DETERMINATION OF HEAVY METAL ACCUMULATION IN IPOMEA AQUATICA USING MICROWAVE DIGESTION METHOD

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JUDUL : DETERMINATION OF HEAVY METAL ACCUMULATION IN IPOMEA AQUATICA USING MICROWAVE DIGESTION METHOD.

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A report submitted in partial fulfillment of the
requirement for the award of degree the of
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Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

DECEMBER 2010

"I declare that this thesis is the result of
my own research except as cited in the references.
The thesis has not been accepted for any degree and is not concurrently
submitted in candidature of any other degree."

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Author 's Name : NABILA SYAZREEN BINTI
ABDUL RAZAK
Date : 1st DECEMBER 2010

I dedicate this to:

My parents:
En. Abdul Razak bin Hj. Abdul Aziz and Pn. Asmah binti Hj. Kassim
My siblings:
Nur Azyla Syazreen, Nur Zulaikha, Mohd Ashrul and Norsyahirah Suhaila
My supervisor and friends.

Without their patience, understanding and support and most of all love,
the completion of this work would not have been possible.

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ABSTRACT

For several decades, farmers have been applied landfill materials as fertilizer to their fields. The aim of this study is to compare the heavy metal uptakes by *Ipomea Aquatica* with both types of the soil cultivated (from Ulu Tualang and from market) and to predict whether the plants cultivated could be used as food and feed. The concentrations of heavy metals in plant cultivated in soil taken from Ulu Tualang and from market were tested with the standard laboratory method by using atomic absorption spectrophotometer (AAS). Sample preparation was conducted by using microwave digestion method. High recovery values of spiked samples confirmed that microwave digestion method gives the best and most accurate result where Pb (9.18 and 9.55 mg/kg) appeared to be the most abundant metal while Mn exhibited the lowest abundance (2.57 and 2.95 mg/kg). It was found that some metal content in *Ipomea Aquatica* (Cd, Cr, and Pb) showed that it had over the maximum limit permitted by WHO. Hence, it is suggested that fertilizers used by farmers should be tested first before being used for plantation purpose. Hence, on the basis of present study, the best possible treatment of soil and fertilizer used in cultivating plants is to remove the sources of those elements of heavy metals in the environment.

ABSTRAK

Selama beberapa dekad, petani telah mengaplikasikan tanah dari tapak pelupusan sampah sebagai baja untuk ladang mereka. Tujuan kajian ini adalah untuk membandingkan penyerapan logam berat oleh *Ipomea Aquatica* yang ditanam di dalam kedua-dua jenis tanah (dari Ulu Tualang dan dari pasaran) dan untuk meramalkan jika tanaman yang ditanam boleh digunakan sebagai makanan. Kadar kepekatan logam berat di dalam tanaman yang ditanamkan di tanah yang diambil dari Ulu Tualang dan dari pasar diuji dengan kaedah makmal standard dengan menggunakan *atomic absorption spectrophotometer* (AAS). Persiapan sampel dilakukan dengan menggunakan kaedah *microwave digestion*. Nilai pemulihan yang tinggi sampel *spike* menegaskan bahawa kaedah *microwave digestion* memberikan keputusan yang terbaik dan paling tepat di mana Pb (9.18 dan 9.55 mg/kg) tampaknya menjadi logam paling banyak, manakala Mn wujud dalam kuantiti terendah (2.57 dan 2.95 mg/kg). Didapati bahawa beberapa kadar logam dalam *Ipomea Aquatica* (Cd, Cr, dan Pb) menunjukkan bahawa mereka telah melebihi had maksimum yang ditetapkan oleh WHO. Oleh kerana itu, disarankan supaya baja yang digunakan oleh petani harus diuji terlebih dahulu sebelum digunakan untuk tujuan perkebunan. Oleh kerana itu, berdasarkan kajian ini, rawatan terbaik tanah dan baja yang digunakan dalam penanaman tanaman adalah untuk menghapuskan sumber unsur-unsur logam berat dalam persekitaran.

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LIST OF ABBREVIATIONS

AAS - Atomic Absorption Spectrophotometry

Cd - Cadmium

Cu - Copper

Cr - Chromium

Fe - Iron

Mn - Manganese

Ni - Nickel

Pb - Lead

Zn - Zinc

ML - Maximum Limit

FAO-WHO - Food and Agriculture Organization of the United Nations – World Health Organization

LIST OF SYMBOLS

ξ^* - metastable energy state

λ_E - wavelengths

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Ipomea Aquatica is a common aquatic plant in Malaysia. In certain areas, both *Ipomea Aquatica* and water hyacinths thrive together (K. S. Low And C. K. Lee, 1981). Wood *et al.* (1976) used *Ipomea Aquatica* to remove wastes from rubber effluents. Its use as a bioagent for heavy metal removal has not been previously reported. *Ipomea Aquatica* is a common food eaten by all social groups. As mentioned by D. Austin (2007), there are several ways people consume these herbs, although the most frequent is a cooked vegetable. A common method is to lightly fry the young tips, including stems and leaves. However, tips are also eaten boiled, steamed, or added to soups, stews, curries, sambals, and even pickled.

As with many plants this is considered a food with medicinal effects, D. Austin (2007) stated that *Ipomea Aquatica* is considered a laxative, which is also recommended for piles and in certain nervous conditions with sleeplessness and head-ache. At one period, *Ipomea Aquatica* has been extremely important in Malaysia and also at western Indonesia. Many consider water spinach second in importance to Pak Choi, the ubiquitous Chinese vegetable (Tay & Toxopeus 1993).

Figure 1.1: *Ipomea Aquatica* plant

For many years, landfills have been one of the most comprehensive forms of disposal of municipal solid waste because of 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 (A. Kumar *et al*., 2009). These inorganic fertilizers are expensive and for farmers whom cannot afford to buy them, they will take the soil from landfill sites to use as fertilizer. Landfill site contains heavy metals, which could accumulate in the agricultural fields where the landfill material is applied. Moreover, I. Baranowska *et al.* (2002) mentioned that 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 (G D Laing *et al.*, 2003). 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.

Figure 1.2: Newly closed landfill in Ulu Tualang

A 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 (http://www.permacultureactivist.net/nurseries/PlntNursrys.htm, 2010). 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 (http://www.plantsupply.com.my/, 2010).

Figure 1.3: Plant nursery in Malaysia

1.2 Problem Statement

Human activity could be the possible main cause of the increasing levels of heavy metals contamination 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).

Nordberg et al. (2003) stated that human nowadays are exposed primarily through contaminated food. As observed by chronic occupational, in the late 1940's in Sweden, cadmium poisoning had occurred in the production of alkaline batteries. Increasing of cadmium is found in the land fill because of the disposal of nickel-cadmium rechargeable batteries. These batteries are usually used in portable consumer products like hand vacuum and food mixers. When the batteries are no longer used, the entire device, plastic case and battery are usually tossed into the trash. Toxic metals could be toxic to living organisms in relatively low concentrations. Incineration and land filling of refuse is an important source of cadmium, mercury, lead, vanadium, copper, and zinc (Herman K. and Michael B., 2003). This is why heavy metals in plants is very dangerous because even at low doses of toxic trace elements taken by human for over a long period of time could cause organ disfunction and lead to poisoning. Since metals do not break down, a metal stays in the body until it is excreted. The metal in the body may be transformed into another more toxic or less toxic species. Hence, it is important to disseminate information on metals and their occurrence and toxicity.

1.3 Objectives

The objectives of this research are to determine total heavy metals uptakes in *Ipomea Aquatica* cultivated in two types of soil by using AAS and to estimate whether a given soil is suitable for cultivation of plants used as food or feed. This research was also carried out to compare the best digestion methods between microwave digestion and wet digestion method and to compare the heavy metal uptakes by *Ipomea Aquatica* with both types of the soil cultivated in.

1.4 Scope of Research

1.4.1 Area (newly closed landfill in Ulu Tualang, Temerloh Pahang and plant nursery in Kuantan, Pahang)

Soil sample used to grow the *Ipomea Aquatica* were taken randomly from landfill area in Ulu Tualang, Temerloh Pahang and were compared with the *Ipomea Aquatica* grown in the soil sample available at the market which were taken from a random plant nursery in Kuantan, Pahang.

Figure 1.4: Location of the newly closed landfill in Ulu Tualang, Temerloh Pahang.

1.4.2 Green plant

The green plant species used to cultivate in the soil samples is *Ipomea Aquatica* (wild spinach). The plant was cultivated from the seeds that had been bought from the market.

1.4.3 Elements of Heavy Metal

In this research, only eight heavy metals out of all of the elements of heavy metals were studied. The concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in soil and *Ipomea Aquatica* samples were determined by using the AAS.

1.4.4 Digestion Method and Analysis

In this study, acid digestion method was used to digest the soil samples. As for the *Ipomea Aquatica* samples, microwave digestion method was used in this study. Concentrations of each element of heavy metals in both soil and *Ipomea Aquatica* were then determined by using Flame Atomic Absorption Spectrometry (FAAS).

CHAPTER 2

LITERATURE REVIEW

2.1 Uses of *Ipomea Acuatica*

Despite being used as vegetables in food, in Africa *Ipomea Aquatica* is used to treat diabetes as it is in Sri Lanka (Iwu 1993, Malalavidhane *et al*. 2000). Plants contain insulin-like compounds clinically shown to be effective. For West Africa, Iwu (1993) gives no references for this species. Elsewhere the species is used to treat abscesses, mental illness (Tanzania), and intestinal problems (Somalia) (Haerdi 1964, Samuelsson *et al*. 1992). There are records of *Ipomea Aquatica* being used medicinally in at least Africa, Borneo, Burma, Cambodia, China, India, Indonesia, the Philippines, and Sri Lanka. Medical use focuses around southeastern Asia. Scarcity of use suggests that the plants began being used as medicine in Africa comparatively recently.

Ipomea Aquatica is also fodder for animals, in limited quantity as it is somewhat laxative. These herbs are often grown in fish ponds by Chinese, particularly as food for their pigs (Ly *et al*. 2002, Westphal 1993), although they are also fed to cattle and fish (Edie & Ho 1969). In Vietnam, giàu muông is fed to chickens, ducks, and pigs (Ogle *et al*. 2003).

2.2 Importance of Heavy Metal Analysis

The progress of analysis of heavy metals in green plants has essentially been carried out by researchers to identify and control contamination of samples during the collection, storage and analysis. The importance of heavy metal control was first realized in the 1960's and for the effort of that, we need to convince an establishment of scientists, governments and corporations that heavy metals pollution was a real and is a forthcoming threat to the society and the environment. As stated by S.Shallri (1998), plants growing on metal-loaded soils respond by exclusion, indication or accumulation of metals (Baker, 1981). Hence, in order to determine their potential for management of polluted soils and especially for metal extraction, a study about plants grew on soils in high metals was conducted by S.Shallri (1998).

2.3 Heavy Metal Toxicity

Heavy metals such as Zn, Cu, fe and Mn could be considerd as useful trace elements for crop growth. However, as discussed by Wijewardena and Gunaratne, (2004), continuous application of organic manure to soil could increase heavy metal contents to the toxic levels. Phototoxicity of Zn occurs in leaves at levels of 100-400 mg/kg while in the case of Cu, the most prominent symptom of u toxicity is chlorosis. The toxic level of Cu to plants is range from 20-100 mg/kg. For Fe, its toxicity can be seen on the entire leaves where the color becomes purplish brown. However, Jones (1972) said that incidences of Fe toxicity to plants have not been reported under upland conditions. The most observable Mn toxicity symptoms is brown speckling of older leaves and it will appear when the concentrations of the heavy metal reach the level of 400-100mg/kg.

