A PRE-STUDY ON THE POTENTIAL PLANTS TO TREAT HEAVY METALS IN CONTAMINATED SOIL

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Name of Supervisor : MR. AZIZAN BIN RAMLI

Date : 19 JANUARY 2012

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

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JANUARY 2012

I declare that this thesis entitled "*A Pre-Study on the Potential Plants to Treat Heavy Metals in Contaminated Soil*" 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."

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Date : 19 JANUARY 2012

DEDICATION

Special dedication to my beloved mother (*Pn. Ruhani Binti Deraman*), father (*En. Yusoff Bin Abdullah*) and my family members (*Mohd Azhar, Roselawati, Mohd Azrul, Mohd Azman, Mohamad Azfar*) for their love and encouragement.

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Sincerely,

Nurul Izyatulikma Binti Yusoff

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ABSTRACT

Heavy metals are among the most important sorts of contaminant in the environment. Several methods already used to clean up the environment from these kinds of contaminants, but most of them are costly and difficult to get optimum results. Currently, phytoremediation is an effective and affordable technological solution used to extract or remove metal pollutants from contaminated soil and water. This study was aimed to evaluate the potential of 4 types of green plants for phytoremediation purpose and also to study the effect of contact time to uptake heavy metals such as Pb, Cd, Cr and Zn. Four types of green plants namely *Cosmos caudatus (ulam raja)*, *Centella asiatica (pegaga)*, *Emilia sonchifolia (bayam praksi)* and *Oenanthe javanica (daun selom)* were selected because they are fast-growth plant species, cheap in price and also have a substantial supply. Plant materials were acid digested and analyzed with the standard laboratory method by using Flame-Atomic Absorption Spectroscopy (AAS) to determine the total metal concentrations in plant samples. This study was perhaps one of the first to evaluate the feasibility of these green plants species for phytoremediation purpose. Results showed that all the studied green plant species possessed different metal concentrations where lead appeared to be the most metal being accumulated by *C.caudatus* (0.905 mg/L), cadmium was found to be highly accumulated by *O.javanica* (0.913 mg/L), chromium highly accumulated by *C.asiatica* (0.340 mg/L) and zinc was the most metal being accumulated by *E.sonchifolia* (2.460 mg/L). It also was found that the percentage of metal uptake by the plant increased with the increasing of time.

ABSTRAK

Logam berat merupakan salah satu bahan yang paling penting dalam pencemaran alam sekitar. Beberapa kaedah telah digunakan untuk membersihkan alam sekitar daripada pencemaran jenis ini, tetapi kebanyakan dari kaedah ini adalah mahal dan sukar untuk mendapatkan keputusan yang optimum. Pada masa ini, teknologi phytoremediation adalah salah satu penyelesaian yang paling berkesan untuk mengestrak atau mengeluarkan bahan pencemar logam dari tanah mahupun air yang tercemar. Kajian ini dijalankan bertujuan untuk menilai potensi empat jenis tumbuh-tumbuhan hijau untuk tujuan phytoremediation dan juga untuk mengkaji kesan masa terhadap pengambilan logam berat seperti Pb, Cd, Cr dan Zn. Empat jenis tumbuh-tumbuhan hijau iaitu ulam raje (*Cosmos caudatus*), pegaga (*Centella asiatica*), bayam praksi (*Emilia sonchifolia*) dan daun selom (*Oenanthe javanica*) telah dipilih kerana mereka merupakan spesis tumbuhan yang mempunyai pertumbuhan pantas, murah dan mempunyai bekalan yang besar. Tumbuh-tumbuhan ini akan dihadam menggunakan asid dan dianalisis menggunakan AAS untuk menentukan tahap kepekatan logam dalam sampel tumbuhan. Kajian ini juga mungkin kajian pertama yang menggunakan spesis tumbuh-tumbuhan hijau ini untuk tujuan phytoremediation. Keputusan menunjukkan bahawa semua spesis tumbuhan hijau ini mempunyai kepekatan logam yang berlainan di mana plumbum merupakan logam yang paling tinggi dalam *C.caudatus* (0.905 mg/L), kadmium paling tinggi dalam *O.javanica* (0.913 mg/L), kromium paling tinggi dalam *C.asiatica* (0.340 mg/L) dan zink paling tinggi dalam *E.sonchifolia* (2.460 mg/L). Keputusan juga telah menunjukkan bahawa peratusan pengambilan logam oleh jenis tumbuhan ini semakin bertambah seiring dengan pertambahan masa.

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LIST OF ABBREVIATIONS

AAS - Atomic Absorption Spectroscopy

Cd - Cadmium

Cr - Chromium

DNA - Deoxyribonucleic acid

HCL - Hallow Cathode Lamp

Pb - Lead

Zn - Zinc

CHAPTER 1

INTRODUCTION

1.1 Background of Study

A major environmental concern due to dispersal of industrial and urban wastes generated by human activities is the contamination of soil. Controlled and uncontrolled disposal of wastes, accidental and process spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils are responsible for the migration of contaminants into non-contaminated sites as dust or leachates and contribute towards contamination of our ecosystem (Ghosh and Singh, 2005).

A wide range of inorganic and organic compounds will cause contamination which includes heavy metals, combustible substances, hazardous wastes, explosives and petroleum products. Major component of inorganic contaminates are heavy metals (Adriano, 1986 & Alloway, 1990). Inorganic contaminants present a different problem than organic contaminants. Soil microorganisms can degrade organic contaminants, while inorganic contaminants (heavy metals) need immobilisation or physical removal. Although many metals are essential, all metals are toxic at higher concentrations because they can cause oxidative stress by formation of free radicals. Another reason why metals may be toxic is that they can replace essential metals in pigments or enzymes disrupting their function (Henry, 2000). Metal contaminated soil can be remediated by chemical, physical and biological techniques (Baker and Walker, 1990).

The concept of using plants to clean up contaminated environments (soils, waste water, etc.) is not something new because plants were proposed for use in the treatment of waste water for about 300 years ago (Hartman, 1975). At the end of 19th Century, Alpine Pennycress; *Thlaspi caerulescens* and Yellow Calamine Violet; *Viola calaminaria* were the first wild flower plant species documented to accumulate high levels of metals in leaves (Baumann, 1885). Utsunamyia (1980) and Chaney (1983) were reintroduced and developed the idea of using plants to uptake metals from contaminated soil and the first field trial on Zn and Cd phytoextraction was conducted in 1991 (Baker et al.).

1.2 Problem Statement

Basic need of life for every living thing in the world is land and water which are very precious natural assets, which rely on the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum utilization and severely degraded or contaminated because of human activities. In sequence to sustain good quality of soils and waters, and also to keep them free from pollution, continuous efforts have been made to build up technologies that are easy to use, sustainable and the most important thing is very economical. Physicochemical advances have been extensively used to remediate and recover contaminated soils and waters especially on small scale (Mukhtar et al., 2010). However, these practises are become more complicated for a large scale of remediation due to high costs and side effects.

The use of different plant species for cleaning contaminated soils and waters which namely as phytoremediation has gained increasing awareness since last decade, as an emerging cheaper technology. In public attitude, phytoremediation technology is more favourable due to its potential for cleaning up environment and the overall aesthetic perfection of the contaminated sites (Chen & Cutright, 2002; Fayiaga et al., 2004). For this study, four different types of green plants were selected to evaluate their potential to uptake Pb, Cd, Cr and Zn from contaminated soil.

1.3 Objectives

The objectives of this study are:

1. To evaluate the feasibility or potential of 4 types of green plants which are cosmos plant; *Cosmos caudatus (ulam raja)*, asiatic pennywort; *Centella asiatica (pegaga), Emilia sonchifolia (bayam praksi)* and selom leaves; *Oenanthe javanica (daun selom)* for phytoremediation purpose.
2. To determine the concentration of lead (Pb), cadmium (Cd), chromium (Cr) and zinc (Zn) uptake by these plants.
3. To study the effect of contact time to uptake heavy metal.

1.4 Research Scope

This research study covers the determination of heavy metals concentration in four types of green plants. The study was focused on 4 types of heavy metals (Pb, Cd, Cr and Zn) since they are the most commonly occurring metals at contaminated sites and pose a significant impacts to human health. Samples have been collected randomly from 3 different places to check its average and accuracy in heavy metal uptake. The collected samples were washed and dried before digested with solvent dosage. The sample solution then will undergo dilution before being analyze using Atomic Absorption Spectroscopy (AAS) to determine the concentrations of heavy metals. By using the data obtained from AAS, their ability to uptake the heavy metals from contaminated soil will be analyzed. This research study also will covers on the effect of contact time to uptake heavy metals.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The increasing use of wide variety of heavy metals in industries and agriculture has caused a serious concern of environmental pollution such as soil pollution (Sinhal et al., 2010). Soil polluted by heavy metals has increased due to human activities and the removal of such pollutants has also been a great concern (Uwumarongie et al., 2008; Fang et al., 2006; Agunbiade and Fawale, 2009). Metals contamination is a continual problem at many contaminated sites. According to Wuana and Okiemen (2011), heavy metals constitute an ill-defined group of inorganic chemical hazards and the most commonly occurring metals at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu) and mercury (Hg). The presence of metals in soils can pose a significant threat to human health and ecological systems because most of them do not undergo microbial or chemical degradation (Marcus et al., 2006).

Now faced with these overly extensive environmental problems, a cost effective means of remediation relevant to the contaminated areas must be found. Several methods of conventional remediation technologies such as solidification, soil washing and permeable barriers have been used to clean up the environment from these kinds of metals but most of them are expensive and not achieve the optimum performance (Tangahu et al., 2011). More than that, it has cause further disturbance to the already damaged environment (Henry, 2000). Recent concerns regarding the environmental contamination have initiated the development of appropriate technologies to assess the presence and mobility of metals in soil (Shtangeeva and Ayrault, 2004), water and waste water. Phytoremediation is evolving as a cost effective which can be an alternative way to high energy and high cost of conventional methods. It is considered to be a ‘Green Revolution’ in the field of innovative clean up technologies (Henry, 2000).

2.2 Phytoremediation Technology

2.2.1 Overview of Phytoremediation

Phytoremediation is a developing technology that can potentially address the problems of polluted areas affected by urban or industrial activities. This technology is using the plants to clean up contaminated soil, sludges, sediments, and ground water through contaminant removal, degradation and containment (USEPA, 1997). The idea of using metal-accumulating plants to uptake heavy metals was first introduced in 1983, but the concept has actually been implemented for the past 300 years on waste water discharges (Chaney et al., 1997).

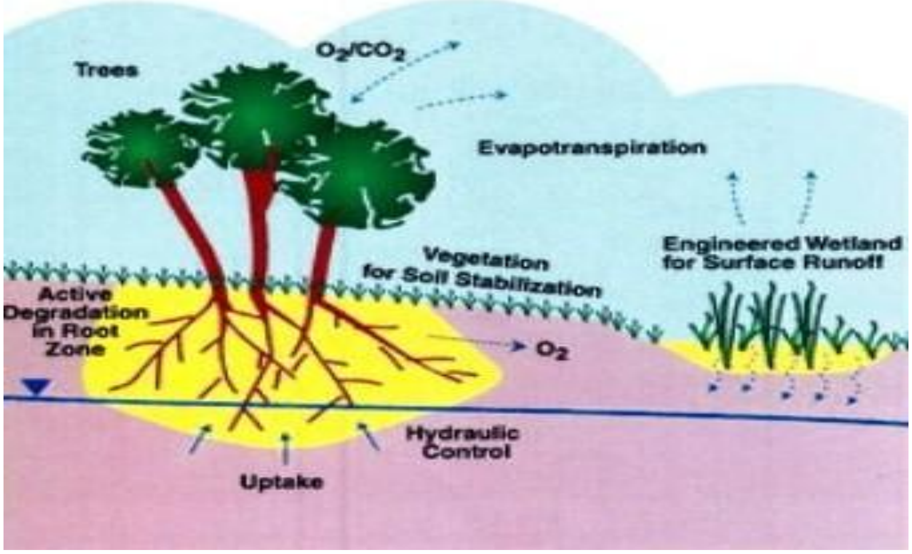


Figure 2.1: Plant processes leading to environmental remediation

Source: www.the-scientist.com

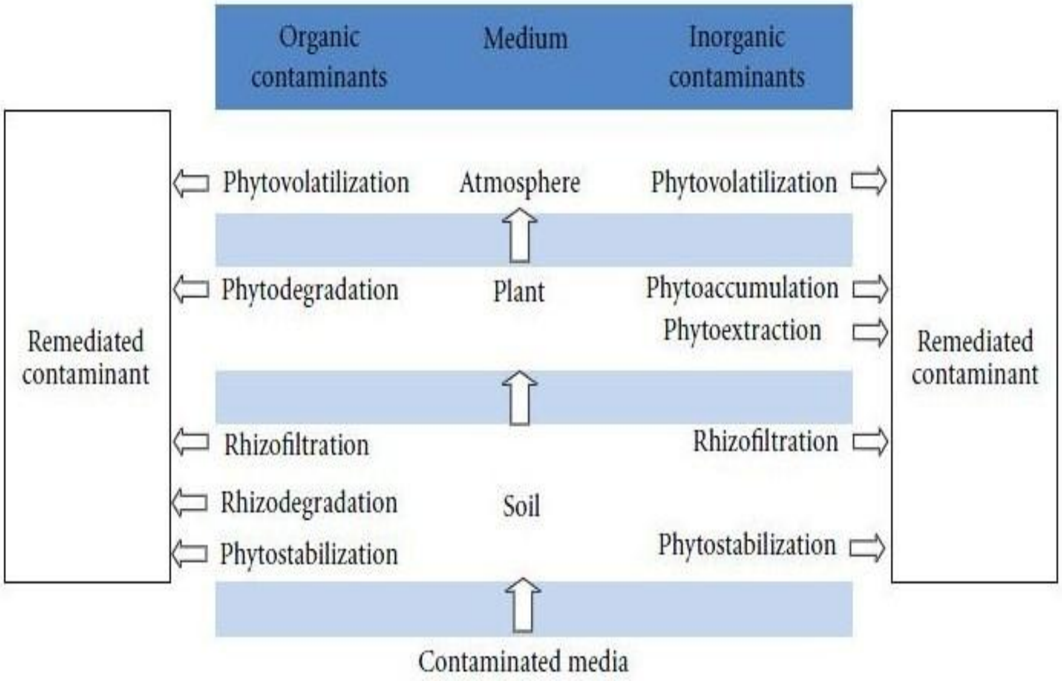


Figure 2.2: Uptake mechanisms on phytoremediation technology

Source: www.itrcweb.org

Five main subgroups of phytoremediation have been identified are:

i. **Phytoextraction** – plants remove metals from the soil and concentrate them in the harvestable parts of plants (Kumar et al., 1995)
ii. **Phytodegradation** – plants and associated microbes degrade organic pollutants (Burken and Schnoor, 1997)
iii. **Rhizofiltration** – plant roots absorb metals from waste streams (Dushenkov et al., 1995)
iv. **Phytostabilisation** – plants reduce the mobility and bioavailability of pollutants in the environment either by immobilisation or by prevention of migration (Vangronsveld et al., 1995)
v. **Phytovolatilisation** – volatilisation of pollutants into the atmosphere via plants (Banuelos et al., 1997)

Three main strategies that currently exist to phytoextract inorganic materials from soils using plants are:

i. Use of natural hyper accumulators
ii. Enhancement of element uptake of high biomass species by chemical additions to soil and plants
iii. Phytovolatilization of elements – often involves alteration of their chemical form within the plant prior to volatilization to the atmosphere

(McGrath et al., 2002)

While for the purposes of phytoremediation, Punshon et al. (1996) suggested that the following characteristics were beneficial:

- i. Ability to grow on nutrient-poor soil
- ii. Deep root system
- iii. Fast rate of growth
- iv. Metal resistance trait

2.2.2 Advantages of Phytoremediation

Phytoremediation has many advantageous features that make it an appropriate and successful technology, giving practitioners a valuable option for remediation. A significant advantage of phytoremediation is that it can be used either as an *in situ* or *ex situ* application (Raskin and Ensley, 2000; USEPA, 2000) but *in situ* applications are frequently used as considered applications. It is because this application can minimize the soil and surrounding environment disturbance. Other than that, *in situ* applications also can reduce the spread of contamination via air and water bone wastes. Another advantage of this technology is that it is a green technology which is environmentally friendly and aesthetically pleasing to the public if it is properly implemented (Raskin and Ensley, 2000).

