



SELECTED HEAVY METALS (Cr, Pb, Cd) UPTAKE IN *Cymbopogon citratus* PLANT BY USING ATOMIC ABSORPTION SPECTROSCOPY

by

NUR HAMIZAH BINTI AMZAH

A report submitted in fulfillment of the requirements for the degree of
Bachelor of Applied Science (Sustainable Science) with Honours

**FACULTY OF EARTH SCIENCE
UNIVERSITI MALAYSIA KELANTAN**

2019

DECLARATION

I declare that the thesis entitled Selected Heavy Metals (Cr, Pb, Cd) Uptake In *Cymbopogon citratus* Plant By Using Atomic Absorption Spectroscopy is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidate of any other degree.

Signature : _____

Name : NUR HAMIZAH BINTI AMZAH

Date : _____

UNIVERSITI
MALAYSIA
KELANTAN

ACKNOWLEDGEMENT

First of all, thanks to the Almighty after nearly a year of hardships and efforts, I manage to complete my final year project successfully. As the time passed while finishing this project, I have gained a lot of knowledge and great experiences which I believe will be very useful for my future and career life.

Second, I would like to express my greatest attitude towards my supervisor, Dr Musfiroh binti Jani for giving me an opportunity to be her supervisee and put an absolute trust on me to do this project on “Selected Heavy Metals (Cr, Pb, Cd) Uptake In *Cymbopogon Citratus* Plant By Using Atomic Absorption Spectroscopy”. Without her guidance and encouragement, all of this work would be impossible to be accomplished.

A million thanks also goes to my parents, Hj Amzah bin Md Aris and Hjh Fatimah binti Darus, as well as my sisters, Aziela, Nur Anis and Nur Aini, for their continuous support, advice and motivation for me to complete my study.

Last but not least, I wish to express my sincere appreciation to all my friends especially my lab partner, Sharifah Norazreen Suhaila, plus Encik Rohanif as the staff in charge for AAS analysis for their kind assistance and help throughout this project. This thesis would not have been possible without the guidance and help of those I stated above. Thank you very much.

SELECTED HEAVY METALS (Cr, Pb, Cd) UPTAKE IN *Cymbopogon citratus* PLANT BY USING ATOMIC ABSORPTION SPECTROSCOPY

ABSTRACT

Cymbopogon citratus is known as lemongrass in Asia. Usually, people will use stem's part of *Cymbopogon citratus* for cooking and medicinal purpose. This research is to analyze the selected heavy metals distribution such as cadmium (Cd), chromium (Cr) and lead (Pb) in this plant by using Atomic Absorption Spectroscopy (AAS). This research is also to compare the fate and transport of heavy metals by using five different concentrations (5 ppm, 10 ppm, 15 ppm, 20 ppm, and 15 ppm) in parts of this plant which are leaves, stems and roots. From the result, Pb has the highest accumulation and been absorbed a lot at the root which is $0.392 \text{ mg/L} \pm 0.0307$. While Cr, it has been absorbed a lot in root but in certain concentration which is 10 ppm $0.061 \text{ mg/L} \pm 0.0179$. Cd also can be found a lot in root but also in certain concentration such as 10 ppm, 15 ppm and 25 ppm which are the values $0.009 \text{ mg/L} \pm 0.0003$, $0.002 \text{ mg/L} \pm 0.0020$, $0.011 \text{ mg/L} \pm 0.0004$. Sequence of heavy metal accumulation for these selected heavy metals Cd, Cr and Pb is root > leaves > stems. The significant value between concentration and heavy metals for each pot has determined as ($P < 0.05$).

UNIVERSITI
MALAYSIA
KELANTAN

**PENGAMBILAN LOGAM BERAT YANG TERPILIH (Cr, Pb, Cd) DALAM
POKOK *Cymbopogon citratus* DENGAN MENGGUNAKAN PENYERAPAN
ATOMIK SPEKTROSKOPI**

ABSTRAK

Cymbopogon citratus dikenali sebagai serai di Asia. Adalah penting bagi orang mengetahui sama ada tumbuhan itu selamat untuk dimakan atau tidak. Biasanya, orang akan menggunakan bahagian batang *Cymbopogon citratus* untuk tujuan memasak dan perubatan. Kajian ini bertujuan untuk menganalisis pengagihan logam berat terpilih seperti kadmium (Cd), kromium (Cr) dan plumbum (Pb) dalam tumbuhan ini oleh Spektroskopi Penyerapan Atom (AAS). Kajian ini juga dapat membandingkan nasib dan pengangkutan logam berat dengan menggunakan lima kepekatan yang berbeza (5 ppm, 10 ppm, 15 ppm, 20 ppm, dan 15 ppm) di bahagian tumbuhan ini iaitu daun, batang dan akar. Dari hasilnya, Pb mempunyai pengumpulan tertinggi dan diserap banyak pada akar yang adalah $0.392 \text{ mg / L} \pm 0.0307$. Manakala Cr, ia telah banyak diserap dalam akar tetapi dalam kepekatan tertentu iaitu 10 ppm $0.061 \text{ mg / L} \pm 0.0179$. Cd juga banyak ditemui dalam akar tetapi juga dalam kepekatan tertentu seperti 10 ppm, 15 ppm dan 25 ppm iaitu nilai $0.009 \text{ mg / L} \pm 0.0003$, $0.002 \text{ mg / L} \pm 0.0020$, $0.011 \text{ mg / L} \pm 0.0004$. Turutan pengumpulan logam berat untuk logam berat terpilih Cd, Cr dan Pb adalah akar > daun > batang. Nilai signifikan antara kepekatan dan logam berat bagi setiap periuk telah ditentukan sebagai ($P < 0.05$).