Heavy metals such as Cd, Pb, Ni and Hg are not considered as essential for crop growth. However, among the heavy metals, Pb could be considered as the least mobile as lead accumulates in the surface horizons of soils and does not usually leached (Davies, 1990). Since the concentration is low and this is limited amount is available for plant uptake. Despite being identified as not essential for crop growth, Ni might be active hydrogenase and translocation of nitrogen as it is known to be present in urease. Ni is easily translocated between plants and accumulates in leaves and seeds.

Bockman et al. (1990) categorized heavy metals as metallic elements that is often toxic to mammals. Heavy metals could be categorized into three groups based on their toxic levels which are; toxic (Cd), moderately toxic (Pb, Ni, Hg) and low toxic (Zn, Fe,Mn, Cu). It is known that vulnerability of man to heavy metal toxicity is high when compared to other animals and plants. Heavy metals can be taken up by plants and subsequently become incorporated into the food chain once it have introduced to crop lands. Moreover, heavy metals also could contaminate ground water as it leach through the soil. Heavy metals usually accumulate in human and other animals, particularly in kidneys, liver, etc. and could also cause diseases such as heart attack, brain damage, cancer, diseases in digestive system, anemia, gout, chronic nephritis, encephalopathy, etc. (Lagerwerff, 1972).

Table 2.1: Guidelines for maximum limit (ML) of metals in vegetables permitted by FAO-WHO.

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

2.4 Digestion method and analysis

As listed in **Table 2.3** (G. D. Laing, 2003), there are several methods to digest the samples before being analysed. As confirmed by M. Tuzen (2003), the comparison of wet and microwave digestion methods showed no statically significant differences in result. Therefore, the microwave digestion method was preferred since this procedure is more proper with respect both time and recovery compared to wet digestion. Samples were then digested for heavy metals analysis with a hot mixture of concentrated nitric acid and hydrogen peroxide after the method of Krishnamurty *et al.* (1976) and made to volume 50 ml. Metal analysis was carried out on the resultant digests using air/acetylene atomic absorption spectroscopy (G.R. MacFarlanea *et al*, 2003). As for the expected result, the plant species collected at the sites exhibited different concentration in metals (S.Shallri *et al*., 1998) which mean in our case, the plant species grow on different location of soil sample will give result in different levels of heavy metals. Lower level of concentration of investigated metals was found in herbs growing in clean regions than in those in polluted areas. Research done by Agamuthu, P. and Fauziah S. H. (2008) showed a significant high correlation of concentration of Fe in wild spinach while Pb gives a very low concentration that is almost undetected whereas concentration of Zn in the plant cells is a in range of 1500 to 2000mg/kg. However, metal concentrations in plant in M. Tuzen's study are lower than those reported by Agamuthu, P. and Fauziah S. H whereby, the Cd, Zn and Pb were being accumulated at a low ratio by plant.

I. Baranowska *et al*. (2002) found some limitations during the study like incomplete mineralization of samples during the microwave digestion process which may cause difficulty in transferring analytes into solution and, on the other hand, disturb electrochemical measurements. However, by application of concentrated HNO_3 for mineralization of herbs, will leads to the complete digestion of samples, which is proved by determined values of metals concentration (I. Baranowska *et al*., 2002). In addition, it is desirable to use higher ashing temperature in graphite furnace in order to remove the matrix efficiently for many analytes in food, biological and environmental samples (M. Tüzen, 2003).

Table 2.2: Destruction methods recently used in related literature for the analytical determination of metals in plants.

Journal Title, Author and Year	**Destruction method**	**Methodology**	**Comment**
Performance of selected destruction methods for determination of heavy metals in reed plant. (Gijs Du Laing, Filip M. G. Tack, Marc G. Verloo, 2003)	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 until about 1 ml of acid remained. After cooling, the suspension was filtered in a 50 ml volumetric flask and diluted to the mark.	• Long period • Easy handling • Not expensive • Good recovery
Performance of selected destruction methods for determination of heavy metals in reed plant (Gijs Du Laing, Filip M. G. Tack,	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	• Long period (but shorter than method 1 and 4) • Easy

Marc G. Verloo, 2003)		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.	handling and not expensive • Low recovery
Performance of selected destruction methods for determination of heavy metals in reed plant (Gijs Du Laing, Filip M. G. Tack, Marc G. Verloo, 2003)	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.	• Long period (like method 1) • Easy handling and not expensive • Low recovery
Performance of selected destruction methods for determination of heavy metals in reed plant (Gijs Du Laing, Filip M. G. Tack, Marc G. Verloo, 2003)	Wet destruction with HNO_3/H_2O_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_2O_2 was added in aliquots of 1 ml. After cooling, the suspension was filtered in a 50-ml volumetric flask and	• Simple (no need to overnight it) • Easy handling, not expensive • Good recovery

		diluted to the mark.	
Performance of selected destruction methods for determination of heavy metals in reed plant (Gijs Du Laing, Filip M. G. Tack, Marc G. Verloo, 2003)	Dry ashing	Samples (1.000 g) were weighed into porcelain crucibles. They were pre-ashed in a muffle 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.	• Low recovery • Complicated handling • Fast
Determination of heavy metals in soil, mushroom and plant samples by AAS (Mustafa Tuzen, 2003)	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 wastransferred quantitatively to 50 ml volumetric flasks and diluted to the mark.	• Best recovery • Simple • Need experience handling • fast

2.5 Atomic Absorption Spectrophotometer (AAS)

By referring to Chemistry Lab 2 (2010, 20 October), it was defined that atomic absorption (AA) is the standard method for the analysis of specific metals. It is widely practiced in environmental analysis. The general aspects of the techniques are:

- mg/L to µg/L detection is routine
- analysis is generally very specific to a given element (although interferences are common)
- some techniques offer multiple compound analysis for one injection
- the techniques are fast and relatively inexpensive

2.5.1 Principle

When metal cations enter a flame (or high T furnace or plasma), metal is quickly reduced to elemental (atomic) state.

For instance, $Fe^{2+} + 2e^{-}$ ---> Fe^{0}

Kinetic energy from gaseous collisions in a flame excites outer electrons to higher energy level. This excitation is a UV-visible transition. Light at a characteristic wavelength lA is absorbed. A diagram for the energy states of Thallium during AA is shown as an example in **Figure 2.1**.

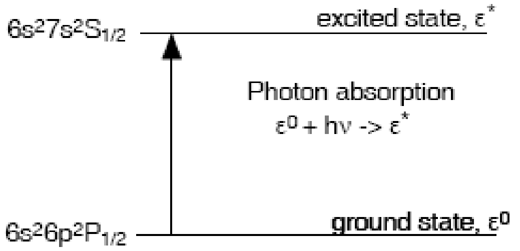


Figure 2.1: Thallium in flame ($\lambda_{A,\ max} = 378$ nm)

2.5.2 Emission spectroscopy

As electrons "fall back" to ground state, photons are emitted for the transition to a metastable energy state (ξ^*) and a portion of the deactivation is without radiation. Photons are emitted at characteristic wavelengths (λ_E) that are usually different than the wavelengths for absorption. Only 1% or so of the atoms are involved in the transition for a flame, and a more energetic source (such as argon plasma) is usually needed to take advantage of emission for chemical analysis. An example energy diagram for Thallium is shown in **Figure 2.2**.

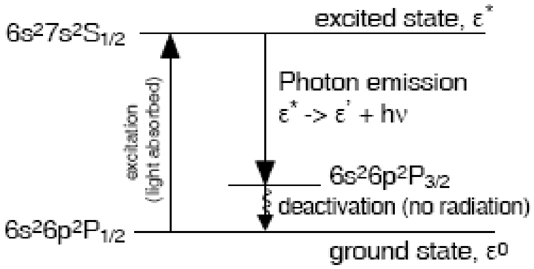


Figure 2.2: Thallium in flame with emission ($\lambda_{E,\max}$ = 535 nm)

2.5.3 Flame Atomic Absorption

A monochromatic beam of light is generated by a cathode ray tube. The lamp is selected from a set of available lamps in order to match the wavelength range with the characteristic wavelength (at maximum absorption) for the specific metal. A metal solution is aspirated into a flame by a nebulizer and burner assembly, shown in **Figure 2.3**. A variety of gas mixtures can be chosen to obtain the best flame temperature for the excitation of the metal. In order to promote the highest number of transitions (more light absorbed), the light should be aligned with the interconal region of the flame. A schematic of an air/acetylene flame is shown in **Figure 2.4**.

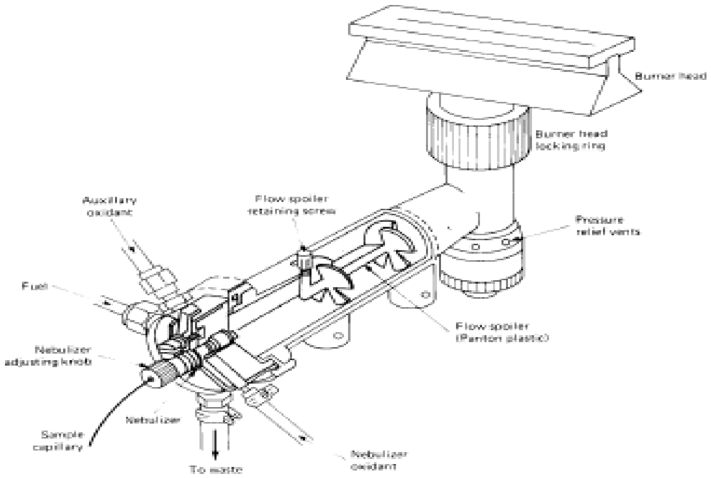


Figure 2.3: Nebulizer and burner assembly for flame atomic absorption. Adapted from Skoog (1984)

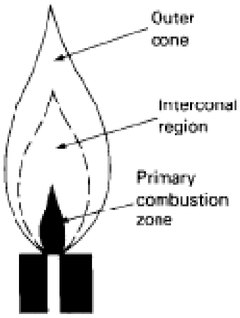


Figure 2.4: Diagram of air/acetylene flame. Adapted from Skoog (1984)

Following Beer's Law (see spectrophotometry notes), the absorbance A is directly proportional to concentration (A = ebC). The molar absorptivity e is a function of the wavelength and flame temperature. The other critical factor is the optical path length b.

This corresponds to the length of the light beam segment that is within the flame. The burner head can be readjusted (see manufacturer's notes) in order to get a different level of sensitivity.

2.6 Previous Result

By referring research that has been done by P. Agamuthu (2008), the research site is located at three landfills in Malaysia. The data obtained for heavy metal contamination in soil that affects the ability of plant heavy metal uptake from soil is shown in **Table 2.2**. This proved that heavy metal contamination occurred in surface soil as well as in deep soil. **Table 2.3** showed that the most commonly heavy metals reported (arsenic, cadmium, lead, chromium and mercury) elements in surface and deep soil from closed-landfill.

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

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 *P. Viridis* from the west coast of Peninsular Malaysia (this study)	Wet	0.12 to 0.22	1.32 to 3.42	0.43 to 1.49	12.80 to 21.90
	Dry	0.68 to 1.25	7.76 to 20.10	2.51 to 8.76	75.10 to 12.90

Table 2.4: 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

CHAPTER 3

METHODOLOGY

3.1 Sample preparation

3.1.1 Soil sampling

The soil samples used in this study were taken from a newly closed land fill in Ulu Tualang, Temerloh. The other soil samples were taken from a random plant nursery in Kuantan. The samples in Ulu Tualang were collected at random from the surface layer (0-20 cm) of the landfill as shown in **Figure 3.1**. The soil samples were air-dried at 150°C and ground to pass through 200-mesh sieve to remove rocks, roots, and other large and small particles and were transferred to polyethylene bottle until analysis. During the sampling, drying, grinding and storage, some precautions were taken in order to avoid contamination (J. Qian et al., 1996).

