

HEAVY METAL ANALYSIS OF DIFFERENT TYPE OF  
AGRICULTURE SOIL BY AAS (ATOMIC ABSORPTION  
SPECTROMETRY)

by

Nor Farah Emyliana Binti Mohd Rusli

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## LIST OF ABBREVIATIONS AND SYMBOLS

AAS	atomic absorption spectroscopy
Ag	silver
As	arsenic
Avg	average
Cd	cadmium
cm	centimeter
Co	cobalt
Cr	chromium
Cu	copper
DI	deionized water
F	fluorine
Fe	iron
g	gram
g/cm <sup>3</sup>	gram per cubic centimetre
Hg	mercury
HCl	hydrochloric acid
HClO <sub>4</sub>	perchloric acid
HF	hydrogen fluoride
HNO <sub>3</sub>	nitric acid
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
H <sub>2</sub> SO <sub>4</sub>	sulfuric acid
ICPMS	inductively coupled plasma mass spectrometry
LOD	limit of detection
m	meter
ml	milliliter

mg/kg	milligram per kilogram
mg/L	milligram per litre
Mn	manganese
Mo	molybdenum
Ni	nickel
Pb	lead
ppm	part per million
ppt	part per trillion
Se	selenium
Sn	tin
Ti	titanium
USEPA	United States Environmental Protection Agency
µg/g	microgram per gram
V	vanadium
XRF	x-ray fluorescence
Zn	zinc

# **ANALISIS LOGAM BERAT DALAM PELBAGAI JENIS TANAH PERTANIAN DENGAN MENGGUNAKAN SPEKTROMETRI SERAPAN ATOM(AAS)**

## **ABSTRAK**

Logam berat adalah jujuk semula jadi kerak Bumi. Ia adalah stabil dan tidak boleh terurai atau musnah, dan oleh itu ia cenderung untuk berkumpul didalam tanah. Komposisi logam berat dalam tanah boleh berfungsi sebagai cap jari tanah dimana tanah boleh digunakan untuk mencari tempat jenayah. Kajian ini menyiasat kepekatan logam berat (Cu, Cd, Cr, Pb dan Zn) dalam sampel permukaan tanah dari jenis tanah pertanian dengan menggunakan Spektrometri Serapan Atom (AAS). Kepekatan Cu, Cd, Cr, Pb dan Zn didalam sampel permukaan tanah dari tanah pertanian jenis kelapa sawit, tebu, getah, limau dan pisang telah dianalisis. Sebelum dianalisis oleh AAS, kaedah sampel pra-rawatan telah dijalankan terlebih dahulu. Kepekatan keseluruhan (mg / kg) Cu, Cd, Cr, Pb dan Zn dalam sampel tanah dari kelapa sawit mengandungi Cu (2.887), Cd (3.468), Cr (2.273), Pb (ND) dan Zn ( 12.194), Tebu: Cu (4.278), Cd (2.918), Cr (7.711), Pb (ND) dan Zn (23.216), getah: Cu (14.735), Cd (4.243), Cr (9.033), Pb (7.890 ) dan Zn (25.525), limau: Cu (27.377), Cd (3.702), Cr (14.212), Pb (27.112) dan Zn (32.222), pisang: Cu (68.162), Cd (4.530), Cr (17.173) , Pb (63.798) dan Zn (40.088). Perbezaan kepekatan logam berat didalam tanah boleh digunakan dalam forensik analisis tanah untuk membezakan jenis tanah pertanian dan data ini boleh membantu dalam siasatan yang melibatkan analisis tanah. Kepekatan Cu, Cd, Cr, Pb dan Zn didalam semua jenis tanah pertanian adalah di bawah piawai USEPA logam berat didalam tanah. Ketepatan prosedur penghadaman asid dinilai dengan menganalisis perolehan semula. Perolehan semula logam adalah dalam lingkungan 64.00% -91.54%.



