

DETERMINATION OF HEAVY METAL ACCUMULATION IN IPOMOEA
AQUATICA USING WET DESTRUCTION METHOD

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UNIVERSITI MALAYSIA PAHANG

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BORANG PENGESAHAN STATUS TESIS[♦]

**JUDUL : DETERMINATION OF HEAVY METAL ACCUMULATION IN
IPOMOEA AQUATICA USING WET DESTRUCTION METHOD**

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**DETERMINATION OF HEAVY METAL ACCUMULATION IN IPOMOEA
AQUATICA USING WET DESTRUCTION METHOD**

MOHD HILMI BIN JAAFAR

**A thesis submitted in fulfillment
of the requirements for the award of the Degree of
Bachelor of Chemical Engineering**

**Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang**

NOVEMBER 2010

“I hereby declare that I have read this thesis and in my/our opinion this thesis has fulfilled the qualities and requirements for the award of Degree of Bachelor of Chemical Engineering”

Signature :

Name of Supervisor : Azizan Bin Ramli

Date : 2 December 2010

I declare that this thesis entitled “*Determination of Heavy Metal in Ipomoea Aquatica using Wet Destruction Method*” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.”

Signature :

Name : Mohd Hilmi Bin Jaafar

Date : 2 December 2010

Dedication,

*To my Beloved Parents,
En. Jaafar Bin Mohamad
Pn. MeK Mah Binti Saleh*

*To my Lovely Brother and Sisters,
Ruhana Binti Jaafar
Fauziah Binti Jaafar
Mohd Amaludin Bin Jaafar
Jazima Binti Jaafar
Mariati Binti Jaafar
Ratinah Binti Jaafar
Mohd Fauzi Bin Jaafar
Nur Azimah Bin Jaafar*

*To my Little Nephew,
Mohd Rusyaidi ,
Mohd Afif Jazimin ,
Nur Faaiqa Dayana ,
Hail Idlan
Marha Irdina, and
Nur Fadhlina Sofia*

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ABSTRACT

The determination of heavy metals in plants is very important since human intake of toxic trace elements which even at low doses over a long period of time can lead to malfunction of organs and could cause chronic toxicity. Hence, it is necessary to obtain more information on the plants which grow on soils that contain high concentration of heavy metals in order to determine their potential for management of polluted soils and for metal extraction. The objectives of this research are to determine heavy metal (Cr, Ni, Cu, Mn, Cd, Fe, Pb and Zn) uptake by *Ipomoea Aquatica* cultivated in closed landfill and nursery soil and to compare the concentration of heavy metal in both soil samples besides in order to estimate whether a given soil is suitable for cultivation of plants used as food or feed based on World Health Organization – Maximum Level standard. Acid Digestion Method and Wet Destruction Method were used for digestion process of soils and plants. After dilution, the samples will undergo analysis by using atomic absorption spectrometry (AAS). From result that obtained, the concentrations of all heavy were detected in plant and soil but with various concentrations. There are several heavy metals that exceed the maximum level of WHO standard, Cr (4.16 and 6.92 mg kg⁻¹), Cd (4.67 and 3.93 mg kg⁻¹) and Pb (9.66 and 8.87 mg kg⁻¹). As a conclusion, monitoring of heavy metal distribution data in soil and plant samples are very useful for become main references or guidelines in order to monitoring and avoid environmental pollution become worst in terms of quality of soil and also safety level for vegetables to be consumed.

ABSTRAK

Penentuan logam berat dalam tumbuhan adalah sangat penting disebabkan pengambilan logam berat oleh manusia walaupun dalam kuantiti yang sangat rendah akan membawa kepada ketidakfungsian organ-organ serta boleh mengakibatkan ketoksikan yang kronik. Oleh itu, ia menjadi keperluan untuk memperoleh maklumat ke atas tumbuhan yang tumbuh dalam tanah yang mengandungi kepekatan logam berat yang tinggi supaya dapat menentukan potensi mereka untuk pengawalan tanah yang tercemar serta penguraian besi. Objektif kajian ini adalah untuk menentukan kandungan logam berat (Cr, Ni, Cu, Mn, Cd, Fe, Pb and Zn) yang diserap oleh kangkung yang ditanam dalam tanah dari tempat pelupusan sampah dan tanah dari pasaran serta ingin membandingkan kepekatan logam berat di dalam kedua-dua tanah tersebut selain juga ingin melihat sama ada tanah tersebut sesuai ataupun tidak untuk penanaman tumbuhan berdasarkan piawaian yang ditentukann oleh Organisasi Kesihatan Dunia. Teknik pencernaan asid dan teknik pencernaan basah masing-masing digunakan untuk proses pencernaan tanah serta tumbuhan. Selepas itu, sampel-sample tersebut akan dianalisis menggunakan Spektrometri Serapan Atom (AAS). Kepekatan kesemua logam berat telah dikesan dalam tumbuhan dan tanah tetapi dengan kepekatan berlainan. Terdapat beberapa logam berat yang melebihi tahap maksimum piawan WHO, Cr (4.16 dan 6.92 mg kg⁻¹), Cd (4.67 dan 3.93 mg kg⁻¹) dan Pb (9.66 dan 8.87 mg kg⁻¹). Kesimpulannya, menyelidik tentang data taburan logam berat dalam tanah serta tumbuhan amat berguna untuk dijadikan rujukan ataupun panduan supaya dapat mengelakkan pencemaran alam sekitar menjadi lebih teruk dalam konteks berkaitan dengan kualiti tanah serta tahap selamat untuk sayur-sayuran yang akan digunakan.

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LIST OF ABBREVIATION

Cr	- Chromium
Cd	- Cadmium
Pb	- Lead
Ni	- Nickel
Zn	- Zinc
Fe	- Iron
Mn	- Manganese
Cu	- Copper
Df	- Dilution Factor
AAS	- Atomic Absorption Spectrometry
HNO ₃	- Nitric Acid
HCl	- Hydrochloric Acid
M	- Concentration
V	- Volume
ppm	- Part per million
WHO	- World Health Organization
ML	- Maximum Level
WDM	- Wet Destruction Method
H ₂ O ₂	- Hydrogen Peroxide
FLAA	- Flame Atomic Absorption Spectrometry
GFAA	- Graphite Furnace Atomic Absorption
ICP-AES	- Inductively Coupled Plasma Atomic Emission Spectrometry

ICP-MS - Inductively Coupled Plasma Atomic Emission Spectrometry

CHAPTER 1

INTRODUCTION

1.1 Research Background

The increasing levels of heavy metals contamination could be caused by human activity which then makes heavy metals as the main sources of pollution on the environment. Heavy metals are considered as a member of an ill-defined subset of elements that reveal metallic properties, which would mainly consist of the transition metals, some metalloids, lanthanides, and actinides. The determination of heavy metals in plants is very important since human intake of toxic trace elements which even at low doses over a long period of time can lead to malfunction of organs and could cause chronic toxicity (Krishnamurty et al., 1976). There are also possibilities which farmers tend to use soil taken from landfill to use for agricultural uses since it is more. Hence, it is necessary to obtain more information on the plants which grow on soils high in metals to determine their potential for management of polluted soils and for metal extraction (S.Shallri, 1998).

1.2 Problem statement

For many years, landfills have been one of the most comprehensive forms of disposal of municipal solid waste due to the operative easiness and the low economic cost. Landfill material contains organic matter and plant nutrients such as nitrogen and phosphorus which is good for fertilizing agricultural soils. These inorganic fertilizers are expensive and will induce the tendency of people to take the soil from

landfill sites for their agricultural activities especially for farmers whom cannot afford to buy the fertilizer. Landfill site contains heavy metals, which could accumulate in the agricultural fields where the landfill material is applied. Moreover, landfill sites also contain an amount of organic and inorganic pollutants include plastics, metals, glasses, fibers and heavy metals which can pollute the agricultural soils in the long term of period. Although these considered as pollution problems, there are still no awareness exists on the potential risk of invisible pollutants like heavy metals. As a matter of fact, heavy metals can be leached through the soil profile; either transported in drainage waters and may pollute groundwater or they can accumulate in the upper soil layer and can be toxic to plants and soil microbial mass. Heavy metals are not only accumulating in the soils but also in the aboveground parts of a plant. Hence, these will give risks for the public health due to the potential exposure to pathogenic agents, toxic substances, gases.

A plant nursery is a place where plants are propagated and let to be grown to a usable size. Nursery also includes retail nurseries, wholesale nurseries. Retail nurseries manage plants that are going to be sold to the general public while wholesale nurseries only sell for business like other nurseries and for commercial. Another type of nursery is private nurseries which supply the needs of institutions or private estates. Despite selling plants, nursery also provides many choices of planting material such as soil, seeds, cuttings, tissue, etc depending on the tree species, the nature of soil and the degree of site preparation. Most of the nurseries apply a highly labor intensive which is mechanized and automated process in order to remain the material's quality and to remain the same condition together. Other nurseries that have not apply the mechanized and automated process might be applying a manual process to provide the planting materials.

1.3 Objectives

- i. To determine total heavy metals uptakes in plants cultivated in two sample of soil (closed landfill and nursery soil).
- ii. To compare the concentration of heavy metal in two sample of soil (nursery soil and closed landfill soil).
- iii. To estimate whether a given soil is suitable for cultivation of plants used as food or feed based on World Health Organization – Maximum Level standard.

1.4 Scope of Research

1.4.1 Determination of Heavy Metal using Wet Destruction Method in Crop (Ipomoea Aquatica)

There are eight types of heavy metal that will be focused in this research. There are:

1. Chromium (Cr)
2. Copper (Cu)
3. Manganese (Mn)
4. Nickel (Ni)
5. Cadmium (Cd)
6. Iron (Fe)
7. Lead (Pb)
8. Zinc (Zn)

Two samples of soils were used. there are nursery soil that were bought from a plant nursery near Kuantan city and the other soil samples were taken randomly from a closed landfill at Ulu Tualang, Temerloh, Pahang. This landfill is the second

biggest of closed landfill in Pahang state of Malaysia. The closed landfill is near to the Temerloh Industrial Estate and Mentakab Industrial Park that focusing in stainless steel fabrication and wood base industry. As additional information, there are 15 landfills are located in Pahang state. 13 of them are operated by Alam Flora including the Ulu Tualang Landfill. The company list that probably using the landfill:

- i. Megaply Industries (M) Sdn Bhd (Plywood & Veneers).
- ii. Intan Suria Sdn Bhd (Frames - Picture, Wood Products).
- iii. SQ Wooden Picture Frame Moulding Sdn Bhd (Picture Frames - Wholesaler & Manufacturers).
- iv. LCS Precast Sdn Bhd (Piling).
- v. Mentakab Stainless Steel Works (Stainless Steel Fabricators).
- vi. Syarikat Perniagaan Boon Wee (Biscuits - Wholesaler & Manufacturers, Food Products).
- vii. Mentakab Agricultural Machinery Sdn Bhd (Agricultural Equipment & Supplies, Tractor Distributors & Manufacturers).

