UNIVERSITI SAINS MALAYSIA

UNIVERSITI SAINS MALAYSIA

Metal Contents in Tobacco Fields of District Bachok near Coastal

Area of South China Sea.

Dissertation submitted in partial fulfillment for the Degree of
Bachelor of Science in Forensic Science

ABDUL HAIL AHMAD TARMIZZI

School of Health Sciences
Universiti Sains Malaysia
Health Campus
16150, Kubang Kerian, Kelantan,
Malaysia

2006

CERTIFICATE

This is to certify that the dissertation entitled

"Metal Contents In Tobacco Fields of district Bachok near Coastal Area of South China Sea"

is the bonafide record of research work done by

Mr Abdul Hail Ahmad Tarmizzi

During the period of **23** rd **Dicember** 2005 to **8** th **April 2006**

under my supervision.

Signature of Supervisor:

Name and Adress of Supervisor: Dr.Syed Waliulllah Shah

Associate Professor

School of Health Science

Universiti Sains Malaysia

Health Campus

16150,Kubang Kerian ,Kelantan,

Malaysia0

Date: 14/05/06

ACKNOWLEDGEMENTS

First of all, I want to thank God for the wisdom and Knowledge He provided me.

I would like to express my deepest gratitude and thank to all the people who supported me completing this research.My thanks to Prof. Zainul Fazirruddin Zainuddin,Dean of the School of Health Science for allowing me to do this research in Forensic laboratory. Thanks toAssociate Prof. R. Kuppuswamy, course coordinator, for his encouragement.I am thankful to Associate Professor Dr.Syed Waliullah Shah who guided me thouroughout my research project and thesis. I deeply appreciate his sacrifices in terms of time and knowledge in order to assist me in completing my project.

I would like to thank all the laboratory personnel from the School of Health Sciences, University Sains Malaysia especially those from analytical laboratory,Forensic Laboratory and Staff of Unit Kemudahan Makmal (UKM) for providing instruments needed for completing the research. Thanks for their kindness in helping me using the instruments as well as their generosity in providing me facilities I required.

Next, I would like to thanks all the landlords who permitted me to collect a soils samples from their lands in Bachok, Kelantan. Finally, I would like to thanks all my fellow course mate for their support, my family and friends who may have noticed a degree if introspection and preoccupation during the interception, development and delivery of the thesis.

CONTENTS

ABBREVIATION

AAS	Atomic Absorption Spectrometry
CV	Coefficient of Variation
FIAS	Flow Injection System
GF	Graphite Furnace
H_2O_2	Hydrogen Peroxide
HCl	Hydrochloric acid.
HF	Hydrofluoric Acid
HREE	Heavy Rare Earth Elements
HNO_3	Nitric Acid
HPDV	High Pressure Digestion Vessels
PTA	Pseudo Total Analysis
PTFE	Polytetrafluoroethylene
ICP-MS	Inductive Coupled Plasma –Mass Spectrometry
MW	Microwave
RM	Reference Materials
SD	Standard Deviation

LIST OF FIGURES AND TABLES

ABSTRACT

A simple acid digestion procedure for soil samples has been developed. This work is focused on studying the experimental conditions for extracting trace metals from soil sample taken from agriculture land cultivating tobacco crop. The area is located in Bachok, Kelantan.

The elements like Cu, Mg, K,Cu and Zn were quantitatively extracted and determined by AAS. The concentration of these metallic element determined in soil samples ranged for Cu (0.0117-0.05)mg/L, Zn (0.136-0.299)mg/L, Na (3.212-7.212)mg/L, K (1.932-6.957) mg/L and Mg (6.43-11.781) mg/L.

INTRODUCTION

Soil is the layer composed of minerals and organic matter with a thickness varying from centimetres to a meter or more, on the land surface. Its main components are rock and mineral matter, organic matter, water, and air. Soils differ in the ratio of these components. Air, trapped in spaces between the various particles, and water, trapped in spaces and on the surface of particles, can comprise up to half of the soil by volume. Based on the texture, soil can be categorized according to particle size as sand (coarsest-2 to 0.02 or 0.05 mm), silt(0.02 or 0.05) or clay (finest-<0.002 mm); Soil texture influences available water capacity and movement, fertility, and workability or tilth, the ratio of these particles to a great degree determines the soil classification and characteristics.