# **HEAVY METAL ANALYSIS OF DIFFERENT TYPE OF AGRICULTURE SOIL BY AAS (ATOMIC ABSORPTION SPECTROMETRY)**

## **ABSTRACT**

Heavy metals are natural constituents of the Earth's crust. They are stable and cannot be degraded or destroyed, therefore they tend to accumulate in soils. Thus heavy metal composition in soil can serve as fingerprint of the land as soil can be used to locate crime scene. This study investigate the concentration heavy metal of Cu, Cd, Cr, Pb and Zn in surface soil sample from different types of agriculture soil by using Atomic Absorption Spectrometer (AAS). The concentration of Cu, Cd, Cr, Pb and Zn in the surface soil sample from different types of agriculture soil of palm oil, sugarcane, rubber, lime and banana were determined. Before analyzed by AAS, sample pre-treatment method was employed. Overall concentration (mg/kg) of Cu, Cd, Cr, Pb and Zn in the soil samples from palm oil was found to be Cu (2.887), Cd( 3.468), Cr (2.273), Pb (ND) and Zn(12.194), Sugarcane: Cu ( 4.278), Cd (2.918), Cr ( 7.711), Pb (ND) and Zn ( 23.216), Rubber: Cu( 14.735), Cd (4.243), Cr ( 9.033), Pb ( 7.890) and Zn (25.525),Lime: Cu (27.377), Cd ( 3.702), Cr (14.212), Pb ( 27.112) and Zn( 32.222), Banana: Cu (68.162), Cd (4.530), Cr (17.173), Pb (63.798) and Zn (40.088). The differences concentration of heavy metal in soil can be used in forensic analysis of soil to differentiate the type of agriculture soil and this data can help in investigation that involved soil analysis. The concentrations of Cu, Cd, Cr, Pb and Zn in all different types of agriculture soil were below the USEPA standard of heavy metal in soil. The accuracy of the optimized acid digestion procedure was evaluated by recovery studies. The recoveries of the metals were in the range of 64.00%-91.54%.



# CHAPTER 1

## INTRODUCTION

### 1.1 Soil

Soils are complex material as it consists of both inorganic and organic components in different proportions. The components of soil may be natural or being introduced by human activities (Dawson and Hillier, 2010). According to Moor *et al.* (2001) soil consists of mineral constituents, organic matter, living organisms, air and water. Soil will regulates the natural cycles of these compounds as it filters, purifies water, degrades and stores materials.

Soil becomes important and fundamental natural resources for human survival as human needs quality food supply and quality environment for sustaining human need (Opaluwa *et al.*, 2012). In addition, soils play an important role as it becomes an essential component in ecosystem as it will provide nutrients and mineral for the growth of plant and becomes support life for diverse groups of organism (Brown and Jasra, 2016).

Besides being important in ecosystem, soils contain a lot of information for forensic use. Soil components and its contact with the people and objects will create a transfer and recovery of soil as possible evidential material. Because of this, soil becomes one of the evidence in courts of law (Dawson and Hillier, 2010). Forensic analysis of soil is the study of soil that uses a wide range of soil information as the solution to legal questions, problems and hypotheses (Fitzpatrick and Raven, 2012).

Soil is one of the physical evidence that can be found at a crime scene. Soil is often use as trace evidence which can link a suspect to a crime scene as soil itself is highly individualistic, easily transferred and retained and can be easily collected and analyzed (Jantzi and Almirall, 2011). According to Fitzpatrick and Raven (2012) the transfer of soil as trace evidence has become known in the Locard Exchange Principle, which states that when two surfaces come into contact with each other there is a mutual exchange of trace evidence between them.

According to Woods *et al.* (2014) soil can be used as an evidence to exclude a suspect, a victim or an object as well as assisting in identifying a scene of crime in a forensic investigation. Forensic soil analysis becomes important in criminal investigation as each soil consists of unique properties from each other and serves as identification markers. Each soil type has a specific composition and characteristics such as elemental composition, color, texture, surface and location of origin. Analysis of soil can help investigators to trace and match soil to each other as connections between differences in soil and collected evidence can be made.

Differences of soil in terms of their composition, physical properties and chemical properties will make the soil from different location has its own unique fingerprint. Soil can be used in many ways. Soil can link some problem relating to environmental accidents, construction failures, pollution and serious crimes (Pye and Croft, 2004). For example, soil has been used to solve a murder case by identifying the similarities between soils.

Each soil has its own characteristics such as morphology, mineralogy and organic matter composition (Fitzpatrick and Raven, 2012). Majority of forensic cases that involve soil materials usually are complex and challenging, often requires the use of laboratory methods (Dawson and Fitzpatrick, 2013).

Besides being one of the important evidence in an investigation, soil also plays an important role as an indicator of environmental quality because soils are main sources for heavy metals and other pollutants (Liu *et al.*, 2015). Soil is a complex, living and dynamic ecosystem. Due to both natural and anthropogenic sources, soil maybe exposed to metal pollution (Varsani and Manoj, 2015). With its inherent capability to absorb and emit substances, soil becomes contaminated with accumulation of heavy metals through many sources. Examples are disposal of high metal wastes, rapidly expanding industrial areas, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Wuana and Okieimen, 2011).