Phytoremediation does not require expensive equipment or highly specialized personnel, and it is relatively easy to implement. It is capable of permanently treating a wide range of contaminants in a wide range of environments. However, the greatest advantage of phytoremediation is it offers a low cost method for soil remediation compared to conventional clean up technologies (USEPA, 2000; Raskin and Ensley, 2000) and some extracted metals may be recycled for value (Chaney et al., 1997).

2.2.3 Disadvantages and Limitations of Phytoremediation

In contrast to its many positive aspects, phytoremediation technology does have a few disadvantages and limitations. It is restricted to the rooting depth of remediative plants. Remediation with plants is a lengthy process, thus it may take several years or longer to clean up a hazardous waste site, and the contamination may still not be fully remediated (USEPA, 2000). The unfavourable climate is another important consideration because it can limit plant growth and phytomass production and then will decreasing the process efficiency (USEPA, 2000).

Table 2.1: Summary of advantages and disadvantages of the phytoremediation technology

Advantages	**Disadvantages/Limitations**
Amendable to a variety of organic and inorganic compounds	Restricted to sites with shallow contamination within rooting zone of remediative plants
In Situ/Ex Situ application	May take up to several years to remediate a contaminated sites
In Situ applications decrease the amount of soil disturbance compared to conventional methods	Restricted to sites with low contaminant concentrations
Reduces the amount of waste to be landfilled (up to 95%)	Lack of remediating plant species
In Situ applications decrease spread of contaminant via air and water	Climatic conditions
Does not require expensive equipment or highly specialized personnel	Introduction to non-native species may affect biodiversity
Low cost compared to conventional treatment methods	Consumption of contaminated plant tissue is also of concern
Easy to implement and maintain	
Environmentally friendly and aesthetically pleasing to the public	

2.3 Sources of Heavy Metals in Contaminated Soils

Heavy metals occur naturally in the soil environment that are regarded as trace (<1000 mg kg^{-1}) and rarely toxic (Kabata-Pendias and Pendias, 2001; Pierzynski et al., 2000). Due to the disturbance and acceleration of nature’s slowly occurring geochemical cycle metals by man, most soils of rural and urban environments may accumulate one or more of the heavy metals above defined background values high enough to cause risks to human health, plants, animals, ecosystems or other media (D’amore et al., 2005). The heavy metals essentially become contaminants in the soil environment because:

i. Their rates of generation via man-made cycles are more rapid relative to natural ones
ii. They become transferred from mines to random environmental locations where higher potentials of direct exposure occur

Metal-bearing solids at contaminated sites can originate from a wide variety of anthropogenic sources in the form of metal mine tailings, disposal of high metal wastes in improperly protected landfills, land application of fertilizer, pesticides, coal combustion residues, petrochemicals and etc (Khan et al., 2008; Zhang et al., 2010; Basta et al., 2005).

2.3.1 Fertilizers

Historically, agriculture was the first major human influence on the soil (Scragg, 2006). To grow and complete the lifecycle, plants must acquire not only macronutrients (N, P, K, S, Ca and Mg), but also essential micronutrients. Some soils are deficient in the heavy metals such as Zn that are essential for healthy plant growth (Lasat, 2000). Large quantities of fertilizers are regularly added to soils in intensive farming systems to provide adequate N, P, and K for crop growth. The compounds used to supply these elements contain trace amounts of heavy metals (e.g. Cd and Pb) as impurities, which after continued fertilizer application may significantly increase their content in the soil (Jones and Jarvis, 1981).

2.3.2 Biosolids

The application of numerous biosolids such as livestock manures and municipal sewage sludge to land inadvertently leads to the accumulation of heavy metals such as As, Cd, Cr, Pb, Zn, etc in the soil (Basta et al., 2005). Heavy metals most commonly found in biosolids are Pb, Ni, Cd, Cr, Cu and Zn and the metal concentrations are governed by the nature and the intensity of the industrial activity, as well as the type of process employed during the biosolids treatment (Mattigod and Page, 1983). Under certain conditions, metals added to soils in applications of biosolids can be leached downwards through the soil profile and can have the potential to contaminate groundwater (McLaren et al., 2005).

2.3.3 Metal Mining and Milling Processes and Industrial Wastes

During mining process, the heavier and larger particles settled at the bottom of the flotation cell during mining which called as tailings are directly discharged into natural depressions, including onsite wetlands resulting to elevated concentrations (DeVolder et al., 2003). Extensive Pb and Zn ore mining and smelting have resulted in contamination of soil that poses risk to human and ecological health.

2.3.4 Air-borne Sources

Air-borne sources of metals are including stack or dust emissions of air, gas or vapour streams and fugitive emissions such as dust from storage areas or waste piles. Some metals such as As, Cd and Pb can be volatize during the high temperature process because of its boiling point. These metals will convert to oxides and condense as fine particles unless a reducing atmosphere is maintained (Smith et al., 1995).

2.4 Background Study of Heavy Metals

2.4.1 Introduction

In general terms, 'heavy metal' has a higher specific weight that is 8 g/cm^3. Generally, the term heavy metal has been often taken to mean all metals and metalloids other than the alkali and alkaline earth elements (Bryan, 1976). This term is frequently used in recent years in association with environmental pollution by such elements where there are connotations of adverse impact to the receiving media (Wagenet et al., 1978). Heavy metals can be dangerous because they cannot be degraded or destroyed but they are bio-accumulate. For example, Cd and Ni inhibit stomata activity and decrease photosynthesis (Prasad, 1995). On the other hand, Delgado et al. (1993) reported that Cd, Cr and Zn can cause neurosis and chlorosis while Cd, Cu Ni and Pb interfere with hormonal balance (Rauser and Dumbroff, 1981).

2.4.2 Zinc

Zinc is a lustrous bluish-white metal which is the first element in group 12 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.

Zinc (Zn) does not occur naturally in elemental form. It is usually extracted from mineral ores to form zinc oxide (ZnO). The primary industrial use for zinc is as a corrosion resistant coating for iron or steel (Smith et al., 1995). Zinc usually occurs in the +II oxidation state and form complexes with a number of anions, amino acids and organic acids. Zinc may precipitate as $Zn(OH)_2$ (s), $ZnCO_3$ (s) or $Zn(CN)_2$ (s).

Figure 2.3: Zinc

Table 2.2: Properties table of zinc

Phase	Solid
Atomic Weight	65.38
Density (near r.t.)	7.14 g cm^{-3}
Liquid density at m.p	6.57 g cm^{-3}
Melting point	692.68 K, 419.53 °C
Boiling point	1180 K, 907 °C
Heat of fusion	7.32 kJ mol^{-1}
Heat of vaporization	123.6 kJ mol^{-1}
Molar heat capacity	25.470 J mol^{-1} K^{-1}

Health effects that can be caused by zinc are (Retrieved from http://www.lenntech.com/periodic/elements/zn.htm):

i. Stomach cramps, skin irritations, nausea and anaemia
ii. Liver or kidney damage
iii. Vomiting and diarrhea are possible symptoms
iv. Damage the pancreas
v. Disturb the protein metabolism and cause arteriosclerosis
vi. Respiratory disorders

The world's zinc production is still rising. This basically means that more and more zinc ends up in the environment. Environment effects that can be caused by zinc are:

i. Water polluted with zinc – it may increase the acidity of waters
ii. Soil polluted – animals will absorb concentrations that are damaging to their health

2.4.3 Cadmium

Cadmium is a lustrous, silver-white, ductile and 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 a highly toxic element and has been described as one of the most dangerous trace elements in the food and environment of human. This highly toxic non-essential metal can accumulate in the kidney of mammals which leads to the kidney dysfunction (Fergusson, 1990). Cadmium also is a ever present non-essential element that possesses high toxicity and is easily accumulated from the environment by organisms (Rahimi and Nejatkhah, 2010).

Cadmium (Cd) occurs naturally in the form of CdS or $CdCO_3$. Cadmium is recovered as a by product from the mining of sulphide ores of lead, zinc and copper. Sources of cadmium contamination include plating operations and the disposal of cadmium containing wastes (Smith et al., 1995). The form of cadmium encountered depends on solution and soil chemistry as well as treatment of the waste prior to disposal. The most common forms of cadmium include Cd^{2+}, cadmium-cyanide complexes or $Cd(OH)_2$ solid sludge (Smith et al., 1995).

Figure 2.4: Cadmium

Table 2.3: Properties table of cadmium

Phase	Solid
Atomic Weight	112.411
Density (near r.t.)	8.65 g cm^{-3}
Liquid density at m.p	7.996 g cm^{-3}
Melting point	594.22 K, 321.07 °C
Boiling point	1040 K, 767 °C
Heat of fusion	6.21 kJ mol^{-1}
Heat of vaporization	99.87 kJ mol^{-1}
Molar heat capacity	26.020 J mol^{-1} K^{-1}

Health effects that can be caused by cadmium are (Retrieved from http://www.lenntech.com/periodic/elements/cd.htm):

i. Severely damage the lungs which may even cause death
ii. Damages filtering mechanisms and further kidney damage
iii. Vomiting, diarrhea and stomach pains
iv. Bone fracture
v. Reproductive failure and possibly infertility
vi. Damage to the central nervous system and immune system
vii. Psychological disorders
viii. Possibly DNA damage or cancer development

Cadmium waste streams from the industries mainly end up in soils. The causes of these waste streams are for instance zinc production, phosphate ore implication and bio industrial manure. Environment effects that can be caused by cadmium are:

i. Water polluted – it may increase the acidity of waters
ii. Soil polluted – the uptake through food will increase

2.4.4 Chromium

Chromium is a lustrous, brittle, hard metal. Its colour is silver-gray and it can be highly polished. It does not tarnish in air, when heated it born and forms the green chromic oxide. Chromium is unstable in oxygen, it immediately produces a thin oxide layer that is impermeable to oxygen and protects the metal below it.

Chromium (Cr) is one of the less common elements and does not occur naturally in elemental form, but only in compounds. Chromium is mined as a primary ore product in the form of the mineral chromite, $FeCr_2O_4$. Major sources of Cr contamination include releases from electroplating processes and the disposal of chromium containing wastes (Smith et al., 1995). Cr(VI) is the form of chromium commonly found at contaminated sites. Chromium mobility depend on sorption characteristics of the soil, including clay content, iron oxide content and the amount of organic matter present.

Figure 2.5: Chromium

Table 2.4: Properties table of chromium

Phase	Solid
Atomic Weight	51.9961
Density (near r.t.)	7.19 g cm^{-3}
Liquid density at m.p	6.3 g cm^{-3}
Melting point	2180 K, 1907 °C
Boiling point	2944 K, 2671 °C
Heat of fusion	21.0 kJ mol^{-1}
Heat of vaporization	339.5 kJ mol^{-1}
Molar heat capacity	23.35 J mol^{-1} K^{-1}

Health effects that can be caused by chromium are (Retrieved from http://www.lenntech.com/periodic/elements/cr.htm):

i. Skin rashes
ii. Upset stomachs and ulcers
iii. Respiratory problems
iv. Weakened immune systems
v. Kidney and liver damage
vi. Alteration of genetic material
vii. Lung cancer
viii. Death

Environment effects that can be caused by chromium are:

i. Water polluted – it will absorb on sediment and become immobile
ii. Soil polluted – the uptake through food will increase

2.4.5 Lead

Lead is 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.

The primary industrial sources of lead (Pb) contamination include metal smelting and processing, secondary metals production, lead battery manufacturing, pigment and chemical manufacturing, and lead contaminated wastes. Widespread contamination due to the former use of lead in gasoline is also of concern. Lead released to groundwater, surface water and land is usually in the form of elemental lead, lead oxides and hydroxides, and lead metal oxynion complexes (Smith et al., 1995).

Figure 2.6: Lead

Table 2.5: Properties table of lead

Phase	Solid
Atomic Weight	207.2
Density (near r.t.)	11.34 g cm^{-3}
Liquid density at m.p	10.66 g cm^{-3}
Melting point	600.61 K, 327.46 °C
Boiling point	2022 K, 1749 °C
Heat of fusion	4.77 kJ mol^{-1}
Heat of vaporization	179.5 kJ mol^{-1}
Molar heat capacity	26.65 J mol^{-1} K^{-1}

Health effects that can be caused by lead are (Retrieved from http://www.lenntech.com/periodic/elements/pb.htm):

i. Disruption of the biosynthesis of haemoglobin and anaemia
ii. Rise in blood pressure
iii. Kidney damage
iv. Miscarriages and subtle abortions
v. Disruption of nervous systems
vi. Brain damage
vii. Behavioural disruptions of children, such as aggression, impulsive behaviour and hyperactivity

Environment effects that can be caused by lead are:

i. Water polluted – body functions of phytoplankton can be disturbed when lead interferes
ii. Soil polluted – soil functions are disturbed by lead intervention, especially near highways and farmlands

2.5 Background Study of Potential Green Plants

2.5.1 Cosmos Plant (*Cosmos caudatus; Ulam raja*)

Cosmos caudatus is an annual, perennial, aromatic herb. It originated from tropical Central America and is now widespread in almost all tropical regions (Shui et al., 2005). It is commonly planted as a decoration plant because its flower is beautiful and colourful. Several bioactive components in cosmos plant have been reported. For instance, Ragasa et al. (1997) have reported several anti-mutagen and anti-fungal compounds from cosmos plant while Zanariah et al. (1986) have reported protein and amino acid compositions of this plant. *C. caudatus* also is used in traditional medication because it can repair the blood flow and purify the blood from toxic substance.