UNIVERSITI
MALAYSIA
KELANTAN

TABLE OF CONTENT

	PAGES
TITLE PAGE	
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
ABSTRAK	iv
TABLE OF CONTENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xii
LIST OF SYMBOLS	xiii
CHAPTER 1 INTRODUCTION	
1.1 Background of Study	1
1.2 Problem Statement	2
1.3 Objectives	3
1.4 Scope of Study	3
1.5 Significance of Study	3
CHAPTER 2 LITERATURE REVIEW	
2.1 <i>Cymbopogon citratus</i>	5
2.2 Heavy Metals	7

2.2.1 Chromium (Cr)	8
2.2.2 Lead (Pb)	8
2.2.3 Cadmium (Cd)	9
2.3 Heavy Metals In Plants	10
2.4 Metals Uptake In Plants	11
2.4.1 Chromium (Cr) Uptake in Plants	12
2.4.2 Lead (Pb) Uptake in Plants	13
2.4.3 Cadmium (Cd) Uptake in Plants	13
2.5 Factors that Affecting the Uptake Mechanisms	14
2.5.1 Plant Species	15
2.5.2 The Root Zone	15
2.5.3 Environmental Conditions	15
2.6 Atomic Absorption Spectroscopy (AAS)	16
CHAPTER 3 METHODOLOGY	
3.1 Materials	17
3.2 Methods	17
3.2.1 Synthetic Mixture in Soil	18
3.2.2 Plant Monitoring	19
3.2.3 Water monitoring	19
3.2.4 Soil monitoring	20
3.2.5 Sample Preparation	22

3.2.6 Wet Digestion Method	22
3.2.7 Standard Series of Dilution	23
3.3.8 Determination Of Heavy Metal Concentration By Using AAS	25
3.2.9 Heavy Metals Distribution	25
3.3 Data Analysis	26
CHAPTER 4 RESULT AND DISCUSSION	
4.1 Soil Physical	27
4.2 pH	28
4.2.1 pH of Water	28
4.2.2 pH of Soil	30
4.3 Growth Observation	32
4.4 Moisture Content	35
4.4.1 Soil Moisture Content	35
4.4.2 Plant Moisture Content	37
4.5 Heavy Metals Accumulations in Three Parts of Plant	39
4.6 Soil Analysis	48
4.7 Water Analysis	51
4.8 Comparison of Three Heavy Metals between Plant, Soil and Water	53
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS	
5.1 CONCLUSION	56
5.2 RECOMMENDATIONS	57

REFERENCES

58

APPENDICES

62



UNIVERSITI

MALAYSIA

KELANTAN

LIST OF TABLES

NO	TITLE	PAGE
3.1	The arrangement of pots for this thesis	18
3.2	The volume of concentrated solution have been obtained after been calculated.	24
4.1	Elements and chemical composition in initial soil by using EDS	27
4.2	The comparison of heavy metals between plant, soil and water	53

LIST OF FIGURES

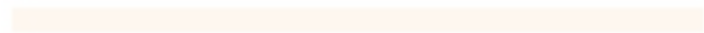
NO	TITLE	PAGE
2.1	<i>Cymbopogon citratus</i>	6
2.2	Factors that Affecting the Uptake Mechanisms	14
4.1	The average of pH for water week 2 and week 4	28
4.2	The average of soil pH for week 2 and week 4	31
4.3	The length of leaves of <i>Cymbopogon citratus</i> from week 1 until week 4	34
4.4	The moisture content for soil at week 2 and week 4	36
4.5	The combination of moisture content of three parts (leaves, stems and roots) of <i>Cymbopogon citratus</i>	38
4.6	The translocation factor for heavy accumulation	40
4.7	The bioaccumulation factor for heavy accumulation	41
4.8	Root accumulation for three heavy metals in five different concentrations	44
4.9	Stem accumulation for three heavy metals in five different concentrations	45
4.10	Leave accumulation for three heavy metals in five different concentrations	46
4.11	Means of average of three heavy metals presence in each of pot	50

4.12 The mean of heavy metals that presence in water
for each of pots.

52



UNIVERSITI



MALAYSIA



KELANTAN

LIST OF ABBREVIATIONS

Hg	Mercury
AAS	Atomic Absorption Spectroscopy
Zn	Zinc
Cr	Chromium
Cd	Cadmium
Pb	Lead
As	Arsenic
Cu	Copper
Fe	Iron
ROS	Reactive Oxygen Species
Hydrogen Peroxide	H ₂ O ₂
Nitric Acid	HNO ₃
Energy Dispersive X ray Spectroscopy	EDS
H ₂ O	Water
Ca	Calcium
WHO	World Health Organization
BF	Bioaccumulation Factor
TF	Translocation Factor

LIST OF SYMBOLS

%	Percentage
°C	Degree celcius
G	Gram
mL	Mililitre
mg/L	milligram per Litre
ppm	parts per million
mm	milimetre
<i>P</i>	Significance level

UNIVERSITI
MALAYSIA
KELANTAN

CHAPTER 1

INTRODUCTION

1.1 Background Of Study

Nowadays, heavy metals have become one of major sources that contribute to the pollution in the environment especially in agriculture. This is because each of heavy metals has their own adverse effect on environment and human. Heavy metals refer to any types of metallic element that has high density and can be poisonous in low concentration. Examples for heavy metals are mercury (Hg), chromium (Cr), cadmium (Cd) and others. They are natural components which cannot be destroyed. Heavy metals are very dangerous and they can give a negative impact to the surrounding including environment and human. Besides that, they are very harmful to humans and also environment. For example, heavy metals can give impact on human health such as cadmium; it can cause kidney failure. In environment, such as plants, it can absorb certain heavy metals and can cause the plants unsafe to consume. Human activities are one of huge contributor to the heavy metal pollution (Alloway & Jackson, 1991). People need to be more cautious about the heavy metals, their behavior and their impact to the environment and human.

The presence of heavy metals in agricultural soils is a major concern in this country. This is because agricultural have an important role or connection with human or animals

and also a part of people diet. Some animals and human depends on agricultural. Heavy metals can be taken up by plants, where it can interrupt the food chain in significant amounts. The heavy metal will be adsorbed by through roots from the soils and direct to the leaf. Hence, it can effect on people's health because they are consuming vegetables from contaminated soils. In general, the uptake is increased in plants that grown up in areas with high soil contamination. Usually agricultural soils become contaminated because of certain farmers use pesticides for their crops to increase the growth of plants without considering any impacts of using pesticides to environment and human.