Some of heavy metals in the soils are released into the environment by microbial actions but most of the heavy metals do not undergo microbial or chemical degradation and their concentrations in soil will persist for a long time even after their introduction in the soil (Wuana and Okieimen, 2011).



## 1.2 Heavy Metals

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed (Monica *et al.*, 2015). Heavy metals such as Fe, Mn, Ni, Co, Zn, Cu, Cr, V, Ti, Cd, Hg, Mo and other trace metals as well as As, Se and F occur naturally in the soils which are formed by alteration and erosion from geological processes (Moor *et al.*, 2001). The group of metals and metalloids are classified as heavy metal if their atomic density either greater than 4 g/cm<sup>3</sup> or is greater than water (Nriagu and Pacyna, 1988).

According to Essoka *et al.* (2006) stated that there are 38 heavy metals and 12 from that are easily influenced by the human activities. Examples are Cd, Cr, Co, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sn and Zn. Heavy metals like Fe, Sn, Cu, Mn, V occur naturally in the environment and serve as plant nutrients depending on their concentrations whereas Hg, Pb, Cd, Ag, Cr and many others which are indirectly distributed as a result of human activities are very toxic even at low concentrations (Opaluwa *et al.*, 2012). Heavy metals and trace elements are also a matter of concern due to their non-biodegradable nature and long biological half-lives (Rahman *et al.*, 2012). Heavy metals which are dangerous to health are Pb, Hg, Cd, As, Cu, Zn and Cr (Essoka *et al.*, 2006).

According to Brown and Jasra (2016) stated that heavy metals composition in soil can serve as a fingerprint of the land as soil can be used to locate a crime scene. Other than that, it will give the information about level of contamination that occur in that soil and has it comply with the environmental protection legislation. Heavy metals in soil exist in different types of binding and chemical forms.

Determination of these forms will give a lot of information about heavy metal mobility, availability and toxicity of heavy metals in comparison with the total element content. Association of heavy metals and their various soil components in different forms have been indicated to affect the mobility and bioavailability of metals in the soil (Huong *et al.*, 2010).

Heavy metal pollution is a worldwide problem for the agriculture lands (Sungur *et al.*, 2015) as concentration of heavy metals in agricultural soil can affect human directly through soil ingestion or through the food web by ingestion of crops and animal. It can cause a severe damage of environmental health and serious threat to human health. Recently the issue of heavy metal concentration in agricultural soils and plants has become a serious problem (Liu *et al.*, 2015). Some of heavy metal exists in a small amount in natural agricultural soil but if the amount of a heavy metal exceeds a certain level of pollutant, contamination of soil can occurs and agricultural products are contaminated (Arao *et al.*, 2010).

The main sources of heavy metals in agricultural soils usually from repeated use of untreated or poorly treated wastewater from industrial and application of chemical fertilizers and pesticides (Rahman *et al.*, 2012). In this study more focus on the analysis heavy metal from different types of agriculture soil to indicate whether different types of agriculture soil have different concentration of heavy metals.



### 1.3 Agriculture Area in Malaysia

Malaysia is a tropical, South East Asian country located between latitudes 20 and 70 N and longitude 920 and 1120 E (Nieuwolt *et al.*, 1983) and Malaysia is divided into Peninsular Malaysia, Sabah and Sarawak (Olaniyi *et al.*, 2011). According to Nieuwolt *et al.* (1983) soils in Malaysia usually are acid and low pH between 3.0 - 4.5; base saturation and nitrogen contents. Malaysia has a total land area of 33.06 million hectares with approximately 14.75 million hectares of the land are suitable for agriculture. Agriculture is one of the main land uses in Malaysia. Of this agriculture land, Peninsular Malaysia has 7.15 million hectares, Sarawak has 4.45 million hectares while Sabah has 3.15 million hectares (Anonymous, 1996).

The agriculture sector in Malaysia has played the dominant role and is dominated by plantation crops. Palm oil covered 2.0 million becomes one of the major crops followed by rubber which is 1.8 million of the land (Anonymous, 1996). Agriculture sector in Malaysia has contributed significantly to the economic development of the country. However its contributions to the Malaysia's economy have declined due to the fact that the industrial sector has increased rapidly. Notwithstanding its declination, the agriculture sector still continues to play a significant role in the development of the country (Olaniyi *et al.*, 2011).