Figure 2.7: Cosmos Plant

2.5.2 Asiatic Pennywort (*Centella asiatica; Pegaga*)

Centella asiatica (L.) is an important traditional medicinal plant (Devkota and Jha, 2008). The plant is native to India, China, Nepal, Indonesia, Sri Lanka, Australia, Madagascar and Southern and Central Africa (Press et al., 2000). The plant can grow in a variety of soils with moist, sandy or clayey loam, rich in humus. *C. asiatica* has also been reported to be useful in the treatment of inflammations, diarrhea, asthma, tuberculosis and various skin lesions and ailments like leprosy, lupus, psoriasis and keloid. In addition, numerous clinical reports verify the ulcer-preventive and anti depressive sedative effects of *C. asiatica* preparations, as well as their ability to improve venous insufficiency and microangiopathy (Zheng and Qin, 2007).

Figure 2.8: Asiatic Pennywort

2.5.3 Selom Leaves (*Oenanthe javanica; Daun selom*)

Oenanthe javanica commonly called “water dropwort” is herbaceous perennial with creeping stolons and pinnate leaves. It occurs naturally in marshes, along streams, and in swampy places in many Asian countries, including China, Japan, Korea, Vietnam, India, Malaysia and Thailand. *O. javanica* plays an important role in vegetative cover due to its rapidly-spreading suckers, improves water quality by its extensive root masses (Hargis 1998), and reduces heavy metal pollution (Deng et al. 2006). In addition, it can be eaten and also can be used medically (Park and Choi 1997; Han et al. 2008).

Figure 2.9: Selom Leaves

2.5.4 (*Emilia sonchifolia; Bayam praksi*)

Emilia sonchifolia is an annual herb of the Amaranthaceae family. The Africans occasionally consume *E.sonchifolia* as vegetable for its laxative properties (Iwu, 2000). It is believed to provide benefits to children, nursing mothers and patients suffering from high fever, anemia, bleeding and those with kidney problems. It also has been advocated in the treatment of various inflammatory conditions including earaches, conjunctivitis and etc. The methanolic extract of *E.sonchifolia* leaves was found to inhibit carrageenan-induced (Shylesh and Padikkala, 1999).

Figure 2.10: *E.sonchifolia*

2.6 Atomic Absorption Spectroscopy (AAS)

2.6.1 Introduction

Atomic absorption spectroscopy is one of the commonest instrumental methods for analyzing metals and some metalloids. It is widely practiced in environmental analysis. Although the atomic absorption spectroscopy is quite expensive, the technique is very wide spread. It is because AAS is possible to determine about 70 elements (mainly metals) at very low concentrations (Skoog et al., 1992). The sample is atomised at a very high temperature (2500°C - 3000°C) and the free atoms have line spectrum which means that they can only absorb the energy of light at discrete energy levels according to the excitations of electrons (Kenkel, 1994).

The general aspects of the technique are:

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

2.6.2 Hallow Cathode Lamp (HCL)

The hallow cathode lamp uses a cathode made of the element of interest with a low internal pressure of an inert gas. A low electrical current (~10 mA) is imposed in such a way that the metal is excited and emits a few spectral lines characteristic of the element. The light is emitted directionally through the lamp's window, a window made of transparent in the UV and visible wavelengths.

2.6.3 Nebulizer

The nebulizer chamber thoroughly mixes acetylene (fuel) and oxidant (air or nitrous oxide) to create a negative pressure at the end of the small diameter, plastic nebulizer tube. This negative pressure acts to suck or uptake the liquid sample up to the tube and into the nebulizer chamber, a process called aspiration to analyze the sample.

2.6.4 Acidic Content and Oxidation State of Samples and Standards

The samples and standards are often prepared with duplicate acid concentrations to replicate the analytes chemical matrix as close as possible. Acid contents of 1% to 10% are common. In addition, high acid concentrations help keep all dissolved ions in solution. The oxidation state of the analyte metal or metalloid is important in AAS.

For instance, AAS analysis of selenium requires the Se(IV) oxidation state (selenite). Therefore, all selenium in Se calibration standards and Se containing samples must be in the Se(IV) form for analysis. This can be accomplished by oxidizing all Se in the sample to selenate using a strong oxidizer such as nitric acid or hydrogen peroxide and then reducing the contained selenate to selenite with boiling HCl.

CHAPTER 3

METHODOLOGY

3.1 Introduction

The experiment was conducted start from the collecting of samples from three different sources in Kota Bharu, Kelantan area and preparation of dried potential green plants (*Cosmos caudatus, Centella asiatica, Emilia sonchifolia* and *Oenanthe javanica*) where the fresh plants were washed, dried, cut, ground and stored in the polyethylene bag. Then, 1000 mg/L of metals (Pb, Cd, Cr and Zn) solution as standard solution were prepared. The dried samples then will be digested with solvent dosage before being analyze using atomic absorption spectroscopy (AAS) to determine the concentrations of heavy metals accumulation in each type of plants. Figure 3.1, 3.2 and 3.3 below show the overall process in this experiment.

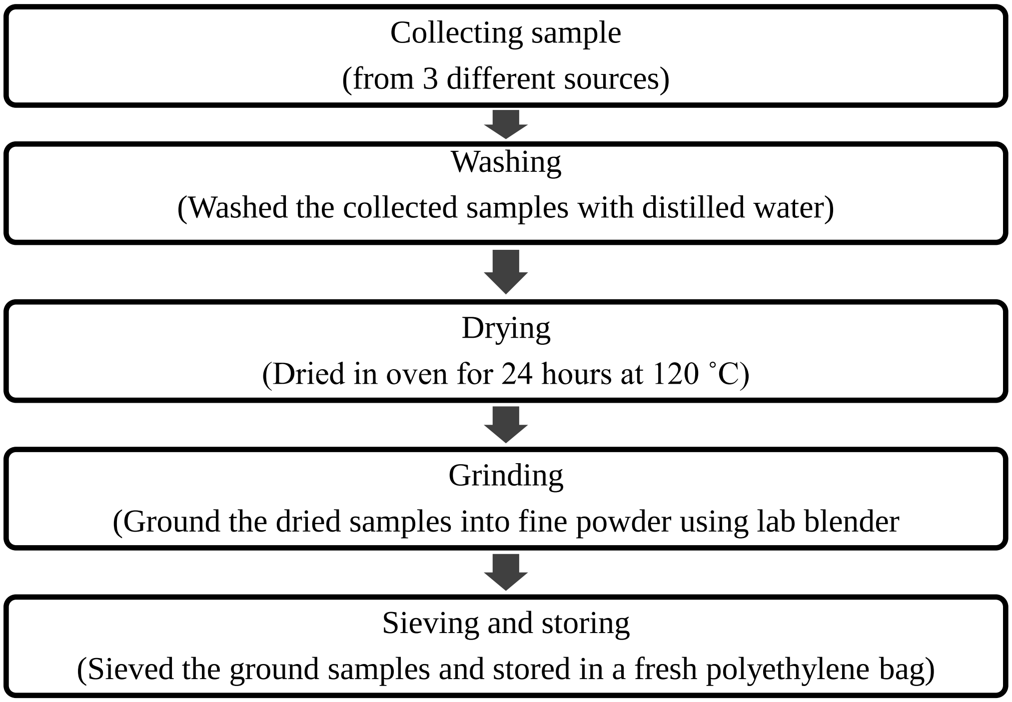


Figure 3.1: Sample preparation

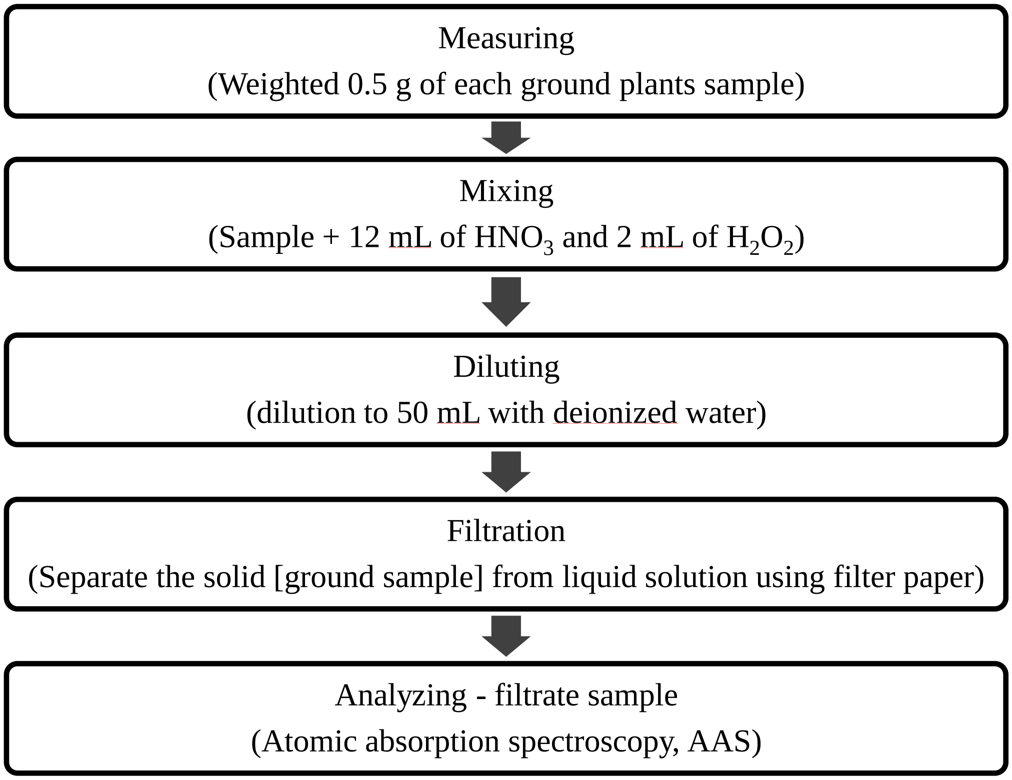


Figure 3.2: Plant analysis

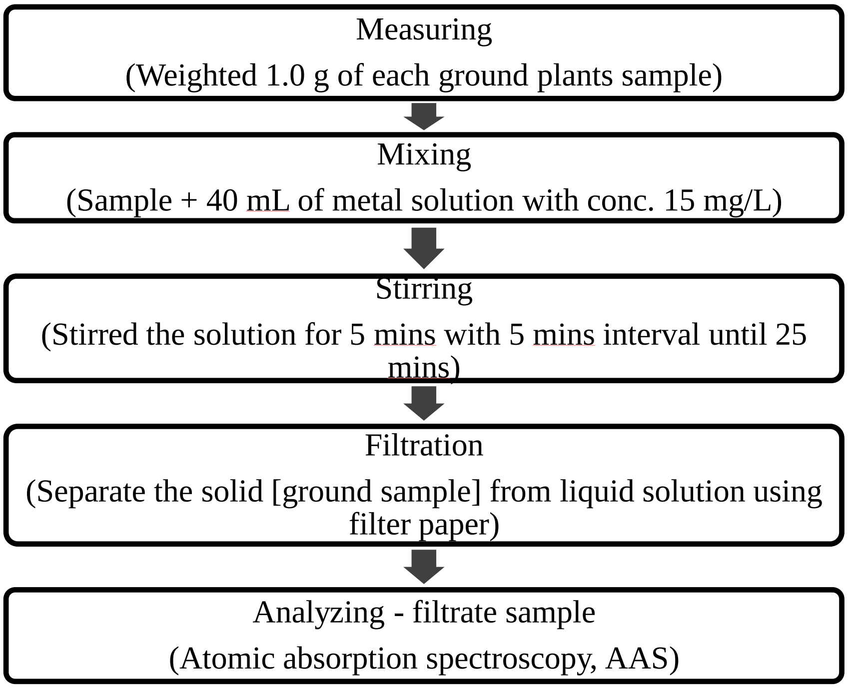


Figure 3.3: Effect of contact time

3.2 Equipments Requirement

Equipments used in this experiment:

i. Z-5000 Polarized Zeeman atomic absorption spectroscopy (AAS)
ii. Stirrer
iii. Magnetic Stirrer
iv. Laboratory blender (WARING COMMERCIAL)
v. Oven (Memmert)
vi. Octagon siever (OCT-DIGITAL 4527-01)
vii. Electric balance Shimadzu (AW 220)
viii. 100 mL volumetric flask
ix. 100 mL conical flask
x. Filter paper

3.3 Chemicals Requirement

Chemicals used in this experiment:

1. Nitric Acid, HNO_3
2. Hydrogen Peroxide, H_2O_2
3. Lead Standard Solution, Pb
4. Cadmium Standard Solution, Cd
5. Chromium Standard Solution, Cr
6. Zinc Standard Solution, Zn
7. Deionised Water
8. Ultra Pure Water

3.4 Experimental Procedure

3.4.1 Sample Preparation

The green plants used in the experimentation were obtained randomly from three different wet markets in Kota Bharu, Kelantan area. The roots, the aerial parts (stems and leaves) were thoroughly washed and rinsed with distilled water as recommended by Tuzen (2003), dried at 120 °C in hot air oven for 24 hours until the weight is constant. Then, the dried plants were ground in a bladed mixer or mechanical grinder to pass through 1mm sieve to remove rocks, roots and other large and small particles (Bigdeli and Seilsepour, 2008). The ground samples were mixed uniformly and stored in a fresh plastic polyethylene bag.

3.4.2 Metals Solution Preparation

A stock solution of metal (Pb, Cd, Cr and Zn) was prepared by diluting analytical reagent (AR) grade quality 1000 mg/L of metal standard solution in ultra pure water. For the experiment, the initial concentration used was at 15 mg/L.

While the standard solutions for each type of heavy metals element used for calibration of atomic absorption spectroscopy (AAS) were prepared by diluting AR grade stock solution of 1000 mg/L (Tuzen, 2003). The standard calibrations were prepared at the range 0 mg/L until 20 mg/L for each element of heavy metals.

The concentration for dilution process was measured as below:

$$M_i V_i = M_f V_f$$

Where;

M = molarity
V = volume
i = initial value
f = final value

Table 3.1: Characteristics of standard solution for calibration

Element	Characteristics
Lead, Pb	0.0 mg/L, 1.0 mg/L, 2.0 mg/L, 4.0 mg/L, 8.0 mg/L, 14.0 mg/L, 16.0 mg/L
Cadmium, Cd	0.0 mg/L, 1.0 mg/L, 2.0 mg/L, 4.0 mg/L, 8.0 mg/L
Chromium, Cr	0.0 mg/L, 1.0 mg/L, 2.0 mg/L, 4.0 mg/L, 8.0 mg/L, 14.0 mg/L, 16.0 mg/L
Zinc, Zn	0.0 mg/L, 1.0 mg/L, 2.0 mg/L, 4.0 mg/L, 8.0 mg/L, 14.0 mg/L, 16.0 mg/L

3.4.3 Plant Analysis – Acid Digestion

The sample of plant parts were chemically analyzed for detection of heavy metals accumulation in each plant. Accurately 0.5 g of ground plant from sample was weighted into a floating boat and digested with mixture of 12 mL of concentrated HNO_3 and 2 mL of H_2O_2 (ratio 6: 1) for 20 minutes. The application of concentrated HNO_3 for mineralization of herbs will leads to the complete digestion of samples (Baranowska et al., 2002). Then, the mixture solution was diluted to 50 mL with deionised water. After dilutions, the sample was then filtered using Whatmann filter paper. The filter sample is then made up to 100 mL with metal-free distilled water and stored in a special container ready to be analyzed by atomic absorption spectroscopy. Another sample of green plants was carried out in the same way.