Generally speaking, though, soil is the uppermost layer of the Earth that can support plant life. It can be subdivided into two general layers or strata; topsoil (the upper few centimeters, where most roots and microorganisms are located) and subsoil which is more dense and less rich in organic matter.

There are plentiful nutrients and trace elements both of which plants require for growth. Soil moves continually in a natural cycle aided by oxygen, water, minerals and decomposing animal and plant matter. These elements create life in the soil, which is ongoing if not disturbed. Good soil consists of 93% mineral and

7% bio organic substances. The bio organic parts are 85% humus, 10% roots, and 5% microbes, fungi, bacteria, earthworms, micro fauna, and macro fauna

There are thirteen essential nutrients which of the plants get from soil. The six that the plants need the most of are called Macronutrients. They are Nitrogen, Phosphorus and

Potassium, which are abbreviated N-P-K, Calcium, Magnesium and Sulfur. The other

nutrients, which are needed only in small amounts, are called Micronutrients. They are

Iron, Manganese, Boron, Zinc, Copper, Molybdenum and Chlorine. Besides, some of the

nutrients get lost naturally through leaching continually wet weather, melting snow,

flooding or through denitrification. Also each cultivated plant takes nutrients from the soil,

as soon as the crops are harvested.

Since, the level of nutrients in soil will decrease with each season of plantation, a soil test

is a tool recommended for monitoring soil nutrient status and fertilizer needs. Efficient

nutrient use can help reduce fertilizer costs and help protect the farm environment without

reducing yield or quality. One of the most important aspects of soil sampling is obtaining a

sample that is representative of the soil conditions. Different soil types should be sampled

separately.

The elements extracts from these cultivation soils can be analyzed by different instruments.

The most appropriate and suggested instrument for detecting most of the metallic elements

is by using Atomic Absorption Spectrometry (AAS).This technique is based on the

principle that atoms of a particular elements absorb light at specific wavelength. Following

the procedures outlined in the equipment manuals, the spectrophotometer is set up,

standards are made for each Cation to be analyzed, masking agents added to both samples

and standard to overcome chemicals and ionization interference and the concentrations

read at specific wavelengths.

LITERATURE REVIEW

The literature on the efficient soil sampling methods, collecting good soil representative sample, varying techniques of extraction for trace elements from solid sample generally and soil sample specifically are very well documented.

According to Vos (1998), heavy metal and trace element content of soils and herbages is estimated with least expenditure of effort and cost by taking a representative sample from a defined area, compositing samples and conducting analysis on a suitably prepared sub sample. For agricultural purposes this is essentially the only method used. The sampled area is generally 2-5 ha (5-13 acres) and should at least be visibly uniform and comprise a single land use class or enclosure. Soil cores generally number 20 – 25. The sampling depth is often 10cm, especially on grassland. (Voss, 1998).However, numerous variants of above are used. For herbage sampling there is a singular lack of protocols but operating procedures appear to mirror those in use for soil sampling.

Vandre (2001) described that poor sampling of soil gives misleading test results. Soil testing can be divided into three major steps: (1) collecting the sample, (2) analyzing the sample, and (3) interpreting the results. Taking the sample is probably the most inaccurate of these three steps. Test results to represent an area can be no more accurate than the sample collected. Poor sampling techniques lead to inaccurate representative sample since just a few grams of soil are actually used in the soil testing procedure, and these several grams must be representative of the total area to be tested. Best decisions can be made only if soil samples are representative of the area sampled and accurately reflect soil conditions. Each soil sample should represent only one soil type, condition or growing situation.