Kelantan state is located in the East Coast of Malaysia Peninsular and is divided into ten provinces, namely Tumpat, Pasir Mas, Kota Bharu, Bachok, Tanah Merah, Pasir Puteh, Machang, Kuala Krai, Gua Musang and Jeli (Enoch, 2012).



In Kelantan, agriculture is one of the main activities and source of income especially for those who live in the rural area. The major agriculture in Kelantan is paddy followed by rubber and palm oil. The agriculture type choose in this study depends on the majority and availability of the agriculture near to the study area and their environment which has high possibilities for a crime to occur.

This present study has been intended to analyses heavy metals in the soil from different types of agriculture soil of palm oil plantation, sugarcane, rubber plantation, lime plantation and banana plantation as well as to ensure its significance role as evidence in a forensic investigation.

## 1.4 Problem Statement

Crime can happen in any places at any time. The places like agriculture area can be a perfect place for a criminal to commit a crime. This is because agriculture area particularly gives remote and hidden environment and commonly located in large and hidden rural area. However there are many types of agriculture areas. Analysis of soils in different types of agriculture areas is necessary and is important in forensic investigation as to narrow down the various types of agriculture soil where crime might have happened. Analysis of soil can provide valuable information which may lead and give a lot of information in forensic investigation.

The analysis of soil in forensic field is significant because there are almost infinite numbers of different types of soil. Soil can provide useful information and becomes evidential value in a criminal investigation. Heavy metal is one of the component of soil that maybe different in different type of agriculture soils. Although analysis of the soil is a very complicated process but the soil's heavy metal profile can be used to link a suspect or victim to a crime scene that having similar soil's heavy metal profile.

In this study, AAS was used to analyse the heavy metal in different type of agriculture soil. AAS gives more information about elemental composition in soil which is really important for this study.

## **1.5 Objective of the Study**

### **General objective:**

General aim of this study was to analyse heavy metals in agriculture soil of palm oil plantation, sugarcane, rubber plantation, lime plantation and banana plantation using Atomic Absorption Spectrometer (AAS).

### **Specific objectives:**

- I. To detect the presence of Cu, Cd, Cr, Pb and Zn in different types of agriculture soils.
- II. To determine the concentration of Cu, Cd, Cr, Pb and Zn in different types of agriculture soils using AAS.
- III. To determine which heavy metal of Cu, Cd, Cr, Pb and Zn have higher amount of concentration in different types of agriculture soils.

## **1.6 Significance of the Study**

The significance of this study is to give the information about estimation value of heavy metal analysis in different types of agriculture soils. Each different types of agriculture soil has different amount of concentration heavy metal in soil. This differences amount of concentration can be an evidential value for the forensic analysis especially in cases that involved the soil analysis. Data about the concentration of heavy metal can be used by forensic analysis in investigating the cases that involved the crime at agriculture area. This study more focus on heavy metals analysis in different types of agriculture soil of palm oil, sugarcane, rubber, lime and banana.



## CHAPTER 2

### LITERATURE REVIEW

Heavy metal analysis must follow a few procedures. In general, sample preparation need to be done for the sample before instrumental analysis. Solid sample need to undergo acid digestion process to change them into liquids form.

#### 2.1 Sample Preparation: Acid Digestion Method

Sample preparation using acid digestion method is necessary step before determine the total element mass concentration in soils. Various digestion methods are used to determine the mass concentration of elements in solid matrices. In other words, sample digestion is a process to quantification of heavy metal includes closed or open digestion system and use of different combinations of acid such as  $\text{HNO}_3$ ,  $\text{HCl}$ ,  $\text{HClO}_4$ ,  $\text{HF}$  as well as oxidants,  $\text{H}_2\text{O}_2$ . The most common digestion methods to determine the concentration of element in solid sample matrices are open beakers heated on hot plates, digestion tubes in a block digester and digestion bombs placed in a microwave oven (Geana *et al.*, 2011).

In a study conducted by Khan *et al.* (2013) used different digestion method for different agriculture soil and agriculture crops. For the agriculture soil, the soil samples were extracted using wet digestion method. The soil sample was digested in 15 mL of aqua-regia in the ratio of 5:1:1 of  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$  and  $\text{HClO}_4$  in 50 mL of conical flask.