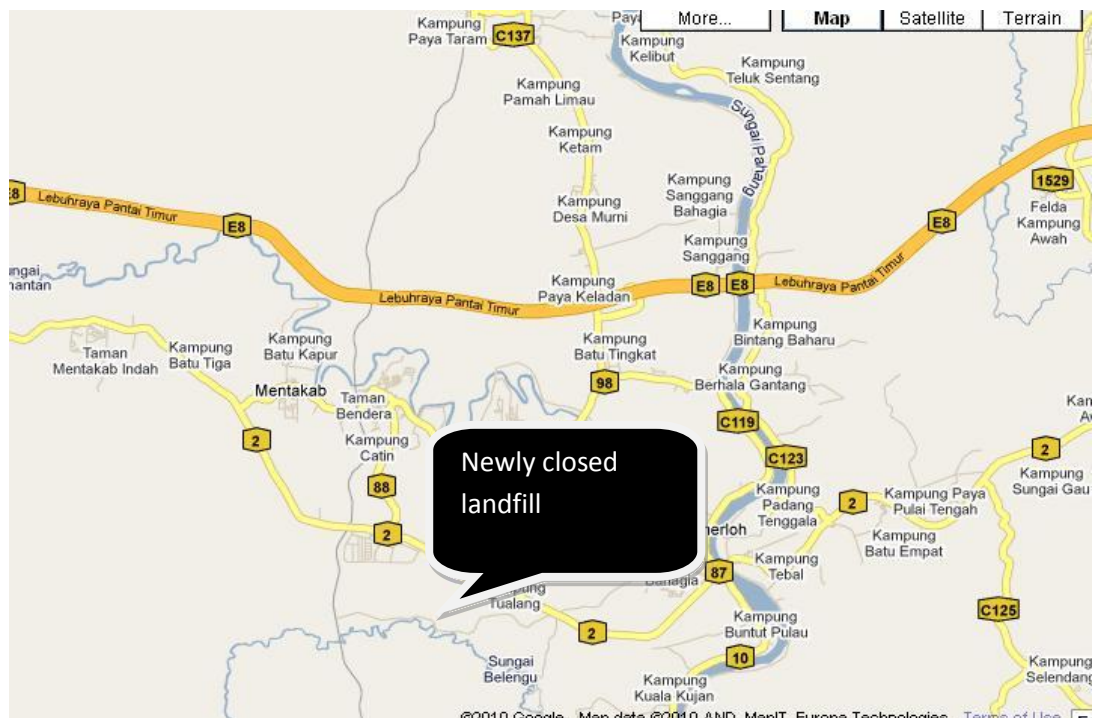


Figure 1.1: Landfill of Ulu Tualang, Temerloh, Pahang



Figure 1.2: *Ipomoea Aquatica*

For plantation, wild spinach (scientific name: *Ipomoea Aquatica*) is selected. *Ipomoea Aquatica* is fast growing crop (Mian-Hao Hu, 2010). The table below shows the characteristic of *Ipomoea Aquatica*:

Table 1.1: Characteristic of *Ipomoea Aquatica*

Plant Type	Single
Plant Height (cm)	30-35
Days to Harvest (DAS)	22-25 / 20-25
Plant vigor	Strong
Degree of Common Disease Tolerant	Good
Features	Bamboo leaf type, Single stem, High yield
Growing Season	All year round

Besides that, this species of *Ipomoea Aquatica* is widely used in Malaysia cuisine and it also available in abundant (substantial available).

For the destruction method of the green plants, wet destruction method (WDM) is used. Wet destruction method was selected because of its advantages. There are:

1. Good in terms of heavy metal recoveries
2. Short time needed
3. Easy handling and cheap

(Gijs Du Laing *et al.*, 2003)

For the soil samples, Acid Digestion Method (SCL Extraction Method) is used in order to digest both soil samples. After that, the samples will be diluted and hence, the diluted samples will undergo analysis by using atomic absorption spectrometry (AAS) in order to determine the concentration of heavy metal in plant and also soil.

1.4.2 Compare the Concentration of Heavy Metal with WHO-ML Standard

After all the data is obtained, the concentration of heavy metal in plant will be compare to the standard of maximum level permissible in vegetation that set by World Health Organization (WHO).

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

This chapter reviews types of heavy metal followed by their toxicology. Besides that, this chapter also covers about the heavy metal in plants, digestion method and analyzer that selected and some previous result that obtained from previous researcher.

2.2 Types of Heavy Metal

There are eight types of metal that will be focused in this research. There are Chromium (Cr), Manganese (Mn), Copper (Cu), Nickel (Ni), Ferum (Fe), Plumbum (Pb), Cadmium (Cd) and Zinc (Zn). Chromium is a lustrous, brittle and hard metal. Its colour is silver-gray and it can be highly polished. Other than that, it does not tarnish in air but it burns and forms the green chromic oxide when heated. Chromium is unstable in oxygen, it immediately produces a thin oxide layer that is impermeable to oxygen and protects the metal below.

Manganese is a pinkish-gray, chemically active element. It is a hard metal and is very brittle. Besides that, it is hard to melt but easily oxidized. When it is in pure condition, manganese is reactive but it will burn in oxygen when it in powder form besides it reacts with water (it rusts like iron) and dissolves in dilute acids.

Copper is a reddish metal with a face-centered cubic crystalline structure. It reflects red and orange light and absorbs other frequencies in the visible spectrum due to its band structure, so it has a nice reddish colour. Copper is malleable, ductile and an extremely good conductor of both heat and electricity. It is softer than iron but harder than zinc and can be polished to a bright finish. It is found in group Ib of the periodic table, together with silver and gold. Copper has low chemical reactivity. In moist air, it slowly forms a greenish surface film called patina; this coating protects the metal from further attack.

Nickel is silvery-white, hard, malleable and ductile metal. It is of the iron group and it takes on a high polish. It is a fairly good conductor of heat and electricity. Nickel is bivalent in its familiar compounds even though it assumes other valences. It also forms a number of complex compounds. Most nickel compounds are blue or green. Nickel dissolves slowly in dilute acids but like iron, becomes passive when treated with nitric acid.

Iron is a lustrous, ductile, malleable, silver-gray metal (group VIII of the periodic table). It is known to exist in four distinct crystalline forms. Iron rusts in damp air but not in dry air. It dissolves readily in dilute acids. Iron is chemically active and forms two major series of chemical compounds, the bivalent iron (II), or ferrous, compounds and the trivalent iron (III), or ferric, compounds.

Lead is a bluish-white lustrous metal. It is very soft, highly malleable, ductile, and a relatively poor conductor of electricity. It is very resistant to corrosion but tarnishes upon exposure to air. Lead isotopes are the end products of each of the three series of naturally occurring radioactive elements.


Cadmium is a lustrous, silver-white, ductile, very malleable metal. Its surface has a bluish tinge and the metal is soft enough to be cut with a knife, but it tarnishes in air. It is soluble in acids but not in alkalis. It is similar in many respects to zinc but it forms more complex compounds.





Zinc is a lustrous bluish-white metal. It is found in group IIb of the periodic table. It is brittle and crystalline at ordinary temperatures, but it becomes ductile and malleable when heated between 110°C and 150°C. It is a fairly reactive metal that will combine with oxygen and other non-metals, and will react with dilute acids to release hydrogen (www.lentech.com, 2010).




2.3 Toxicology of Heavy Metal

The determination of heavy metals in soil samples and also plant samples are very important in monitoring environmental pollution. Metals are toxic even in traces. The ability of the plant to absorb heavy metals can cause human health or ecological become worst. For example, human take their meal (especially crops) that contain a lot of toxic heavy metals will cause them get lung cancer. The soils at the landfill and nursery soil widely used in agricultural activities but we do not know about the quality of both soil. The soils are containing a lot of essentials metals that important in biological system cause human attracted to use the contaminant soils as their fertilizer for their agriculture activity. The table below describes the toxicology of four heavy metals that stated before:

Table 2.1: Toxicology of Heavy Metal (www.lentech.com, 2010)

Heavy Metal	Toxicology
 <p data-bbox="558 1680 598 1713">Cr</p>	<ul style="list-style-type: none"> <li data-bbox="885 1478 1053 1512">- Skin rashes <li data-bbox="885 1534 1252 1568">- Upset stomachs and ulcers <li data-bbox="885 1590 1189 1624">- Respiratory problems <li data-bbox="885 1646 1268 1680">- Weakened immune systems <li data-bbox="885 1702 1236 1736">- Kidney and liver damage <li data-bbox="885 1758 1284 1792">- Alteration of genetic material <li data-bbox="885 1814 1069 1848">- Lung cancer <li data-bbox="885 1870 981 1904">- Death

 <p>Mn</p>	<ul style="list-style-type: none"> - Fatness - Glucose intolerance - Blood clotting - Skin problems - Lowered cholesterol levels - Skeleton disorders - Birth defects - Changes of hair colour - Neurological symptoms
 <p>Cu</p>	<ul style="list-style-type: none"> - Headaches - Stomachaches - Dizziness - Vomiting - Diarrhoea - May cause liver and kidney damage
 <p>Ni</p>	<ul style="list-style-type: none"> - Higher chances of development of lung cancer, nose cancer, larynx cancer and prostate cancer - Sickness and dizziness after exposure to nickel gas - Lung embolism - Respiratory failure - Birth defects - Asthma and chronic bronchitis - Allergic reactions such as skin rashes, mainly from jewellery - Heart disorders
 <p>Cd</p>	<ul style="list-style-type: none"> - Diarrhoea, stomach pains and severe vomiting - Bone fracture - Reproductive failure and possibly even infertility - Damage to the central nervous system

	<ul style="list-style-type: none"> - Damage to the immune system - Psychological disorders - Possibly DNA damage or cancer development
 <p style="text-align: right; margin-right: 20px;">Pb</p>	<ul style="list-style-type: none"> - Disruption of the biosynthesis of haemoglobin and anaemia - A rise in blood pressure - Kidney damage - Miscarriages and subtle abortions - Disruption of nervous systems - Brain damage - Declined fertility of men through sperm damage - Diminished learning abilities of children - Behavioural disruptions of children, such as aggression, impulsive behavior and hyperactivity
 <p style="text-align: right; margin-right: 20px;">Fe</p>	<ul style="list-style-type: none"> - Conjunctivitis - Choroiditis - Retinitis
 <p style="text-align: right; margin-right: 20px;">Zn</p>	<ul style="list-style-type: none"> - Stomach cramps - Skin irritations - Vomiting - Nausea - Anemia - Damage the pancreas - Disturb the protein metabolism - Cause arteriosclerosis

2.4 Heavy Metal in Plants

Heavy metals are metallic elements with high atomic weights, examples mercury, chromium, magnesium and zinc. Heavy metals are widely distributed in nature, in places such as water, soil, air and various forms of organisms (Manyin Zhang *et al.*, 2009). Increased heavy metal concentrations in the soil (mostly from anthropogenic activities such as sewage sludge application) are considered to pose possibly serious hazards in the soil-plant-animal system (Jin Qian *et al.*, 1996). Plants have ability to absorb heavy metals in soil. Potential health risks to humans and animals from consumption of crops can be due to heavy metal uptake from contaminated soils via plant roots as well as direct deposition of contaminants from the atmosphere onto plant surfaces (Ping Zhuanga *et al.*, 2009). Simultaneously, some micronutrient elements (e.g. Cu, Cr, Mn, Ni, Cd, Fe, Pb and Zn) may be toxic to both plants at high concentration (K. Chojnacka *et al.*, 2004). So, the plant that has a lot of heavy metals can give greatest impact and carry the highest risk to human health such carcinogenic, muscular failure and arteriosclerosis. Actually, vegetation has its maximum level of heavy metal accumulation in order to avoid public take the vegetable that contain more heavy metal. The table below shows the maximum level of heavy metal from World Health Organization (WHO) in vegetation for several heavy metals:

Table 2.2: World Health Organization Standard in Vegetation (Mohsen Bigdeli & Mohsen Seilsepour, 2008).

Heavy Metal	WHO-ML
Cd	0.10
Pb	0.30
Mn	500.00
Fe	425.00
B	-
Zn	100.00
Cu	73.00
Ni	67.00
Co	50.00

*WHO-ML; Codex Alimentarius Commission (FAO/WHO). Food additives and contaminants. Joint FAO/WHO Food

Standards Program 2001; ALINORM 01/12A:1-289

Table 2.3: Guidelines on heavy metals for food safety set by different countries
(C.K. Yap *et al.*, 2004)

Location	WB	Cd ($\mu\text{g/g}$)	Cu ($\mu\text{g/g}$)	Pb ($\mu\text{g/g}$)	Zn ($\mu\text{g/g}$)
Permissible limits by Malaysian Food Regulation (1985)	Wet	1.00	30.00	2.00	100.00
International Council for the Exploration of the Sea (ICES, 1988) for status: 'increased contamination'	Dry	1.80	-	3.00	-
Maximum permissible levels established by Brazilian Ministry of Health (ABIA, 1991)	Dry	5.00	150.00	10.00	250.00
Permissible limit set by Ministry of Public Health, Thailand (MPHT, 1986)	Dry	-	133.00	6.67	667.00

Food and Drug Administration of the United States (USFDA, 1990)	Dry	25.00	-	11.50	-
	Wet	3.70	-	1.70	-
Australian Legal Requirements (NHMRC, 1987)	Dry	10.00	350.00	-	750.00
Permissible limit set by the Hong Kong Environmental Protection Department (HKEPD, 1997)	Wet	2.00	-	6.00	-

Table 2.3 above shows the guidelines on heavy metals for food safety set by different countries. The maximum level is different for each country.