Ramsey & Argyraki (1997) stated that reliability of estimate is usually expressed as CV (coefficient of variation) or SD (standard deviation/mean) x100. Variability (bias or uncertainty) in estimates is considered to arise at sampling, sample preparation and analysis stages. Sampling variability is also subdivided into geochemical and sampling components. In general, geochemical and sampling variabilities are considered to be large and analytical variability small except for some elements such as sulphur by nephelometry (Cameron et al., 1994) and little is known of variability arising during sample preparation.

Raij et al (2005) on their work of routine soil testing of various agricultural region in Brazil to monitor heavy metal and boron has successfully extracted metals like copper, iron, manganese, zinc, cadmium, chromium and lead from soils with a DTPA solution at pH 7.3 and determined by plasma emission spectrometry . Using standard procedures of soil testing for advisory purposes, the analyses were made using scoop volume measurements of the soil samples, a convenient and accurate procedure according to the previous works by Raij & Grohmann (1989).

Gutwerk (2006) on his view on pressure digestion of sample preparation described that the advantage of this procedure in comparison with open digestion in a recycling device or with the traditional hot plate, lies in the significantly higher working temperatures which can be achieved. While in open systems these temperatures are limited by the acid solution's boiling point, temperatures in the 200-260°C range can be typically achieved in sealed digestion vessels. This results in a dramatic acceleration of the reaction kinetics, allowing digestion reactions to be carried out in a matter of hours (pressure digestion using Tölg bombs) or in less than an hour (microwave digestion). However, these methods also make it clear that the temperature itself actually represents the most significant reaction parameter. It is the ultimate determinant of the digestion quality, but also results in a pressure increase in the vessel and therefore in a potential safety hazard. Therefore, the

pressure must ultimately also be considered. These effects are independent of the heating method.

Hödrejärv & Vaarmann (1999) analyzed metallic elements in siliceous soil by acid digestion and flame atomic absorption spectrometry. They carried out the analysis using digestion of siliceous soil with HNO_3 / H_2O_2 and aqua regia and detected by atomic absorption spectrometry. The dissolved silicate was found to interfere with the absorbance of Ca, Mg, Mn, Zn and Fe. No interference was found with Cu and Pb. The effect of silicate can be decreased by dilution of solutions and/or using lanthanum as the releasing element. The gain in the cations released by pseudo total analysis (PTA) of soil depends on the mineral composition of the soil. The degree of dissolution of siliceous soil depends on the acid system used. The difference between the digestion efficiency of $HNO_3 H_2O_2$ and aqua regia is statistically significant for calcium, and partly so for iron and magnesium. No differences were found for copper, manganese, and zinc. Silicate is more soluble in aqua regia than in the nitric acid/hydrogen peroxide system. The stability of a silicate solution depends on the salinity and the acidity of the solution. Formation of silicate gel is probably the reason why the silicate concentration in solution diminished when the digestion time was increased. The optimum time of digestion varies for different elements. Already at the end of the first day, about 80–90% of the total products of a 5-day digestion are dissolved. In some cases, the concentration of calcium decreased when the digestion time increased.

Sastre et al (2002) made an attempt to compare the efficiency of microwave-assisted total digestion versus aqua regia and nitric acid extraction for the determination of Cd, Cu, Pb and Zn in environmental samples. Their goal in validating these two protocols, along with a nitric acid digestion was to propose a rapid, cheap and easily automated digestion method for monitoring heavy metal content in environmental samples. They applied the digestion protocols to samples with a wide range of organic matter such as sediments, soils, sludges

6

and plant material. The use of HF acid ensures a total digestion of the aluminosilicate matrix, and its use with microwave has shortened the time devoted to this step. However, other approaches such as aqua regia extraction in reflux conditions in an aluminium block, also allow digesting a high number of samples simultaneously and the extractable metal amounts are similar to the total content. Comparing the microwave (MW) and aqua regia protocols for Cd, Zn, Cu, and Pb in the environmental samples studied, both methods led to obtain similar results. Therefore, aqua regia offers a good choice for monitoring low organic matter content samples as it is cheaper, and relatively faster than the microwave-assisted total digestion. For samples with a high organic matter content (for instance, about 70%),nitric acid may be an alternative to microwave total digestion since the mean results did not differ from those obtained with microwave for Cd, Cu and Pb, although for Zn an underestimation of the heavy metal content was observed in some samples.