Figure 3.1: Soil collected at 0-20cm of the surface layer in Ulu Tualang.

3.1.2 Plant sampling

The remaining of the soil samples were then used to cultivate plants. Seedlings of *Ipomea Aquatica* were cultivated separately in both of the soil samples. After 2 weeks, the mature trees were harvested to analyse the uptake of heavy metals in the soil samples by the *Ipomea Aquatica*. The *Ipomea Aquatica* samples were washed with distilled water and dried at 105°C for 24 h. The dried *Ipomea Aquatica* samples were ground then homogenized using an agate pestle and were keep stored in polyethylene bottles until analysis.

3.2 Reagents

All chemicals used were of analytical reagent grade quality unless otherwise stated. Double deionized water (Mili-Q Milipore 18.2 MΩ/cm resistivity) was used for all dilutions. Nitric acid, 65% were used for preparing the digestion solutions. All the plastic and glassware were cleaned by soaking in dilute HNO_3 (1+9) and were rinsed with distilled water prior to use.

The standard solutions for each of the heavy metals element used for calibration were prepared by diluting a stock solution (1000ppm). For each element of heavy metals, the standard calibrations were prepared at 0.1ppm, 0.5ppm, 1ppm and 10ppm. Metal standard solutions (1000ppm) used for spiking the soil samples were prepared in deionized water by dissolving the appropriate amount of standard solutions.

3.3 Apparatus

A Perkin Pelmer Analyst 700 Atomic Absorption Spectrometer was used to analyse the concentration of each of the heavy metals in soil and *Ipomea Aquatica* samples. Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in soil and *Ipomea Aquatica* samples were determined by graphite furnace using nitrogen as the inert gas. Other measurements were carried out in an air/acetylene flame. The instrumental parameters and operating condition for each of the elements were given in Table 1 (M. Tuzen, 2003).

3.4 Digestion Methods

3.4.1 Acid Digestion Method

The soil samples were digested by extraction using digestion method. One gram of dried soil samples from Ulu Tualang was weighted into a floating boat. The dried samples was digested into a mixture of 9 ml of concentrated (100%) HCl and 3 ml of concentrated (65%) HNO_3, (ratio 3:1) in a beaker. The beaker were heated at 95°C and continued until the disappearance of NO_2 (reddish brown) fumes and no more changes in appearance. Watch glass was used during the heating for vapor recovery. The mixture was mark up to 100 ml of volumetric flask with deionized water. Another sample of soil samples from the market was carried out the same way. Both of the soil samples prepared were kept to be stored in a veil.

3.4.2 Microwave Digestion Method

A distillation microwave (**Figure 3.2**) was used in this study. One gram of *Ipomea Aquatica* sample prepared was placed into an Erlenmeyer bulb (100 ml capacity) and digested with 10 ml of concentrated (65%) HNO_3. The mixture of digested *Ipomea Aquatica* sample was placed in microwave digestion system for 10 min at 300W and diluted to 100 ml volumetric flask. Another sample of *Ipomea Aquatica* samples from the market was carried out the same way. Both of the *Ipomea Aquatica* samples prepared was keeping stored in a veil.

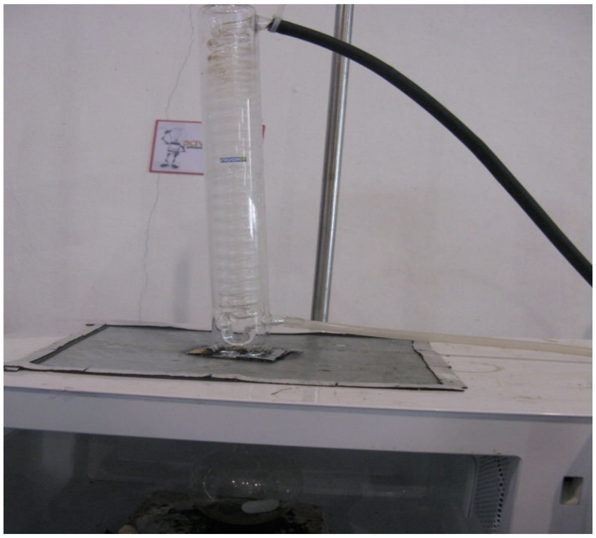


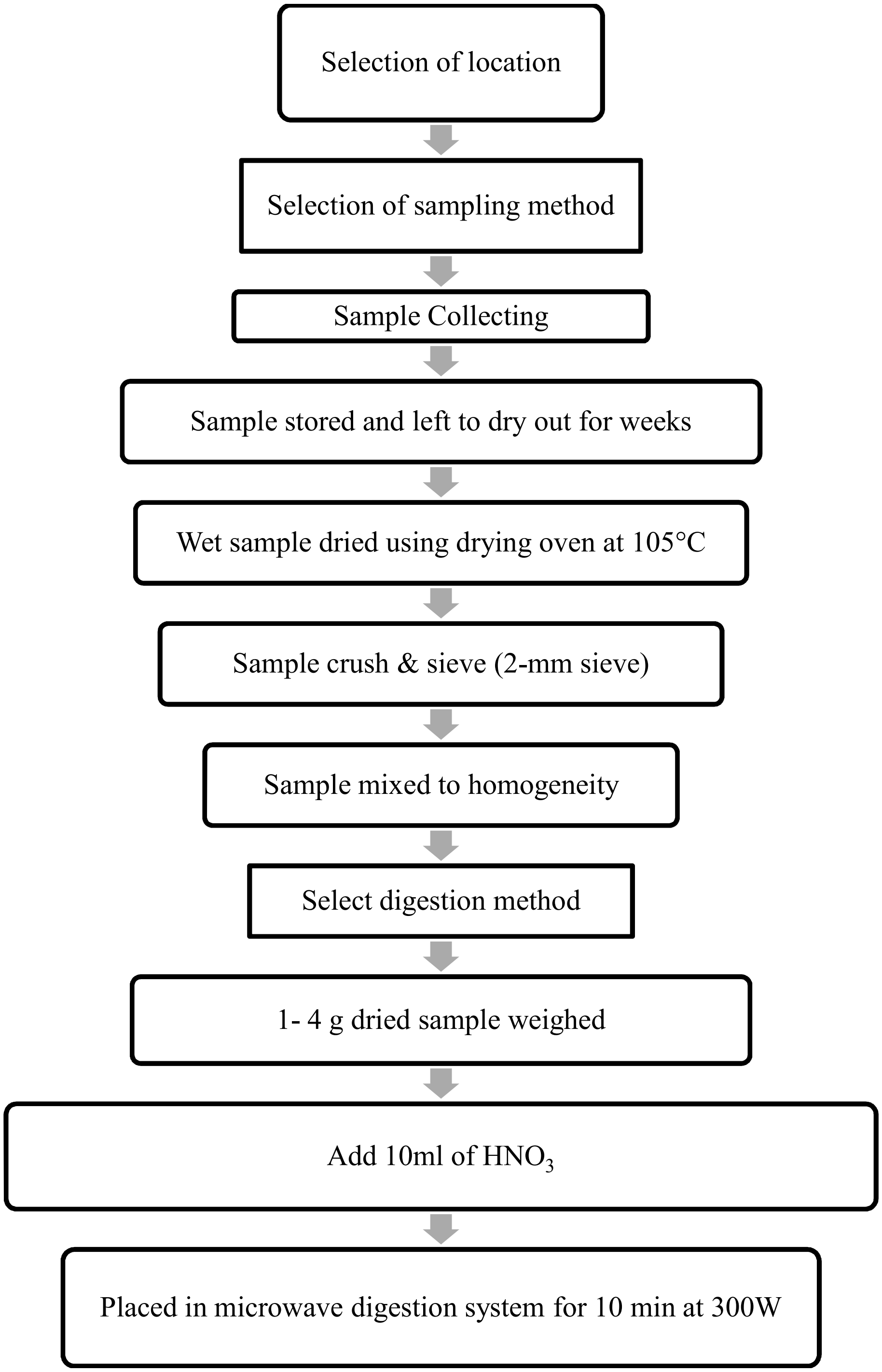
Figure 3.2: Distillation Microwave.

3.5 Elemental Analysis

By referring to J. Qian *et al*. (1996) method, the concentration of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were determined by atomic absorption spectrophotometer (**Figure 3.3**). Calibration techniques follow closely with those used for spectrophotometers. Since the aspirator sometimes gets clogged, the rate of sample introduction into the flame can be uneven, and standards should be checked often. The most common method is by filtering samples and keeping the solution acidic. Therefore, all samples standards, and rinsing solution should contain a background acid concentration near 0.1N. Note that acid varies depending on the analysis. Also, the rinse solution should be aspirated during the periods between the samples (C. Suros, 1997).

Figure 3.3: Atomic Absorption Spectrophotometer (AAS)

3.6 Summary of Methodology



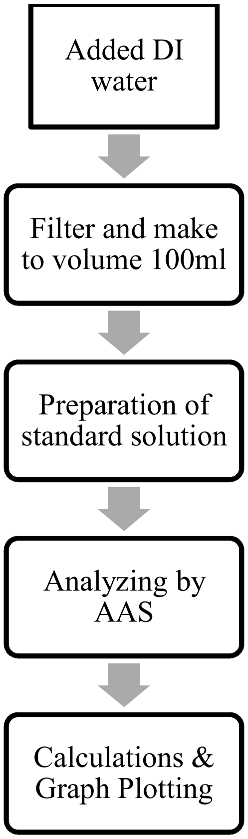


Figure 3.4: Summary of Methodology for *Ipomea Aquatica*

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Observation

Observation was made on the physical properties of the soil as well as Ipomea Acuatica cultivated in soil from Ulu Tualang and from market. A significant difference in color was observed as in **Figure 4.1** and **Figure 4.2** where soil from Ulu Tualang has a light brown grey color while the color of the soil from market is reddish brown. It was also shown that soil from Ulu Tualang has larger particles compared to soil from market which has small particles. For soil from market, 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 structure tends to be better agricultural soils (Ritter, Michael E, 2010). In contrast; soil from Ulu Tualang has blocky structure since it has larger particles of soil. As shown in **Figure 4.3** and **Figure 4.4**, *Ipomea Aquatica* cultivated in soil from market showed they had grown quite well compared to plants cultivated in soil from Ulu Tualang. This is because the *Ipomea Aquatica* grown in soil from Ulu Tualang have a maximum height of 13 cm where as the *Ipomea Aquatica* grown in soil from market have a maximum height about 16 cm. However, both *Ipomea Aquatica* cultivated in both soils gave different color where the color of *Ipomea Aquatica* grown in soil from market is dark green while the color of *Ipomea Aquatica* grown in soil from Ulu Tualang is light green.

Figure 4.1: Soil bought from available market, plant nursery in Kuantan.

Figure 4.2: Soil collected from newly closed landfill in Ulu Tualang.

Figure 4.3: *Ipomea Aquatica* cultivated in soil from market.

Figure 4.4: *Ipomea Aquatica* cultivated in soil from Ulu Tualang.

4.2 Concentration of Heavy Metals in Soil

In general, based on summary result in **Table 4.1**, soil from Ulu Tualang has higher concentration of heavy metals compared to the soil from the market. Even though concentrations of total heavy metals in soil do not show the availability of heavy metals for plant uptakes, Rosazlin A. *et al*. (2006) reported that it can provide information on potential heavy metal pools that can become available in a period of time as the availability of plant uptakes is indirectly depending on the content of heavy metal in the soil. Based on the summary result in **Table 4.1**, Ni appeared to be the most abundant metal and again Pb exhibited the lowest abundance for both soil from Ulu Tualang and from market (7.4 and 7.85 mg/kg). Three heavy metals, Ni, Cu and Fe are shown to be high in the soil and need to be monitored. Ni content in the soil from Ulu Tualang and from market (102.95 and 113.75 mg/kg) while Cu content (57.45 and 63.95 mg/kg). Fe content in both soils from Ulu Tualang and from the market did have same concentration where the metal content is 12.37 mg/kg. However, among all elements of heavy metal, only Cu showed a different content where the concentration of soil from market is higher compared to soil from Ulu Tualang.