1.2 Problem Statement

Heavy metals can be taken up by plants, where heavy metal can interrupt the food chain in significant amounts. The heavy metal will be adsorbed by through roots from the soils and direct to the leaf. Hence, it can effect on people's health because they are consuming vegetables from contaminated soils in their daily life. Generally, the uptake is increased in plants that grown up in areas with high soil contamination. Previously, elements of heavy metals that can be found such as Cd, Pb and Cr in *Cymbopogon citratus* plants (Anal, 2014). They are heavy metals that can give negative impact to human health even at low concentrations (Anal, 2014). *Cymbopogon citratus* plant act as herbs that commonly used in cooking and daily dietary. Hence, it is important for us to know whether the plant is safe to be consumed or not when they are contaminated with the heavy metals.

1.3 Objectives

The objectives of this study are:

- 1) To analyze the heavy metals distribution in different parts of *Cymbopogon citratus* (roots, stems, leaves) by using Atomic Absorption Spectroscopy (AAS).
- 2) To compare the distribution of heavy metals Cr, Pb and Cd in *Cymbopogon citratus* plants by using Atomic Absorption Spectroscopy (AAS).

1.4 Scope Of Study

This study focuses mainly on the analysis of three types of selected heavy metals (Cr, Pb, Cd) distribution in different parts of *Cymbopogon citratus* plants namely roots, leaves and stems by using Atomic Absorption of Spectroscopy (AAS). Next, the fate and transport of heavy metals (Cr, Pb, Cd) in *Cymbopogon citratus* will be compared using Atomic Absorption of Spectroscopy (AAS).

1.5 Significance Of Study

Cymbopogon citratus is one of daily herbs that usually used in cooking. *Cymbopogon citratus* is also known as *Serai* by local and can be found abundantly especially in Malaysia. This study is essential and will focus on how the selected heavy metals fate and also transport into different parts of *Cymbopogon citratus* plants (roots, stems, leaves). Besides that, this research will help people to be more careful in

consuming *Cymbopogon citratus* plants and other herbal plants and did not get any of negative impacts from the contamination soils. In this study, it will be more focus on these three selected heavy metals (Cr, Pb, Cd). Hence, the condition of soil and the transport in plants will be examined. This study will come out with huge help for the researchers to find a solution to overcome this problem which is connects with the heavy metals contamination in *Cymbopogon citratus* plants.

CHAPTER 2

LITERATURE REVIEW

2.1 *Cymbopogon citratus*

Plants are multicellular and photosynthetic eukaryotes of Kingdom Plantae. Plants play an important role in the world. Plants or vegetables give human, animals food to survive such as tasty nuts and sweet fruits. Plants also contribute a huge help to human and animal by producing oxygen in air. Human use the plants for food or as feed for domestic animals. Plants also can be used for medicines. Medicines from plants are sources of organic compounds. A lot of medicines derived from plants such as aspirin, taxol and morphine.

Cymbopogon citratus plant is commonly known as lemongrass in Asia. It is native from southwest Asia but now it grows constantly and spontaneously around the world including Malaysia. It is widely used herb in a lot of tropical countries. *Cymbopogon citratus* also can be used in aromatherapy. However, *Cymbopogon citratus* can be used or function in synergy with other plants and also can act alone (Lawal et al., 2017). *Cymbopogon citratus* also has citral which is an essential oil and it has repellent activity. *Cymbopogon citratus* also act as aromatic healer and used in culinary. In culinary, *Cymbopogon citratus* offers medical benefits and it is in extensive demand because of its anti-bacterial and others. This plant also can help to reduce diarrhea and

stomachaches. *Cymbopogon citratus* also has been used in perfumery, cosmetic and pharmaceutical industries to control pathogens (Bachiega & Sforcin, 2011).

The health benefit of *Cymbopogon citratus* is to lower the cholesterol. Some studies have shown that regular consumption of *Cymbopogon citratus* is good in sustaining healthy levels of triglycerides and reducing LDL cholesterol. Next health benefit is detoxification the body. *Cymbopogon citratus* helps in cleansing harmful toxic wastes out of body. Other benefit is *Cymbopogon citratus* plant can help to prevent cancer. *Cymbopogon citratus* is effective in treating cancers without affecting any healthy normal cell of body (Philion et al., 2017).



Figure 2.1 : *Cymbopogon citratus*

2.2 Heavy Metals

The definition or term of heavy metal is any types of metallic element that has a high density and can be poisonous even at low concentration. Besides that, heavy metal is one of globally recognition in environmental issues because they cannot be degraded or destroyed. There are more than 20 known heavy metals in the world but the most gained attention are the presence of cadmium (Cd), lead (Pb), chromium (Cr) and mercury (Hg). For example, zinc (Zn) and cadmium (Cd), they can lead to decreasing metabolic activity while copper (Cu) can induce oxidative stress (Edelstein & Ben-Hur, 2018). Heavy metal is one of big contributors to pollution in the world. It can affect plants, animals and human. Heavy metals such as arsenic (As), mercury (Hg) and lead (Pb) are not essential for animals and plants. The essential metals can produce very toxic and hazardous effects when the metal intake is highly elevated (Duran, Tuzen, & Soylak, 2008).

Sources of heavy metals in environment can be caused by natural event and anthropogenic activities such as mining, using fertilizers that containing heavy metals for crops and metallurgy (Megateli, Semsari, & Couderchet, 2009). For example, one of the sources of heavy metals from anthropogenic activities is agriculture. Certain farmers will use fertilizer to increase of plant growth and productivity but at the same time, the farmers will lead to pollution which is presence of heavy metals in soil. Heavy metals also easily to be transported and accumulated in environment (Demirezen, Aksoy, & Uruc, 2007). While from natural processes such as emissions include forest fires and rock weathering. It can lead to release of metals to the environment. These heavy metals can be found in form of sulphides, phosphates and others.