3.4.4 Effect of Contact Time

Contact time is one of the important parameter needed in order to remove metal (Pb, Cd, Cr and Zn) ions from soil. In this experiment, 5 samples of 15 mg/L metal solution were prepared with 1.0 g of green plant sample at 25 °C. Each sample was stirred using stirrer with varies time started from 5 minutes with 5 minutes interval time until 25 minutes.

3.5 Analytical Analysis

3.5.1 Analyzing the Potential Plant to Uptake Heavy Metal

Concentration of Pb, Cd, Cr and Zn were determined by using absorbance value at 228.8 nm with Z-5000 Polarized Zeeman atomic absorption spectroscopy. 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 and standards solution should contain a background acid (normally HNO_3) concentration near 0.1N. Note that acid varies depending on the analysis.

3.5.2 Analyzing the Metal Uptake

Concentration of Pb, Cd, Cr and Zn were determined by using absorbance value at 228.8 nm with Z-5000 Polarized Zeeman atomic absorption spectroscopy. The following equation was used to compute the percent of metal uptake by the sorbent (Hanif et al., 2007).

$$\%\ \text{uptake} = \frac{(C_i - C_f)}{C_i} \times 100\%$$

Where;

C_i = initial concentration of metal

C_f = final concentration of metal

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

The purposes of this research are to evaluate the potential of 4 types of green plants for phytoremediation purpose and also to study the effect of contact time to uptake heavy metals such as Pb, Cd, Cr and Zn.

4.2 Experiment 1: Determination of Heavy Metals Concentration in Green Plants

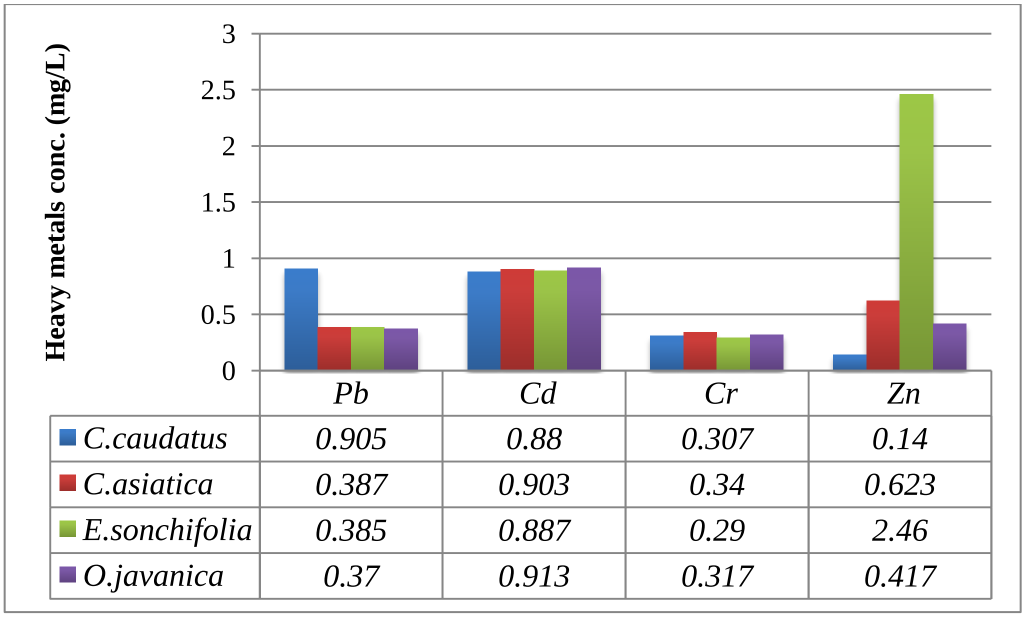
Table 4.1 below presented a summary of concentration data of heavy metals (Pb, Cd, Cr and Zn) contain in 4 types of green plants. It can be seen that all the studied green plant species possessed different metal concentrations and the highest levels of metals concentrations also were found in different types of plants for different elements.

Results showed that all the studied green plants species were able to accumulate heavy metals but in different concentrations. From the data obtained below, lead appeared to be the most metal being accumulated by *C.caudatus* which is 0.905 mg/L followed by *C.asiatica* (0.387 mg/L), *E.sonchifolia* (0.385 mg/L) and *O.javanica* (0.370 mg/L). For cadmium metal, it was found to be highly accumulated by *O.javanica* with concentration of 0.913 mg/L which closely followed by *C.asiatica* (0.903 mg/L), *E.sonchifolia* (0.887 mg/L) and *C.caudatus* (0.880 mg/L). On the other hand, chromium metal was highly accumulated by *C.asiatica* with concentration 0.340 mg/L followed by *O.javanica* (0.317 mg/L), *C.caudatus* (0.307 mg/L) and lastly by *E.sonchifolia* (0.290 mg/L) but the concentrations value was not significantly different. For the last element which is zinc, it was the most metal being accumulated by *E.sonchifolia* at 2.460 mg/L followed by *C.asiatica* (0.623 mg/L), *O.javanica* (0.417 mg/L) and *C.caudatus* (0.140 mg/L).

Table 4.1: Mean concentrations (mg/L) of heavy metals in green plants

Types of plants	**Lead (Pb)**	**Cadmium (Cd)**	**Chromium (Cr)**	**Zinc (Zn)**
Cosmos caudatus (ulam raja)	0.905	0.880	0.307	0.140
Centella asiatica (pegaga)	0.387	0.903	0.340	0.623
Emilia sonchifolia (bayam praksi)	0.385	0.887	0.290	2.460
Oenanthe javanica (daun selom)	0.370	0.913	0.317	0.417

Figure 4.1 below showed the mean concentrations of heavy metals being accumulated by the *Cosmos caudatus (ulam raja)*, *Centella asiatica (pegaga)*, *Emilia sonchifolia (bayam praksi)* and *Oenanthe javanica (daun selom)*.



	Pb	*Cd*	*Cr*	*Zn*
🟦 *C.caudatus*	*0.905*	*0.88*	*0.307*	*0.14*
🟥 *C.asiatica*	*0.387*	*0.903*	*0.34*	*0.623*
🟩 *E.sonchifolia*	*0.385*	*0.887*	*0.29*	*2.46*
🟪 *O.javanica*	*0.37*	*0.913*	*0.317*	*0.417*

Figure 4.1: Heavy metals concentration in studied plants

This study indicates that *E.sonchifolia* had accumulated Zn very effectively than others (Figure 4.1). Although amount of Zn was quite little in 3 other plants compared to *E.sonchifolia*, but it still considered as an effective accumulators. Zinc also plays essential metabolic roles in the plant, of which the most significant is its activity as a component of a variety of enzymes such as proteinases and peptidases. Shkolnik and Leringrad (1974) indicated that the basic Zn functions in plants are related to the metabolisms of carbohydrates, proteins and phosphate.

Analysis of chromium, Cr in this experimental plants showed that *C.caudatus, C.asiatica*, *E.sonchifolia* and *O.javanica* have quite same value of Cr concentrations. According to Figure 4.1, it was clearly seen that the concentration of Cd also significantly same for all plant species. As known, the exchangeable form Cd is easily absorbed by plant. According to Zhang et al. (2010), the exchangeable form Cd was partly removed by plant uptake that accompanied with the intake of nutrition.

This data shave howed that *C.asiatica* can absorbed and accumulate Pb, Cd, Cr and Zn efficiently and was chosen as a most effective plant regarding as heavy metal accumulator for this study because it is a common plant that can accumulate most of heavy metals effectively compared to others.

The high potential of metal accumulation by *C.asiatica* in the polluted soils indicated that it is a good source for bioaccumulation of heavy metals and therefore it is good for phytoremediation (Barthwal et al., 2008), besides being a good biomonitoring plant. On the other hand, since it is a medicinal herb plant, this preliminary finding should give a great concern about heavy metal contamination of herbal raw material using *C.asiatica*.

4.3 Experiment 2: Effect of Contact Time

This experiment was conducted with different contact time at range from 0 minute until 25 minutes with 5 minutes time interval. From table 4.2, the optimum sorption of lead uptake by *C.caudatus* was observed at 25 minutes with percent uptake of 87.5%.

Table 4.2: Lead, Pb uptake by cosmos plant (*Cosmos caudatus; Ulam raja)*

Sample	Contact time (min)	Initial conc. (mg/L)	Final conc. (mg/L)	Metal uptake (%)
1	5	15	10.36	30.9
2	10	15	8.56	42.9
3	15	15	4.05	73.0
4	20	15	3.55	76.3
5	25	15	1.87	87.5

Figure 4.2 shows the effect of contact time for removal of Pb. As illustrated below, sorption took place very rapidly from 10 to 15 minutes and then it continued at a relatively slower rate up to maximum sorption.

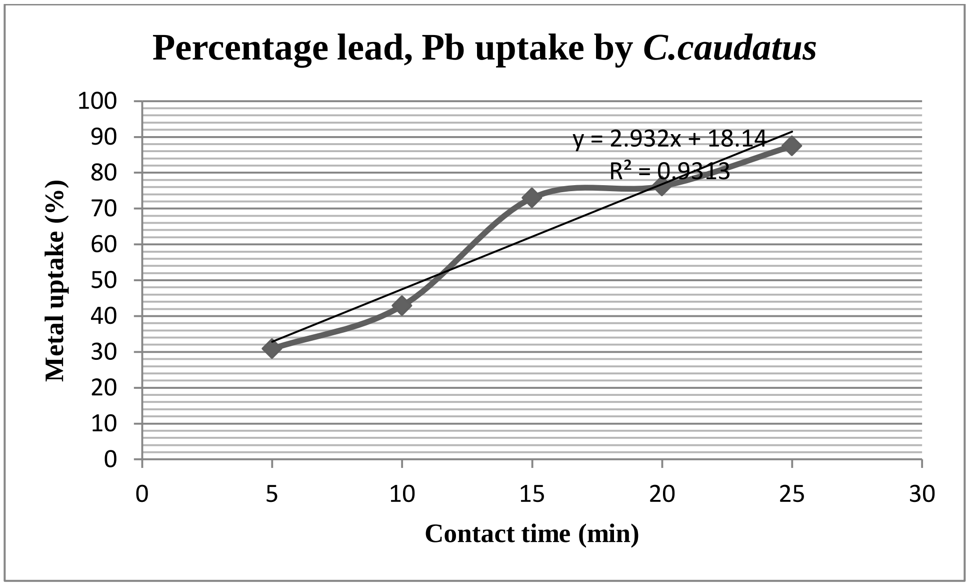


Figure 4.2: Effect of *C.caudatus* contact time on Pb removal

As seen from table 4.3, the cadmium metal uptake by *O.javanica* increased with the increasing of contact time. For Cd removal, at 25 minutes was found to be the appropriate time for maximum adsorption. It is because the entire adsorbents surfaces are having an optimum contact with the adsorbate (cadmium).

Table 4.3: Cadmium, Cd uptake by selom leaves (*Oenanthe javanica; Daun selom*)

Sample	**Contact time (min)**	**Initial conc. (mg/L)**	**Final conc. (mg/L)**	**Metal uptake (%)**
1	5	15	12.97	13.5
2	10	15	4.32	71.2
3	15	15	3.42	77.2
4	20	15	2.58	82.8
5	25	15	2.38	84.1

As illustrated in Figure 4.3, *O.javanica* biosorbent absorbed cadmium ions very rapidly from 5 to 10 minutes and then it continued with slower rate up to the optimum percentage metal uptake which is 84.1% at 25 minutes.

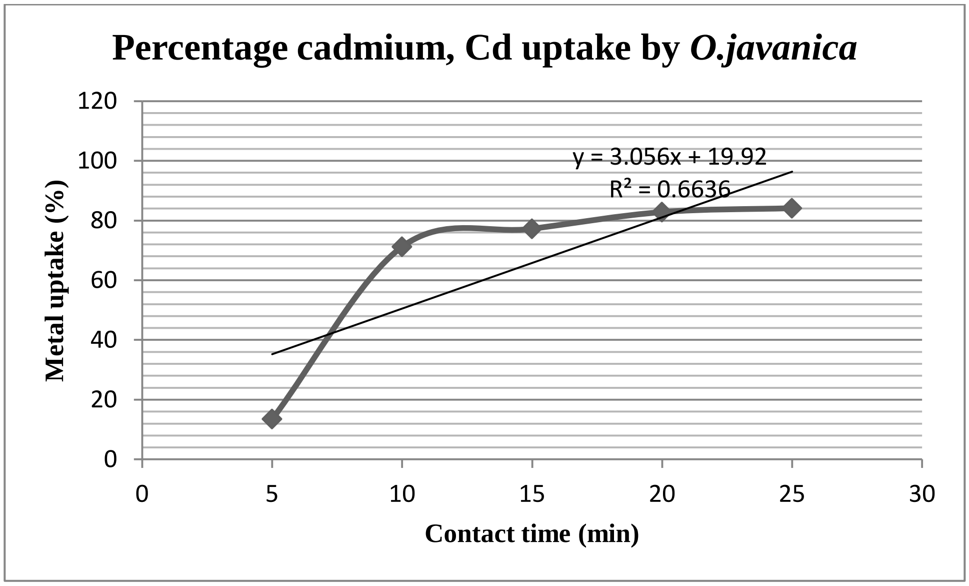


Figure 4.3: Effect of *O.javanica* contact time on Cd removal

Table 4.4 shown that the optimum sorption of chromium uptake by *C.asiatica* was observed at 25 minutes with the percent Cr uptake is 61.0%.

Table 4.4: Chromium, Cr uptake by asiatic pennywort (*Centella asiatica; Pegaga*)

Sample	Contact time (min)	Initial conc. (mg/L)	Final conc. (mg/L)	Metal uptake (%)
1	5	15	10.78	28.1
2	10	15	8.71	41.9
3	15	15	7.61	49.3
4	20	15	6.33	57.8
5	25	15	5.85	61.0

As illustrated in Figure 4.4, *C.asiatica* biosorbent absorbed chromium ions rapidly from 5 to 10 minutes and then it continued with slower rate up to the optimum percentage metal uptake which is 61.0% at 25 minutes.