J. Dai et al. (2004) reported that the bioavailability of heavy metals depend on their chemical forms in the soils. 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. Overall, there is no significant difference between observed between the studied sites (KoÈhler *et al*., 1995), except for Fe, indicating that accumulation effects must occur in later stages. The concentration in the soil from 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 market, comparable to effects observed by many authors in heavy metal contaminated soils.

Table 4.1: Heavy metal concentrations in soil

Sample (mg/kg)	Market	Ulu Tualang
Cd	74.4	83.2
Cr	39.2	48.15
Cu	63.95	57.45
Fe	9.65	86.85
Mn	11.45	11.65
Ni	102.95	113.75
Pb	7.4	7.85
Zn	24.75	24.95

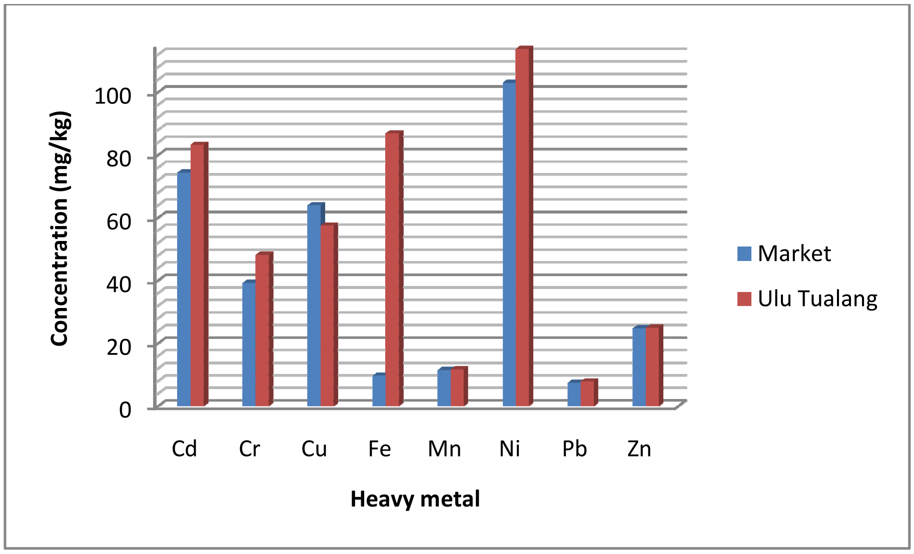


Figure 4.5: Comparison concentration of heavy metal of soil from Ulu Tualang and from market.

4.3 Concentration of Heavy Metals in Ipomea Acuatica

Table 4.2 presents a collection data of concentration of Cd, Cu, Cr, Fe, Mn, Ni, Pb and Zn analysed for *Ipomea Aquatica* samples cultivated in soil from Ulu Tualang and from market. Based on the summary result, it was found that the total concentration for Pb was the highest for both *Ipomea Aquatica* cultivated in soil from Ulu Tualang and *Ipomea Aquatica* cultivated in soil from the market (9.18 and 9.55 mg/kg) followed by Ni (4.79 and 8.84 mg/kg) and Zn (5.93 and 5.93 mg/kg). However *Ipomea Aquatica* cultivated in soil from market contained higher concentration of Pb, Ni and Zn compared to *Ipomea Aquatica* cultivated in soil from Ulu Tualang. Pb appeared to be the most abundant metal and again Mn exhibited the lowest abundance (2.57 and 2.95 mg/kg). The other content of metal showed an average content of metal are Cr (5.68 and 9 mg/kg) followed by Fe (3.08 and 5.47 mg/kg), Cu (2.22 and 4.78 mg/kg) and Cd (2.62 and 3.93 mg/kg).

As Cd was found at low concentration in the *Ipomea Aquatica*, Jarvis et al.(1976) reported that Cd was easily taken up by plants and transported to different organs thou it had no advantages effects to plants and animals. The non-woody character of the *Ipomea Aquatica* supported by vigorous root system close to the ground surface might have provided an excellent opportunity to absorb heavy metal pollutants from the sediment on a higher rate.(G. Agoramoorthy *et al.,* 2008) In Malaysia, fertilizer used was mainly rock phosphate, which is known to contribute appreciable amounts of Cd to agricultural soils especially spinach, oat, ryegrass and carrot (Am-Euras, 2009). E. I. Hozhina *et al*. found that a certain amount of each elements of metal enters the crops organ by the flow of nutrients. Furthermore, a certain amount of trace elements of metal stays in the plant, whereas the rest is released outside.

Table 4.2: Concentration of heavy metals in *Ipomea Aquatica* cultivated in soil from Ulu Tualang and soil from market.

Sample (mg/kg)	**Market**	**Ulu Tualang**
Cd	2.62	3.93
Cr	5.68	9
Cu	2.22	4.78
Fe	3.08	5.47
Mn	2.57	2.95
Ni	8.84	4.79
Pb	9.55	9.18
Zn	5.99	5.93

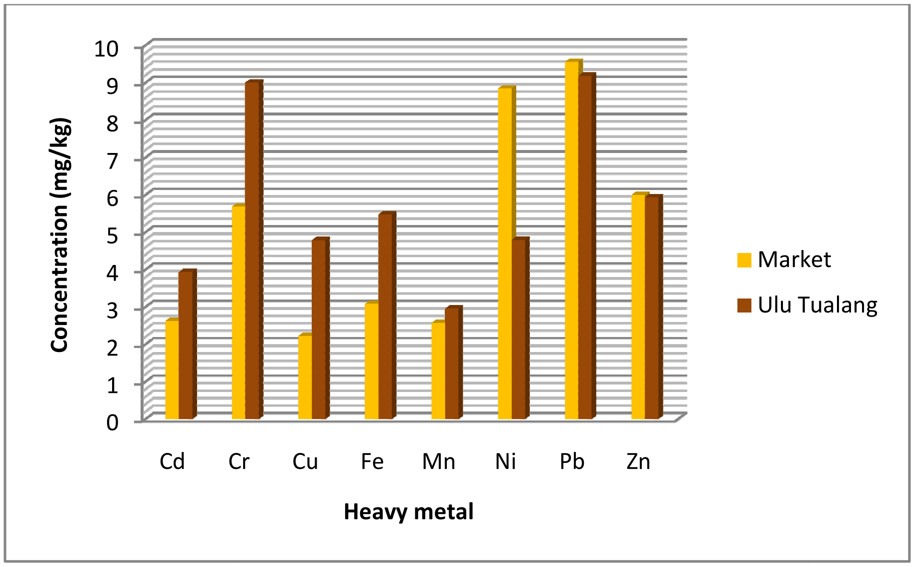


Figure 4.6: Comparison concentration of heavy metals in *Ipomea Aquatica* cultivated in soil from Ulu Tualang and from market.

4.4 Guidelines for Maximum Limit of metals in vegetables by FAO-WHO

Meanwhile, comparison between concentrations of both *Ipomea Aquatica* cultivated in soil from Ulu Tualang and from market was compared with WHO-ML (Am-Euras, 2008). Data showed in **Table 4.3** presents the guidelines for maximum limit (ML) of metals in vegetables from Food and Agriculture Organization of the United Nations – World Health Organization (FAO-WHO). From eight of the elements heavy metals studied, three of them have a concentration over the maximum limit permitted by WHO which are Pb (0.1mg/kg), Cr (0.1 mg/kg) and Cd (1mg/kg). Am-Euras (2008) discussed that although plants do not have a visible change in their appearance or yield; plants usually have the ability to accumulate large amounts of lead. Lead (Pb) is a toxic element that can be harmful to humans since Pb accumulation in several plants can exceed few hundred times the threshold of maximum level permissible for human (Wierzbicka, M., 1995). As for Cd, metal content in both plants cultivated in soil from Ulu Tualang and from market showed similar result with Pb where the concentration exceeded maximum limit (ML). Fazeli, M.S (1998) reported that Cd is highly mobile metal which can be easily absorbed by the plants through root surface and transferred to wood tissue and to upper part of the plants. . Foodstuffs that are rich in Cd can greatly increase the cadmium concentration in human bodies. Cd will then accumulate in kidneys, where it damages filtering mechanisms. This causes the excretion of essential proteins and sugars from the body and further kidney damage. Crops usually contain systems that could arrange the Cr uptake to be low enough so that it will not cause any harm or negative effects. However, when the amount of Cr in the soil rises, this can still lead to higher concentrations in plants. Another factor that could influence the Cr uptake by plants is acidification of soil. Ingestion of Cr may affect human's kidney and liver function (http://www.lenntech.com/, 2010).

Table 4.3: Comparison between concentrations of metal in *Ipomea Aquatica* and maximum limit (ML) of metals in vegetables permitted by FAO-WHO.

Metal	Ulu Tualang	Market	Maximum Limit (ML, mg/kg)	Exceeded Limit
Cd	3.93	2.62	0.1	✓
Cr	9	5.68	1	✓
Cu	4.78	2.22	70	✗
Fe	5.47	3.08	425	✗
Mn	2.95	2.57	500	✗
Ni	4.79	8.84	67	✗
Pb	9.18	9.55	0.3	✓
Zn	5.93	5.99	100	✗

CHAPTER 5

CONCLUSION AND RECOMMENDATION

As for the conclusion, it can be concluded that metal content in plants affect the accumulation of heavy metals by *Ipomea Aquatica*. This is because *Ipomea Aquatica* cultivated in soil will accumulate the heavy metals content from the soil. By comparing both *Ipomea Aquatica* cultivated in both soils, it is proven that *Ipomea Aquatica* cultivated in soil from Ulu Tualang contained higher concentration of heavy metals compared to *Ipomea Aquatica* cultivated in soil from market. However, some metal content in *Ipomea Aquatica* (Cd, Cr, Pb) showed that it had over the maximum limit permitted by WHO.

Therefore, transfer of metals to food which has exceeded the maximum level is one of the major concerns due to its toxicity to human health. This is why plant or vegetables sample as well as soil samples should be periodically analyzed for heavy metals. It is recommended to monitor heavy metal content in soils as well as vegetable products grown in soil available at the market. 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). Hence, further study is needed to investigate these characteristics in order to understand the availability and toxicity of the heavy metals in green plants.