2.2.1 Chromium (Cr)

People can be exposed with chromium by inhaling, drinking, eating and direct skin contact with chromium. Chromium can be found in soils, plants, rocks and animals. Chromium also can be found in three forms which are liquid, gas and solid. Chromium can be relentless in sediments. Chromium may cause disturbance in the nutrient level of the plant (Miroślawski & Paukzto, 2018). Chromium (Cr) also can affects photosynthesis process in term of carbon dioxide fixation, photophosphorylation and enzyme activities (Edelstein & Ben-Hur, 2018). Chromium is used to harden the steel and also to manufacture stainless steel. Chromium also used to produce metal alloys. Chromium has been classified as essential trace elements in human because it can help in glucose usage. However, it is poisonous and dangerous at high concentration. There are health effects which are caused by chromium such as skin allergies and developmental problems. When the chromium is excess from the standard, it can cause death too.

2.2.2 Lead (Pb)

Lead has atomic number 82, atomic weight 207.19 and has a specific gravity of 11.34. It means is a bluish or silvery grey metal. Lead has a melting point of 327.5 °C and also boiling point at atmospheric pressure (Tangahu et al., 2011). Lead can be found in all part of human activities such as fossil fuel burning. Lead has no main function in human. Mostly lead (Pb) exposure was because of industrial processes, smoking and domestic sources (Thurmer, Williams, & Reutt-Robey, 2002). Lead can be used in a lot

of ways. It can be used to produce metal products such as solder, batteries and X-ray shielding services. Nowadays, the most known sources of lead exposure are contaminated soil, household dust, lead crystal and waste from battery industries. Lead also can affect human body's system. Lead also can affect a long term exposure of adults which is weakness in fingers, ankles and increases the blood pressure. When the lead is high can damage the kidneys, brain and also can cause a death (Griswold, 2009). Lead also one of highly toxic heavy metals that can interrupt processes in plants. When there is high level of lead (Pb) in plant, it can cause fastens the production reactive oxygen species (ROS). This ROS can cause damage of chlorophyll so it will prevent from the plant keep growing and damage to cells (Najeeb et al., 2017). Lead (Pb) also can give variety of unwanted effects in human life such as miscarriages and subtle abortions, declined fertility of men which is through the sperm, slow down the children's ability to learn and can cause hyperactive behavior towards the children. Mostly, people will receive their larger Pb intake via food. Lead can get into to foods during storage such as canned food (Mudgal et al., 2010).

2.2.3 Cadmium (Cd)

Cadmium is one of very toxic heavy metals. Cadmium is naturally present or usually found in an environment. It can be found in all types of soils and rocks. It also can be found in coal and fertilizers. Cadmium also can be emitted to air by mines. Cadmium can be used in batteries, metal coatings and plastics. In electroplating, Cadmium also can be used extensively. This heavy metal can be remain in soils for

several decades. Cadmium or cadmium compounds are also known as human carcinogens. Tobacco smoke is one of the biggest sources to the cadmium. Smoking is one of the activities that can get too exposed to significantly higher cadmium level than non-smokers. If human are breathing in high level of cadmium, it can cause damage to the lung. Cadmium also can affect in a long term exposure with low of cadmium level which are leads to kidney disease, fragile bones and lung damage. Cadmium (Cd) can lead to decreasing plant metabolic activity and generate damage of oxidative (Edelstein & Ben-Hur, 2018).

2.3 Heavy Metals In Plants

Heavy metals are not only contributes to soil contamination but it also can affect food production of agriculture, safety and quality of plants (Muchuweti et al., 2006). Plants or vegetables are sources that really important to living things. Plant is very important source of human diets. Many of plants are sensitive with excessive heavy metals (Nagajyoti, Lee, & Sreekanth, 2010). However, if the level of heavy metals is high or the contamination is high, it can cause a very serious health problem such as retardation and kidney damage (Edelstein & Ben-Hur, 2018). Certain heavy metals are very toxic to the plants even at low concentration (Verkleij et al., 2009). Green plants has a properties which is the accumulation of heavy metals in their tissues without exhibiting any toxicity symptoms (Intawongse & Dean, 2006). Heavy metals can give a lot of impact to the plants. For example, heavy metals can make the plant has low quality of plants and not safe for plant growth. High concentration of heavy metals in

plants can affect the growth, productivity and yield of many crops. Plants which is used for medicinal that growing in polluted area may eventually can cause the quality of the plants and also the quantity decreased (Asgari Lajayer, Ghorbanpour, & Nikabadi, 2017). Heavy metals that can be found in plants are such as zinc (Zn), chromium (Cr) and iron (Fe). Zinc (Zn) and cadmium (Cd) can decrease plant metabolic while iron (Fe) can cause damage of membranes.

2.4 Metals Uptake In Plants

Contamination of heavy metals in agricultural is a major concern. Excessive heavy metals accumulations in agriculture not only result in soil pollution but also lead to heavy metal uptake by crops. It can also give negative impact which are effect on quality of product and food safety (Muchuweti et al., 2006). Plants have an ability which is they can absorb or uptake the heavy metals as a nutrient from the soil which are very important for their growth (Anal, 2014) . When the plant absorbs excess heavy metals it may enter to the food chain act as one of the main pathways for human exposure. Hence, human will get health effect from consuming plants or vegetables that grown in contamination soil (Intawongse & Dean, 2006). Human are too exposed to high level of heavy metals so they have high probability to get various disease such as cancers, gastrointestinal and renal failure Sources that can influence the contamination in crops are type of cultivar and cultivation method (Wang, Takematsu, & Ambe, 2000). Normally metal uptake is increased in plants that grown at the areas that filled with increased contaminated soils.

Metal uptake by plants taken up by the roots is considered as bioavailable fraction. This term of bioavailability refers to where any chemicals that can be absorbed by plants. The roots of the plants are a part that has special interest which means that the contaminants can be adsorbed by the root. Heavy metals can get into the xylem stream via the root symplasm but the metals ions must cross plasma membrane to make this happen (Tester & Leigh, 2001). Heavy metals uptake at leaf can occur through the stomata, lenticels, aqueous pores and cuticular cracks (Fernández & Brown, 2013).