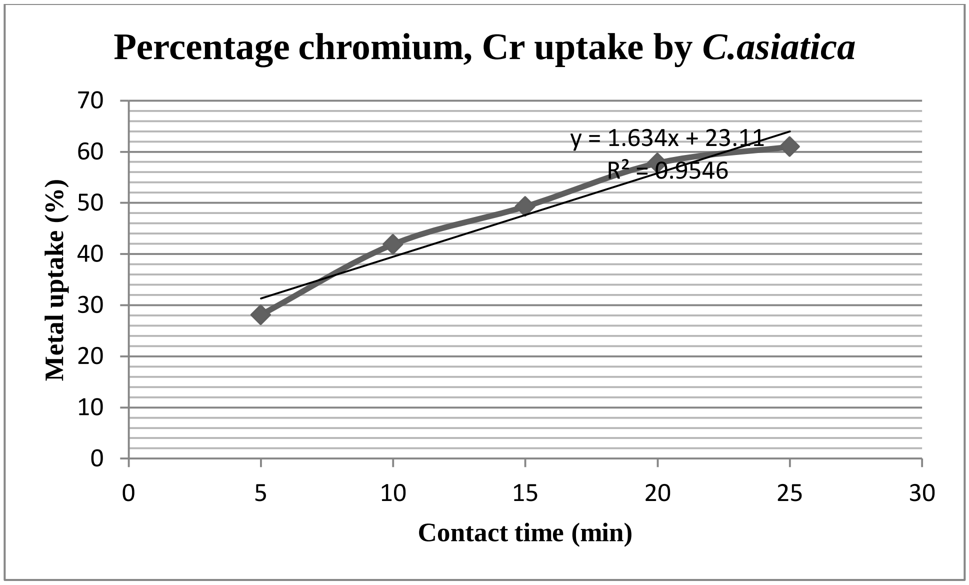


Figure 4.4: Effect of *C.asiatica* contact time on Cr removal

From table 4.5, it shown that the optimum sorption of zinc uptake by *E.sonchifolia* was observed at minutes 25 with percent uptake of 67.6%.

Table 4.5: Zinc, Zn uptake by *Emilia sonchifolia (bayam praksi)*

Sample	Contact time (min)	Initial conc. (mg/L)	Final conc. (mg/L)	Metal uptake (%)
1	5	15	14.49	3.4
2	10	15	8.66	42.3
3	15	15	8.06	46.3
4	20	15	5.96	60.3
5	25	15	4.86	67.6

Figure 4.5 shows the effect of contact time for removal of Zn. As illustrated below, sorption took place very rapidly from 5 to 10 minutes and then it continued at a relatively slower rate up to maximum sorption.

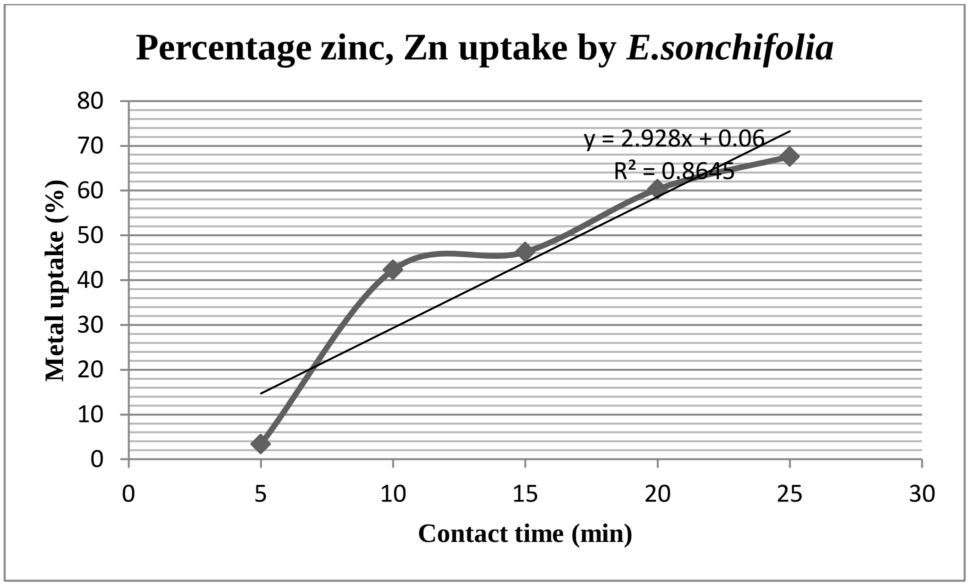


Figure 4.5: Effect of *E.sonchifolia* contact time on Zn removal

As seen from all figures, data showed that the percentage metal uptake by plant increased with the increasing of time as recommended by Kacaoba (2007). For Pb uptake by *C.caudatus,* the optimum sorption was observed at 25 minutes with the percentage uptake of 87.5%. As illustrated in Figure 4.5, sorption took place very rapidly from 10 to 15 minutes and then it continued at a relatively slower rate up to maximum sorption. It is because initially a large number of vacant sites may be available for adsorption and after some time, the remaining vacant surface sites may be difficult to occupy due to forces between the solute molecules of solid and bulk phase (Fogler, 2006).

On the other hand, optimum Cd uptake by *O.javanica* was found at 25 minutes too followed by Cr uptake by *C.asiatica* and Zn uptake by *E.sonchifolia* but with different percentage metal uptake. The percentage uptakes are 84.1%, 61.0% and 67.6% respectively. Fazeli (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.

From the result obtained, it showed *C.caudatus* is the most able species in the removing of heavy metals from polluted soil in certain time. It is because the percentage metal uptake for *C.caudatus* is the highest among another species in this study.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

As for the conclusion, it can be concluded that *C.caudatus*, *C.asiatica*, *E.sonchifolia* and *O.javanica* has the potential of being used as biomonitoring plant for heavy metal pollution in the polluted soils. They also are good sources for bioaccumulation of heavy metals and therefore it is good for phytoremediation purpose where *C.asiatica* is the most efficient. The obtained results also showed that percentage of metal uptake is directly proportional with the contact time which is in line with Kacaoba (2007). The contact time increased the uptake of heavy metals in plant that could enhance the phytoremediation of Pb, Cd, Cr and Zn from contaminated soil. This study using these 4 types of green plants as a biomonitor have provides important baseline information which can be used to monitor changes in heavy metal contamination and bioavailability.

5.2 Recommendation

Much research work is required in this respect of study such as metal uptake studies at cellular level including efflux and influx of different metal ions by different cell organelles and membranes. Further studies by using the plant parts and their growth for biomonitoring work are very useful and should be conducted regularly. Other than that, parameter such as temperature, pH, moisture and organic matter in soil also need to be conducted in order to improve the removal efficiencies.

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APPENDIX A

TABLES OF AAS RESULTS

Results for zinc (Zn)

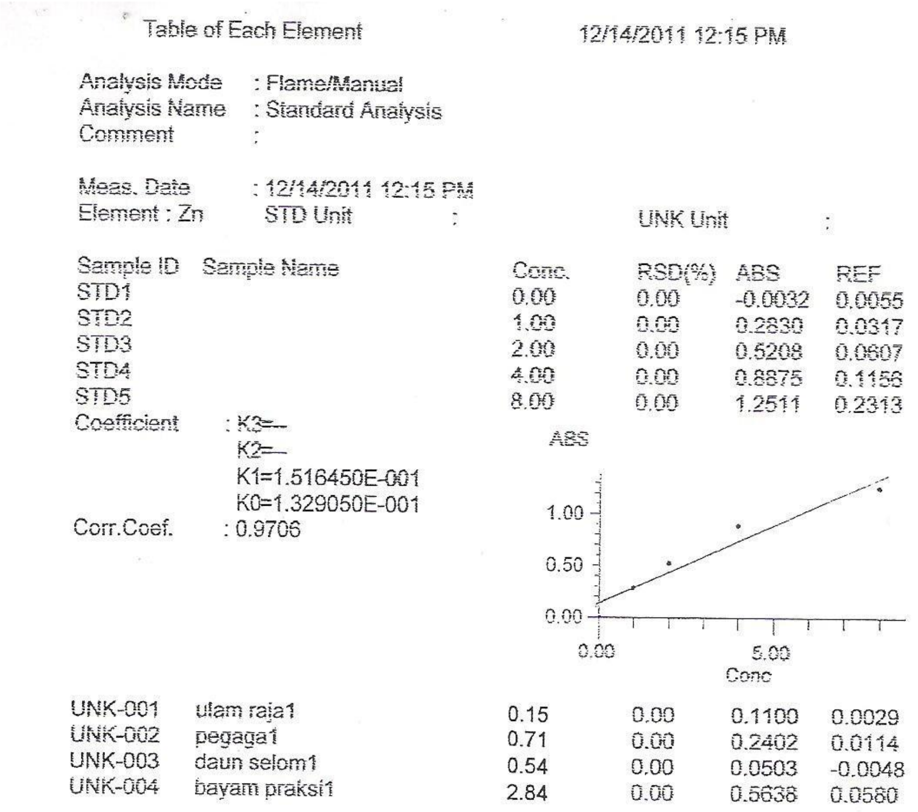

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Table of Each Element                        12/14/2011 12:15 PM

Analysis Mode    : Flame/Manual
Analysis Name    : Standard Analysis
Comment          :

Meas. Date       : 12/14/2011 12:15 PM
Element : Zn       STD Unit          :              UNK Unit        :

Sample ID   Sample Name                Conc.      RSD(%)   ABS       REF
STD1                                   0.00       0.00     -0.0032   0.0055
STD2                                   1.00       0.00     0.2830    0.0317
STD3                                   2.00       0.00     0.5208    0.0607
STD4                                   4.00       0.00     0.8875    0.1156
STD5                                   8.00       0.00     1.2511    0.2313
Coefficient     : K3=--
                  K2=--
                  K1=1.516450E-001
                  K0=1.329050E-001
Corr.Coef.      : 0.9706

UNK-001     ulam raja1                 0.15       0.00     0.1100    0.0029
UNK-002     pegaga1                    0.71       0.00     0.2402    0.0114
UNK-003     daun selom1                0.54       0.00     0.0503    -0.0048
UNK-004     bayam praksi1              2.84       0.00     0.5638    0.0580
```

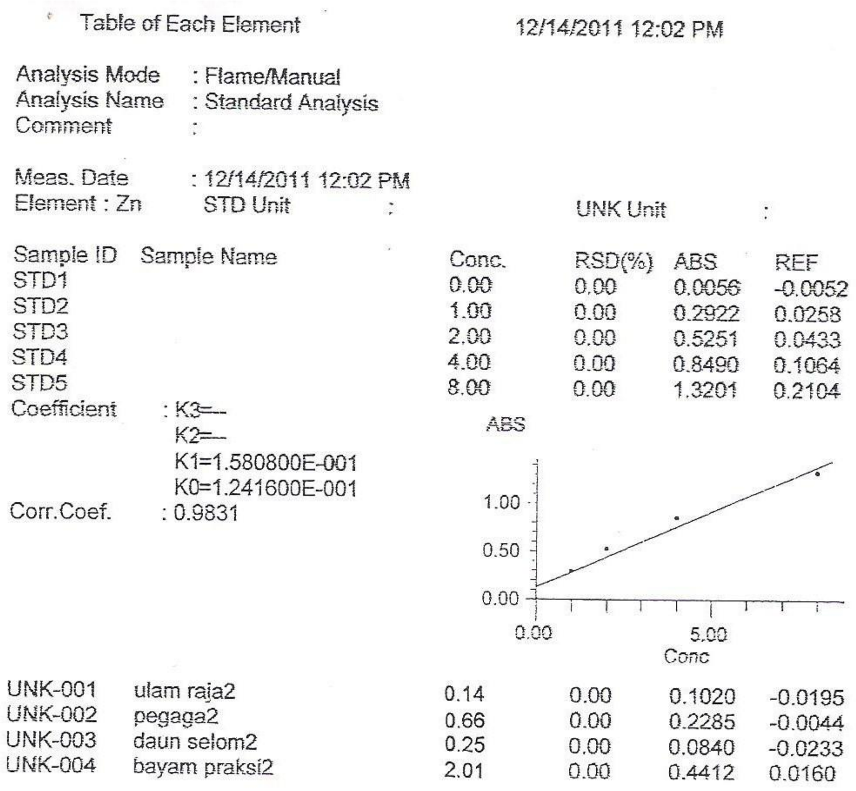

Table of Each Element 12/14/2011 12:02 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment :

Meas. Date : 12/14/2011 12:02 PM
Element : Zn STD Unit : UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0056	-0.0052
STD2		1.00	0.00	0.2922	0.0258
STD3		2.00	0.00	0.5251	0.0433
STD4		4.00	0.00	0.8490	0.1064
STD5		8.00	0.00	1.3201	0.2104

Coefficient : K3=--
K2=--
K1=1.580800E-001
K0=1.241600E-001
Corr.Coef. : 0.9831

UNK-001	ulam raja2	0.14	0.00	0.1020	-0.0195
UNK-002	pegaga2	0.66	0.00	0.2285	-0.0044
UNK-003	daun selom2	0.25	0.00	0.0840	-0.0233
UNK-004	bayam praksi2	2.01	0.00	0.4412	0.0160

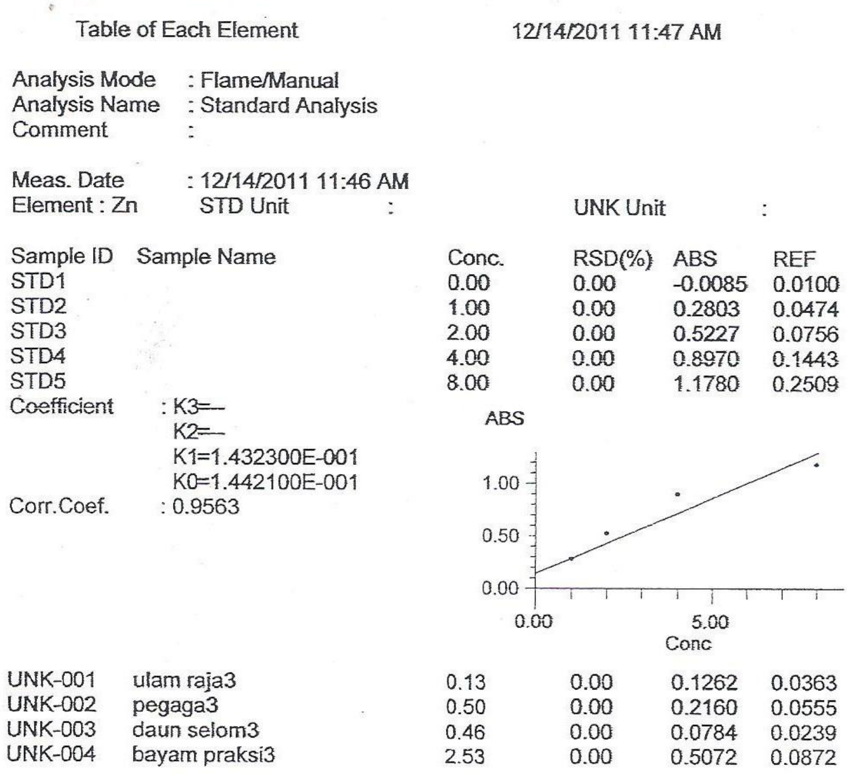

Table of Each Element 12/14/2011 11:47 AM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment :

Meas. Date : 12/14/2011 11:46 AM
Element : Zn STD Unit : UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0085	0.0100
STD2		1.00	0.00	0.2803	0.0474
STD3		2.00	0.00	0.5227	0.0756
STD4		4.00	0.00	0.8970	0.1443
STD5		8.00	0.00	1.1780	0.2509

Coefficient : K3=--
K2=--
K1=1.432300E-001
K0=1.442100E-001
Corr.Coef. : 0.9563

UNK-001	ulam raja3	0.13	0.00	0.1262	0.0363
UNK-002	pegaga3	0.50	0.00	0.2160	0.0555
UNK-003	daun selom3	0.46	0.00	0.0784	0.0239
UNK-004	bayam praksi3	2.53	0.00	0.5072	0.0872

Results for lead (Pb)

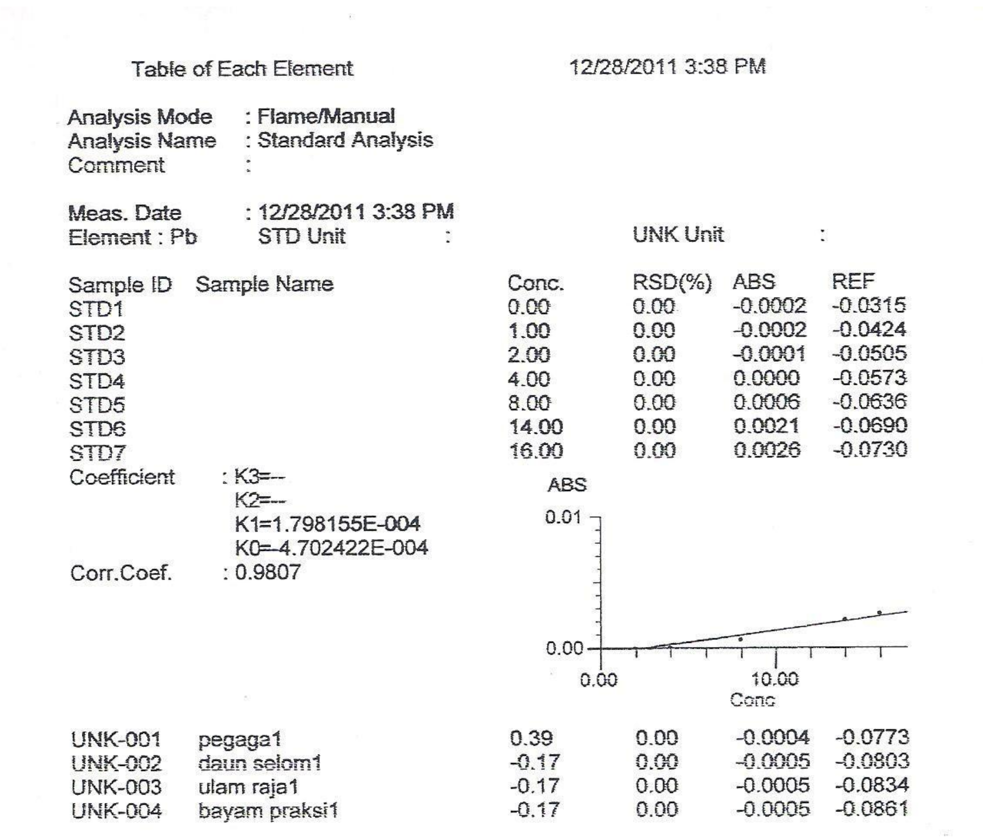

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       Table of Each Element                       12/28/2011 3:38 PM

Analysis Mode    : Flame/Manual
Analysis Name    : Standard Analysis
Comment          :

Meas. Date       : 12/28/2011 3:38 PM
Element : Pb       STD Unit          :                UNK Unit          :

Sample ID   Sample Name                     Conc.     RSD(%)  ABS       REF
STD1                                        0.00      0.00    -0.0002   -0.0315
STD2                                        1.00      0.00    -0.0002   -0.0424
STD3                                        2.00      0.00    -0.0001   -0.0505
STD4                                        4.00      0.00    0.0000    -0.0573
STD5                                        8.00      0.00    0.0006    -0.0636
STD6                                        14.00     0.00    0.0021    -0.0690
STD7                                        16.00     0.00    0.0026    -0.0730
Coefficient     : K3=--
                  K2=--
                  K1=1.798155E-004
                  K0=-4.702422E-004
Corr.Coef.      : 0.9807

UNK-001     pegaga1                         0.39      0.00    -0.0004   -0.0773
UNK-002     daun selom1                     -0.17     0.00    -0.0005   -0.0803
UNK-003     ulam raja1                      -0.17     0.00    -0.0005   -0.0834
UNK-004     bayam praksi1                   -0.17     0.00    -0.0005   -0.0861
```