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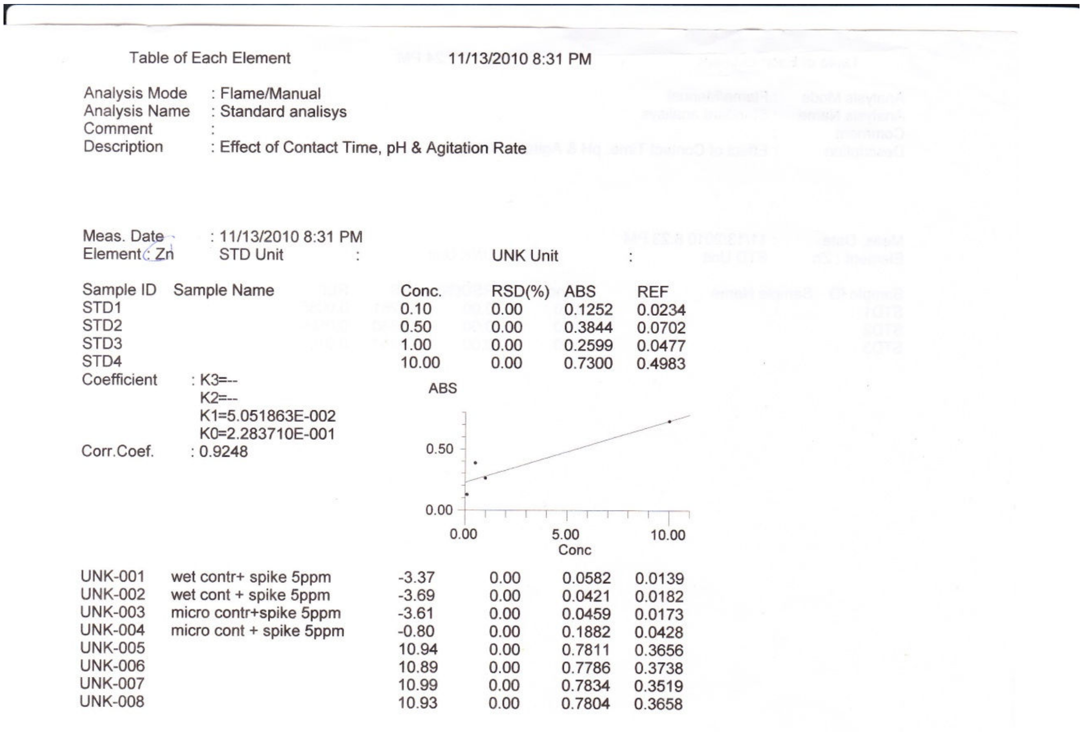
APPENDIX A

AAS analysis: *Ipomea Aquatica*



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Description : Effect of Contact Time, pH & Agitation Rate

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Element : Zn STD Unit : UNK Unit :

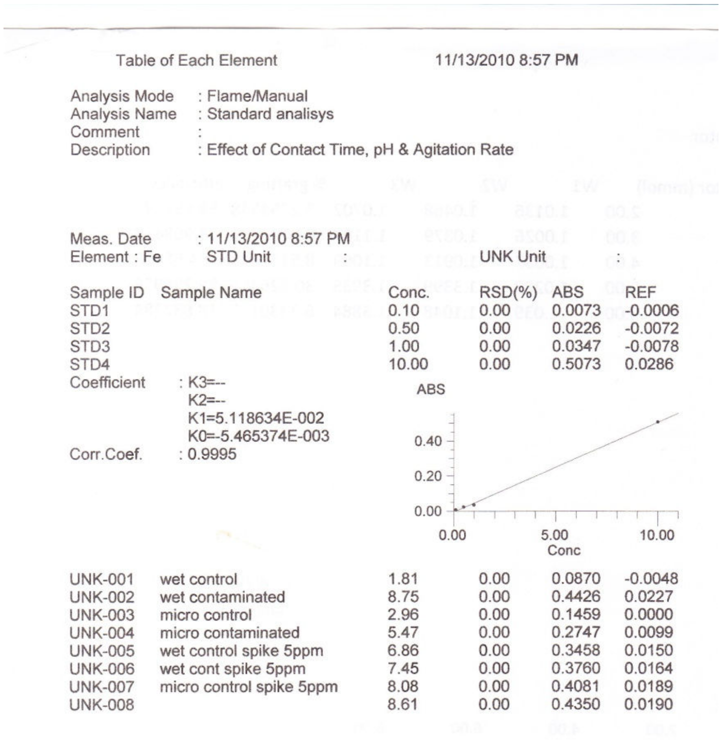
Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.10	0.00	0.1252	0.0234
STD2		0.50	0.00	0.3844	0.0702
STD3		1.00	0.00	0.2599	0.0477
STD4		10.00	0.00	0.7300	0.4983

Coefficient : K3=--
K2=--
K1=5.051863E-002
K0=2.283710E-001
Corr.Coef. : 0.9248

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
UNK-001	wet contr+ spike 5ppm	-3.37	0.00	0.0582	0.0139
UNK-002	wet cont + spike 5ppm	-3.69	0.00	0.0421	0.0182
UNK-003	micro contr+spike 5ppm	-3.61	0.00	0.0459	0.0173
UNK-004	micro cont + spike 5ppm	-0.80	0.00	0.1882	0.0428
UNK-005		10.94	0.00	0.7811	0.3656
UNK-006		10.89	0.00	0.7786	0.3738
UNK-007		10.99	0.00	0.7834	0.3519
UNK-008		10.93	0.00	0.7804	0.3658

APPENDIX B

AAS analysis : *Ipomea Aquatica*



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Comment           :
Description       : Effect of Contact Time, pH & Agitation Rate


Meas. Date        : 11/13/2010 8:57 PM
Element : Fe        STD Unit        :              UNK Unit       :

Sample ID  Sample Name                   Conc.     RSD(%)   ABS      REF
STD1                                     0.10      0.00     0.0073   -0.0006
STD2                                     0.50      0.00     0.0226   -0.0072
STD3                                     1.00      0.00     0.0347   -0.0078
STD4                                     10.00     0.00     0.5073   0.0286
Coefficient     : K3=--
                  K2=--
                  K1=5.118634E-002
                  K0=-5.465374E-003
Corr.Coef.      : 0.9995

UNK-001    wet control                   1.81      0.00     0.0870   -0.0048
UNK-002    wet contaminated              8.75      0.00     0.4426   0.0227
UNK-003    micro control                 2.96      0.00     0.1459   0.0000
UNK-004    micro contaminated            5.47      0.00     0.2747   0.0099
UNK-005    wet control spike 5ppm        6.86      0.00     0.3458   0.0150
UNK-006    wet cont spike 5ppm           7.45      0.00     0.3760   0.0164
UNK-007    micro control spike 5ppm      8.08      0.00     0.4081   0.0189
UNK-008                                  8.61      0.00     0.4350   0.0190
```

APPENDIX C

AAS analysis : *Ipomea Aquatica*

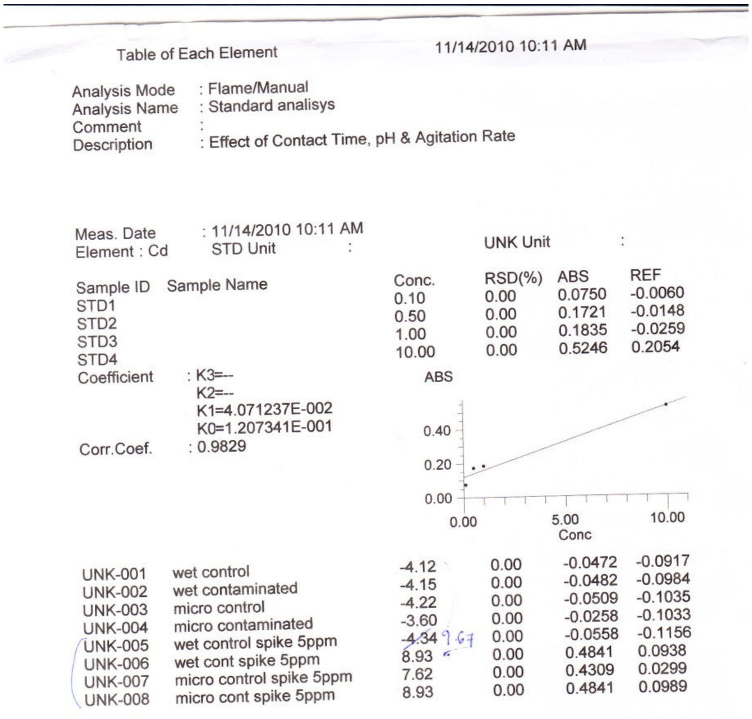


Table of Each Element 11/14/2010 10:11 AM

Analysis Mode : Flame/Manual
Analysis Name : Standard analisys
Comment :
Description : Effect of Contact Time, pH & Agitation Rate

Meas. Date : 11/14/2010 10:11 AM
Element : Cd STD Unit : UNK Unit :

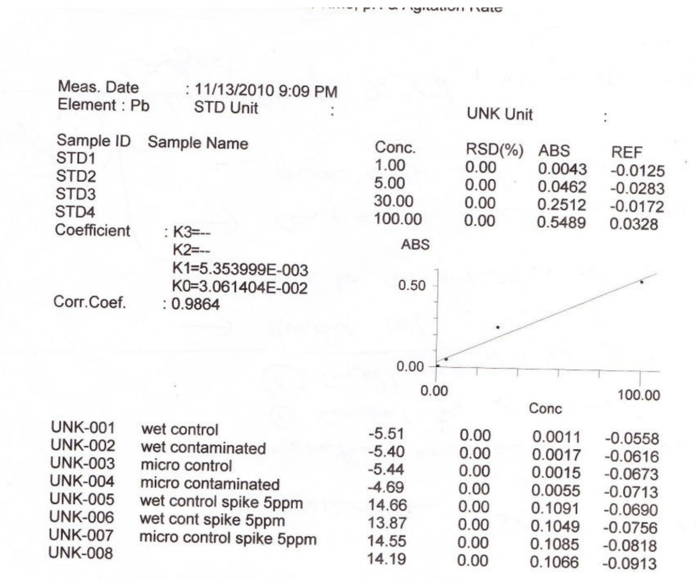
Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.10	0.00	0.0750	-0.0060
STD2		0.50	0.00	0.1721	-0.0148
STD3		1.00	0.00	0.1835	-0.0259
STD4		10.00	0.00	0.5246	0.2054

Coefficient : K3=--
K2=--
K1=4.071237E-002
K0=1.207341E-001
Corr.Coef. : 0.9829

UNK-001	wet control	-4.12	0.00	-0.0472	-0.0917
UNK-002	wet contaminated	-4.15	0.00	-0.0482	-0.0984
UNK-003	micro control	-4.22	0.00	-0.0509	-0.1035
UNK-004	micro contaminated	-3.60	0.00	-0.0258	-0.1033
UNK-005	wet control spike 5ppm	~~-4.34~~ 9.67	0.00	-0.0558	-0.1156
UNK-006	wet cont spike 5ppm	8.93	0.00	0.4841	0.0938
UNK-007	micro control spike 5ppm	7.62	0.00	0.4309	0.0299
UNK-008	micro cont spike 5ppm	8.93	0.00	0.4841	0.0989

APPENDIX D

AAS analysis : *Ipomea Aquatica*



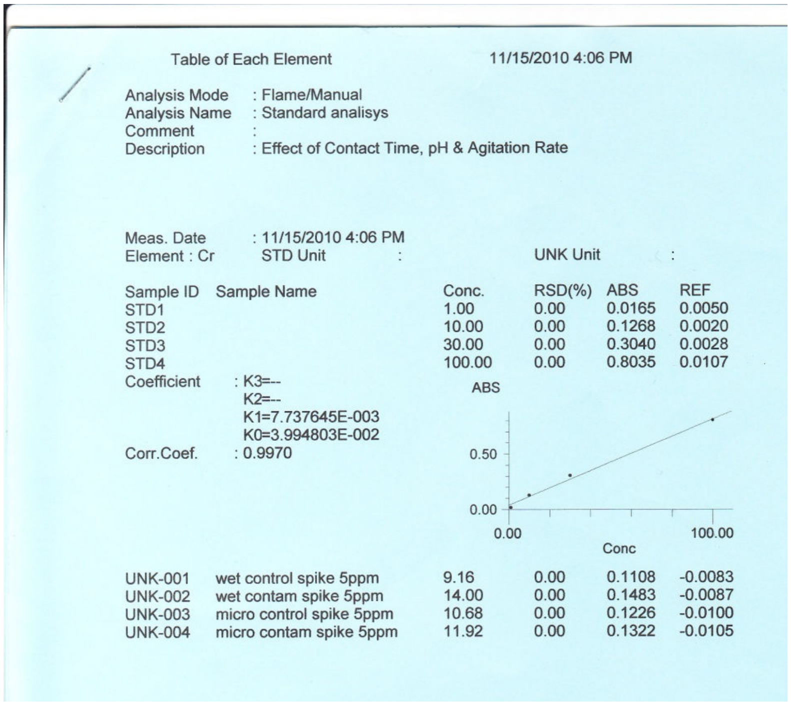
```
Meas. Date        : 11/13/2010 9:09 PM
Element : Pb        STD Unit          :              UNK Unit          :