2.5 Digestion Method

2.5.1 Plant Destruction Method

Determination of heavy metals concentration requires matrix destruction. The reliability of the heavy metal determination in its complex matrices mainly depend on the dissolution process used (Mustafa tuzen, 2003). There are six of destruction methods for determination of heavy metals that are appropriate for this research.

Table 2.4: Destruction Method of Plant

Destruction method	Methodology	Comment
Wet destruction overnight with HNO ₃	10mL of concentrated HNO ₃ was added to 1.000 g plant sample and allowed to stand overnight at room temperature. The sample was then heated for 4 h at 120 °C, after which the temperature was increased to 140 °C. The digestion was continued at this temperature	-Take long time -Easy handling -Cheap -Good recovery

	until about 1 ml of acid remained. After cooling, the suspension was filtered in a 50 ml volumetric flask and diluted to the mark.	
Wet destruction with HNO ₃	10mL of concentrated HNO ₃ was added to 1.000 g plant sample and the suspension was heated for 45 min at 90 °C. The sides of the beaker were occasionally washed down with distilled water. The temperature was increased to 140 °C and the digestion continued at this temperature until about 1 ml of acid remained. After cooling, the suspension was filtered in a 50 ml volumetric flask and diluted to the mark.	-Take long time (but shorter than method 1 and 4) -Easy handling and cheap -Low recovery
Wet destruction with HNO ₃ /HClO ₄	10mL of concentrated HNO ₃ and 1 ml of 70% HClO ₄ were added to 1.000 g plant sample and the suspension was allowed to stand overnight at room temperature. The sample was then heated for 1 h at 120 °C, and then at 175 °C until about 2 ml of acid remained. The temperature was then further increased to 225 °C for 10 min. After cooling, the suspension was filtered in a 50-ml volumetric flask and diluted to the mark.	-Take long time (like method 1) -Easy handling and cheap -Low recovery
Wet destruction with HNO ₃ /H ₂ O ₂	Samples (1.000 g) were weighed into 100 ml pyrex beakers and treated with 10 ml concentrated HNO ₃ . The beaker was covered with a watch-glass and the suspension was heated up to 130 °C for 1 h. A total amount of 4 ml 20% H ₂ O ₂ was added in aliquots of 1 ml. After cooling, the suspension was filtered in a 50-ml volumetric flask and diluted to the mark.	-Simple (no need to overnight it) -Easy handling, cheap -Good recovery
Dry ashing	Samples (1.000 g) were weighed into porcelain crucibles. They were pre-ashed in a muffle	-Low recovery -Complicated

	furnace at a temperature of 250 °C and subsequently ashed during 3 h at 450 °C. The crucible was transferred to a hot plate, 5 ml of 6 M HNO ₃ was added and the mixture was evaporated to a low volume. The residual was dissolved in 5 ml of 3 M HNO ₃ , filtered and diluted to 50 ml.	(hard handling) -fast
Microwave destruction	Samples (0.500 g) were weighed into 45 ml Teflon destruction bombs. 10mL of concentrated HNO ₃ was added. The recipients were placed during 15 min in an ultrasonic bath. Closed bombs were then heated in a microwave at 300 W and 190 °C during 10 min. Recipients were cooled and the solution was transferred quantitatively to 50 ml volumetric flasks and diluted to the mark.	-Good recovery -Simple -But need experience (handling) -fast

The microwave destruction procedure (method 6) yielded the best overall recoveries. Method 1 (Wet destruction overnight with HNO₃) and method 4 (Wet destruction with HNO₃/H₂O₂) may however substitute for microwave destruction for determination of Cd, Cu, Pb, Zn, Fe or Mn as they are relatively easy and fast to carry out in the laboratory for a numerous amount of samples (Gijs Du Laing *et al.*, 2003). The preferred method for this study wet destruction method because good recoveries in terms of heavy metal recoveries and besides that, this method is simple, cheap, and easy in handling and short time needed.

2.5.2 Digestion Method of Soil

The sample that obtained from the first step must be digesting to extract the heavy metal compound from the soil. The best method needed to make sure the heavy metal is fully extracted and give the real concentration level when conduct the analysis stage. For pollutant inputs are not silicate-bound, a ‘pseudo total’ analysis of

strong acid digest such as aqua-regia digestion method is sufficient (Sabiene *et al.*, 2004). The alternative way is digesting the sample with HNO₃ and H₂O₂ using the Method 3050B suggested by USEPA (Chen *et al.*, 2004).

2.6 Analysis

For the part of analysis, atomic absorption spectrometry (AAS) is used to determine the concentration of heavy metals in plant. Slurry sampling AAS can be successfully used for the simple, rapid and reproducible determination of single metals in tea leaf samples (J. Mierzwa, 1998). More recently, several improvements in AAS spectrometers have also contributed to partially or totally solving these problems and by facilitating direct analysis of solids thereby enhancing the capacity of AAS to compete with atomic emission spectrometry in terms of sensitivity and detection limit (Jordi Sardans *et al.*, 2009). Besides that, as stated by Ashok *et al.* (2009), this method is simple and very selective. So, AAS is the best equipment to analyze heavy metals because easy handling and also very fast in order to get result.

2.7 Previous Result

From the previous research that has been done by P. Agamuthu (2008), his research site located at three landfills of Malaysia (Sungai Kundang, Sungai Sedu and Sungai Panchang). He managed to obtain the data about heavy metal contamination in soil that also affect plant which has ability the heavy metal from the soil. The table below shows the overall result that he obtained from his research:

Table 2.5: Average concentration of metal and non-metal (P. Agamuthu, 2008)

Parameter	Unit	Surface soil (5cm from ground surface)	Deep soil (5cm below the ground surface)	Dutch Intervention Standard
Phosphate	mg/kg	2.5 – 5.5	0 – 13.6	-
Fluoride	mg/kg	2.4 – 7.0	0.5 – 0.9	-
Sulphate	mg/kg	30.2 – 946.3	4.2 – 10.2	-
pH	na	5.8 – 9.9	7.3 – 8.2	-
Chloride	mg/kg	6.3 – 238.3	2.1 – 8.1	-
Nitrate	mg/kg	4.7 – 83.3	0.5 – 5.0	-
Nitrite	mg/kg	1.1 – 2.9	Not detected	-
Zn	mg/kg	7.7 – 129.8	Not detected	720
Sb	mg/kg	0 – 3.0	Not detected	15
Cd	mg/kg	0 – 0.6	Not detected	12
Cr	mg/kg	0.5 – 14.1	Not detected	380
Cu	mg/kg	2.3 – 17.3	Not detected	190
Pb	mg/kg	2.7 – 148.0	Not detected	530
Ni	mg/kg	0.3 – 5.0	0 – 9.0	210
Ag	mg/kg	0 – 1.2	Not detected	15
Ti	mg/kg	0 – 58.0	Not detected	15
As	mg/kg	8.8 – 64.5	0.3 – 2.7	55
Hg	mg/kg	0 – 1.4	8.5 – 11.5	10

According to P. Agamuthu (2008), heavy metal contamination occurs in surface soil and deep soil. The table of average concentration of metal and non-metal elements in surface and deep soil from ex-landfill proves heavy metals most commonly reported are arsenic, cadmium, lead, chromium and mercury. Table below shows the previous result that prepared by C.K. Yap et al., (2004):

Table 2.6: Comparison Metal levels of *P. Viridis* with Standard

Location	WB	Cd (µg/g)	Cu (µg/g)	Pb (µg/g)	Zn (µg/g)
Permissible limits by Malaysian Food Regulation (1985)	Wet	1.00	30.00	2.00	100.00
International Council for the Exploration of the Sea (ICES, 1988) for status: 'increased contamination'	Dry	1.80	-	3.00	-
Maximum permissible levels established by Brazilian Ministry of Health (ABIA, 1991)	Dry	5.00	150.00	10.00	250.00
Permissible limit set by Ministry of Public Health, Thailand (MPHT, 1986)	Dry	-	133.00	6.67	667.00
Food and Drug Administration of the United States (USFDA, 1990)	Dry	25.00	-	11.50	-
	Wet	3.70	-	1.70	-
Australian Legal Requirements (NHMRC, 1987)	Dry	10.00	350.00	-	750.00
Permissible limit set by the Hong Kong Environmental Protection Department (HKEPD, 1997)	Wet	2.00	-	6.00	-
Metal levels of <i>P. Viridis</i> from the west coast of Peninsular Malaysia (this study)	Wet	0.12	1.32	0.43	12.80
		to 0.22	to 3.42	to 1.49	to 21.90
	Dry	0.68	7.76	2.51	75.10
		to 1.25	to 20.10	to 8.76	to 12.90

According to Mohsen Bigdeli and Mohsen Seilsepour (2008), heavy metal depositions are associated with a wide range of sources such as small scale industries (including battery production, metal products, metal smelting & Cable coating industries) and diesel generator sets. These can all be important contributors to the contamination found in vegetables. Additional potential sources of heavy metals in field locations in urban and per urban areas include irrigation water contaminated by industrial effluent leading to contaminated soils and vegetables.

CHAPTER 3

METHODOLOGY

3.1 Overview

This chapter will cover about the methodology of this research. There are 8 stage of work flow for this research, starting with identifying problem and ended with analyzing result data.

3.2 Research Activities

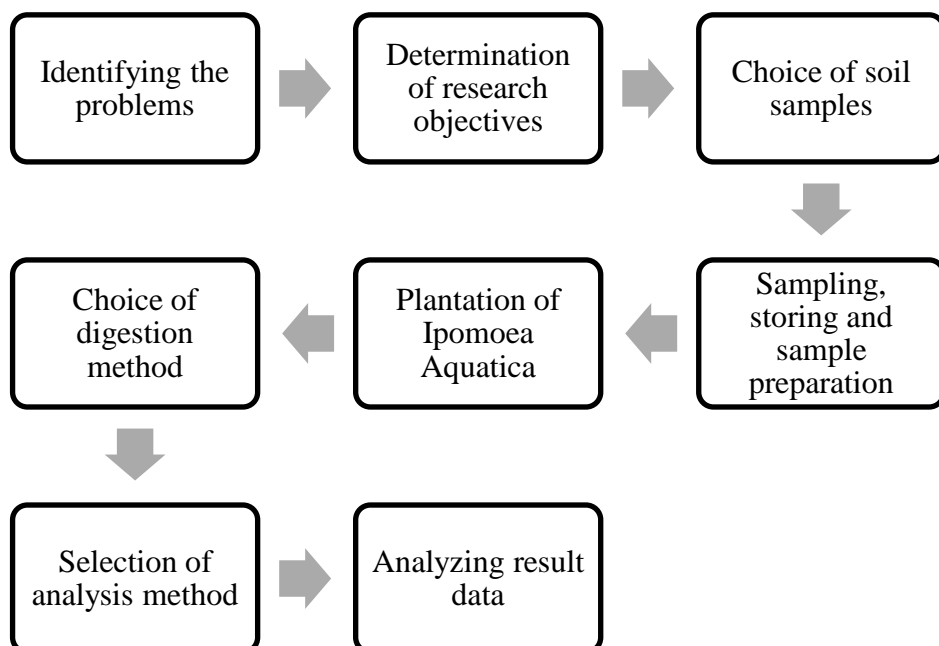


Figure 3.1: Schematic Diagram of Research Activities

3.2.1 Identifying the Problem

First of all, field of the research must be determined and selected before confirmation of research's title. The environmental section had been chosen for this research. Basically, selection of problem usually based on problem that our country faced. After further reading, problem about excess of heavy metals in crops problem had been selected and discussed for title selection.

3.2.2 Determination of Research Objectives

Before conducting the experimental works, the objectives of this research must be clearly stated. This objective will guide the research to achieve the main purpose of this research and prevent unnecessary works that can interrupt the quality of the collected data.

3.2.3 Choice of Soil Samples

In order to conduct an environmental case study research, the first thing that must be considered is the case scenario and a target of research. So, for this research, choice of soil samples was very critical. The first criteria that should be filled are the targets must be difference source of soil. In this research, two types of soil are selected (closed landfill soil and nursery soil).

3.2.4 Sampling, storing and sample preparation

There are two types of soil samples used in this study. Closed landfill soil samples were taken from a newly closed land fill in Ulu Tualang, Temerloh. While for other soil samples were taken from a nearby plant nursery in Kuantan city. The soil samples in Ulu Tualang were collected at random from the surface layer (0-20 cm). In order to remove larger particle such as rocks, roots or other large particle, the soil samples were air-dried at 150°C and ground to pass through 200-mesh sieve

before transferred to polyethylene bottle until analysis (Mohsen Bigdeli & Mohsen Seilsepour, 2008). There are some precautions that should be taken such as the steel equipment was reduced during the sampling, drying, grinding and storage of soil sample in order to avoid contamination (J. Qian *et al.*, 1996).