Nearly similar experiment were conducted by Bettinelli et al (2000) .They determined the efficiency of heavy metals in soils and sediments by microwave-assisted digestion and inductively coupled plasma optical emission spectrometry analysis . A complete digestion of soils and sediments was achieved by using an acid mixture of $HF/HCl/HNO_3$ (1:3:1 v/v/v); the microwave-irradiated closed vessel system used for the determination of aqua regia leachable quota, proved to be a viable alternative to the traditional reflux system. They obtained good results for the extraction of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in soils .Besides, contamination is minimized, sample preparation times were significantly shorter and the extraction efficiency was guaranteed.

An alternative technique which is suitable for extraction of metal elements from soil sample others than acid or base digestion has also been studied. Al-Merey et al (2001) attempted to extract Cu, Pb and Zn from soil sample using cold ultrasonic acid extraction. The soil sample was placed in a small beaker and small volume of concentrated HCl added

to each beaker. The beakers was placed in the position of maximum wave pulse inside the ultrasonic cleaning bath. The beakers was exposed to ultrasound waves for 4 h and the samples was filtered. The filtrates was evaporated to dryness and the residues dissolved in 5 mL of nitric acid. The volume was made to 50 ml with distilled water for atomic absorption spectrometry and anodic stripping voltammetric measurements. Based on the results of the analysis, they reported that cold ultrasound is a clean technique and could be used to increase chemical reactions rates which lead to sample dissolution. This study indicates that the performance of this technique is equal to the hot-plate digestion method and significantly reduces the hazardous wastes and fume-hood emissions. The optimum experimental conditions for the extraction of Cu, Pb and Zn from some Syrian soil samples were established using concentrated HCl, and short exposure time (maximum 4 h) compared to other conventional acid or base digestion. However, it has been found that the extraction conditions of this technique depend on the sample matrix and the element to be determined. Under these conditions Sr, Mn, Fe, Al, Cr, Co, and Ni were only partially extracted from the studied samples.

One of the problem that causes difficulty during digestion is presence of several elements in the soil is most of siliceous type soil is hard to be digested using ordinary technique of extraction. This problem will lead to negative result when the extract is being analyzed by spectrometry . To overcome this problem, Diegor et al (2001) tested the applicability of high pressure digestion technique to the analysis of sediment and soil samples by inductively coupled plasma-mass spectrometry. In their experiment, the use of high pressure dissolution was examined by using High pressure digestion vessels (HPDVs). Their use was compared with the existing method that utilizes screw top PTFE jars for digesting reference materials (RMs). ICP-MS analysis of solutions demonstrated complete digestion of three marine sediment RMs, indicated by higher Y, Zr, Nb, heavy rare-earth

element (HREE), Hf, and U concentrations when the HPDV was used. The results indicate better decomposition of resistant minerals, especially zircons that were likely in high abundance in the sediments. To evaluate its suitability to real samples, the method was applied to the ICP-MS analysis of sediment and soil samples from Cebu, Philippines. Good correlation for Nb and Pb, among other elements, was found from ICP-MS and X-ray fluorescence spectrometry.

OBJECTIVE OF THE STUDY

The objective of this study are:

1. To learn about proper sampling methods in order to obtain the best representative sample.

2. To learn and practice acid digestion method as an appropiate technique for extracting trace elements form soil sample.

3. To learn and use AAS for the detection and quantification of elements present in the digested sample.

4. To determine the concentration of selected elements and from that, deduce whether the selected soil areas contain sufficient nutrient for the tobacco plantations. The elements are Cuprum, Zinc, Potassium, Magnesium and Sodium.

MATERIALS AND METHODS

3.1 Chemical and reagents

All the chemicals and reagents were analaR grade and obtained from Fluka/Merck (Germany).