Sample ID   Sample Name                    Conc.      RSD(%)   ABS       REF
STD1                                       1.00       0.00     0.0043    -0.0125
STD2                                       5.00       0.00     0.0462    -0.0283
STD3                                       30.00      0.00     0.2512    -0.0172
STD4                                       100.00     0.00     0.5489    0.0328
Coefficient     : K3=--
                  K2=--
                  K1=5.353999E-003
                  K0=3.061404E-002
Corr.Coef.      : 0.9864

UNK-001   wet control                      -5.51      0.00     0.0011    -0.0558
UNK-002   wet contaminated                 -5.40      0.00     0.0017    -0.0616
UNK-003   micro control                    -5.44      0.00     0.0015    -0.0673
UNK-004   micro contaminated               -4.69      0.00     0.0055    -0.0713
UNK-005   wet control spike 5ppm           14.66      0.00     0.1091    -0.0690
UNK-006   wet cont spike 5ppm              13.87      0.00     0.1049    -0.0756
UNK-007   micro control spike 5ppm         14.55      0.00     0.1085    -0.0818
UNK-008                                    14.19      0.00     0.1066    -0.0913
```

APPENDIX E

AAS analysis : *Ipomea Aquatica*



```
          Table of Each Element                   11/15/2010 4:06 PM

Analysis Mode     : Flame/Manual
Analysis Name     : Standard analisys
Comment           :
Description       : Effect of Contact Time, pH & Agitation Rate

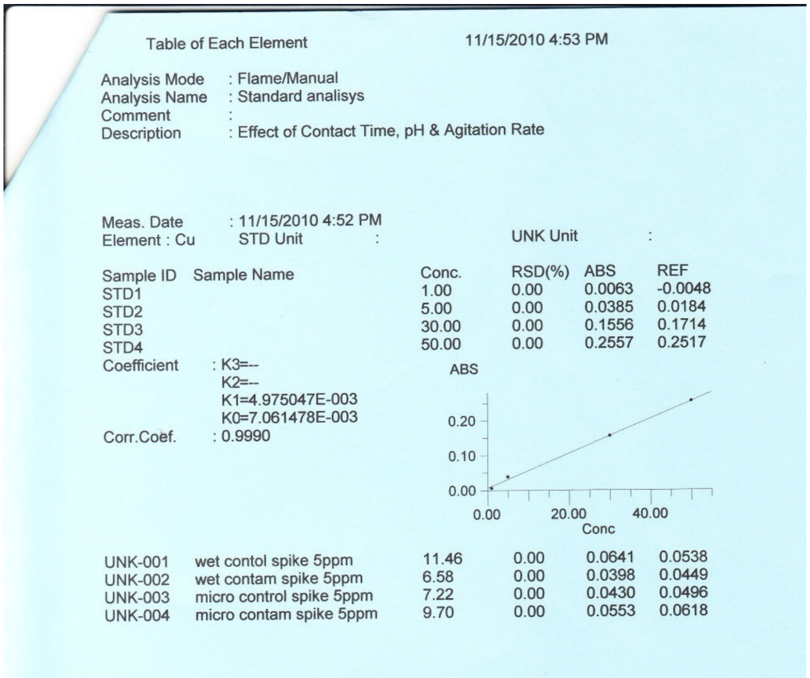

Meas. Date        : 11/15/2010 4:06 PM
Element : Cr        STD Unit          :                 UNK Unit        :

Sample ID   Sample Name                 Conc.       RSD(%)   ABS      REF
STD1                                    1.00        0.00     0.0165   0.0050
STD2                                    10.00       0.00     0.1268   0.0020
STD3                                    30.00       0.00     0.3040   0.0028
STD4                                    100.00      0.00     0.8035   0.0107
Coefficient    : K3=--
                 K2=--
                 K1=7.737645E-003
                 K0=3.994803E-002
Corr.Coef.     : 0.9970

UNK-001     wet control spike 5ppm      9.16        0.00     0.1108   -0.0083
UNK-002     wet contam spike 5ppm       14.00       0.00     0.1483   -0.0087
UNK-003     micro control spike 5ppm    10.68       0.00     0.1226   -0.0100
UNK-004     micro contam spike 5ppm     11.92       0.00     0.1322   -0.0105
```

APPENDIX F

AAS analysis : *Ipomea Aquatica*



```
      Table of Each Element                      11/15/2010 4:53 PM

Analysis Mode     : Flame/Manual
Analysis Name     : Standard analisys
Comment           :
Description       : Effect of Contact Time, pH & Agitation Rate

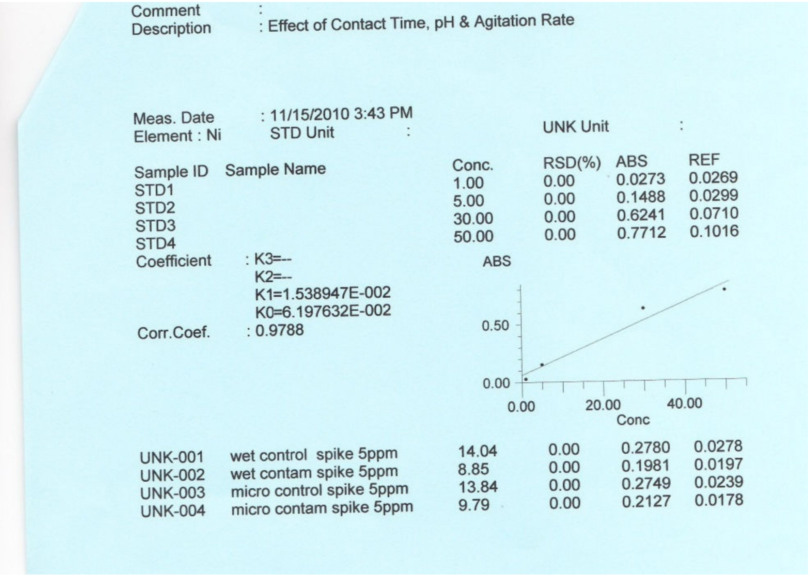

Meas. Date        : 11/15/2010 4:52 PM
Element : Cu        STD Unit          :          UNK Unit          :

Sample ID   Sample Name              Conc.     RSD(%)   ABS      REF
STD1                                 1.00      0.00     0.0063   -0.0048
STD2                                 5.00      0.00     0.0385   0.0184
STD3                                 30.00     0.00     0.1556   0.1714
STD4                                 50.00     0.00     0.2557   0.2517
Coefficient     : K3=--
                  K2=--
                  K1=4.975047E-003
                  K0=7.061478E-003
Corr.Coef.      : 0.9990


UNK-001     wet contol spike 5ppm     11.46     0.00     0.0641   0.0538
UNK-002     wet contam spike 5ppm     6.58      0.00     0.0398   0.0449
UNK-003     micro control spike 5ppm  7.22      0.00     0.0430   0.0496
UNK-004     micro contam spike 5ppm   9.70      0.00     0.0553   0.0618
```

APPENDIX G

AAS analysis : *Ipomea Aquatica*



```
Comment          :
Description      : Effect of Contact Time, pH & Agitation Rate

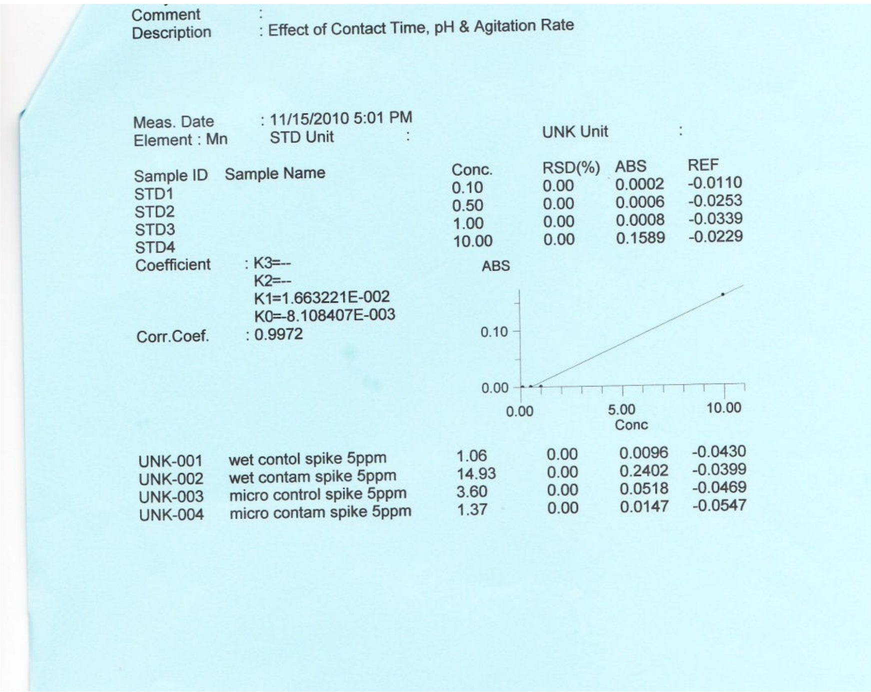

Meas. Date       : 11/15/2010 3:43 PM
Element : Ni       STD Unit          :                UNK Unit          :

Sample ID   Sample Name                     Conc.     RSD(%)   ABS      REF
STD1                                        1.00      0.00     0.0273   0.0269
STD2                                        5.00      0.00     0.1488   0.0299
STD3                                        30.00     0.00     0.6241   0.0710
STD4                                        50.00     0.00     0.7712   0.1016
Coefficient     : K3=--
                  K2=--
                  K1=1.538947E-002
                  K0=6.197632E-002
Corr.Coef.      : 0.9788


UNK-001     wet control  spike 5ppm         14.04     0.00     0.2780   0.0278
UNK-002     wet contam spike 5ppm           8.85      0.00     0.1981   0.0197
UNK-003     micro control spike 5ppm        13.84     0.00     0.2749   0.0239
UNK-004     micro contam spike 5ppm         9.79      0.00     0.2127   0.0178
```

APPENDIX H

AAS analysis : *Ipomea Aquatica*



```
Comment          :
Description      : Effect of Contact Time, pH & Agitation Rate

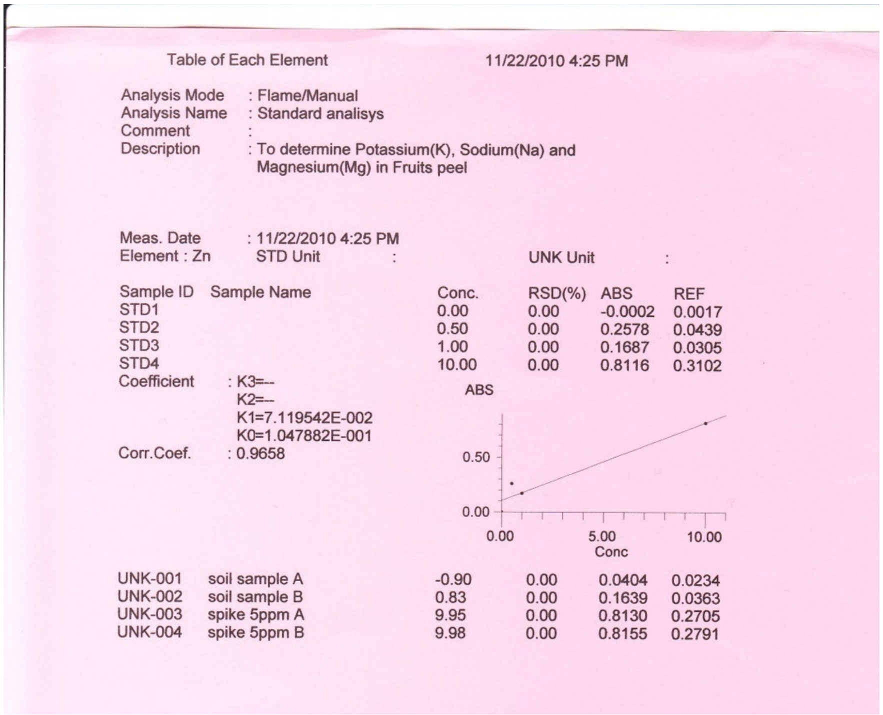

Meas. Date       : 11/15/2010 5:01 PM
Element : Mn       STD Unit          :                UNK Unit          :