3.2.5 Plantation of *Ipomoea Aquatica*

The remaining of the soil samples were then used to cultivate plants. The seed of *Ipomoea Aquatica* was taken from agricultural land. Seedlings of wild spinach were cultivated separately in both of the soil samples. After 5 weeks, the mature of wild spinach were harvested in order to analyze the heavy metals uptake from the soil samples by the plant. The plant samples were washed with deionized water and dried at 105°C for 24 h (K. Chojnacka *et al.*, 2005). The dried plant samples were ground then homogenized using an agate pestle and were keep stored in polyethylene bottles until analysis.

3.2.6 Digestion Process

There are several acid digestion methods that can be chosen for extracting the heavy metal content in the soil samples such as US EPA method 3050, ASTM method 9.3.4, SCL method and US EPA draft method 6020. The purpose of the acid digestion is to solubilize all elements of interest. To achieve the purpose this acid digestion method must perform two distinct tasks, the first one it must decompose the sample matrix to expose the entire mass to the acid cocktail and second is it must react with the elements of interest to form water-soluble compound (Kimbrough and Wakakuwa, 1992). Each of this method has different extraction capability. For this research, SCL method had been chosen due its high capability of extraction and this method also faster than other method (Kimbrough and Wakakuwa, 1992).

This method required 1.00-4.00 g of dried sample to be digested in a mixture of 9 mL of concentrated hydrochloric acid and 3 mL of concentrated acid at ambient air in a 50 mL digestion vessel. This mixture must be prepared in a fume hood due to

the volatility of the acids. The sample and reaction mixture were slowly heated to 95°C to prevent an overly vigorous reaction. For the vapor recovery, a watch glass had been used. The digestion was continued until the disappearance of NO₂ (reddish brown) fumes and no more changes in appearance (Kimbrough and Wakakuwa, 1992).

In addition, the sample must be brought to near dryness and if necessary 10 ml of deionized water added to enhance the solubility of the metal chloride and metal nitrate. The times taken for the fumes disappear vary due to the type of soil. Most of the samples take 30 minutes to 2 hours to achieve the condition. The digestate is filtered using AAS grade filter paper (Whatman 41 or equivalent) and collected in a 100 mL volumetric flask. To enhance the filtration rate, a vacuum filter had been used for replacing the conventional filtration method. Then, the filter paper is washed with no more than 5 mL of hot (95 °C) concentrated hydrochloric acid and then 20 mL of hot deionized water.

There are several plant destruction methods that can be chosen for extracting the heavy metal content in the soil samples such as microwave digestion method, dry ashing and wet destruction method. Wet destruction method using HNO₃ and H₂O₂ were used due to short time needed, easy in handling, cheap and also good in terms of heavy metal recoveries.

Plant samples (1.000 g) were weighed into 100 ml pyrex beakers and treated with 10 ml concentrated HNO₃. The beaker was covered with a watch-glass for vapour recovery in order to make sure that no heavy metal were vaporized into surroundings and the suspension was heated up to 130 °C for 1 h. A total amount of 4 ml 20% H₂O₂ was added in aliquots of 1 ml. After cooling, the suspension was filtered in a 50-ml volumetric flask and diluted to the mark.

3.2.7 Analysis Using AAS

In order to determine the concentration of heavy metal in slurry mixture, there are many type of analysis equipment that can be use such as flame atomic absorption spectrometry (FLAA), graphite furnace atomic absorption (GFAA), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma atomic emission spectrometry (ICP-MS). FLAA had been used due to the equipment availability for this research.

The standard solutions for each of the element (Cr, Cu, Mn, Ni, Pb, Fe, Cd and Zn) used for calibration were prepared by diluting from a stock solution (1000ppm). Calibration or standard curve is important in determining the unknown concentration of heavy metal in the solution by using Atomic Adsorption Spectrometry (AAS). The concentration of single heavy metal such as Cr can be determined if it is detected (occur) within the range of standard solution of Cr that had been prepared. For each element of heavy metals, the standard calibrations were prepared at 0.1ppm, 0.5ppm, 1ppm and 10ppm. The most accurate formula for making dilutions of solutions is the following equation:

$$M_1V_1 = M_2V_2$$

M_1 = percentage you have

M_2 = percentage you want

V_1 = unknown volume

V_2 = volume wanted

The time estimation for every analysis of specific metal in the wastewater is about 15 minutes using Atomic Adsorption Spectrometry (AAS). The graph and the value of specific metal concentration are then print out automatically after the analysis finished.

Metal standard solutions (1000ppm) used for spiking the soil samples were prepared in deionised water by dissolving the appropriate amount of standard solutions. The spike method was used if the value that detected by AAS found in negative value.

3.2.8 Analyzing Result Data

The data that obtained from the Atomic Absorption Spectrometry (AAS) is in part per million (ppm). So, when convert it to concentration unit, it will become mg/kg. If the sample spiked, the value must be multiply with the Dilution factor (Df). Dilution factor equation is shown below:

$$Df = \frac{\text{volume of dilution}}{\text{volume of sample used}}$$

Before the data from AAS were taken, the value of the correlation coefficient must be determined first. The acceptable value is 0.9-0.99. If the values lower than 0.9 that means that the standard solutions have contamination and the data cannot be taken. For this research, the correlation coefficient for all heavy metal is in range 0.96 to 0.99. Hence, there is no problem with the standard curve and the data is valid to become the result.

This calculation will show the example of calculation for soil sample (closed landfill soil) for Nickel:

$$\begin{aligned} 22.75 \text{ ppm} &= \frac{22.75 \mu\text{g}}{L} \times \frac{\text{dilution volume}}{\text{sample weight}} \times \frac{1000 \text{ g}}{1 \text{ kg}} \times Df \\ &= \frac{22.75 \mu\text{g}}{L} \times \frac{100 \text{ mL}}{1 \text{ g}} \times \frac{1000 \text{ g}}{1 \text{ kg}} \times 50 \\ &= 113.75 \text{ mg/kg} \end{aligned}$$

3.3 Apparatus and Materials

- i. Shovel.
- ii. Steel ruler.
- iii. Poly bag.
- iv. Drying ovens – able to maintain $30^{\circ}\text{C} \pm 4^{\circ}\text{C}$.
- v. Mortar & pestle.
- vi. Sieve.
- vii. Analytical balance.
- viii. Digestion vessels – 50ml
- ix. Filter paper.
- x. Vacuum filter.
- xi. Funnel or equivalent.
- xii. Graduated cylinder.
- xiii. Heating source – adjustable and able to maintain a temperature of 90-95C.
- xiv. Temperature measurement device – capable of measuring to at least 125°C .
- xv. Vapor recovery device (ribbed watch glasses, appropriate refluxing device, and appropriate solvent handling system).
- xvi. Volumetric flask
- xvii. Ultrasonic bath
- xviii. micropipette

3.4 Reagent

- i. Ultra pure water.
- ii. Nitric acid (concentrated), HNO_3 , 65 %. CAS no. 7697-37-2, Fisher Scientific.
- iii. Hydrochloric acid (concentrated), HCL , 37%. CAS no. 2315-95-7, Fisher Scientific.

3.5 Analytical Equipment

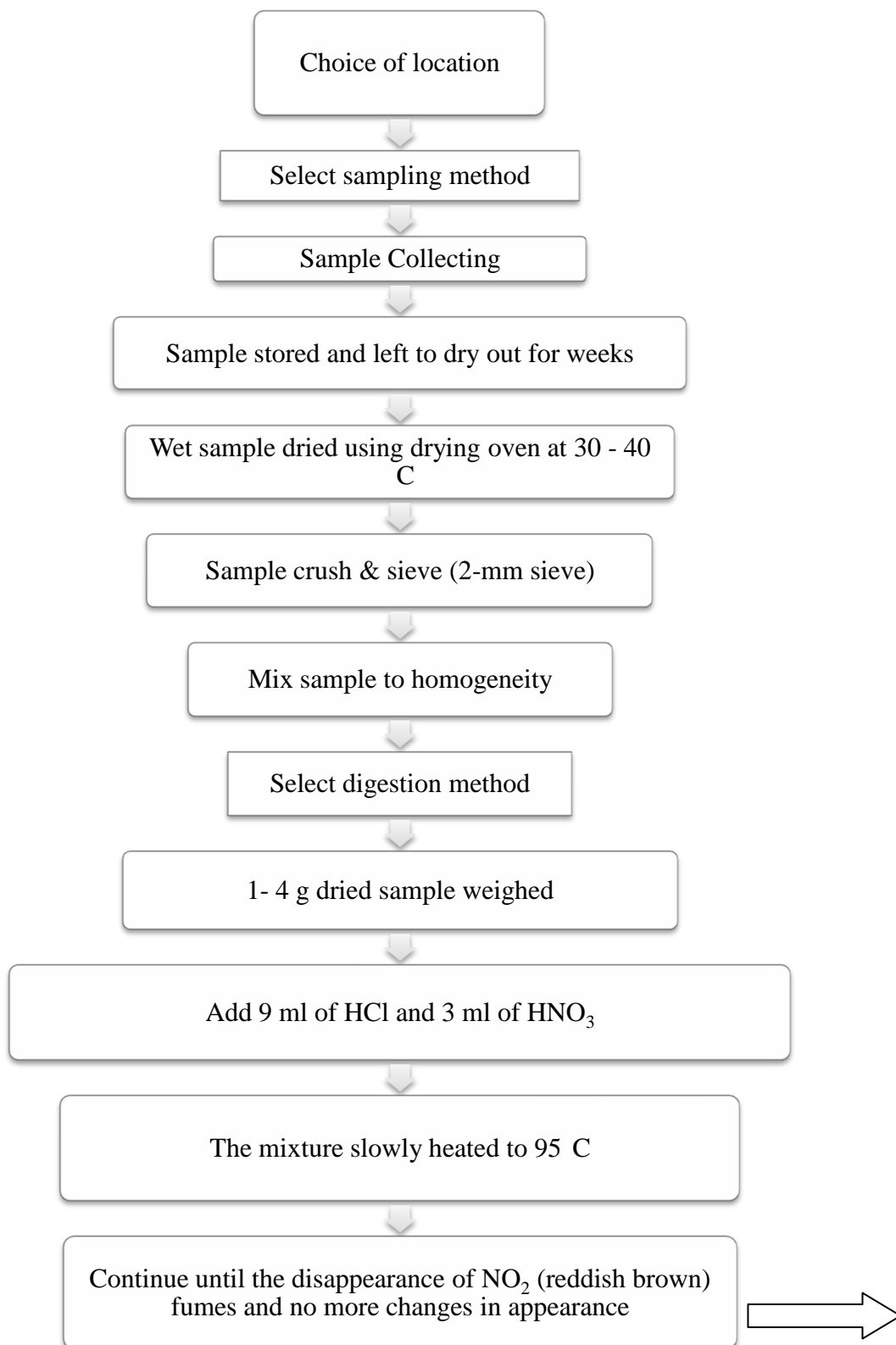
- i. Hitachi's Polarized Zeeman Atomic Adsorption Spectrometry (Flame Version)

3.6 Precaution

There are several precautions that should be concerned when conducting this research. It is very important to ensure the data that obtained from this research are accurate and valid for future reference. The things that we need to consider are;

- i. Plastic equipment must be used for handling, storing and analyzing the sample to prevent metal-contamination in the sample.
- ii. All equipment should be cleaned before treating the next sample to minimize the potential of cross-contamination.
- iii. Make sure heat the slurry mixture (sample + digester) not exceed 95°C to prevent an overly vigorous reaction.
- iv. Only used ultra pure water when using the AAS equipment to ensure the AAS reading did not interrupted by outside contamination.
- v. When handling the concentrated HCl and HNO₃, used glove, mask and do the wwork inside the fume hood.