After that, the contaminants will be subsequently stored or metabolized by the plants. For a lot of contaminants, the passive uptake of heavy metals is via microspores in the root cell walls that may be a major route into the root. Heavy metals can affect plant growth even at low concentrations. The higher the concentration of heavy metals, the plant growth will be decreased drastically (Helena et al., 2018). Heavy metals induced oxidative stresses which can have effects on photosynthetic processes. While elevated concentration of heavy metals can cause disturbance the coordination mechanisms between essential elements.

2.4.1 Chromium (Cr) Uptake in Plants

Chromium (Cr) is known as top seven most abundant element on the earth and sixth place for most abundant transition metal (Mohan & Pittman, 2006). Chromium (Cr) can be found or detected in any environmental problems such as air, water and soil pollution (Velma, Vutukuru, & Tchounwou, 2009). There are several factors that influencing Cr absorption in plants such as temperature, plant size, type of plants and

moisture content (Yamamoto & Kozlowski, 1987). Chromium (Cr) have some effects to plants such as it can causes reduction on growth and plant productivity (Zayed et al., 1998). It is also effect on photosynthesis and mineral nutrition (Nagajyoti, Lee, & Sreekanth, 2010).

2.4.2 Lead (Pb) Uptake in Plants

Lead (Pb) is one of the most known as has highly toxicity and It is usually used in many industries (Cecchi et al., 2008). Sources of lead contamination are usually comes from smelting, sewage sludge and fertilizers (Gupta et al., 2009). Lead (Pb) absorption by the roots and once it was absorbed onto rhizoderm roots, it can enter to the root passively. Once the lead (Pb) has permeate into the root system, it can accumulate aerial parts of plants. Mostly, absorbed lead was accumulated in the root and only a few fraction accumulated in aerial plants (Piechalak et al., 2002). Transportation of any heavy metals in plants including lead (Pb) will be transported from the roots to shoots and it must pass by xylem (Verbruggen, Hermans, & Schat, 2009).

2.4.3 Cadmium (Cd) Uptake in Plants

Cadmium (Cd) is known as a metal with unknown essential function but it is easily be absorbed by plants (Staessen et al., 1992). Cadmium (Cd) also act as plant nutrient. Excessive consumption of Cd in contaminated food crops can lead to toxicities to animal, humans and environment. Cd also can alter the uptake of minerals in plant by

its effect on availability of minerals. Cadmium ions are readily absorbed by plants and can be equally distributed over the plant. Cadmium can be taken up from the roots to the upper of plant which is leaves. After that, people are more exposed to cadmium because they are consuming plants. Seafood also one of sources of cadmium such as molluscs (Mudgal et al., 2010).

2.5 Factors that Affecting the Uptake Mechanisms

There are several factors that can affect the uptake mechanism of heavy metals is shown in Figure 2.2.

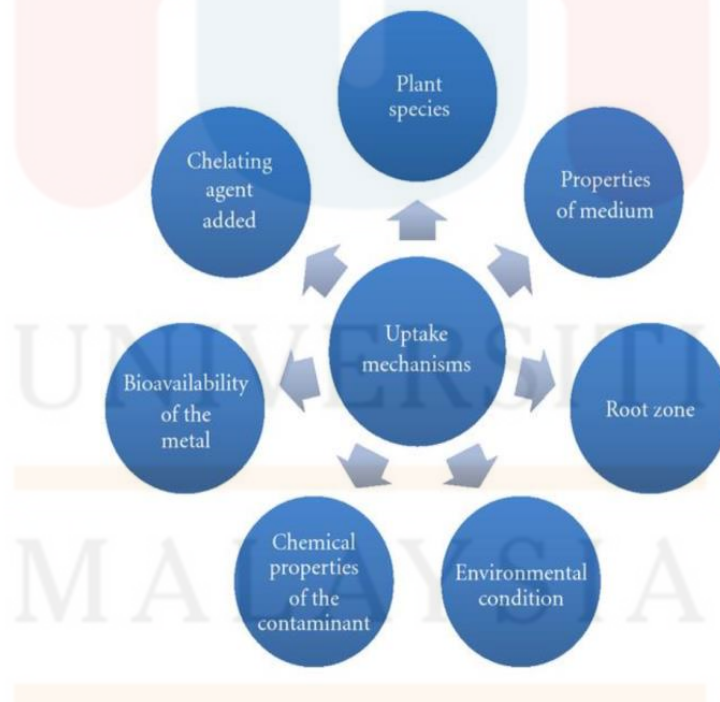


Figure 2.2 : Factors that Affecting the Uptake Mechanisms (Tangahu et al., 2011)

2.5.1 Plant Species

The uptake of heavy metals can be affected by characteristics of the plants (Joel Burken & Jerald Schnoor, 1996). Plants have potential as heavy metals bioaccumulator from soil and water that contaminated. Some plants have different responses to different heavy metals exposure. Some of plants are very sensitive to the heavy metals while some of the plants can tolerate with heavy metals and keep growth as well (Sumiahadi & Acar, 2018).

2.5.2 The Root Zone

Root is the crucial part in a plant. A plant can grow healthy if the root is growing healthy. Roots take water and nutrients via osmosis and can act as support or storage. Roots can absorb the contaminants and store it inside the plant tissue (Tangahu et al., 2011).

2.5.3 Environmental Conditions

The uptake of heavy metals in plant can be affected by environmental conditions. For example, temperature which is means can affect the growth of plants and the crucial part of plant which is root. Heavy metals uptake by plants also depends on the bioavailability of metal. Furthermore, when the heavy metals are bound to soil, pH and others, they will affect the tendency of heavy metals to exist in plants (Tangahu et al., 2011).

2.6 Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectroscopy is commonly technique used in a lot of analytical laboratories in determination of trace elements (Soodan et al., 2014). AAS also widely used for industrials for metal content, quality control of metals, testing water quality for heavy metals.