3.2 Glassware

All the glasswares was cleaned by soaking overnight in 1:1 v/v nitric acid:hydrochloric acid mixture and then rinsed with deionized water and dried at ambient temperature in a clean environment. The glassware as well as sieves and agate pestle and mortar were wasted with 10% HNO_3 followed distilled water and rinsed with purified water.

3.3 Apparatus

10 mL measuring pipettes,128 μm sieve, pestle and mortar, an electronic microbalance, oven and hot plate were used.

3.4 Instrumentation

A Perkin-Elmer Model A800 Atomic Absorption Spectrophotometer was used to analyze the acid digested soil samples. This unit has five basic components, light sources, absorption cell, monochromator, detector and a display. This instrument provides flexible trace metal analysis (ppm to ppb) by graphite furnace atomic absorption, flame, or hydride generation. It consists an autosampler for all modes of operation, FIAS (flow injection system), Power Supply (for arsenic, selenium), and Zeeman background correction. The Hallow Cathode Lamps for several elements are also available.

Figure 1.Perkin –Elmer A800 AAS.

3.5 Sampling and pre-treatment of samples

After surveying the area of Bachok, 10 tobacco agricultural sites were selected for soil

sampling. These sites are top tobacco growing areas of east coast of peninsular

Malaysia.They are located at a distance of 5-15 km distance from each other.

BANCI PENDUDUK DAN PERUMAHAN MALAYSIA, 1991
POPULATION AND HOUSING CENSUS OF MALAYSIA, 1991

KELANTAN

PETA 3.3: PETA MENUNJUKKAN SEMPADAN JAJAHAN DAN KAWASAN PIHAK
BERKUASA TEMPATAN
MAP 3.3: MAP SHOWING JAJAHAN BOUNDARY AND LOCAL AUTHORITY AREA

Disediakan dan diterbitkan oleh Jabatan Perangkaan, Malaysia 1992.
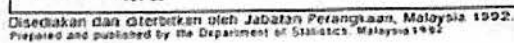Prepared and published by the Department of Statistics, Malaysia 1992.
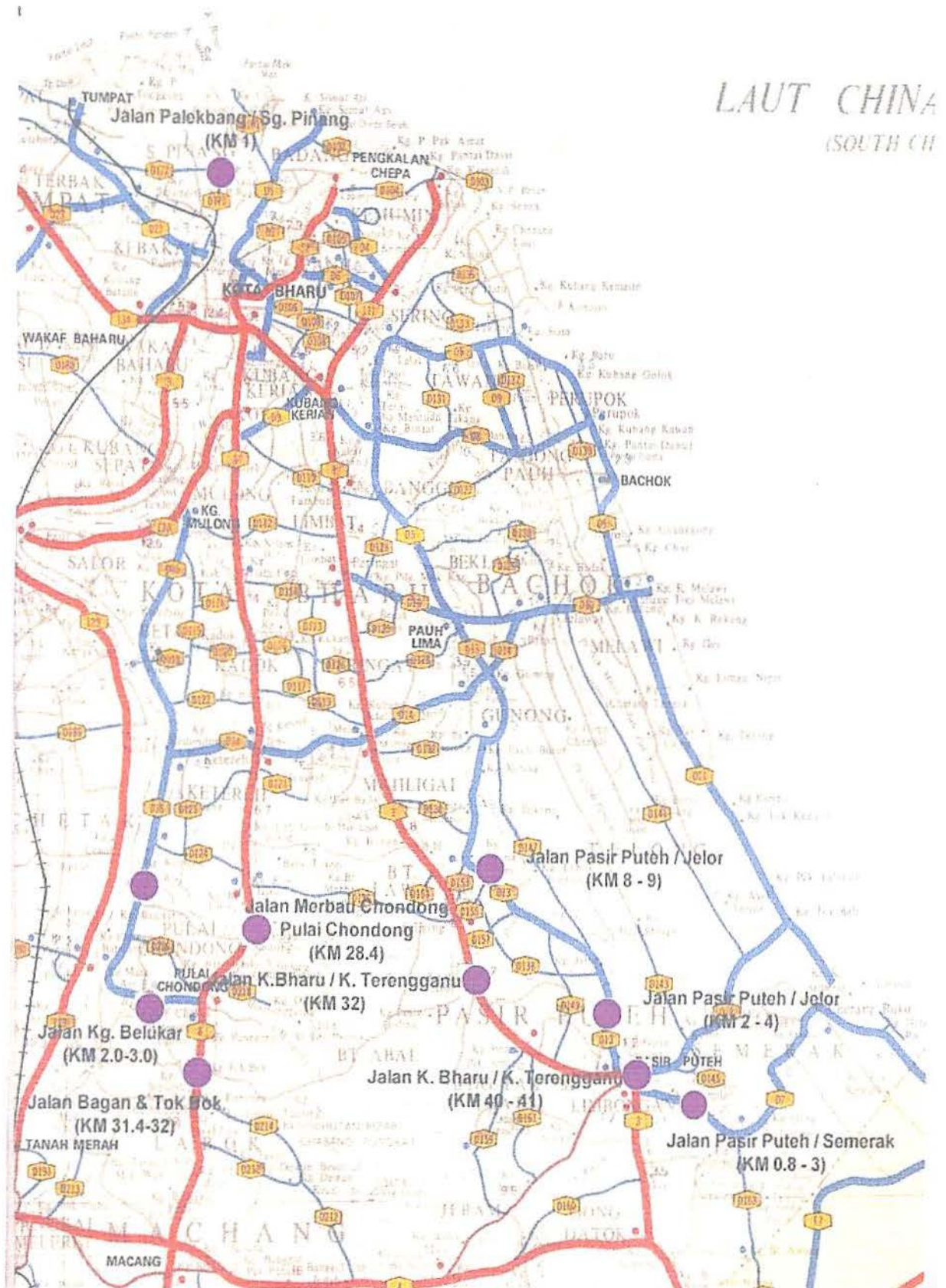
65