3.7 Summary of Procedures



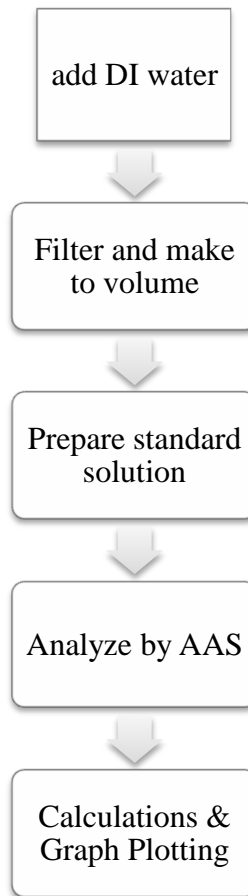
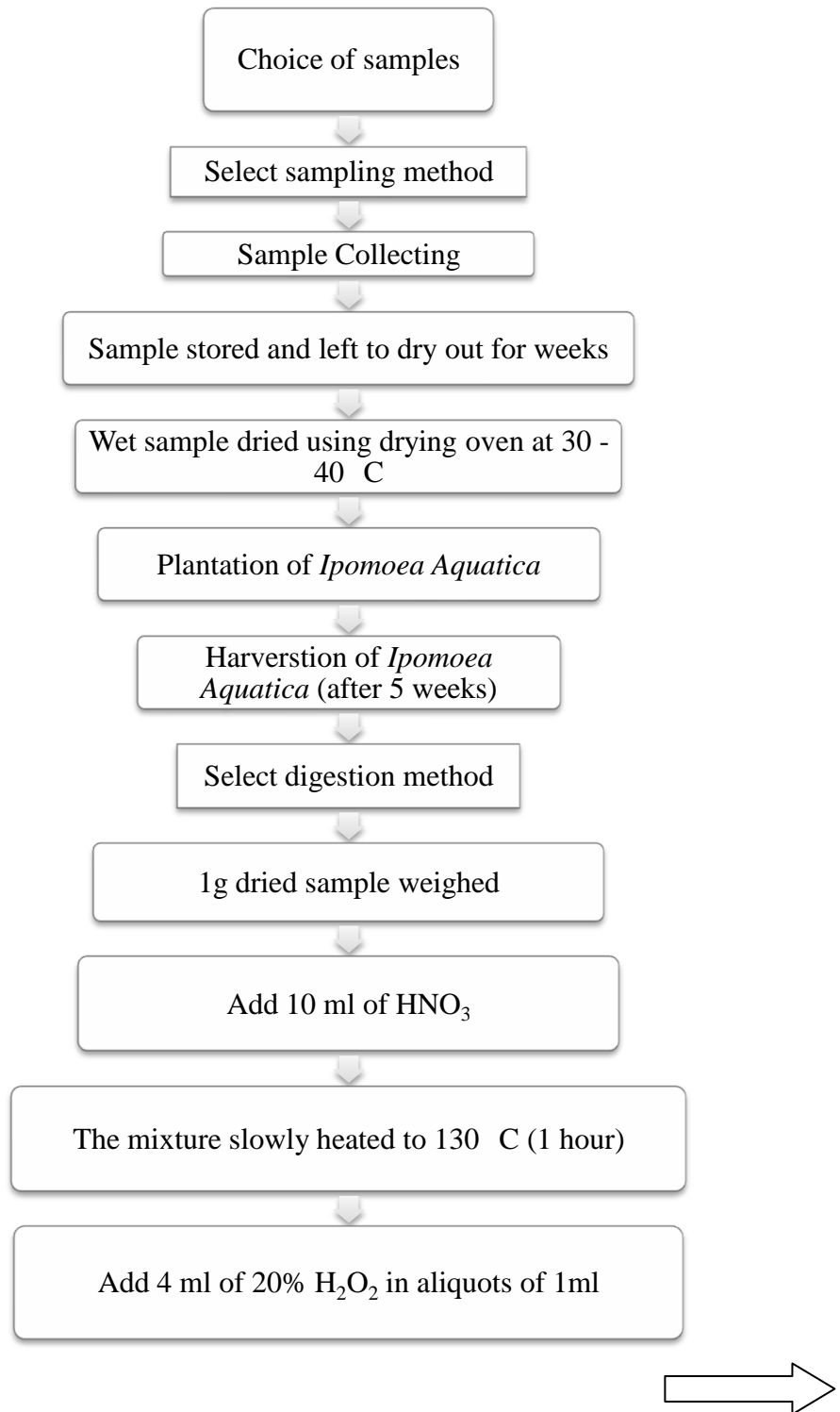


Figure 3.2: Summary of Methodology for Soil



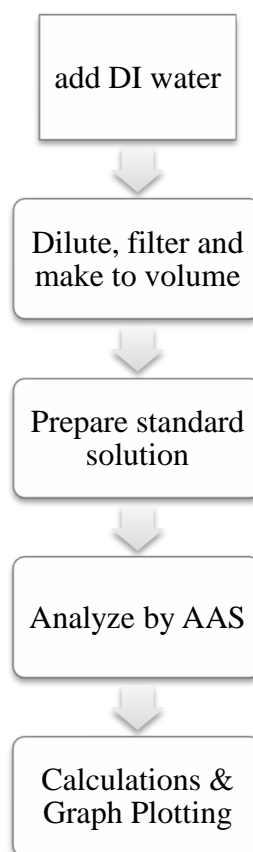


Figure 3.3: Summary of Methodology for Plant

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Overview

Introduction

After analyzing all plant samples and soil samples using Atomic Absorption Spectrometry (AAS), all the result that obtained are in negative values for the first time running test. K. Christidis *et al* (2007) stated that the majority of samples that had been analyzed are typically negative. So, Spike method 5ppm was used in order to get the actual value of heavy metal concentration.

The procedure of Spike 5 ppm Method

- Dilute 1ml of filtered sample + 250 μ L of 1000 ppm stock solution in 50 ml volumetric flask. After that, shake well.
- Analyze using AAS

Spike 5 ppm calculation

$$(A \text{ ppm}) = \frac{B \text{ mg}}{1000 \text{ L}} \times \frac{\text{volume of volumetric flask}}{\text{weight of sample}} \times \frac{1000 \text{ g}}{1 \text{ kg}} \times \text{dilution factor}$$

A = actual value

B = detected value (in AAS) – 5 ppm

$$\text{Dilution factor} = \frac{\text{volumetric flask volume}}{\text{volume of sample}}$$

This is the example of calculation of spike 5ppm method:

$$\begin{aligned} (12.37 - 5) \text{ ppm} &= \frac{9.37 \text{ mg}}{1000 \text{ L}} \times \frac{0.1 \text{ L}}{1 \text{ g}} \times \frac{1000 \text{ g}}{1 \text{ kg}} \times \frac{50 \text{ ml}}{1 \text{ g}} \\ &= 36.85 \text{ mg kg}^{-1} \end{aligned}$$

The figure below shows the characteristic (physical appearance) and observation of both types of soil and plants that cultivated:



Types of soil	Description
 <p data-bbox="492 919 659 951">Nursery Soil</p>	<ul style="list-style-type: none"> • Color of the soil from nursery is reddish brown • Has smaller particles compare to the landfill soil • The soil structure is granular which also known as crumb structure tends to form an open structure that allows water and air to penetrate the soil which is why open structures tend to be better agricultural soils (Ritter, Michael E, 2010).
 <p data-bbox="350 1621 800 1652">Closed Landfill Soil (Ulu Tualang)</p>	<ul style="list-style-type: none"> • Color of the soil from Ulu Tualang has a light brown grey color • Has larger particles compare to nursery soil • Has blocky structure since it has larger particles of soil

Figure 4.1: Nursery soil and Closed landfill soil



Ipomoea Aquatica (after 5 weeks)	Observation
 <p data-bbox="386 730 764 762">Cultivation using nursery soil</p>	<ul data-bbox="943 310 1437 625" style="list-style-type: none"> • Had grown quite well • Have a maximum height about 16 cm • The color of <i>Ipomoea Acuatica</i> grown in soil from nursery is dark green
 <p data-bbox="342 1255 808 1287">Cultivation using closed landfill soil</p>	<ul data-bbox="943 842 1437 1157" style="list-style-type: none"> • The <i>Ipomoea Acuatica</i> grown in soil from Ulu Tualang have a maximum height of 13 cm • The color of <i>Ipomea Acuatica</i> grown in soil from Ulu Tualang is light green

Figure 4.2: Cultivation in nursery soil and closed landfill soil

Table 4.1: Heavy Metal Concentration in Plant and Soil

Soil Sample / Heavy Metal		Cr	Cu	Mn	Ni	Cd	Fe	Pb
Nursery Soil	Plant	4.16	6.46	2.85	9.04	4.67	1.81	9.66
	Soil	39.20	63.95	11.45	102.95	ND	9.65	7.40
Closed Landfill Soil	Plant	6.92	1.58	2.80	3.85	3.93	8.75	8.87
	Soil	48.15	57.45	11.65	113.75	ND	86.85	7.85

Concentration of all eight heavy metal found in both soils and both plants that cultivated in both soils are summarized in table above. From the table, we can see the comparison between nursery soil and closed landfill soil in terms of heavy metal accumulation in samples (plant samples and soil samples).

4.2 Heavy Metal in *Ipomoea Aquatica*

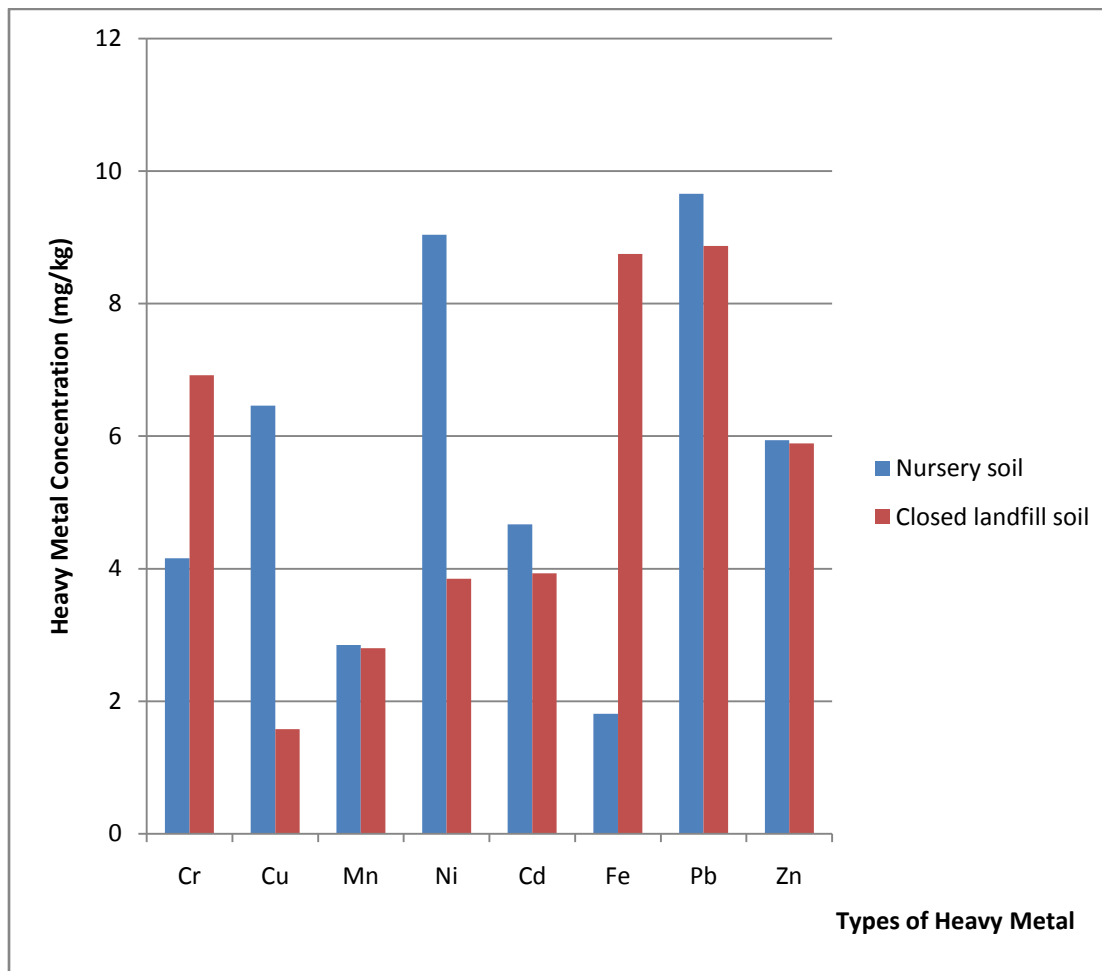


Figure 4.3: Graph heavy metal concentration in *I. Aquatica* versus types of heavy metal

Figure 4.3 represents a collection data of concentration of Chromium, Copper, Manganese, Nickel, Cadmium, Iron, Lead and Zinc analysed for plant samples that cultivated in soil from closed landfill soil and also nursery soil. From the graph, we can see that the concentration of heavy metal in nursery soil is higher than closed landfill soil in terms of the number of heavy metal. Plantation using closed landfill soil that taken from Ulu Tualang shows only shows more indication of Chromium and Iron in plant samples compare to the nursery soil.