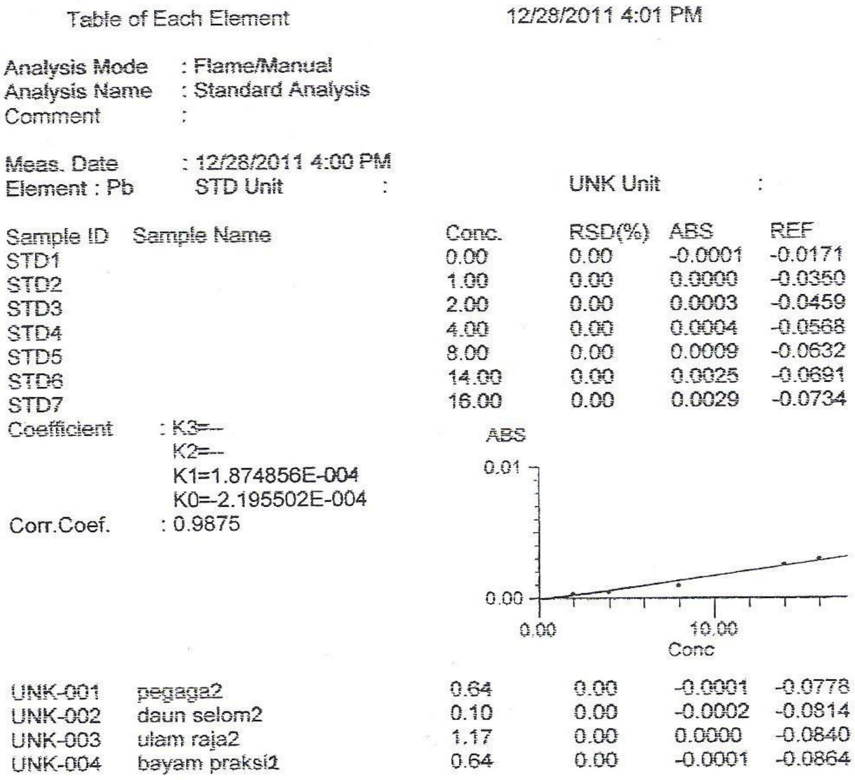

Table of Each Element 12/28/2011 4:01 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment :

Meas. Date : 12/28/2011 4:00 PM
Element : Pb STD Unit : UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0001	-0.0171
STD2		1.00	0.00	0.0000	-0.0350
STD3		2.00	0.00	0.0003	-0.0459
STD4		4.00	0.00	0.0004	-0.0568
STD5		8.00	0.00	0.0009	-0.0632
STD6		14.00	0.00	0.0025	-0.0691
STD7		16.00	0.00	0.0029	-0.0734

Coefficient : K3=--
K2=--
K1=1.874856E-004
K0=-2.195502E-004
Corr.Coef. : 0.9875

UNK-001	pegaga2	0.64	0.00	-0.0001	-0.0778
UNK-002	daun selom2	0.10	0.00	-0.0002	-0.0814
UNK-003	ulam raja2	1.17	0.00	0.0000	-0.0840
UNK-004	bayam praksi2	0.64	0.00	-0.0001	-0.0864

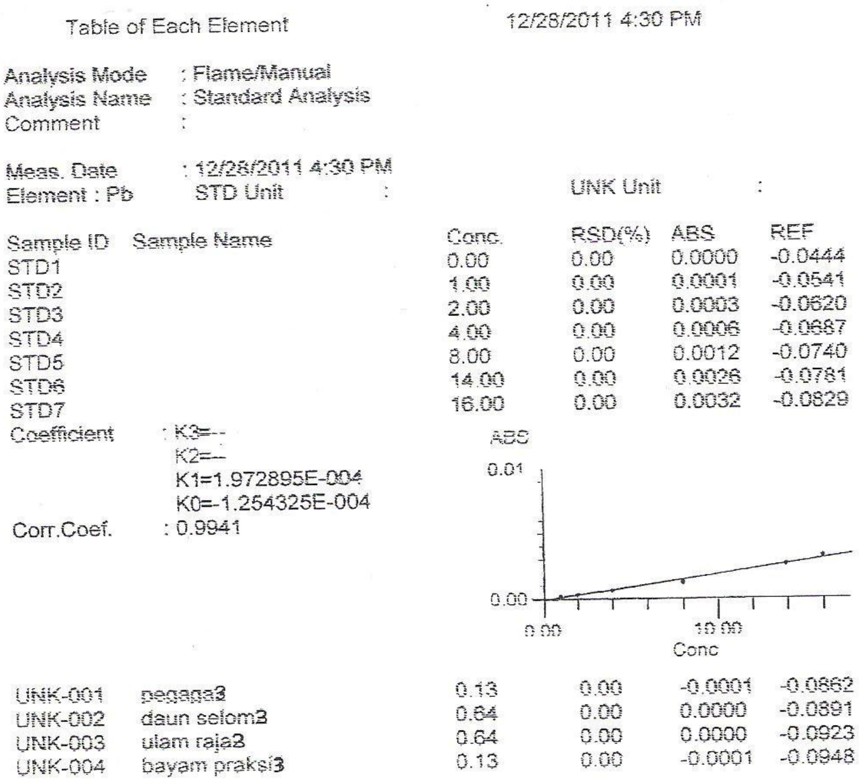

Table of Each Element 12/28/2011 4:30 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment :

Meas. Date : 12/28/2011 4:30 PM
Element : Pb STD Unit : UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0000	-0.0444
STD2		1.00	0.00	0.0001	-0.0541
STD3		2.00	0.00	0.0003	-0.0620
STD4		4.00	0.00	0.0006	-0.0687
STD5		8.00	0.00	0.0012	-0.0740
STD6		14.00	0.00	0.0026	-0.0781
STD7		16.00	0.00	0.0032	-0.0829

Coefficient : K3=--
K2=--
K1=1.972895E-004
K0=-1.254325E-004
Corr.Coef. : 0.9941

UNK-001	pegaga3	0.13	0.00	-0.0001	-0.0862
UNK-002	daun selom3	0.64	0.00	0.0000	-0.0891
UNK-003	ulam raja3	0.64	0.00	0.0000	-0.0923
UNK-004	bayam praksi3	0.13	0.00	-0.0001	-0.0948

Results for chromium (Cr)

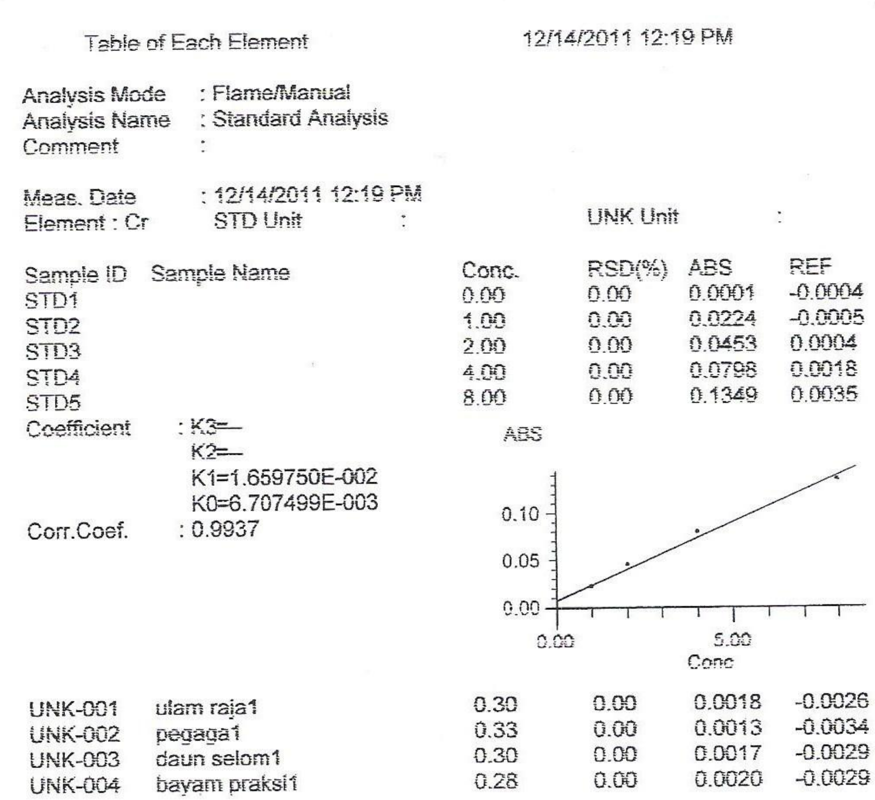

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     Table of Each Element                        12/14/2011 12:19 PM

Analysis Mode     : Flame/Manual
Analysis Name     : Standard Analysis
Comment           :

Meas. Date        : 12/14/2011 12:19 PM
Element : Cr        STD Unit          :               UNK Unit           :

Sample ID   Sample Name                    Conc.      RSD(%)   ABS      REF
STD1                                       0.00       0.00     0.0001   -0.0004
STD2                                       1.00       0.00     0.0224   -0.0005
STD3                                       2.00       0.00     0.0453    0.0004
STD4                                       4.00       0.00     0.0798    0.0018
STD5                                       8.00       0.00     0.1349    0.0035
Coefficient       : K3=—
                    K2=—
                    K1=1.659750E-002
                    K0=6.707499E-003
Corr.Coef.        : 0.9937

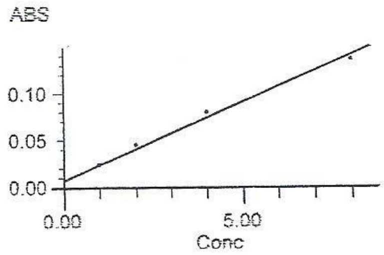
UNK-001     ulam raja1                      0.30       0.00     0.0018   -0.0026
UNK-002     pegaga1                         0.33       0.00     0.0013   -0.0034
UNK-003     daun selom1                     0.30       0.00     0.0017   -0.0029
UNK-004     bayam praksi1                   0.28       0.00     0.0020   -0.0029
```

```
        Table of Each Element                          12/14/2011 12:06 PM

Analysis Mode     : Flame/Manual
Analysis Name     : Standard Analysis
Comment           :

Meas. Date        : 12/14/2011 12:06 PM
Element : Cr        STD Unit          :              UNK Unit           :