The mixture was kept overnight and heat gently on a hot plate at 80°C until transparent extract was obtained before being filtered and diluted. For the agricultural crop, samples were mixed with 15 mL HClO<sub>4</sub> and HNO<sub>3</sub> solution in the ratio 1:4. After overnight cold digestion, the crop samples were heated on a hot plate at different temperature until transparent solution was obtained. In this study, stated that different digestion methods were used for different types of sample. Research done by Opaluwa *et al.* (2012) used different sample treatment to the soil as soil sample was digested using 15.0 mL HNO<sub>3</sub>, 20.0 mL HClO<sub>4</sub> and 15.0 mL HF on a hot plate for 3 hours. Compared to other research, this study more focus on the use of HNO<sub>3</sub>, HClO<sub>4</sub> and HF.

In addition to study carried out by Yusuf *et al.* (2015) stated that the soil sample was digested with 5mL of HNO<sub>3</sub>, 15 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and 0.3 mL of HClO<sub>4</sub> using dropping pipette. The mixture was digested in a fume cupboard, heating until a dense white fume appeared then ingested for 15 minutes. After that the mixture was diluted with distilled water to diluted mark volume.

Research conducted by Monica *et al.* (2015) used three different digestion method of soil sample to offer a proper method for determination of heavy metal. For example, first digestion method used was the combination of 6 mL of 65% HNO<sub>3</sub>, 3 mL of 37% HCl and H<sub>2</sub>O<sub>2</sub>. Second digestion method only used HNO<sub>3</sub> whereas lastly used the mixture of HNO<sub>3</sub> and HCl. In this study, low pressure microwave heating programmers modified were used for HNO<sub>3</sub> and HNO<sub>3</sub> with HCl. For HNO<sub>3</sub> with HCl and H<sub>2</sub>O<sub>2</sub> does not use pressure microwave.



Researcher conducted by Zauro *et al.* (2013) used different acid in different amount of volume in digested soil sample. The soil samples were treated with the mixture of 60%  $\text{HClO}_4$ , concentrated  $\text{HNO}_3$  and concentrated  $\text{H}_2\text{SO}_4$  in the ratio 5: 1: 0.5. After that, the mixture was swirled gently and digested for 15 minutes, allowed to cool and diluted.

In addition, research done by Matthews-Amune and Samuel (2012) study on comparison of digestion method in determination of metal level in soil. In this study, research used various types of different digestion method in digested soil. For example, the digestion methods used were  $\text{HNO}_3$ ,  $\text{HCl}$  and different combinations of acids such as  $\text{HClO}_4$ ,  $\text{HCl}$ ,  $\text{HNO}_3$  and  $\text{HF}$ . This research more focus on comparison of various digestion method to determine the best method of extracting metals from soils.

According to Edgell (1989), this study used method 3050B Acid Digestion of Sediments, Sludges and Soils which involved the use of three different acid which were concentrated  $\text{HNO}_3$ ,  $\text{HCl}$  and  $\text{H}_2\text{O}_2$  using hot plate in different amount of value. The soil sample was heated in temperature of  $95^\circ\text{C}$ . Each digestion methods have their own advantages and disadvantages. According to Sastre *et al.* (2002) the use of microwaves in wet digestion has really useful in determination of elements as the risk of contamination and loss of volatile chemical elements can be reduced. However, the open digestion system allows large number of sample to be analysis and facilitate evaporation of acid and drying of the digests as well as analytical problem in digestion can be reduced. Research done by Zheljazkov and Warman (2002) verified that  $\text{HNO}_3$  with  $\text{HClO}_4$  digestion can provide a higher recovery of Cd and Pb and study by Hseu (2004) also verified that good result obtained with  $\text{HNO}_3$  digestion.

Based on the previous study, several methods have been used by different researcher in the digestion of soil samples to the determination of heavy metal. Usually the selected acid digestion method depends on the type of soil or sample and which one of acid digestion method provides the best method in sample preparation. Sample preparation using acid digestion method is important step before the sample being analyzed by selected instrument.

## **2.2 Heavy Metal Analysis**

There are many instruments that can be used to analyze elemental composition. Examples of this instrument are X-ray fluorescence XRF, inductively coupled plasma ICP spectrometry and atomic absorption spectroscopy AAS (Dawson and Hillier, 2010). This instrument can also be used for heavy metal analysis in soil. There is a lot of research related to the heavy metal analysis that had been done in other countries including Malaysia.

XRF has been applied in metal determination in environmental sample such as soil, sediment, dust and rocks. The accuracy of measurement by XRF can be affected by the sample characteristics such as moisture content, density, flatness of the surface, particle size, and soil type (Lu *et al.*, 2010). XRF has some potential advantages for soil heavy metal analysis as it is nondestructive with rapid throughout and simple sample preparation without acid digestion. According to Kodom *et al.* (2012) the XRF is a very sensitive technique, hence great caution was taken to avoid contamination of the pellets ahead of the analysis.