Sample ID   Sample Name                       Conc.      RSD(%)   ABS      REF
STD1                                          0.10       0.00     0.0002   -0.0110
STD2                                          0.50       0.00     0.0006   -0.0253
STD3                                          1.00       0.00     0.0008   -0.0339
STD4                                          10.00      0.00     0.1589   -0.0229
Coefficient     : K3=--
                  K2=--
                  K1=1.663221E-002
                  K0=-8.108407E-003
Corr.Coef.      : 0.9972


UNK-001     wet contol spike 5ppm             1.06       0.00     0.0096   -0.0430
UNK-002     wet contam spike 5ppm             14.93      0.00     0.2402   -0.0399
UNK-003     micro control spike 5ppm          3.60       0.00     0.0518   -0.0469
UNK-004     micro contam spike 5ppm           1.37       0.00     0.0147   -0.0547
```

APPENDIX I

AAS analysis : Soil



```
        Table of Each Element                    11/22/2010 4:25 PM

Analysis Mode    : Flame/Manual
Analysis Name    : Standard analisys
Comment          :
Description      : To determine Potassium(K), Sodium(Na) and
                   Magnesium(Mg) in Fruits peel

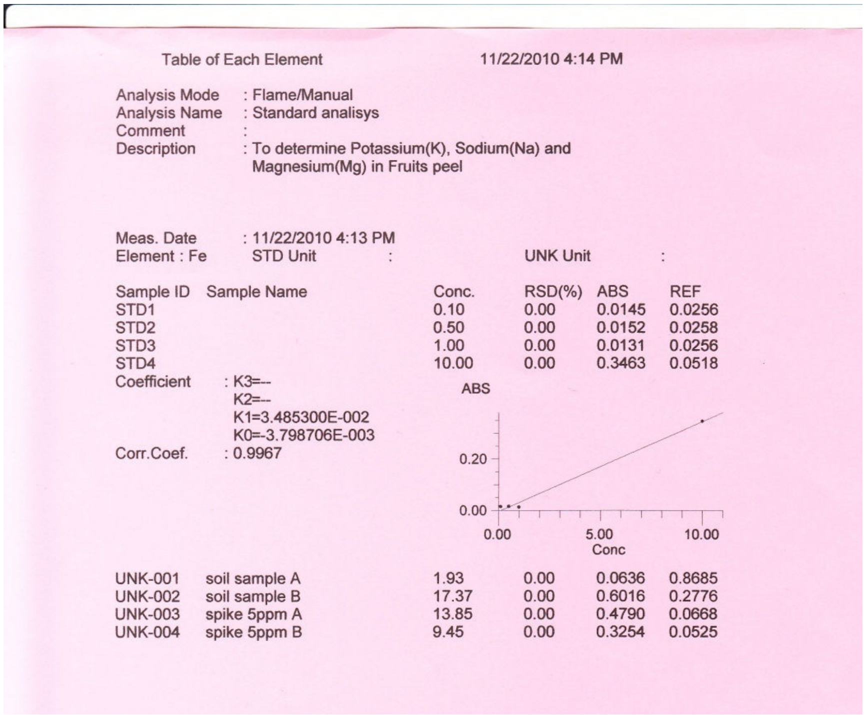

Meas. Date       : 11/22/2010 4:25 PM
Element : Zn       STD Unit          :          UNK Unit           :

Sample ID  Sample Name                Conc.     RSD(%)  ABS      REF
STD1                                  0.00      0.00    -0.0002  0.0017
STD2                                  0.50      0.00    0.2578   0.0439
STD3                                  1.00      0.00    0.1687   0.0305
STD4                                  10.00     0.00    0.8116   0.3102
Coefficient     : K3=--
                  K2=--
                  K1=7.119542E-002
                  K0=1.047882E-001
Corr.Coef.      : 0.9658

UNK-001    soil sample A              -0.90     0.00    0.0404   0.0234
UNK-002    soil sample B              0.83      0.00    0.1639   0.0363
UNK-003    spike 5ppm A               9.95      0.00    0.8130   0.2705
UNK-004    spike 5ppm B               9.98      0.00    0.8155   0.2791
```

APPENDIX J

AAS analysis : Soil



```
Table of Each Element                    11/22/2010 4:14 PM

Analysis Mode    : Flame/Manual
Analysis Name    : Standard analisys
Comment          :
Description      : To determine Potassium(K), Sodium(Na) and
                   Magnesium(Mg) in Fruits peel

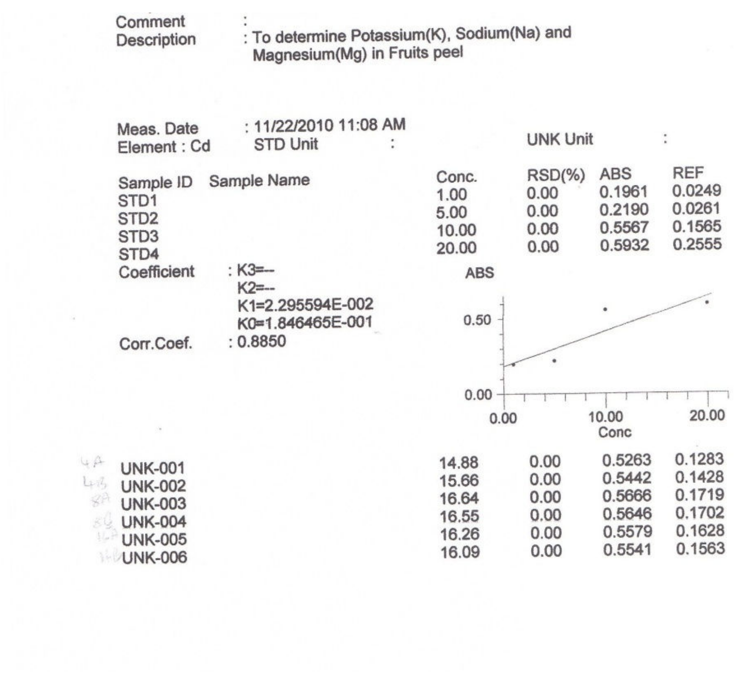

Meas. Date       : 11/22/2010 4:13 PM
Element : Fe       STD Unit          :           UNK Unit          :

Sample ID  Sample Name               Conc.     RSD(%)  ABS     REF
STD1                                 0.10      0.00    0.0145  0.0256
STD2                                 0.50      0.00    0.0152  0.0258
STD3                                 1.00      0.00    0.0131  0.0256
STD4                                 10.00     0.00    0.3463  0.0518
Coefficient    : K3=--
                 K2=--
                 K1=3.485300E-002
                 K0=-3.798706E-003
Corr.Coef.     : 0.9967

UNK-001    soil sample A             1.93      0.00    0.0636  0.8685
UNK-002    soil sample B             17.37     0.00    0.6016  0.2776
UNK-003    spike 5ppm A              13.85     0.00    0.4790  0.0668
UNK-004    spike 5ppm B              9.45      0.00    0.3254  0.0525
```

APPENDIX K

AAS analysis : Soil



Comment :
Description : To determine Potassium(K), Sodium(Na) and Magnesium(Mg) in Fruits peel

Meas. Date : 11/22/2010 11:08 AM
Element : Cd STD Unit : UNK Unit :

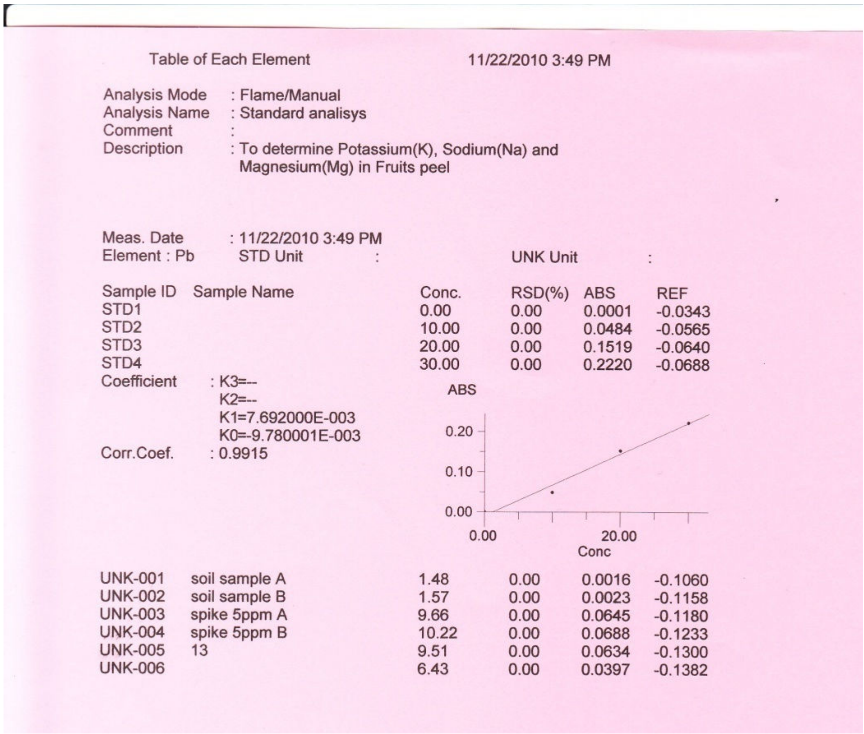
Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		1.00	0.00	0.1961	0.0249
STD2		5.00	0.00	0.2190	0.0261
STD3		10.00	0.00	0.5567	0.1565
STD4		20.00	0.00	0.5932	0.2555

Coefficient : K3=--
K2=--
K1=2.295594E-002
K0=1.846465E-001
Corr.Coef. : 0.8850

	Sample ID	Conc.	RSD(%)	ABS	REF
4A	UNK-001	14.88	0.00	0.5263	0.1283
4B	UNK-002	15.66	0.00	0.5442	0.1428
8A	UNK-003	16.64	0.00	0.5666	0.1719
8B	UNK-004	16.55	0.00	0.5646	0.1702
16A	UNK-005	16.26	0.00	0.5579	0.1628
16B	UNK-006	16.09	0.00	0.5541	0.1563

APPENDIX L

AAS analysis : Soil



```
     Table of Each Element                          11/22/2010 3:49 PM

Analysis Mode    : Flame/Manual
Analysis Name    : Standard analisys
Comment          :
Description      : To determine Potassium(K), Sodium(Na) and
                   Magnesium(Mg) in Fruits peel