Atomic absorption spectroscopy can be used to measure concentrations of heavy metals in soil extract and plants, and air. The atomic absorption spectroscopy can also measure the amount of energy in the form of photons and light absorbed. Atomic absorption spectroscopy (AAS) can be in qualitative and quantitative analysis. Quantitative means to determine the amount of analyte in the sample while qualitative is refers to determine whether the analyte is present in the sample or not.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Materials

The apparatus used in this research were 50 mL volumetric flask, beakers, micropipette, filter papers and 50 mL and 15 mL falcon tubes, filter funnel and syringe filter, pestle and mortar and blender. For materials, *Cymbopogon citartus* plants, soils, pots, sample bags and porcelain crucibles also have been used in order to complete all the processes that needed for this research. The chemicals that have been used such as hydrogen peroxide (H_2O_2), nitric acid (HNO_3) and distilled water.

3.2 Methods













There are three methods have been applied in this research which are wet digestion, standard series of dilution and determination of heavy metal by using Atomic Absorption Spectroscopy (AAS). First process was started with synthetic mixture of soil sample. Second process was continued with plant monitoring and sample preparation. The first method was done by using wet digestion method which is acid digestion method for plants. Further method was a standard series of dilution. Last method was applied identification or analysis of heavy metals in different parts of *Cymbopogon*

*citratu*s plants which are leaves, roots, stems by using Atomic Absorption Spectroscopy, AAS which is based on standard condition.

3.2.1 Synthetic Mixture in Soil

In this process, the soils was collected from Kg Kusial, Tanah Merah, Kelantan. The soils also were analyzed first as initial by using Energy Dispersive X ray Spectroscopy (EDS). After that, the soils were polluted by using these three selected heavy metals which are Pb, Cr and Cd. These heavy metals were injected by using syringe with amount 5, 10, 15, 20 and 25 mg/L into the pots which is containing soils that have been collected. All the pots were labelled as pot 1 until pot 12. Pot 1 act as control which is containing plant and soil only while pot 12 also acts as control but containing soil and heavy metals only. The arrangement of pots which was located at UMK Jeli's nursery had shown in Table 3.1;

Table 3.1 : The arrangement of pots for this thesis.

Control	Concentration	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
 Pot 1(Plant)	Main pot	 Pot 2	 Pot 3	 Pot 4	 Pot 5	 Pot 6
 Pot 12 (Heavy metal)	Duplicate pot	 Pot 7	 Pot 8	 Pot 9	 Pot 10	 Pot 11

3.2.2 Plant Monitoring

Cymbopogon citratus plants were planted in a pot within up to 30 days. The pots were placed at herbal area in Universiti Malaysia Kelantan, Jeli. The plants were chosen by using its characteristics and features. The all pots were put at the same place but arranged to two rows because of limitation space and to differentiate between main and duplicate. The temperature of the plants depends on the surrounding of the area where the plant is planted. Usually the temperature will be at 27 °C until 29 °C based on the weather on the day. For watering steps, around 200 mL of tap water was used for each pot in order to make sure that the plants will get sufficient water at morning and evening. The plants were watered for twice for first and second week because they are still early grow but after two weeks, the watering step became once in a day because the plant already matured. Every 2 weeks, the length of leaves has been recorded in order to know their growth.

3.2.3 Water monitoring

Below each of pots, a plastic container was put to collect the water from watering steps. This is because not all of water will be absorbed by plant and soils. Next, the collected water in week 4 and was run by using Atomic Absorption Spectroscopy (AAS) to know exactly the amount of heavy metal in that water. Other reason by put the container is to avoid all of the heavy metals flow to the ground and contaminate the soils around the pot and the nursery. The water collected also was tested the pH by using pH

meter. Before the water was tested by pH, the water was filtered by using filter paper into 50 mL of falcon tubes.

3.2.4 Soil monitoring

Soil was collected for twice which are in week 2 and week 4. This is because to monitor the distribution of heavy metals in soil in week 2 and week 4. This is also to know the amount of heavy metals in soil because some of heavy metals are not going straight down to the soil in the pot and may be the heavy metals are being absorbed by plants or water under pot. Soil monitoring also done because of to know whether the amount of heavy metals in soil is tally with the concentration that have been decided to put in each pot which are 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm. Next, the soils were collected by using different sample bag to avoid from the soil fall to the ground. 50 mL beaker and pH meter was used for determination of pH. 8 g of soil sample was weighed and put into 50 mL beaker. 30 mL of distilled water was poured into the beaker and stirred it for two minutes. The beaker was put aside for ten minutes to get a dissolved soil. Then, pH meter was put into the distilled water first to get neutral reading and it was put into the 50 mL beaker that containing soil sample.

Moisture content of collected soils from every pots have been calculated. In determining of soil moisture content, gravimetric method was the most accurate method and also preferred method based on (Lunt, 2005). This soil moisture content was used to determine water presence in the soil. It was measured by using gravimetric method that involved weighing the wet soil that wet or before the soil was inserted into the oven for

5 days with 60 °C to get a completely dried soil. After being inserted in an oven, the sample was reweighed again to determine the amount of moisture removed.

Generally, soil moisture is important because it can help to determine amount of water in soil. Water is also important to the plant especially for growth because water can act as nutrient itself. It also can regulate the soil temperature and helps in chemical and biological activities in soil and plant (Walt, 2015).

Soil moisture was determined by using a formula which is dividing the difference between wet and dry masses from the soil sample. After that, it was multiplied by 100 in order to get the percentage of water. The gravimetric soil moisture content method was calculated by using the formula below (Equation 4.1);

$$\text{Moisture content \%} = \frac{W_2 W_3}{W_3 W_1} \times 100 \quad (\text{Equation 4.1})$$

Where;

W_1 = Weight of aluminium foil (g)

W_2 = Weight of wet soil + aluminium foil (g)

W_3 = Weight of dried soil + aluminium foil (g)

Next, the soil was inserted into the oven for 5 days with 60 °C to get a complete dried and to get moisture content. While for pH by using pH meter, the dried sample was crushed by using pestle and mortar and sieved through 2 mm screens. 8 g of dried

sample was taken and put into a 100 mL of beaker and was filled up with distilled water. Then, the beaker was shaken vigorously and stirred. The solution was kept for 10 minutes in order to get a complete dissolve of solution. Next, the pH was tested with pH 7 buffer solution before any readings was taken.