Figure 2.Overall map of Kelantan

Figure 3.Map of Bachok

The soil samples in each area were taken in a zigzag pattern, 2 soil samples per acre. For

example, if the land area is 5 acres, the number soil samples (n) should be 10. At each

sampling location, a total of about 1 kg of soil sample was taken from 10cm depth using a spade. The sample was then placed into an ethylene bag and labeled properly with the name of the field, the crop that is grown, the numbers of the sample for the area and the date of sampling.

Thus the number of sub-samples taken by farmers should be *realistic*, considering the particular field situation.
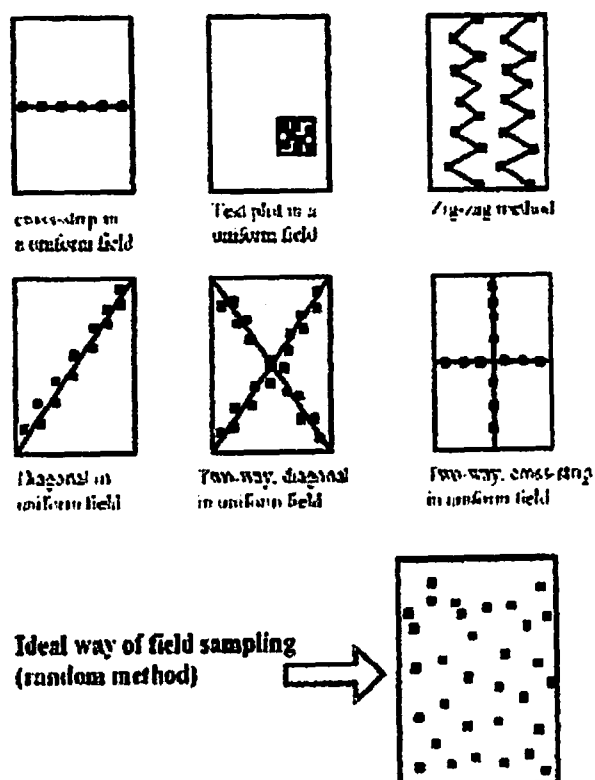