Table 4.2: Heavy metal concentrations in *I. Aquatica*

Sample (mg/kg)	Nursery Soil	Closed Landfill Soil
Cr	4.16	6.92
Cu	6.46	1.58
Mn	2.85	2.80
Ni	9.04	3.85
Cd	4.67	3.93
Fe	1.81	8.75
Pb	9.66	8.87
Zn	5.94	5.89

It was found that the total concentration for Lead was the highest for both *I. Aquatica* cultivated in soil from closed landfill and *I. Aquatica* cultivated in soil from the plant nursery (8.87 and 9.66 mg kg⁻¹) followed by Nickel (3.85 and 9.04 mg kg⁻¹) and Copper (1.58 and 6.46 mg kg⁻¹). While, heavy metal that found lowest concentration level in *I. Aquatica* samples is Manganese that exhibited in range 2.80 mg kg⁻¹ (using closed landfill soil) and 2.85 mg kg⁻¹ (using nursery soil). *I. Aquatica* that cultivated in soil from plant nursery contained higher concentration of Copper, Cadmium, Manganese, Iron, Zinc and Nickel compared to plant cultivated in soil from Ulu Tualang. However, the availability of heavy metals in soil to plants is influenced by the soil characteristics such as pH, cation exchange, redox condition and chlorine contents (Du Laing *et al.*, 2002).

4.3 Heavy Metal in Soil

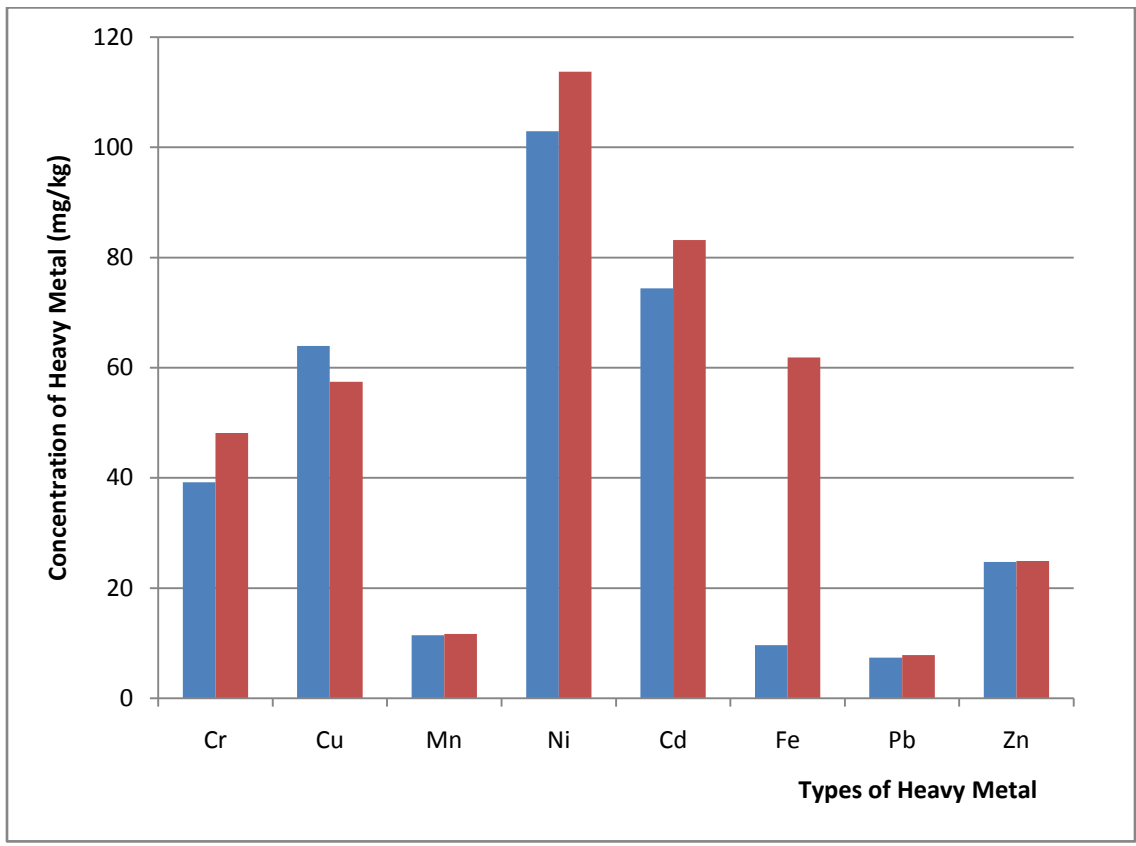


Figure 4.4: Graph heavy metal concentration in soil versus types of heavy metal

From the graph, we can see that the heavy metal accumulation in closed landfill soil is higher than the soil that available at plant nursery in terms of number of heavy metal. Copper is the only one that higher in nursery soil rather than closed landfill soil.

Table 4.3: Heavy metal concentrations in soil

Sample (mg/kg)	Nursery Soil	Closed Landfill Soil
Cd	74.40	83.20
Cr	39.20	48.15
Cu	63.95	57.45
Fe	9.65	61.85
Mn	11.45	11.65
Ni	102.95	113.75
Pb	7.40	7.85
Zn	24.75	24.95

Based on table above, Lead exhibited the lowest abundance 7.4 mg kg^{-1} for nursery soil and 7.85 mg kg^{-1} for closed landfill soil while Nickel appeared to be the most abundant metal. There are several heavy metals are shown to be high in the soil and need to be monitored. Firstly, Nickel that content in the nursery soil and Ulu Tualang soil (102.95 and $113.75 \text{ mg kg}^{-1}$ respectively) while Cadmium is the second higher after Nickel because the concentration of Cadmium in nursery soil and closed landfill are 74.40 and 83.20 mg kg^{-1} respectively. Iron content in both soil from closed landfill of Ulu Tualang and soil that available in the market did not give a big difference whereby the average content is 61.85 mg/kg . However, among all elements of heavy metal, only Copper showed a different content where the concentration of soil from market is higher compared to soil from Ulu Tualang.

The bioavailability of heavy metals depends on their chemical forms in the soils (J. Dai *et al.*, 2004). A few components of the soil act as the reservoirs of heavy metals. According to M. Karvelas *et al.* (2003), the metal content of soil often fluctuates due to irregular inputs from urban and industrial sources. The observed concentration differences between the heavy metal from closed landfill of Ulu Tualang and plant nursery strongly imply a relative accumulation of soil organic matter in the polluted soils (D.J. Russell, G. Alberti, 1998). The concentration in the soil from closed landfill of Ulu Tualang lies in the range typical for soils with impaired decomposition. It is suggested

that a tendency for reduced organic matter decomposition relative to the soil from plant nursery, comparable to effects observed by many authors in heavy metal contaminated soils.

4.4 Comparisons with World Health Organization (Permissible Maximum Level)

Table 4.4: Comparison with maximum level of World Health Organization (WHO)

Types of heavy metal	<i>Ipomoea Aquatica</i> (Nursery Soil)	<i>Ipomoea Aquatica</i> (Closed Landfill Soil)	WHO-ML
Cr	4.16	6.92	1.00
Cu	6.46	1.58	73.00
Mn	2.85	2.80	500.00
Ni	9.04	3.85	67.00
Cd	4.67	3.93	0.10
Fe	1.81	8.75	425.00
Pb	9.66	8.87	0.30
Zn	5.94	5.89	100.00

Chromium: Concentrations of Chromium in *Ipomoea Aquatica* that cultivated in nursery soil and closed landfill soil are found 4.16 mg kg⁻¹ and 6.92 mg kg⁻¹ respectively. This values are exceeded the limitation of WHO that just permitted only 1.00 mg kg⁻¹ of Chromium in vegetables. So, the plant is in dangerous range that may lead to the following symptoms (www.lentech.com, 2010):

1. Skin rashes
2. Upset stomachs and ulcers
3. Respiratory problems
4. Weakened immune systems
5. Kidney and liver damage
6. Alteration of genetic material
7. Lung cancer

Cadmium: While for Cadmium, WHO fixed the maximum level of Cadmium in vegetation is 0.10 mg kg⁻¹. So, as we can see, the concentration of Cadmium in both cultivated of *Ipomoea Aquatica* shows 4.67 mg kg⁻¹ and 3.93 mg kg⁻¹ respectively that indicate the big differences in terms of value after compare to the WHO standard. If human absorb more Cadmium in body, this will cause significant symptoms likes psychological disorders, bone fracture and possibly DNA damage or cancer.

Copper: The concentration of Copper found in the green plants was within safe limits in both soil samples. It means that the copper level was under maximum level of permissible World Health Organization.

Lead: Lead (Pb) in the plant sample that also a toxic metal has low maximum level set by World Health Organization (0.30 mg kg⁻¹). From the table above, we can see that Lead is the heavy metal that absorbs more by *Ipomoea Aquatica*, 9.66 mg kg⁻¹ (using nursery soil) and 8.87 mg kg⁻¹ (using closed landfill soil). Data shows that in both *Ipomoea Aquatica*, concentration of Lead is more than permitted level. The major health problems of Pb are manifested in three organ systems namely the haematological, nervous and renal systems (Hutton, 1987) and so, they are not suitable for consumption.

Zinc: The maximum level of Zinc that permitted by World Health Organization is 100 mg kg⁻¹ which is the same value as permissible limits set by Malaysian Food Regulation 1985 (C.K.Yap et al, 2004). Data shows that there was not any pollution in both *Ipomoea Aquatica* samples after compare with World Health Organization standard because the concentration of Zinc in both plant samples is under the maximum level. Even though the maximum level for Zinc is high, but we need avoid the uptake of Zinc in food. Knowledge about toxicology of Zinc in humans is minimal (Mohsen Bigdeli and Mohsen Seilsepour, 2008). Zinc is a trace element that is essential for human health. When people absorb too little zinc they can experience a loss of appetite, decreased sense of taste and smell, slow wound healing and skin sores. High concentrations of Zinc can be accumulated in vegetables that growing on heavy metal contaminated soils can cause serious health risk to consumers (Mohsen Bigdeli and Mohsen Seilsepour, 2008).

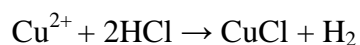
Manganese: From the result that obtained, the values of manganese in both *Ipomoea Aquatica* samples are 2.85 mg kg⁻¹ (using nursery soil) and 2.80 mg kg⁻¹ (using closed landfill soil). 500.00 mg kg⁻¹ is the permissible maximum level for manganese in vegetation that fixed by World Health Organization (WHO), so, from that, we can justify that the concentration of both plant samples is under maximum level and within safe range.

Nickel: Nickel is also can bring many negatives effects to our health and the ecosystem if in excessive amount. Similar with the other heavy metal, the Nickel level in soil can be enhancing by mining activity, industrial waste and open burning of coal. Plant grown in high Ni containing soil showed impairment of nutrient of nutrient balance and resulted in disorder of cell membrane functions (Yadav, 2009). The concentrations of Nickel in both *Ipomoea Aquatica* samples are within safe condition after compare to the World Health Organization's standard in vegetation.

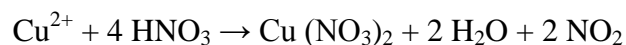
Iron (Ferum): The maximum level of iron that permitted by World Health Organization is second biggest after manganese, 425.00 mg kg⁻¹. The concentration of Iron that found in *Ipomoea Aquatica* after analyzing are very low compare to the limitation that fixed by WHO. So, concentration of Iron is in *Ipomoea Aquatica* also in safe range.

4.5 Metal Chloride and Metal Nitrate

In this research, the acid digestion method and wet destruction method had been used. This can be explained using chemical reaction of heavy metal with the concentrated acid. The chemical reaction for Copper had shown below;



This equation shows that copper ion will react with the hydrochloride acid to form water soluble copper (II) chloride.



