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

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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
HNO3	-	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_2O_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 borns 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 as 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 dump 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**
 Cr	- Skin rashes - Upset stomachs and ulcers - Respiratory problems - Weakened immune systems - Kidney and liver damage - Alteration of genetic material - Lung cancer - Death

 Mn	- Fatness - Glucose intolerance - Blood clotting - Skin problems - Lowered cholesterol levels - Skeleton disorders - Birth defects - Changes of hair colour - Neurological symptoms
 Cu	- Headaches - Stomachaches - Dizziness - Vomiting - Diarrhoea - May cause liver and kidney damage
 Ni	- 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
 Cd	- Diarrhoea, stomach pains and severe vomiting - Bone fracture - Reproductive failure and possibly even infertility - Damage to the central nervous system

	- Damage to the immune system - Psychological disorders - Possibly DNA damage or cancer development
 Pb	- 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
 Fe	- Conjunctivitis - Choroiditis - Retinitis
 Zn	- 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 (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	-

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