Sample ID   Sample Name                   Conc.     RSD(%)  ABS      REF
STD1                                      0.00      0.00    0.0000   0.0004
STD2                                      1.00      0.00    0.0238   0.0016
STD3                                      2.00      0.00    0.0451   0.0021
STD4                                      4.00      0.00    0.0797   0.0029
STD5                                      8.00      0.00    0.1359   0.0051
Coefficient     : K3=--
                  K2=--
                  K1=1.666250E-002
                  K0=6.912499E-003
Corr.Coef.      : 0.9943
```



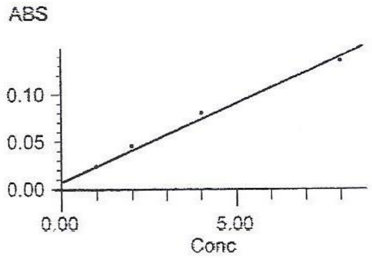
```
UNK-001    ulam raja2                     0.31      0.00    0.0017   -0.0010
UNK-002    pegaga2                        0.35      0.00    0.0011   -0.0006
UNK-003    daun selom2                    0.33      0.00    0.0014   -0.0006
UNK-004    bayam praksi2                  0.28      0.00    0.0023   -0.0004
```

```
        Table of Each Element                          12/14/2011 11:51 AM

Analysis Mode     : Flame/Manual
Analysis Name     : Standard Analysis
Comment           :

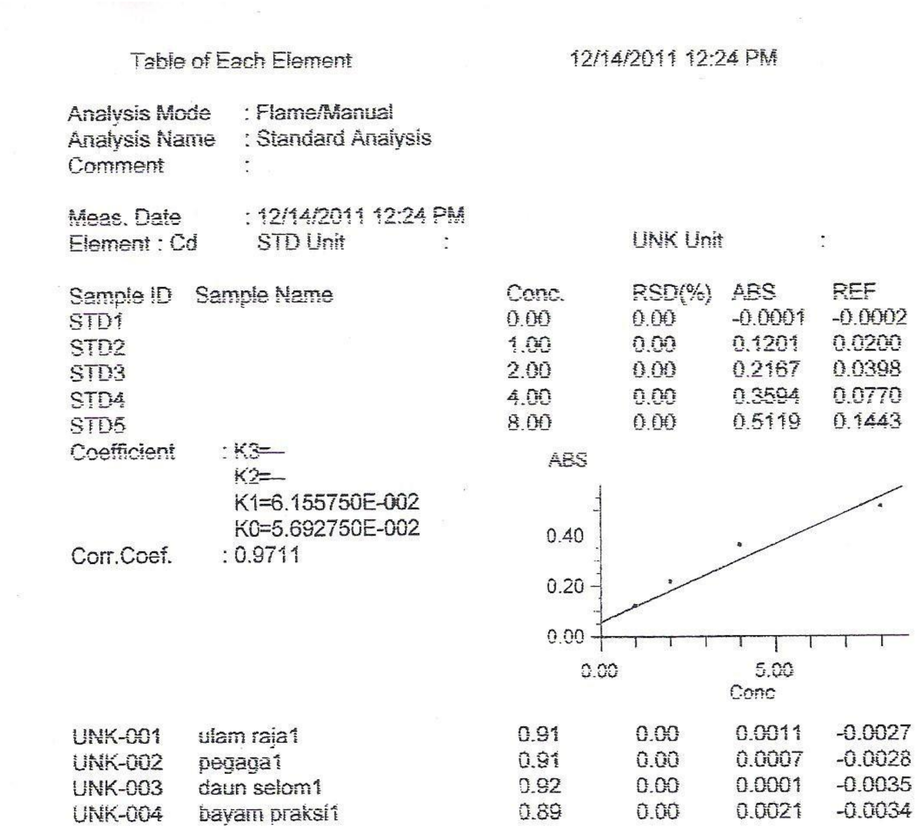
Meas. Date        : 12/14/2011 11:51 AM
Element : Cr        STD Unit          :              UNK Unit           :

Sample ID   Sample Name                   Conc.     RSD(%)  ABS      REF
STD1                                      0.00      0.00    0.0002   0.0001
STD2                                      1.00      0.00    0.0236   0.0017
STD3                                      2.00      0.00    0.0454   0.0021
STD4                                      4.00      0.00    0.0803   0.0043
STD5                                      8.00      0.00    0.1359   0.0055
Coefficient     : K3=--
                  K2=--
                  K1=1.666500E-002
                  K0=7.085000E-003
Corr.Coef.      : 0.9940
```



```
UNK-001    ulam raja3                     0.31      0.00    0.0019   0.0002
UNK-002    pegaga3                        0.34      0.00    0.0015   -0.0001
UNK-003    daun selom3                    0.32      0.00    0.0017   0.0001
UNK-004    bayam praksi3                  0.31      0.00    0.0019   0.0004
```

Results for cadmium (Cd)



```
     Table of Each Element                    12/14/2011 12:24 PM

Analysis Mode    : Flame/Manual
Analysis Name    : Standard Analysis
Comment          :

Meas. Date       : 12/14/2011 12:24 PM
Element : Cd       STD Unit         :          UNK Unit          :

Sample ID   Sample Name               Conc.     RSD(%)   ABS       REF
STD1                                  0.00      0.00     -0.0001   -0.0002
STD2                                  1.00      0.00     0.1201    0.0200
STD3                                  2.00      0.00     0.2167    0.0398
STD4                                  4.00      0.00     0.3594    0.0770
STD5                                  8.00      0.00     0.5119    0.1443
Coefficient    : K3=—
                 K2=—
                 K1=6.155750E-002
                 K0=5.692750E-002
Corr.Coef.     : 0.9711

UNK-001     ulam raja1                0.91      0.00     0.0011    -0.0027
UNK-002     pegaga1                   0.91      0.00     0.0007    -0.0028
UNK-003     daun selom1               0.92      0.00     0.0001    -0.0035
UNK-004     bayam praksi1             0.89      0.00     0.0021    -0.0034
```

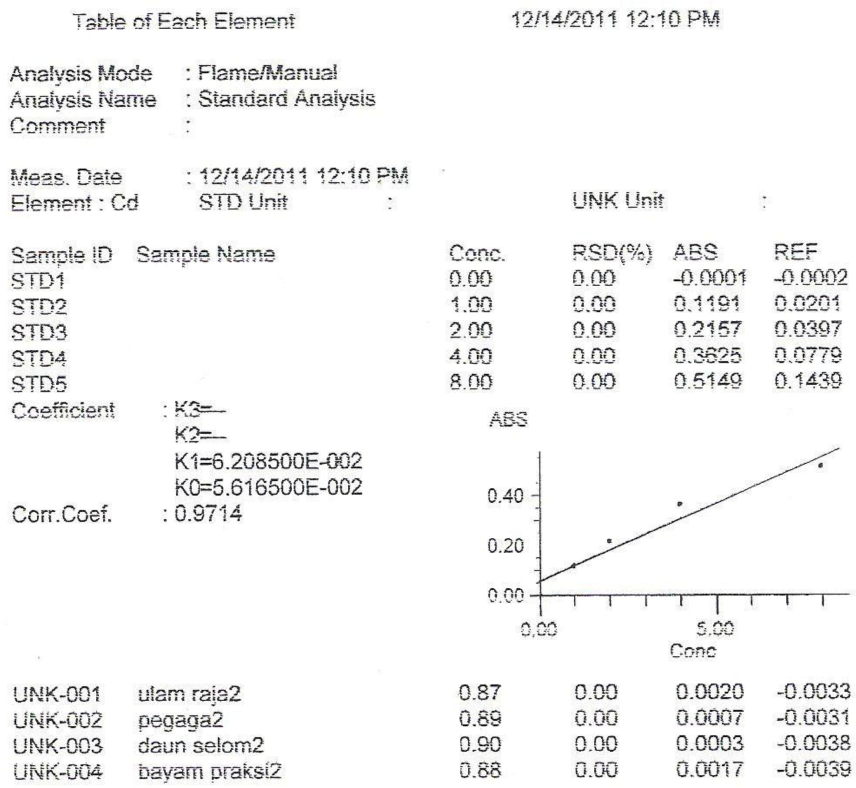


Table of Each Element 12/14/2011 12:10 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment :

Meas. Date : 12/14/2011 12:10 PM
Element : Cd STD Unit : UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0001	-0.0002
STD2		1.00	0.00	0.1191	0.0201
STD3		2.00	0.00	0.2157	0.0397
STD4		4.00	0.00	0.3625	0.0779
STD5		8.00	0.00	0.5149	0.1439

Coefficient : K3=--
K2=--
K1=6.208500E-002
K0=5.616500E-002
Corr.Coef. : 0.9714

UNK-001	ulam raja2	0.87	0.00	0.0020	-0.0033
UNK-002	pegaga2	0.89	0.00	0.0007	-0.0031
UNK-003	daun selom2	0.90	0.00	0.0003	-0.0038
UNK-004	bayam praksi2	0.88	0.00	0.0017	-0.0039

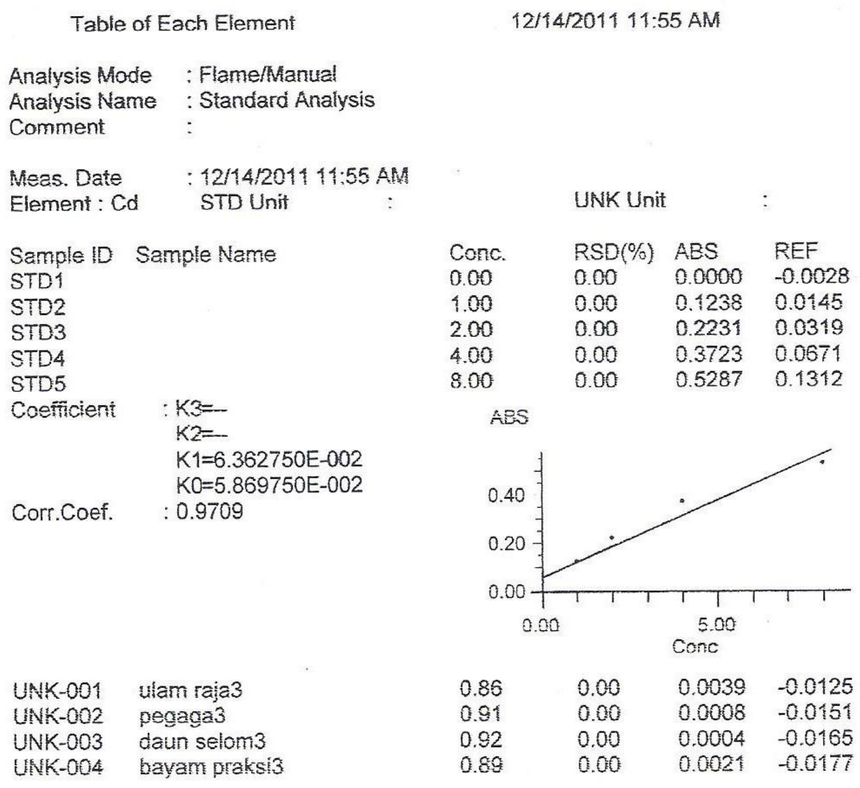


Table of Each Element 12/14/2011 11:55 AM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment :

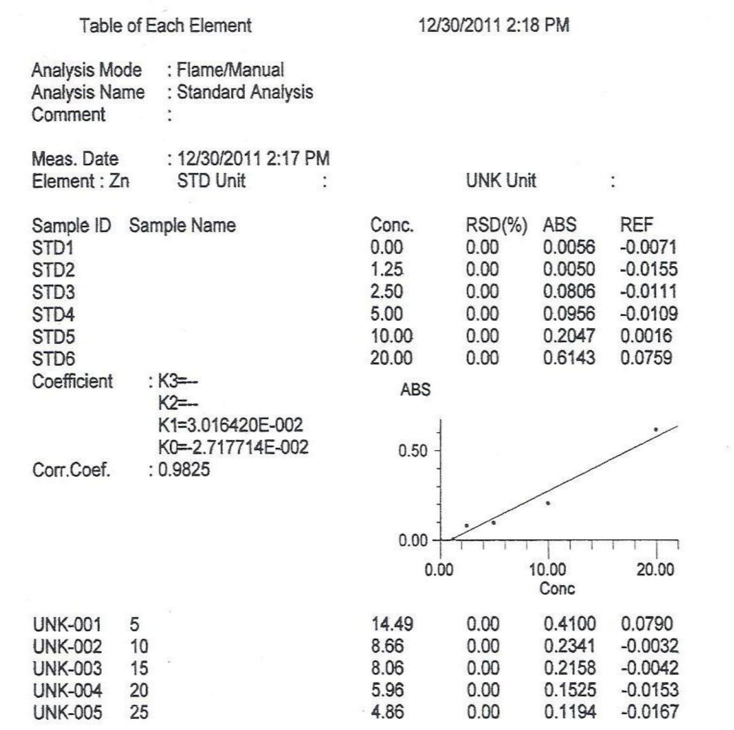
Meas. Date : 12/14/2011 11:55 AM
Element : Cd STD Unit : UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0000	-0.0028
STD2		1.00	0.00	0.1238	0.0145
STD3		2.00	0.00	0.2231	0.0319
STD4		4.00	0.00	0.3723	0.0671
STD5		8.00	0.00	0.5287	0.1312

Coefficient : K3=--
K2=--
K1=6.362750E-002
K0=5.869750E-002
Corr.Coef. : 0.9709

UNK-001	ulam raja3	0.86	0.00	0.0039	-0.0125
UNK-002	pegaga3	0.91	0.00	0.0008	-0.0151
UNK-003	daun selom3	0.92	0.00	0.0004	-0.0165
UNK-004	bayam praksi3	0.89	0.00	0.0021	-0.0177

Results for heavy metal uptake in green plants



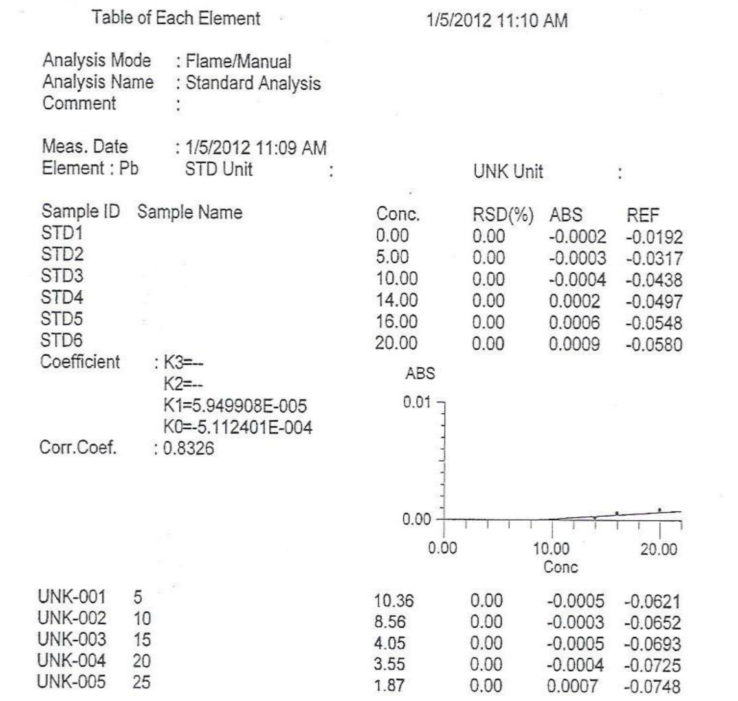
```
        Table of Each Element                 12/30/2011 2:18 PM

Analysis Mode    : Flame/Manual
Analysis Name    : Standard Analysis
Comment          :