Equation above shows that copper ion will form copper (II) nitrate that also soluble in water when reacted with nitric acid. For other heavy metals, the equations are the same like Copper. From this chemical reaction, adding water after digestion the sample will help increase the heavy metal extraction.

CHAPTER 5

CONCLUSION AND FUTURE WORKS

5.1 Conclusion

As a conclusion, the determination of heavy metals in *Ipomoea Aquatica* and soil samples collected from closed landfill of Ulu Tualang, Temerloh, Pahang was run successfully. Starting with first stage which is sample collecting, pre-treatment procedure and plantation of *Ipomoea Aquatica*, followed by second stage which is digestion procedure and end by third stage which is Atomic Absorption Spectrometry (AAS) is used in order to analyze the samples, it show that all the samples contain all the eight types of heavy metal (Cr, Cu, Mn, Ni, Pb, Fe, Zn and Cd) but with various concentration. Based on result, it shows that the concentration of heavy metal in nursery soil is higher than closed landfill soil in terms of the number of heavy metal. Besides that, this research also shows that the plantation in closed landfill in closed landfill soil that taken from Ulu Tualang shows only shows more indication of Chromium and Iron rather than the nursery soil. After compare the result with maximum level of World Health Organization in vegetation, the concentration of Chromium, Lead and Cadmium were exceeded the limit with 4.16 and 6.92mg kg⁻¹, 4.67 and 3.93 mg kg⁻¹ and 9.66 and 8.87 mg kg⁻¹ respectively. The heavy metal distribution data in soil and plant samples are very useful for become main references or guidelines in order to monitoring and avoid environmental pollution become worst in terms of quality of soil and also safety level for vegetables to be consumed.

5.2 Future Works

5.2.1 Method (The Destruction Method of Plant)

For the improvement of this research, other digestion method of plant samples can be used in order to compare with the wet destruction method in terms of heavy metal recoveries. There are some other methods that suitable to compare with wet destruction method such as microwave digestion method and dry ashing.

5.2.2 Plant

For this research, ipomoea aquatic (wild spinach) is used. So, other green plants are recommended to be used in future study in order to compare the green plants with wild spinach in terms of ability in absorbing the heavy metal. The plant that been selected must be fast grow crops, available in abundant and also widely used in Malaysia cuisine.

5.2.3 Soil sample

This study will be more interesting if the soil sample that taken randomly from Ulu Tualang closed landfill and gridding is used in order to get more soil for plantation of the green plants and also for analysis of soil sample. So, the comparison will be made in terms of the distribution of heavy metal accumulation.

Besides that, this study will be improved by using a lot of soil that taken from other place like near the roadside and near textile plants. So, the comparison can be made between the places in terms of the distribution of heavy metal concentration.

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APPENDIX A

Result AAS (Plant)