Poor handling of the samples could seriously affect the results of the analysis due to high sensitivity of the spectrometer, which was sensitive enough to detect fingerprints on the pellet's surface layer.

In the study of soil samples collected in Kumasi, Ghana, analyses qualitatively and quantitatively the concentrations of heavy metals Zn, Pb, Cr, Cu, Co, Ni, Cd, Hg, and As from an industrial cluster that present in the surface soils range from 0-15 cm by using XRF spectroscopy analysis. The result obtained in this study were Zn 189.2–908.6 mg/kg, Pb 133.7–571.3 mg/kg, Cr 91.3–545.8 mg/kg, Cu 62.9–334.6 mg/kg, Co 38.6–81.9 mg/kg, Ni 12.4–30.9 mg/kg, Cd 6.9–13.2 mg/kg, Hg 5.5–10.4 mg/kg, and As 2.3–18.6 mg/kg (Kodom *et al.*, 2012).

ICPMS is a multi-element technique which characterized with high sensitivity, selectivity and has detection limits much lower than other multi element techniques. In addition, ICPMS are powerful techniques for trace analysis of elements (Voica *et al.*, 2012) and can analyzed simultaneous elements. Besides that, ICPMS detection limits are very impressive as most detection limits are in the 1-10 part per trillion ranges for solutions. Research conducted by Ripin *et al.* (2014) studied 18 soil sample at depth of 0-15cm around Perlis was determine the concentration of heavy metal such as Cu, Cr, Ni, Cd and Pb by using ICPMS. The objective of this study was to determine whether the heavy metals contamination distribution were due to industrialization, urbanization and agricultural activities. Overall concentrations of Cu, Cr, Ni, Cd and Pb in the soil samples ranged from 0.38-240.59, 0.642-3.921, 0.689-2.398, 0-0.63 and 0.39-27.47 mg/kg respectively. The concentration of heavy metals in the soil display the following decreasing trend: Cu > Pb > Cr > Ni > Cd.

In this study, the use of ICPMS to make sure that analysis of soil will be faster and several elements can be analyzed simultaneously. In addition, ICPMS is widely accepted analytical techniques which useful for detection and quantification of elements that are present.

AAS is a useful technique for measuring the quantities of chemical elements that present in the samples. AAS is a technique for determining the concentration of a particular metal element within samples as AAS can be used to analyze the concentration of over 62 different metals in a solution (Garcia and Baez, 2012). The advantages of using AAS are greater sensitivity and detection limits, direct analysis of some types of liquid sample and require only small sample size. AAS offers a destructive analytical technique and can only analyzed one element in single analysis and time consuming but requires at least 1-2 mL of solution each analysis. Research carried out by Coskun *et al.* (2006) state that the concentration of Pb, As and Cd were determined by using graphite furnace AAS while Cu, Co, Mn, Ni and Zn were determined by using flame AAS. In this study, all heavy metal in sample being analyzed by AAS but using different types of AAS such as flame and graphite furnace. The differences between furnace and graphite were flame can analyze 4 seconds per elements whereas graphite can analyze 2-3 minutes per elements.

Study by Okeyode and Rufai (2011), used flame AAS to determine the elemental composition in the sample. AAS can only analyze solution, so solid soil sample will be digested to be a solution before being analyzed by AAS. Before the concentrations of heavy metal were determined, different standard solution were prepared for different elements.



In addition, this study more focus analysis heavy metal in soil samples of dumpsites and control site and the result showed that at dumpsites, the concentrations of Fe, Mn, Zn, Pb, Ni, Mg, Na and Cu ranged from 86.89-277.33 mg/L, 1.9731-5.6644 mg/L, 0.3299-4.847 mg/L, 0.4132-2.8137 mg/L, 0.0896-0.1376 mg/L, 1.9731-5.6644 mg/L, 0.9203-29.00 mg/L and 0.1645-5.4191 mg/L respectively, while at control sites 20m away from dumpsite, the concentrations ranged from 37.64-109.94 mg/L, 0.5342-1.7910 mg/L, 0.1015-0.3692 mg/L, 0.2150-0.7595 mg/L, 0.0590-0.0881 mg/L, 0.0927-0.3976 mg/L, 0.4385 and 0.9876 mg/L and 0.004 and 0.416 mg/L respectively.