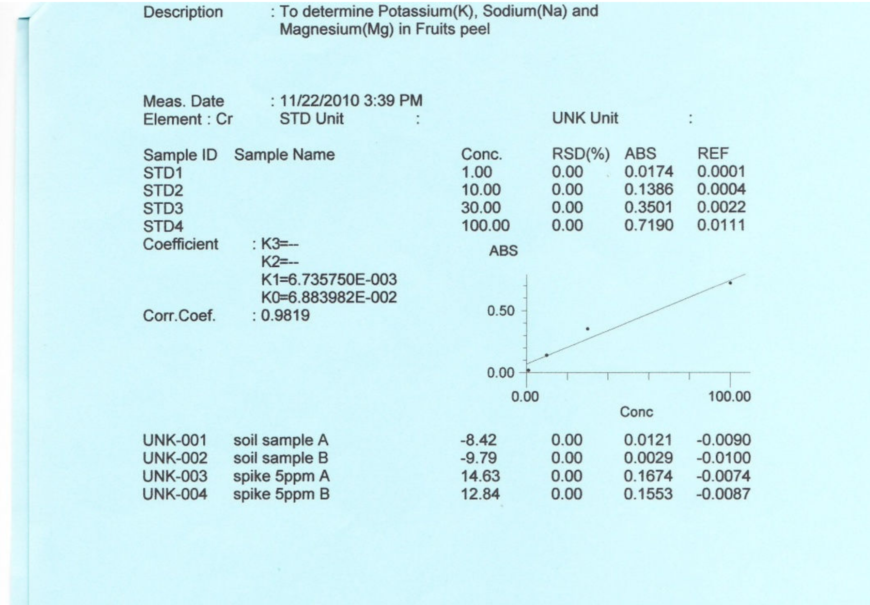

Meas. Date       : 11/22/2010 3:49 PM
Element : Pb       STD Unit          :               UNK Unit          :

Sample ID   Sample Name                       Conc.     RSD(%)   ABS      REF
STD1                                          0.00      0.00     0.0001   -0.0343
STD2                                          10.00     0.00     0.0484   -0.0565
STD3                                          20.00     0.00     0.1519   -0.0640
STD4                                          30.00     0.00     0.2220   -0.0688
Coefficient    : K3=--
                 K2=--
                 K1=7.692000E-003
                 K0=-9.780001E-003
Corr.Coef.     : 0.9915


UNK-001   soil sample A                       1.48      0.00     0.0016   -0.1060
UNK-002   soil sample B                       1.57      0.00     0.0023   -0.1158
UNK-003   spike 5ppm A                        9.66      0.00     0.0645   -0.1180
UNK-004   spike 5ppm B                        10.22     0.00     0.0688   -0.1233
UNK-005   13                                  9.51      0.00     0.0634   -0.1300
UNK-006                                       6.43      0.00     0.0397   -0.1382
```

APPENDIX M

AAS analysis : Soil



```
Description        : To determine Potassium(K), Sodium(Na) and
                     Magnesium(Mg) in Fruits peel

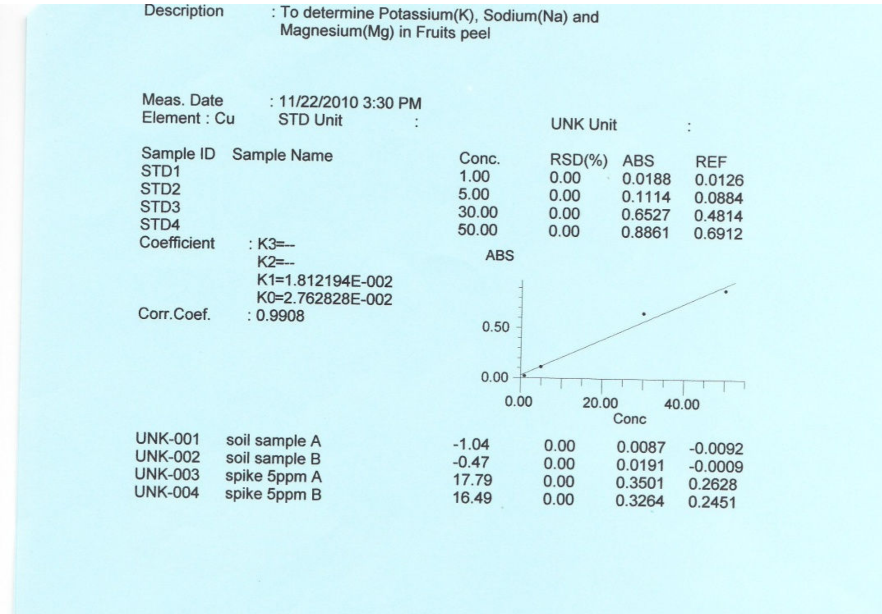

Meas. Date         : 11/22/2010 3:39 PM
Element : Cr         STD Unit          :            UNK Unit           :

Sample ID   Sample Name                 Conc.       RSD(%)   ABS      REF
STD1                                    1.00        0.00     0.0174   0.0001
STD2                                    10.00       0.00     0.1386   0.0004
STD3                                    30.00       0.00     0.3501   0.0022
STD4                                    100.00      0.00     0.7190   0.0111
Coefficient      : K3=--
                   K2=--
                   K1=6.735750E-003
                   K0=6.883982E-002
Corr.Coef.       : 0.9819


UNK-001     soil sample A               -8.42       0.00     0.0121   -0.0090
UNK-002     soil sample B               -9.79       0.00     0.0029   -0.0100
UNK-003     spike 5ppm A                14.63       0.00     0.1674   -0.0074
UNK-004     spike 5ppm B                12.84       0.00     0.1553   -0.0087
```

APPENDIX N

AAS analysis : Soil



```
Description     : To determine Potassium(K), Sodium(Na) and
                  Magnesium(Mg) in Fruits peel

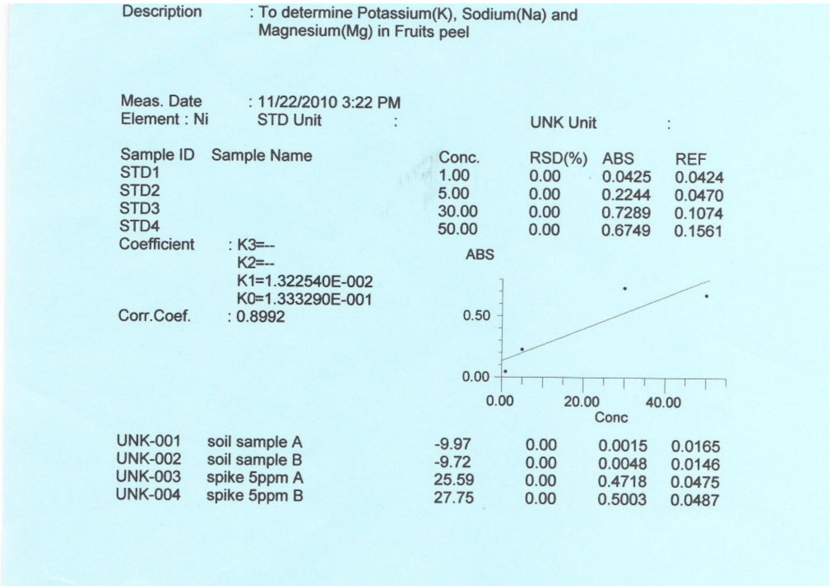

Meas. Date      : 11/22/2010 3:30 PM
Element : Cu      STD Unit          :              UNK Unit          :

Sample ID  Sample Name                   Conc.     RSD(%)  ABS      REF
STD1                                     1.00      0.00    0.0188   0.0126
STD2                                     5.00      0.00    0.1114   0.0884
STD3                                     30.00     0.00    0.6527   0.4814
STD4                                     50.00     0.00    0.8861   0.6912
Coefficient    : K3=--
                 K2=--
                 K1=1.812194E-002
                 K0=2.762828E-002
Corr.Coef.     : 0.9908


UNK-001    soil sample A                 -1.04     0.00    0.0087   -0.0092
UNK-002    soil sample B                 -0.47     0.00    0.0191   -0.0009
UNK-003    spike 5ppm A                  17.79     0.00    0.3501   0.2628
UNK-004    spike 5ppm B                  16.49     0.00    0.3264   0.2451
```

APPENDIX O

AAS analysis : Soil



```
Description        : To determine Potassium(K), Sodium(Na) and
                     Magnesium(Mg) in Fruits peel

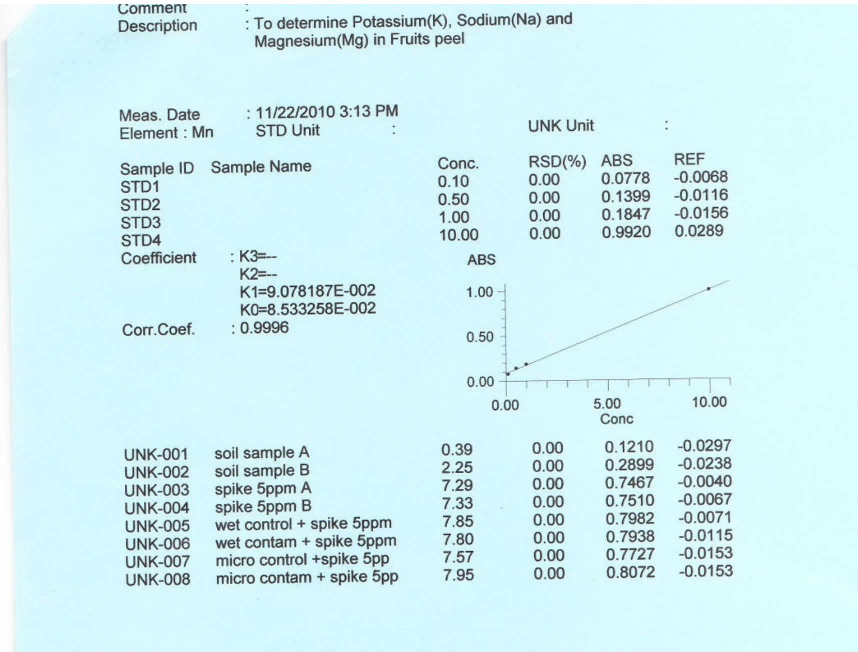

Meas. Date         : 11/22/2010 3:22 PM
Element : Ni         STD Unit          :               UNK Unit          :

Sample ID   Sample Name                   Conc.     RSD(%)   ABS      REF
STD1                                      1.00      0.00     0.0425   0.0424
STD2                                      5.00      0.00     0.2244   0.0470
STD3                                      30.00     0.00     0.7289   0.1074
STD4                                      50.00     0.00     0.6749   0.1561
Coefficient       : K3=--
                    K2=--
                    K1=1.322540E-002
                    K0=1.333290E-001
Corr.Coef.        : 0.8992

UNK-001     soil sample A                 -9.97     0.00     0.0015   0.0165
UNK-002     soil sample B                 -9.72     0.00     0.0048   0.0146
UNK-003     spike 5ppm A                  25.59     0.00     0.4718   0.0475
UNK-004     spike 5ppm B                  27.75     0.00     0.5003   0.0487
```

APPENDIX P

AAS analysis : Soil



Comment :
Description : To determine Potassium(K), Sodium(Na) and Magnesium(Mg) in Fruits peel

Meas. Date : 11/22/2010 3:13 PM
Element : Mn STD Unit : UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.10	0.00	0.0778	-0.0068
STD2		0.50	0.00	0.1399	-0.0116
STD3		1.00	0.00	0.1847	-0.0156
STD4		10.00	0.00	0.9920	0.0289

Coefficient : K3=--
K2=--
K1=9.078187E-002
K0=8.533258E-002
Corr.Coef. : 0.9996

UNK-001	soil sample A	0.39	0.00	0.1210	-0.0297
UNK-002	soil sample B	2.25	0.00	0.2899	-0.0238
UNK-003	spike 5ppm A	7.29	0.00	0.7467	-0.0040
UNK-004	spike 5ppm B	7.33	0.00	0.7510	-0.0067
UNK-005	wet control + spike 5ppm	7.85	0.00	0.7982	-0.0071
UNK-006	wet contam + spike 5ppm	7.80	0.00	0.7938	-0.0115
UNK-007	micro control +spike 5pp	7.57	0.00	0.7727	-0.0153
UNK-008	micro contam + spike 5pp	7.95	0.00	0.8072	-0.0153