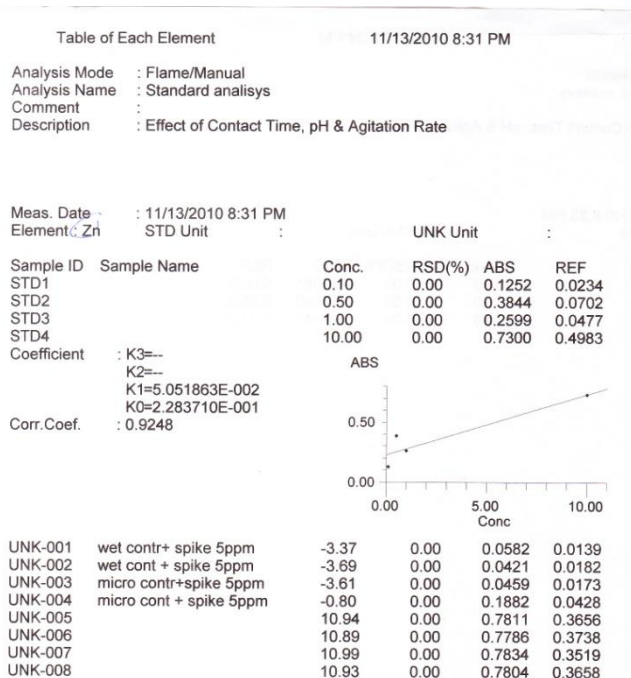


Figure A.1: Result of Zinc

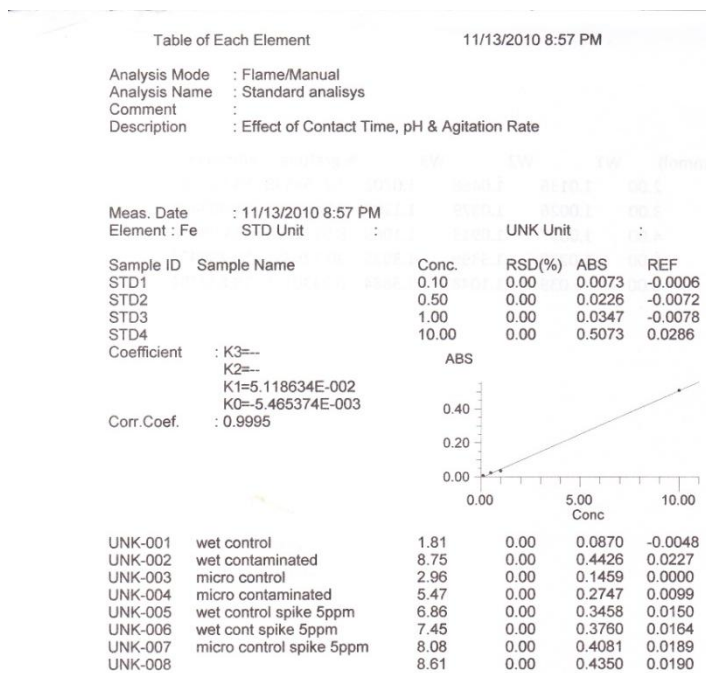


Figure A.2: Result of Iron

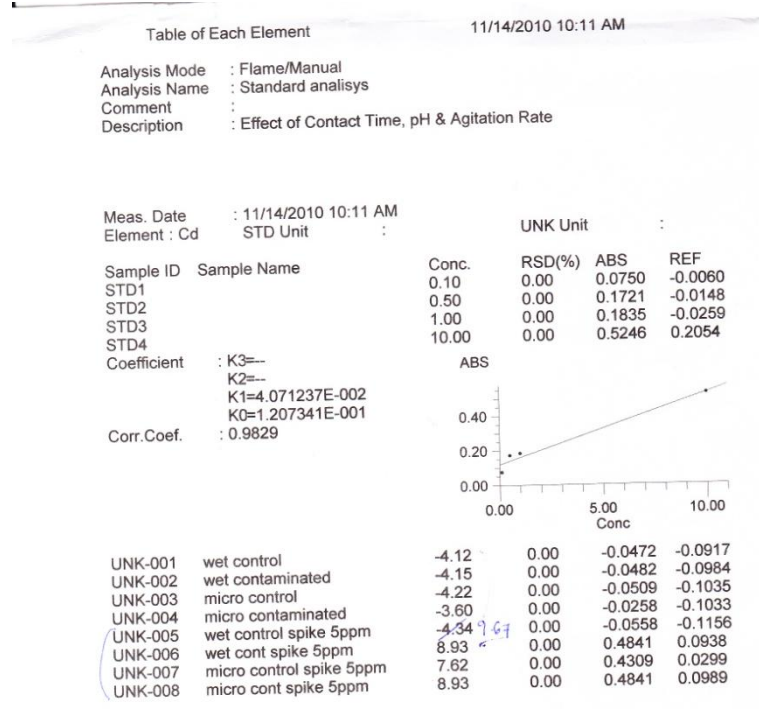


Figure A.3: Result of Cadmium

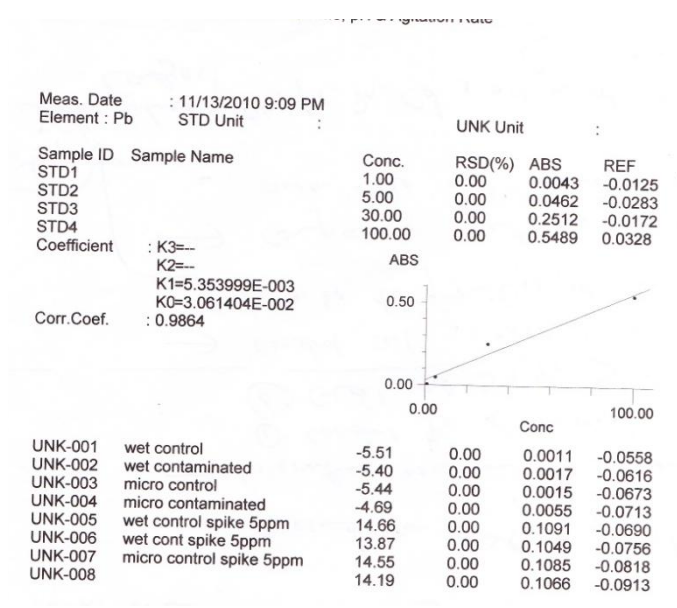


Figure A.4: Result of Lead

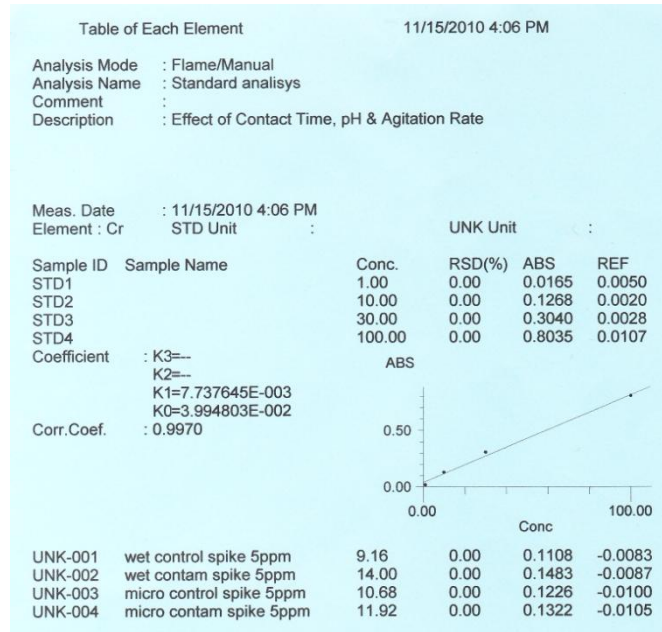


Figure A.5: Result of Chromium

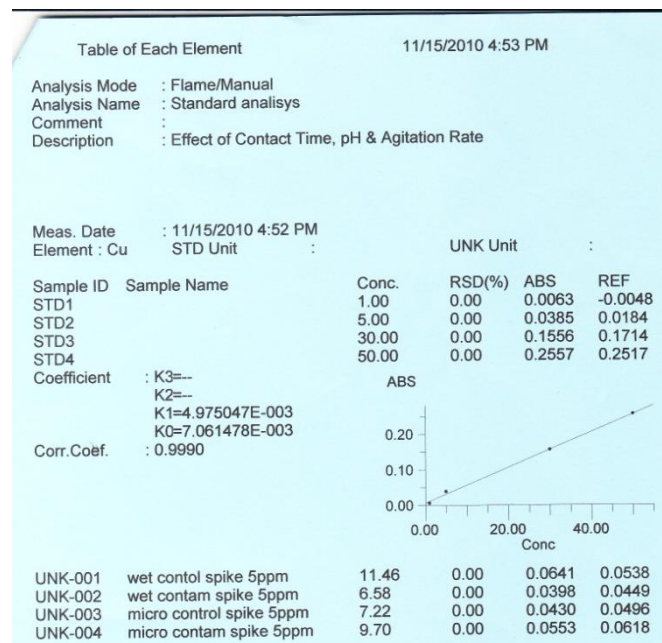


Figure A.6: Result of Copper

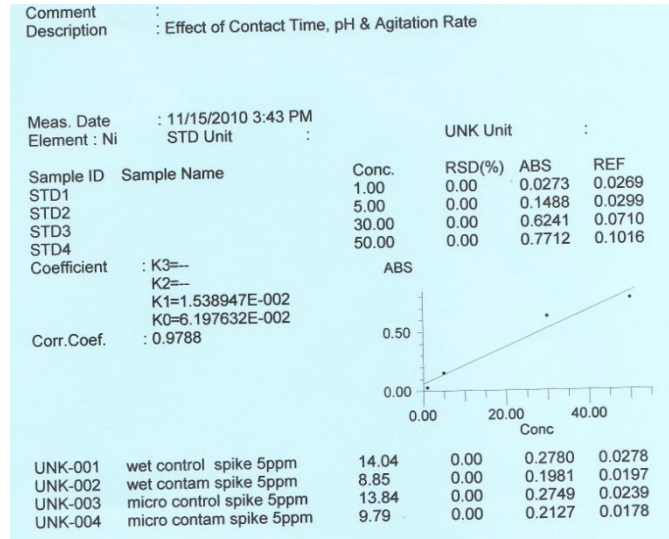


Figure A.7: Result of Nickel

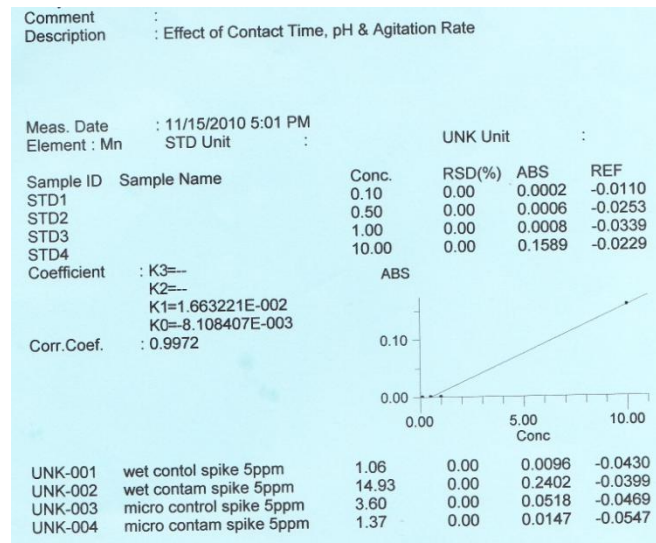


Figure A.8: Result of Manganese

APPENDIX B

Result AAS (Soil)

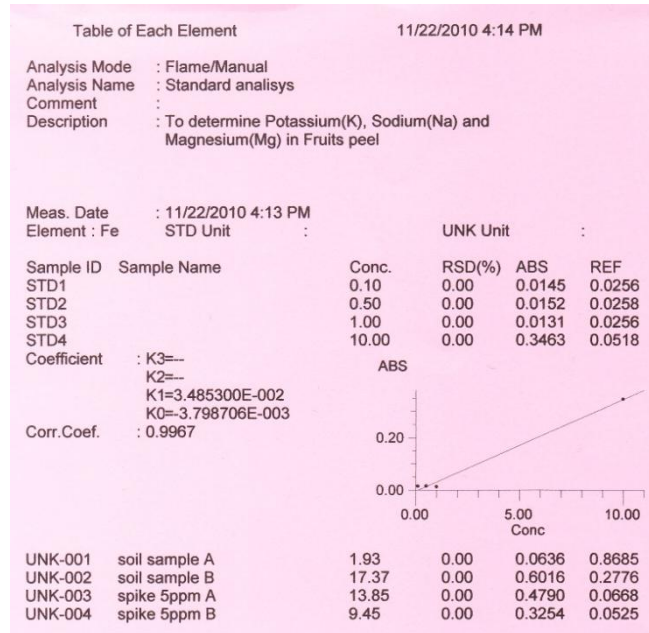


Figure B.1: Result of Iron

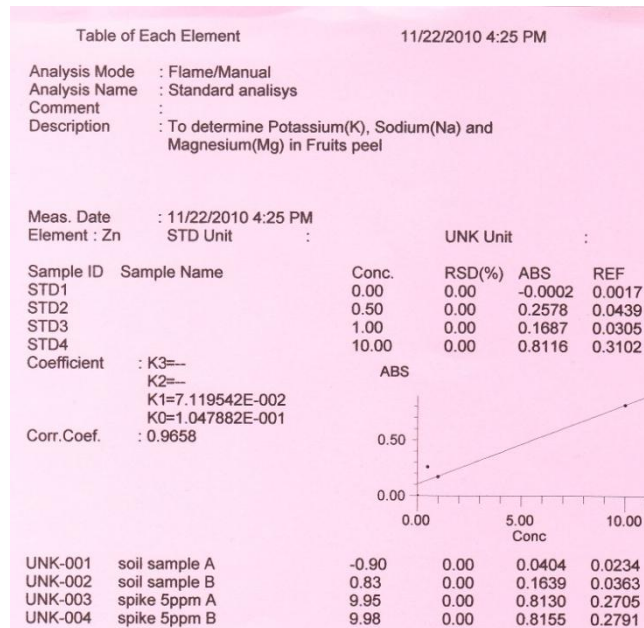


Figure B.2: Result of Zinc

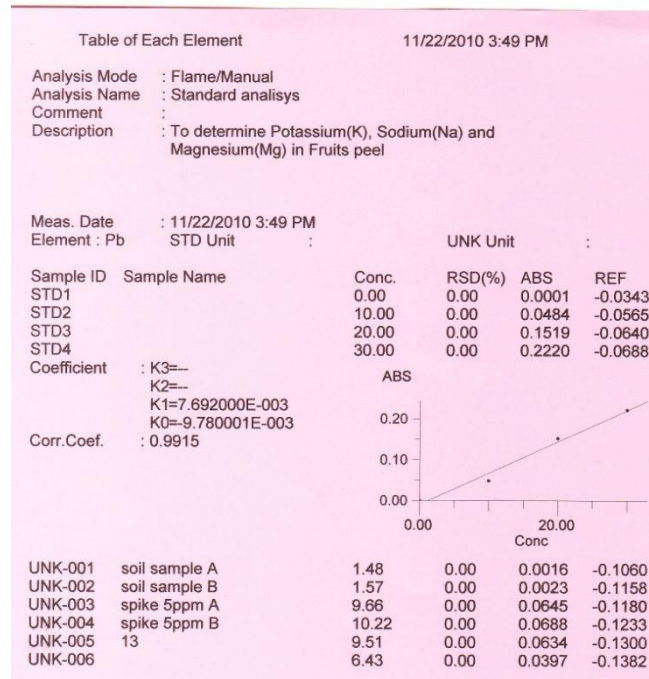


Figure B.3: Result of Lead

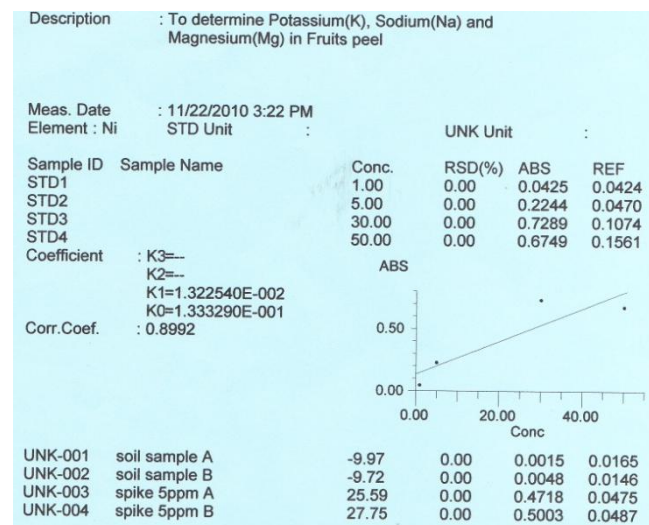


Figure B.4: Result of Nickel

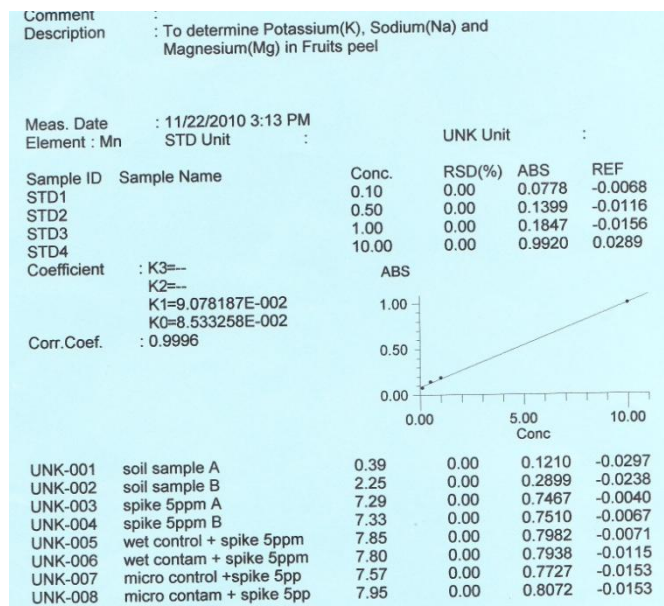


Figure B.5: Result of Manganese

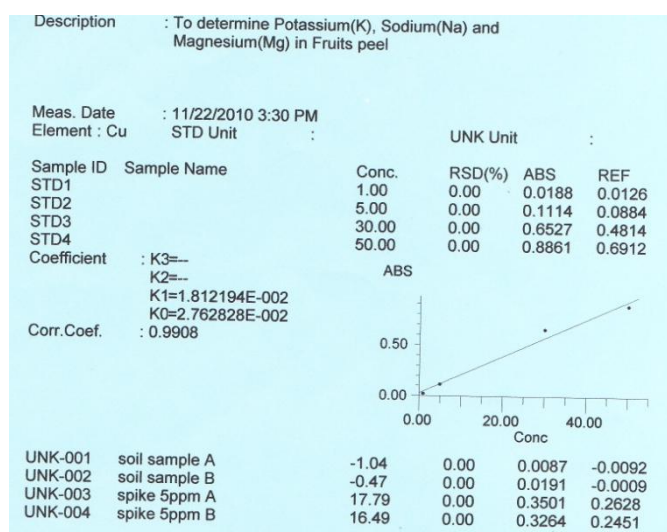


Figure B.6: Result of Copper

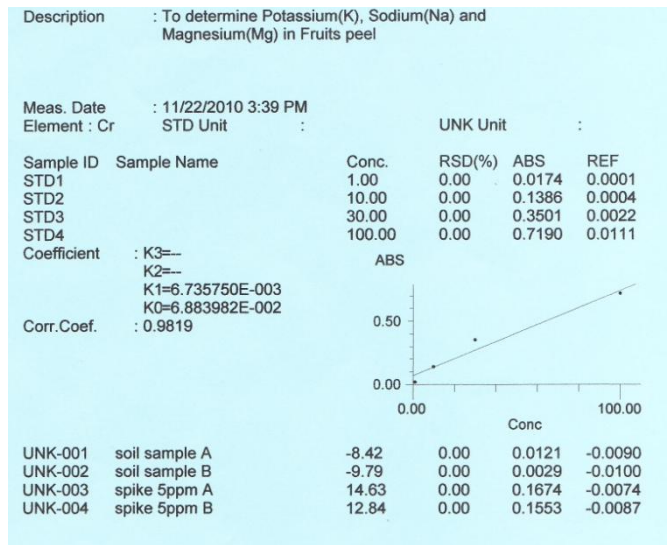


Figure B.7: Result of Chromium

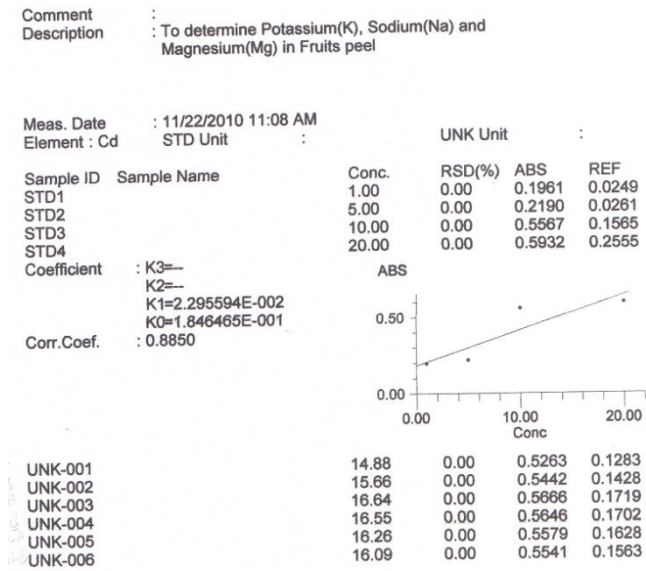


Figure B.8: Result of Cadmium