Analysis by AAS is well-known methods to determine the concentration of metal in soil sample although involve length process. Calibration was carried out using standard solutions and if sample has a high levels of contamination, the digested soil solution were diluted by a factor of 10 to make sure it within the calibration range (Radu and Diamond, 2009). Determination of elemental concentrations in soil samples was performed using the method of calibration curve and several standard solution of different known concentration was prepared and concentration of unknown sample was determined from calibration curve (Ene *et al.*, 2009).

A recent research by Brown and Jasra (2016) studies the estimation of heavy metal concentration in soil for forensic investigation. Based on this study, showed that the land use type does not affect the concentration of heavy metal as each location has own unique land history and soil properties. This research more focus on analysis heavy metal of As, Cd, Cr, Cu, Fe, Hg, Pb and Zn by using ICP in six sampling location of three land use type which were cemetery, industrial such as waste management and petrol and lastly control.

There was two sampling location were chosen in each of three land use type. The result showed that industrial for waste management has highest concentration of each heavy metal except for Fe and V whereas industrial for petrol has lowest concentration for each heavy metal than the control location. This is unusual as it was expected that control locations should have the lowest concentration of heavy metal. Heavy metal for cemetery in both locations showed the differences. The concentrations of heavy metal vary with locations as heavy metal associated with soils in different ways as it depends on its chemical and physiochemical properties. Research study by Martin *et al.* (2006), revealed that the concentration levels of heavy metal depend on pH and organic matter content. The concentrations of heavy metal were low in areas of high pH and areas of low organic matter content concentration.

Based on the previous research, there are many different instruments that can be used to analyze the concentration of heavy metal in sample. Sometimes this instrument is characterized by time as it consuming acid digestion, the high analysis costs and maybe takes a week to complete. Different instrument used usually depend on the availability of that instrument and which instrument that can provide the best result in analysis.

Overall, AAS is a technique which most suitable to analyze heavy metal concentration in soil compared to ICPMS and XRF. This technique is more expensive than the XRF method and requires a prior digest to obtain the sample in the form of a solution. Despite this, it is widely used to analyze heavy metal concentration. The advantages of this technique include its robust interface, speed of analysis and ease to use although detection limits are moderate compared to the ICPMS analysis.



## CHAPTER 3

### METHODOLOGY

Sample preparation using acid digestion method and the preparations of standard solutions as well as the analysis of heavy metal concentration by Atomic Absorption Spectrometer (AAS) were done at the Analytical Laboratory and Forensic Laboratory of Universiti Sains Malaysia.

#### 3.1 Chemical Reagents

The chemicals and reagents used in the laboratory for sample analysis were deionized water, concentrated nitric acid, concentrated hydrochloric acid and hydrogen peroxide (Table 3.1). The chemical and reagent were analytical grade.

Table 3.1: List of chemical reagents

Chemical reagent	Brands
Nitric acid, $\text{HNO}_3$ (65%)	HmbG ® chemicals in Darmstadt, Germany
Hydrochloric acid, $\text{HCl}$ (37%)	HmbG ® chemicals in Darmstadt, Germany
Hydrogen peroxide, $\text{H}_2\text{O}_2$ (30%)	MERCK Schuchardt OHG, Germany
Deionized water, DI	-

### 3.2 Instrument

The instruments that were used in this study are listed in Table 3.2. All the instruments used were handled with care to produce the precise and accurate result.

Table 3.2: List of instruments

Instrument	Brand
Grinder	IKA ®, A11 basic
Analytical balance	Shimadzu, Kyota Japan
Hot plate	ERLA ®, EMS-HP-7000
AAS	Perkin Elmer , AAnalyst800

### 3.3 Soil Samples

This study was carried out in five different agriculture areas. Example of agriculture soils that used are listed in Table 3.3. The selected heavy metals used in this study are Cu, Cd, Cr, Pb and Zn. The soils in the agriculture were focused only on the surface of soil.

Table 3.3: List of agriculture soil

Sites	Agriculture types	Location
Soil 1	Palm oil	Tanah Merah
Soil 2	Sugarcane	Bachok
Soil 3	Rubber	Bachok
Soil 4	Lime	Bachok
Soil 5	Banana	Bachok

### **3.4 Methodology**

The experiment was divided into two phases. The first phase, the sample pre-treatment by acid digestion method and the second phase was the analysis of samples to determine the concentration of heavy metals in the different types of agriculture soils using AAS.