Figure 4.Ideal ways of field sampling

The chosen sites are:

Area	Sites	Size (acres)	Number of samples(n)
1	Kampung Pintu Gerbang	6	12
2	Kubang Golok	6	12
3	Kampung Sungai	6	12
4	Kampung Nelayan Kandis,Telong	6	12
5	Serdang Baru,Sg Gali,Telong	6	12
6	Kampung Paruh,Sungai Dua,	6	12
7	Kampung Pantai Melawi	6	12
8	Telok	7	14
9	Kampung Kemudi,Tanjung Pauh	6	12
10	Beris Kubur Besar	6	12

Table 1.Sampling sites and sizes of the agriculture land.

The soils were spread to dry on flat trays at ambient temperature. The recommended trays should be made from wood, zinc, fiberglass or plastics. It is important to maintain the identity of each sample and subsamples at all stages of sample preparation. The temperature should not be allowed to exceed 35 °c and the relative humidity should be between 30° c and 70 °c. Oven drying a soil can cause such a change that it is not recommended as a preparatory procedure in spite of its convenience and reproducibility.

All the soils samples were ground using pestle and mortar and sieved through a 128μm sieve The soil, that is less than 128μm size is then used in the chemical analysis. The sieved sample were subjected to 'quartering' where the soil is spread uniformly over a

sheet of brown paper or polythene and divided into four equal portion as illustrated in figure 5 below.
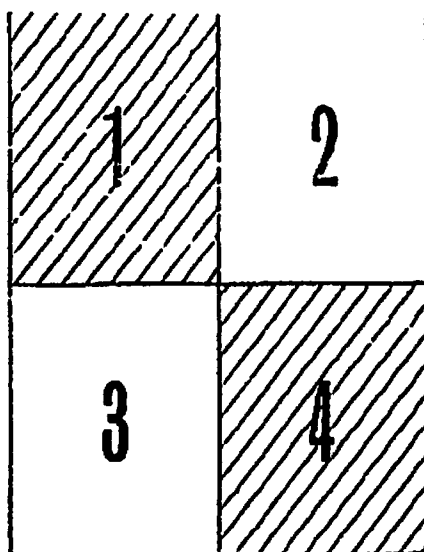
Reducing a soil sample by quartering

Figure 5.Reducing soil sample by quartering

The portions marked 1 and 4 were discarded and the remaining portions mixed together, spread out again and reduced to half by the same procedure This process was repeated until a required sample was obtained.

3.6 Solution preparation

Water was doubly distilled and further purified using a Milli-Q water purification system (Millipore,USA). A 10% HNO_3 solutions was prepared by adding 20-40 mL of doubly distilled deionised water into 50 mL volumetric flask, then 10 mL of concentrated HNO_3 stock solution was added into the flask followed by addition of sufficient doubly distilled water to bring the total solution volume to 100 mL

3.7 Acid Digestion procedure

1) From the powdered sieved grain size fraction, 3 sub samples of 0.20 g soil from each bulk(1 bulk for one field area) were weighed.

2) Each subsample is then placed directly into 25-ml beakers and 2 ml of concentrated HCl (37%) was then added into the beaker.

3) The mixture was then heat on a hot plate at 80 °c near to drying .The operation should be conducted in ventilated fume hood for safety.

4) Then after cooling, 5 ml of concentrated HNO_3 (70%) was then added into the beaker and step (3) was repeated.

5) Let the beakers to cool for few minutes. After that, 2 ml of 10 % HNO_3 followed by 10 ml of distilled water were then added into the beakers.

6) The final digestion product was then filtered, to remove the silica content, through an ashless 90mm diameter Whattman type 41 filter paper, England, and transferred to 50 ml volumetric flask to be diluted with distilled water to 50 ml. The digests were stored at 4 °C until analysis.