Meas. Date       : 12/30/2011 2:17 PM
Element : Zn       STD Unit          :            UNK Unit         :

Sample ID   Sample Name                  Conc.    RSD(%)   ABS      REF
STD1                                     0.00     0.00     0.0056   -0.0071
STD2                                     1.25     0.00     0.0050   -0.0155
STD3                                     2.50     0.00     0.0806   -0.0111
STD4                                     5.00     0.00     0.0956   -0.0109
STD5                                     10.00    0.00     0.2047   0.0016
STD6                                     20.00    0.00     0.6143   0.0759
Coefficient    : K3=--
                 K2=--
                 K1=3.016420E-002
                 K0=-2.717714E-002
Corr.Coef.     : 0.9825

UNK-001     5                            14.49    0.00     0.4100   0.0790
UNK-002     10                           8.66     0.00     0.2341   -0.0032
UNK-003     15                           8.06     0.00     0.2158   -0.0042
UNK-004     20                           5.96     0.00     0.1525   -0.0153
UNK-005     25                           4.86     0.00     0.1194   -0.0167
```



```
        Table of Each Element                 1/5/2012 11:10 AM

Analysis Mode    : Flame/Manual
Analysis Name    : Standard Analysis
Comment          :

Meas. Date       : 1/5/2012 11:09 AM
Element : Pb       STD Unit          :            UNK Unit         :

Sample ID   Sample Name                  Conc.    RSD(%)   ABS       REF
STD1                                     0.00     0.00     -0.0002   -0.0192
STD2                                     5.00     0.00     -0.0003   -0.0317
STD3                                     10.00    0.00     -0.0004   -0.0438
STD4                                     14.00    0.00     0.0002    -0.0497
STD5                                     16.00    0.00     0.0006    -0.0548
STD6                                     20.00    0.00     0.0009    -0.0580
Coefficient    : K3=--
                 K2=--
                 K1=5.949908E-005
                 K0=-5.112401E-004
Corr.Coef.     : 0.8326

UNK-001     5                            10.36    0.00     -0.0005   -0.0621
UNK-002     10                           8.56     0.00     -0.0003   -0.0652
UNK-003     15                           4.05     0.00     -0.0005   -0.0693
UNK-004     20                           3.55     0.00     -0.0004   -0.0725
UNK-005     25                           1.87     0.00     0.0007    -0.0748
```

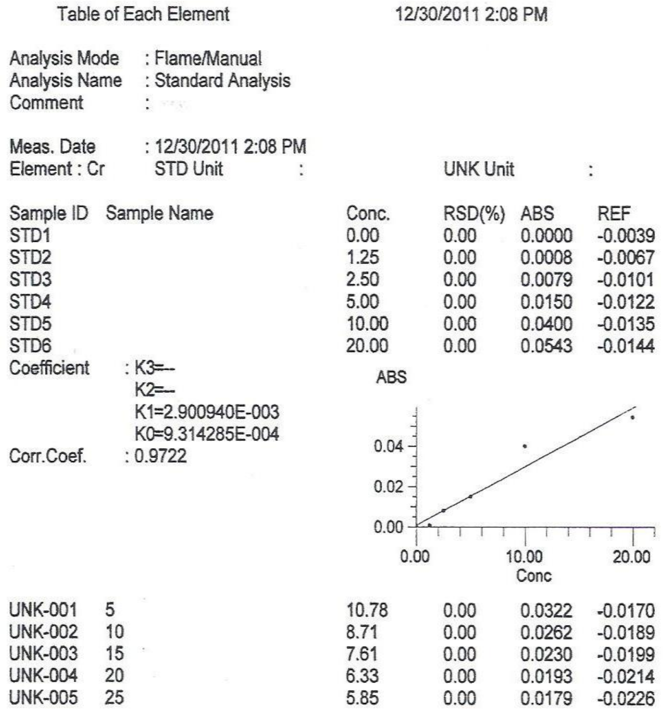


Table of Each Element 12/30/2011 2:08 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment :

Meas. Date : 12/30/2011 2:08 PM
Element : Cr STD Unit : UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0000	-0.0039
STD2		1.25	0.00	0.0008	-0.0067
STD3		2.50	0.00	0.0079	-0.0101
STD4		5.00	0.00	0.0150	-0.0122
STD5		10.00	0.00	0.0400	-0.0135
STD6		20.00	0.00	0.0543	-0.0144

Coefficient : K3=--
K2=--
K1=2.900940E-003
K0=9.314285E-004
Corr.Coef. : 0.9722

UNK-001	5	10.78	0.00	0.0322	-0.0170
UNK-002	10	8.71	0.00	0.0262	-0.0189
UNK-003	15	7.61	0.00	0.0230	-0.0199
UNK-004	20	6.33	0.00	0.0193	-0.0214
UNK-005	25	5.85	0.00	0.0179	-0.0226

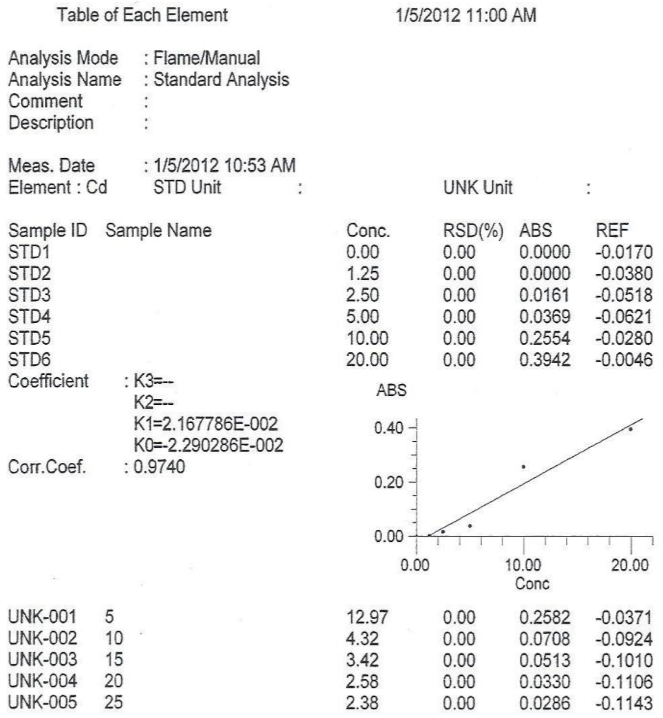


Table of Each Element 1/5/2012 11:00 AM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment :
Description :

Meas. Date : 1/5/2012 10:53 AM
Element : Cd STD Unit : UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	0.0000	-0.0170
STD2		1.25	0.00	0.0000	-0.0380
STD3		2.50	0.00	0.0161	-0.0518
STD4		5.00	0.00	0.0369	-0.0621
STD5		10.00	0.00	0.2554	-0.0280
STD6		20.00	0.00	0.3942	-0.0046

Coefficient : K3=--
K2=--
K1=2.167786E-002
K0=-2.290286E-002
Corr.Coef. : 0.9740

UNK-001	5	12.97	0.00	0.2582	-0.0371
UNK-002	10	4.32	0.00	0.0708	-0.0924
UNK-003	15	3.42	0.00	0.0513	-0.1010
UNK-004	20	2.58	0.00	0.0330	-0.1106
UNK-005	25	2.38	0.00	0.0286	-0.1143

APPENDIX B

CALCULATIONS

Calculation:

Heavy metals (Pb, Cd, Cr and Zn) standard solution concentration for AAS calibration

i. Stock solution of lead (Pb)

Initial concentration from stock solution, M_i = 1000 mg/L
Final concentration needed, M_f = 20 mg/L
Final volume needed, V_f = 100 mL

Using formula,

$$M_iV_i = M_fV_f$$

$$V_i = [(20\ \text{mg/L})(100\ \text{mL})]/(1000\ \text{mg/L})$$

$$V_i = 2\ \text{mL}$$

ii. The final concentration above now will be the initial concentration for lower concentration e.g: 1 mg/L, 2 mg/L, 4 mg/L, 8 mg/L, 14 mg/L, etc.

Initial concentration, M_i = 20 mg/L
Final concentration, M_f = 14 mg/L
Final volume, V_f = 50 mL

Using formula,

$$M_iV_i = M_fV_f$$

$$V_i = [(14\ \text{mg/L})(50\ \text{mL})]/(20\ \text{mg/L})$$

$$V_i = 35\ \text{mL}$$

Notes:

a) For final volume, V_f it can be changed depend on the volume of beaker or volumetric flask used in the experiment.

b) Apply the same method for another element (Cd, Cr and Zn).

APPENDIX C

ANALYSIS OF RESULTS

Calculation:

i. Average in heavy metal uptake

$$\mu = \frac{\sum \bar{x}}{N}$$

Where;

μ = mean, average

$\sum \bar{x}$ = summation of each value in the population

N = the number of values (the populations)

a) Average lead, Pb uptake by green plants

Types of plants	**Pb concentration (mg/L)**			**Average concentration (mg/L)**
	1	**2**	**3**	
Cosmos caudatus (ulam raja)	**-**	**1.17**	**0.64**	**0.905**
Centella asiatica (pegaga)	0.39	0.64	0.13	0.387
Emilia sonchifolia (bayam praksi)	-	0.64	0.13	0.385
Oenanthe javanica (daun selom)	-	0.10	0.64	0.370

b) Average cadmium, Cd uptake by green plants

Types of plants	**Cd concentration (mg/L)**			**Average concentration (mg/L)**
	1	**2**	**3**	
Cosmos caudatus (ulam raja)	0.91	0.87	0.86	0.880
Centella asiatica (pegaga)	0.91	0.89	0.91	0.903
Emilia sonchifolia (bayam praksi)	0.89	0.88	0.89	0.887
Oenanthe javanica (daun selom)	**0.92**	**0.90**	**0.92**	**0.913**

c) Average chromium, Cr uptake by green plants

Types of plants	**Cr concentration (mg/L)**			**Average concentration (mg/L)**
	1	**2**	**3**	
Cosmos caudatus (ulam raja)	0.30	0.31	0.31	0.307
Centella asiatica (pegaga)	**0.33**	**0.35**	**0.34**	**0.340**
Emilia sonchifolia (bayam praksi)	0.28	0.28	0.31	0.290
Oenanthe javanica (daun selom)	0.30	0.33	0.32	0.317

d) Average zinc, Zn uptake by green plants

Types of plants	**Zn concentration (mg/L)**			**Average concentration (mg/L)**
	1	**2**	**3**	
Cosmos caudatus (ulam raja)	0.15	0.14	0.13	0.140
Centella asiatica (pegaga)	0.71	0.66	0.50	0.623
Emilia sonchifolia (bayam praksi)	**2.84**	**2.01**	**2.53**	**2.460**
Oenanthe javanica (daun selom)	0.54	0.25	0.46	0.417

ii. Percentage metal uptake for the effect of contact time

$$\% \text{ uptake} = \frac{(C_i - C_e)}{C_i} \times 100\%$$

Where;

C_i = 15 mg/L

a) Lead, Pb uptake by cosmos plant (*Cosmos caudatus; Ulam raja)*

Time (min)	**C_e = Pb concentration (mg/L)**	**Pb uptake (%)**
5	10.36	$\frac{15.0 - 10.36}{15.0} \times 100\% = 30.93\%$
10	8.56	$\frac{15.0 - 8.56}{15.0} \times 100\% = 42.9\%$
15	4.05	$\frac{15.0 - 4.05}{15.0} \times 100\% = 73.0\%$
20	3.55	$\frac{15.0 - 3.55}{15.0} \times 100\% = 76.3\%$
25	1.87	$\frac{15.0 - 1.87}{15.0} \times 100\% = 87.5\%$

b) Cadmium, Cd uptake by selom leaves (*Oenanthe javanica; Daun selom*)

Time (min)	**C_e = Cd concentration (mg/L)**	**Cd uptake (%)**
5	12.97	$\frac{15.0 - 12.97}{15.0} \times 100\% = 13.53\%$
10	4.32	$\frac{15.0 - 4.32}{15.0} \times 100\% = 71.2\%$
15	3.42	$\frac{15.0 - 3.42}{15.0} \times 100\% = 77.2\%$

20	2.58	$\frac{15.0-2.58}{15.0} \times 100\% = 82.8\%$
25	2.38	$\frac{15.0-2.38}{15.0} \times 100\% = 84.1\%$

c) Chromium, Cr uptake by asiatic pennywort (*Centella asiatica; Pegaga*)

Time (min)	**C_e = Cr concentration (mg/L)**	**Cr uptake (%)**
5	10.78	$\frac{15.0-10.78}{15.0} \times 100\% = 28.13\%$
10	8.71	$\frac{15.0-8.71}{15.0} \times 100\% = 41.9\%$
15	7.61	$\frac{15.0-7.61}{15.0} \times 100\% = 49.3\%$
20	6.33	$\frac{15.0-6.33}{15.0} \times 100\% = 57.8\%$
25	5.85	$\frac{15.0-5.85}{15.0} \times 100\% = 61.0\%$

d) Zinc, Zn uptake by *Emilia sonchifolia (bayam praksi)*

Time (min)	**C_e = Zn concentration (mg/L)**	**Zinc uptake (%)**
5	14.49	$\frac{15.0-14.49}{15.0} \times 100\% = 3.4\%$
10	8.66	$\frac{15.0-8.66}{15.0} \times 100\% = 42.3\%$
15	8.06	$\frac{15.0-8.06}{15.0} \times 100\% = 46.3\%$
20	5.96	$\frac{15.0-5.96}{15.0} \times 100\% = 60.3\%$
25	4.86	$\frac{15.0-4.86}{15.0} \times 100\% = 67.6\%$

APPENDIX D

FIGURES IN LABORATORY DURING EXPERIMENTS

Figure D.1: Sample was dried using hot air oven

Figure D.2: Hot air oven

Figure D.3: Dried samples

Figure D.4: Weight balance

Figure D.5: Solution was stirred using hot plate stirrer

Figure D.6: Metal solution for calibration curve

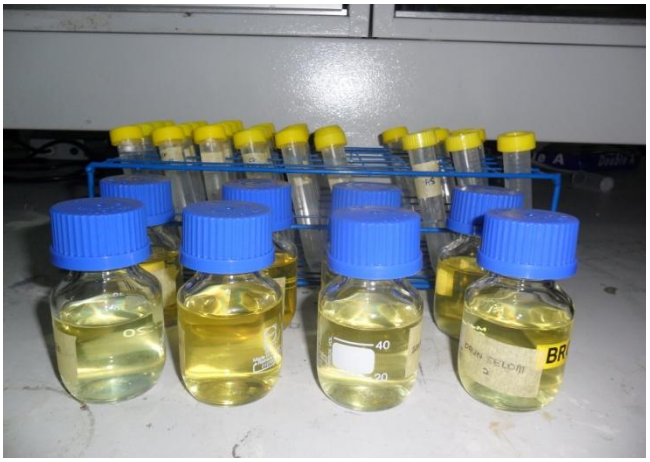


Figure D.7: Sample ready to be analyzed

Figure D.8: Atomic absorption spectrometry (AAS)