 59

Note : ND = not detected

It is well established that metal ions are required for many critical functions in humans. Four main group (Na, K, Mg, and Ca) and ten transition metals ( V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo and Cd ) are currently known or thought to be required for normal biological functions in humans. Deficiency of some metal ions can lead to disease. Well known examples include pernicious anemia resulting from iron

deficiency, growth retardation from insufficient dietary zinc, and heart disease in infants due to copper deficiency. But at the same time metal ions can also induce toxicity in humans, classical examples being the toxic metal poison from mercury and lead. Even essential metal ions can be toxic when present in excess. So when people practicing geophagy, they would take both the beneficial elements and the toxic elements into their bodies at same time. Any way geophagy seemingly provided some benefits and few problems, though human health might be adversely affected if soil was consumed in excess or inappropriately.

#### 4.2.5 Bioavailable iron and zinc

Since iron and zinc are the main elements considered to be related with geophagy behavior (Rose et al., 2000), it is interested to find out how much iron and zinc are available in soil consumed by the geophagists. In the experiment, the amounts of iron and zinc in the form that can be absorbed by human body through the gastrointestinal system, were measured by atomic absorption spectroscopy technique. The human digestive system was simulated using 0.10 M HCl in the extraction of bioavailable iron and zinc. Table 4.7 shows the results of this experiment.

**Table 4.7** Bioavailable iron and zinc.

Sample	Bioavailable iron (mg/kg)	Bioavailable zinc (mg/kg)
1	72.05	0.86
2	79.91	0.67
3	47.32	0.61
4	22.35	0.52

Bioavailable iron varied from 22.35 mg/kg (sample 4) to 79.91 mg/kg (sample 2). Bioavailable zinc varied from 0.52 mg/kg (sample 4) to 0.86 mg/kg (sample 1). It is also interested to compare the concentrations of bioavailable iron and zinc in Sisaket pica soil with the Recommended Daily Dietary Allowances (RDA) for the healthy Thais (The Committee on Recommended Daily Allowances, 1989). Assuming that the Thai geophagists consumed the same average amount of soil as reported by Geissler et al. (1997) of 28 g/day. The RDA values shown are for the young children 7 - 9 years of age and for the women 50 - 59 years of age since they are those who practice geophagy in Sisaket.

**Table 4.8** Comparison of bioavailable iron and zinc from Sisaket soil samples with the RDA for Thais.

Subject	RDA for Thais		28 g of Sisaket soil samples							
	Fe (mg)	Zn (mg)	Sample 1		Sample 2		Sample 3		Sample 4	
			Fe (mg)	Zn (mg)	Fe (mg)	Zn (mg)	Fe (mg)	Zn (mg)	Fe (mg)	Zn (mg)
Children 7-8 years	10	10	2.016	0.0243	2.236	0.0177	1.323	0.0177	0.625	0.0149
Women 50-59 years	10	15								

The data presented in Table 4.8 show that iron in all four pica soil samples appeared to be the major mineral supplementation for humans as compared with the

RDA value. For example, the children who consumed 28 g of sample 1 would receive 2.018 g of iron and 0.0243 g of zinc which were 20.18 % and 0.245 % of the RDA for iron and zinc respectively. Even though it was clear that the geophagists got some useful minerals from eating soil, the benefits of the elements for human body were difficult to assess with any accuracy. Several factors such as age, sex, the composition of a diet (meats or vegetable), and competition interaction between elements might influence the amount of bioavailable elements in human body (Abrahams and Parson, 1997). Moreover, it is well known that soil may contain intestinal parasites and toxic substances such as toxic metals which are harmful to human body.

Table 4.9 shows the comparison of bioavailable concentrations of iron and zinc obtained from the analysis of pica soil samples from various studies. The highest concentration of bioavailable iron and zinc were found in Johns and Duquette (1991) samples. The findings in Table 4.9 suggested that the concentration of bioavailable iron and zinc varied from source to source. In fact, many factors affected the concentration of bioavailable iron and zinc in soil such as parent materials, vegetation, climate and fertilization used. Besides, the analytical methods used in the determination of bioavailable iron and zinc also had an influence on the results obtained. But the results obtained from the study of Abrahams and Parson (1991) on pica soil from the north of Thailand were quite similar to the results from this study. Their bioavailable iron and zinc were 34 mg/kg and 0.3 mg/kg respectively.

**Table 4.9** Comparison of bioavailable concentrations of iron and zinc obtained from the analyses of pica soil samples in other studies with the results obtained from Sisaket pica soil samples.

Researchers / year	Sample name / areas	Bioavailable iron (mg/kg)	Bioavailable zinc (mg/kg)
Hunter and de Kleine (1984) <sup>a</sup>	Tierra santa Central America	43.4	9.90
Johns and Duquette (1991) <sup>b</sup>	California, Hopland	3000	-
	Sardinia, Baunei	5000	-
	Cameroon	9000	-
	Kenya-1	7000	3000
	Kenya -2	12000	5000
	Nigeria	10000	3000
	Togo	5000	-
	Zambia	74000	2000
	Zaire	497000	-
Abrahams (1997) <sup>c</sup>	Uganda	528*	6.7 *
Abrahams and Parsons (1997) <sup>c</sup>	Thailand	34	0.3
	Uganda	326	4.0
	Zaire	380	2.0
Geissler et al., (1998) <sup>d</sup>	Western Kenya	168.9**	2.7**
Results obtained in this study <sup>d</sup>	Sisaket province, Thailand		
	Sample 1	72.05	0.86
	Sample 2	79.91	0.67
	Sample 3	47.32	0.61
	Sample 4	22.35	0.52

- , not determined

\* Median value (n = 13)

\*\* Median value ( n = 48)

<sup>a</sup> Simulated human digestion: extracted 5 g sample with 50 ml 0.1 N HCl ; analysis iron and zinc by atomic absorption spectroscopy technique

<sup>b</sup> Simulated human digestion: extracted 1 g sample with 50 ml 0.1 M NaCl adjusted to pH 2 with HCl; analysis iron and zinc by atomic absorption spectroscopy technique

<sup>c</sup> Simulated human digestion: extracted 1 g sample with 20 ml 0.1 N HCl ; analysis iron and zinc by atomic absorption spectroscopy technique

<sup>d</sup> Simulated human digestion: extracted 10 g sample with 100 ml 0.1 M HCl ; analysis iron and zinc by atomic absorption spectroscopy technique

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## Curriculum Vitae

**Name:** Songkot Utara

**Sex:** Male

**Born:** November 21, 1976

**Place of birth:** Nongkhai, Thailand

**Marital status:** Single

**Citizenship:** Thai

**Education:**

1995-1999 B.Sc. Chemistry

Rajabhat Institute Udon Thani, Thailand

**Experience:**

1998 -1999 Scientist, Department of Medical Sciences

Ministry of Public Health, Udon Thani, Thailand