#### **3.4.1 Sample Preparation using Acid Digestion Method**

The soil samples were weighed in appropriate amount (refer Appendix A) and each of them were placed into 2.5 mL beaker respectively, then, 2.5 mL of deionized water (DI) and 2.5 mL concentrated  $\text{HNO}_3$  were added into the beaker respectively and it was stirred before covered with watch glass. After that, the solution was heated to  $95^\circ\text{C}$  for 10 minutes without boiling. The solution was allowed to cool before 2.5 mL of concentrated  $\text{HNO}_3$  was added and the solution was heated for another 30 minutes. When brown fume was generated, another 2.5 mL of concentrated  $\text{HNO}_3$  was added until no brown fumes appeared.

The solution was then evaporated to 2.5 mL volume without boiling. The solution was cooled before 1 mL of DI and 1.5 mL of 30%  $\text{H}_2\text{O}_2$  was added. The solution in beaker was covered with watch glass and heated for peroxide reaction until bubbling stopped. Then, another 0.5 mL of 30%  $\text{H}_2\text{O}_2$  was added until bubbling reduced, ensuring that the total amount of  $\text{H}_2\text{O}_2$  did not exceed 5 mL. The solution in beaker was covered with a watch glass and was heated until the volume was reduced to 2.5 mL without boiling.



Then, 5 mL of concentrated HCl was added to the solution and covered with a watch glass before heated to 95°C for 15 minutes. After cooling, the solution was filtered through a filter paper using a filter funnel in 25 mL volumetric flask. The volume of filtrate in 25 mL of volumetric flask was made up to mark with DI and then all the solutions of digested soil samples were analyzed by AAS. The same procedure was repeated to the other type of agriculture soils. In each type of agriculture soil triplicate analysis was performed.

### 3.4.2 Calibration Curves

The selected heavy metals in this study were Cu, Cd, Cr, Pb and Zn. Calibration curves for each of the selected metal were prepared using five standard solutions which were prepared by serial dilution from 1000 ppm of standard stock solution as stated in Table 3.4.

Table 3.4: List of five standard solutions for each heavy metal

Heavy metal	Five standard solutions
<b>Cu</b>	0.3125 ppm, 0.625 ppm, 1.25 ppm, 2.50 ppm, 5.00 ppm
<b>Cd</b>	0.125 ppm, 0.25 ppm, 0.50 ppm, 1.00 ppm, 2.00 ppm
<b>Cr</b>	0.3125 ppm, 0.625 ppm, 1.25 ppm, 2.5 ppm, 5.0 ppm
<b>Pb</b>	1.875 ppm, 3.75 ppm, 7.5 ppm, 15 ppm, 30 ppm
<b>Zn</b>	0.0625 ppm, 0.125 ppm, 0.25 ppm, 0.5 ppm, 1.0 ppm

Calibration curves were constructed for Cu, Cd, Cr, Pb and Zn by plotting absorbance versus metal ion concentration. Calibration curves were prepared to determine the concentration of the selected heavy metals in the soil samples.

### **3.4.3 Limit of Detection (LOD)**

In this study, five readings of blank samples were taken to identify the lowest analyte concentration that can be detected. The readings were recorded and standard deviation was calculated. The limit of detection (LOD) was determined by using the equation below.

$$\text{LOD} = 3.3\left(\frac{\text{SD}}{\text{SLOPE}}\right)$$

Where, SD: standard deviation of blank sample

Slope: the slope of the calibration curve

### **3.4.4 Recovery Studies**

Recovery studies were done to evaluate the sample pre-treatment using acid digestion method was suitable to carry out in this study. Each type of agriculture soil was spiked with same concentration of heavy metal which was 0.26 ppm except for Zn in lime and banana soil which was 0.10 ppm. The digestion method for the spiked sample followed the same procedure as the non- spiked sample as described in section 3.4.1.



The amount that was recovered after digestion of the spiked samples was used to calculate % recovery. The % recovery was calculated using the equation below.

$$R = \frac{C_s - C}{s} \times 100\%$$

Where,

s= spiked concentration of heavy metal added to the sample

C<sub>s</sub>= metal content of the spiked sample

c = metal content of non-spiked sample

R = percent recovered

### 3.4.5 Heavy Metal Analysis

All the solutions of digested soil samples were analyzed by AAS to determine their concentration. The absorbance for each type of digested soil sample was compare with its respective standard calibration curve.

The results obtained in this study were in weight per volume (mg/L). Therefore the result should be converted from weight per volume (mg/L) to weight per weight (mg/kg) as soil was a solid sample.

$$\frac{\text{mg}}{\text{kg}} = \frac{\text{concentration of metal} \left( \frac{\text{mg}}{\text{L}} \right) \times \text{volume of sample(L)}}{\text{sample weight(kg)}}$$