Figure 6.Hot plate

Figure 7.An electronic microbalance

Figure 8.Fume hood

3.9 AAS determination

A Perkin-Elmer model A800 flame atomic absorption spectrometer, with a deuterium lamp for continuous background correction, was used for Cu, Zn, Na, Mg and K determination at different wavelength for different elements, respectively. A series of standards for Zn and other respective elements must be prepared before the sample can be analyzed. The conventional parameters of acetylene–air FAAS analyses were applied. The scattering effect of light was avoided by using a deuterium lamp background corrector. The determination limit and optimal concentration range of analytes were:

Zn (0.25–1.0) ppm, Cu (1.25- 5.0) ppm, K (0.5-1.0) ppm, Na (0.25–1.0) ppm and Mg (0.75- 3.0) ppm.

RESULTS AND DISCUSSION

Atomic absorption spectroscopy (AAS) is an important analytical technique based upon the absorption of radiation by free atoms. Virtually all metallic elements can be detected with excellent accuracy, precise quantitation, and very sensitive detection limits. This technique can also be used to indirectly detect and measure the amounts of some nonmetallic elements. The Perkin-Elmer AAS instruments used in this analysis is capable of measuring both atomic absorption and atomic emission process. Emission spectroscopy measures the light emitted by the electrons as they descend back to the ground state. On the other hand, absorption spectroscopy measures the original absorption of energy by ground-state electrons.

Since many more atoms are in the ground state than in an excited state in a sample at any one time, absorption measurements are generally more sensitive than emission measurements When all the digested soil sample in this experiment were analyzed by AAS, the flame of AAS will target all metallic elements which have a specific number of electrons associated with its nucleus. The most normal and stable orbital configuration of atoms is known as the "ground state". If energy is applied to the atom, the energy will be absorbed and outer electron will be promoted to a less stable configuration known as the "excited state". Since this state is unstable, the atom will immediately return to the ground state releasing light energy. The emission spectrum of an element consist of a collection of emission wavelength called emission line because of discrete nature of the emitted wavelengths. The intensity at an emission lines will increase as the number of excited atoms of the elements increase.

The "ground state" atoms absorb light energy of specific wavelengths as it enter the "excited state". As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special light source and careful selection of wavelength allow the specific determination of individual elements

Atomic absorption instruments consist of simple apparatus. The critical components are a hollow cathode lamp capable of emitting the line spectrum of the element to be analyzed, an atomizer connected to a flame where sample ions are converted to free atoms, a monochromator with the capability of wavelength selection, and a detector with an amplifier and readout device

When the light source (hollow cathode lamp or electrodeless discharge lamp) emits a spectrum specific to the elements which it is made, which is focused through the sample cell into the monochromator. The light source must be electronically modulated or mechanically chopped to differentiate between the light from the source and the emission from the sample cells. The monochromator disperse the light and the specific wavelength of the lights isolated passess to the detector, which is usually a photomultiplier tube. An electrical current is produced depending on the light intensity and processed by the instruments electronics. The electronic light will measure the amount of attenuation in the the sample cell and convert those readings to the actual sample concentration.

After the final process, the instrument will provide the final concentrations of desired elements (Cu, Zn, Mg, Na ,and K) from each samples in mg/L. The concentrations of each elements from each field is provided in the table next pages. The mean value and the standard deviation for each elements from each field were also calculated.

Experimental condition for Cu

Wavelength   =213.9 nm

Slit   =0.7nm

Burner head   =10-cm burner head

Technique   =flame

Repl	Standard concentration(mg/L)	Signal
0	0	0
1	0.25	0.063
2	0.5	0.132
3	1.0	0.248

Correlation coefficients, $r = 0.997$

Experimental condition for Zn

Wavelength   =324.8 nm

Slit   =0.7nm

Burner head   =10-cm burner head

Technique   = flame

Repl	Standard concentration(mg/L)	Signal
0	0	0
1	1.25	0.006
2	2.5	0.131
3	5.0	0.258

Correlation coefficient , $r = 0.964$