3.2.5 Sample Preparation

The plants were cut and separated to three different parts which are stems, roots and leaves. After that, these different parts of plant have been rinsed into the distilled water. This is because to clean the three parts which are roots, leaves and stems from the soil. The samples were dried for three days in order to get complete dry sample to calculate the moisture content of plant samples. The sample was dried by using oven at 65 °C. Then, plant samples were grounded by using a blender.

3.2.6 Wet Digestion Method

The dry plant sample was ground by using a blender and weighed for 0.5 gram. Then, 0.5 g of plant samples was put into the crucible and 5 mL 65 % of nitric acid (HNO_3) was added into the crucible in the fume cupboard. The solution in crucible was stirred by using a glass rod. When the all material was wet, then 4 mL of 30 % of hydrogen peroxide (H_2O_2) was carefully added into the crucible and stirred. All this process has done in a fume cupboard in order to avoid release of harmful substances. The crucible was heated on the hot plate at 180 °C. After that, a strong effervescence

was produced. The crucible was heated for 8 minutes and the solution was allowed to cool. For the cooling process, it cooled for 5 minutes beside the hot plate and a slightly yellow dissolution still remained in the crucible.

After that, the solution was filtered by using standard filter paper and was put into 50 mL of volumetric flask. When the solution was filtered, distilled water was added to the reach mark and shake upwards and downwards. Then, the solution that has been diluted in 50 mL volumetric flask was filtered again by using syringe filter into 50 mL of falcon tubes. Usage of syringe filter in this process is for removal of any solid and undissolved material before further to analysis process. The all steps were repeated for each part of plants from different pots (Pequerul et al., 1993).

3.2.7 Standard Series Dilution

The standard series dilutions were prepared for the establishment of AAS analysis calibration graph. The standard series of dilution were conducted with preparation of stock solution by using 50 mL of volumetric flask. From 1000 ppm of pure heavy metals (Cr, Pb, Cd) was diluted in order to obtain 100 ppm and further successive dilution to get 5, 10, 15, 20, 25 mg/L as series of Cr, Pb, and Cd standard solution. In order to get 100 ppm for stock solution, Equation 3.1 was used to obtain the amount of volume that will be pipetted to get standard solutions.

$$M_1V_1 = M_2V_2 \quad \text{Equation (3.1)}$$

Where,

M_1 = The concentration in molarity of concentrated solution

M_2 = The concentration in molarity of diluted solution

V_1 = The volume of concentrated solution

V_2 = The volume of diluted solution

For this formula, same volumetric flask has been used for dilution which is 50 mL and it considered as V_2 . V_2 is the volume of diluted solution. The constant concentration in molarity of concentration solution which is M_1 , 100 mg/L was used to obtain V_1 which is the volume of concentrated solution. Different concentration was used to calculate M_2 which is the concentration in molarity of diluted solution. After that, volume of concentration solution was obtained after all the values have been inserted. Table 3.2 shows the values that have been inserted by following the formula.

Table 3.2: The volume of concentrated solution have been obtained after been calculated.

M_1	M_2	V_2	V_1
100 mg/L	5 mg/L	50 mL	2.5 mL
100 mg/L	10 mg/L	50 mL	5.0 mL
100 mg/L	15 mg/L	50 mL	7.5 mL
100 mg/L	20 mg/L	50 mL	10 mL
100 mg/L	25 mg/L	50 mL	12.5 mL

3.2.8 Determination Of Heavy Metal Concentration By Using AAS

After digestion process, the methods were continued by obtaining diluted solution. This diluted solution was used for determination heavy metal concentration by using Atomic Absorption Spectroscopy (AAS). 50 mL of diluted solution was transferred to 50 mL falcon tubes. Before the diluted solution was transferred into the falcon tubes, the solutions were filtered by using syringe filter. It is act as stock solution. After diluted solution was transferred into the falcon tubes, the tubes were labelled as (A), (B), (C) and (D) respectively for analysis solution. Next process is 15 mL of solution was transferred to 15 mL of falcon tube to be act as analysis solution. 13.5 of distilled water was added into each of 15 mL of falcon tubes. Next, 1.5 mL of solution was transferred into the first falcon tube which is labelled (A). The falcon tube has been shaken gently. After that, 1.5 mL of solution from the tube (A) was transferred into the second falcon tube and became dilution for (B) solution. The tube was shaken gently. The steps were repeated until the forth falcon tube. The heavy metal concentration was determined by using the flame atomic absorption spectroscopy (Intawongse & Dean, 2006).

3.2.9 Heavy Metals Distribution

Three parts of *Cymbopogon citratus* plant was examined by using AAS to determine the amount of heavy metals or heavy metals accumulation in these parts of plant. This is one of the main objectives of this project. The effectiveness of heavy metals movement from roots to leaves can be determined by using translocation factor

formula. While for uptake and bioaccumulation of heavy metals from soil to plant can be determined by using bioaccumulation factor formula (Deng, Ye, & Wong, 2004). Plant with both BF plant and TF values > 1 can be used as a suitable candidate for hyperaccumulation of metals, while plants with TF < 1 can phytostabilize metals in roots (Gołda & Korzeniowska, 2016). The translocation factor and bioaccumulation factor was calculated by using Equation 3.2;

$$\begin{aligned} \text{Bioaccumulation Factor} &= \frac{\text{Concentration of metal in plant}}{\text{Concentration of metal in soil}} \\ \text{Translocation Factor} &= \frac{\text{Concentration of metal in plant shoot}}{\text{Concentration of metal in plant root}} \end{aligned} \quad (\text{Equation 3.2})$$

3.3 Data Analysis

The data obtain was analyzed by using ANOVA analysis. ANOVA analysis is used to test general rather than specific differences among means. ANOVA also used to assess the relationship between the heavy metal concentrations and calibration graph that get from AAS analysis (Qishlaqi & Moore, 2007). The result was obtained from by using ANOVA analysis and the result have revealed the lower or higher values of concentration of three selected heavy metals.

CHAPTER 4

RESULT AND DISUSSION

4.1 Soil Physical

Initial soil has been observed by using SEM-EDS to know the elements in it. The initial soil has 0.0120 mm. This soil has been determined as clay loam. Based on determination of type of soil process, the soil has 40 % clay, 20 % silt and 40 % sand. A jar was used to put original soil and water to determine layer of the soil and know the class of soil. pH of this soil that have been used for this research is pH 6.6. This is common pH used a plant that always been used for agricultural (Lake, 2000). Elements that presences in this soil are Pb, Cd, Cr, Fe and Ca as shown in (Table 4.1);

Table 4.1 : Elements and chemical composition in initial soil by using EDS

Soil		
Element	Weight (%)	Atomic (%)
C K	15.52	24
O K	49.23	57.16
AL K	1.64	1.13
Si K	24.98	16.52
W M	7.34	0.74
Pb M	0.03	0
Cd L	0.1	0.02
Ca K	0.3	0.14
Cr K	0.18	0.06
Fe K	0.69	0.23

4.2 pH

4.2.1 pH of Water

Water that had been collected under a pot was observed and monitored their pH by using pH meter. Water was collected at week 2 and week 4. This process was analyzed due to know the pH of water after put the selected heavy metals into a pot. After that, due to know the difference between pH of water from original pot and the other pots which are containing five different concentrations. The average of pH of water from week 2 and 4 has been recorded in Figure 4.1;

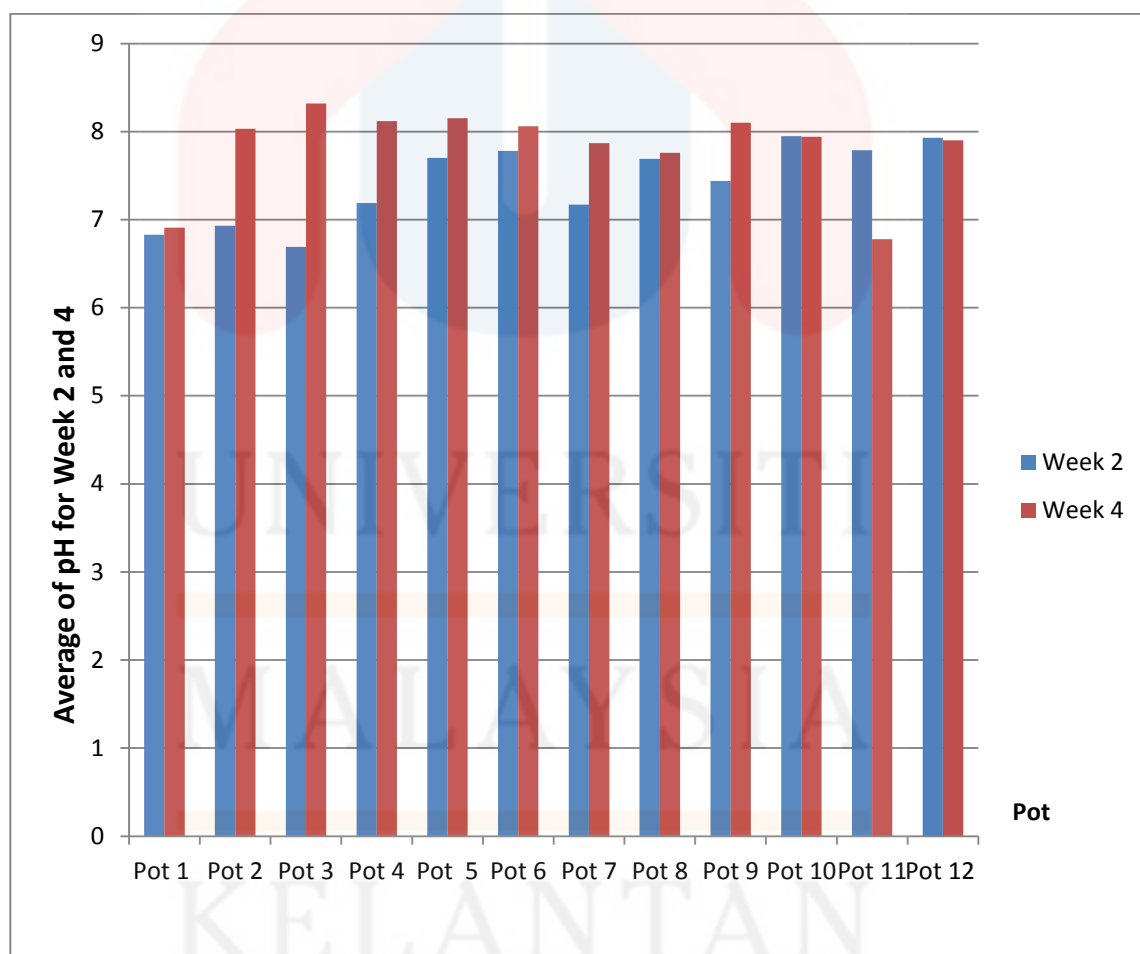


Figure 4.1 : The average of pH for water week 2 and week 4

According to Figure 4.1, there is increasing average of pH for main pot and duplicate pot compared to pot 1 which is contained plant only. Main pots are referring to pot 2 until 6 which is contained 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm respectively. While for duplicate pot was done too for observation or reference for main pots.

Based on Figure 4.1, pot 1 is the control pot which contained plant and soil only without any heavy metals poured into it. Due to monitor and get the difference between the pH from original pot and each of pots that containing different concentration of Cr, Cd and Pb. Pot 2 was contained with 5 ppm of Cr, Cd and Pb and act as main pot for 5 ppm concentration. While pot 7 is act as duplicate for 5 ppm of Cr, Cd and Pb. This research was done duplicate due to get more observation or acts as reference to the main row. Pot 3 is paired with pot 8 which is containing 10 ppm of these three selected heavy metals Cr, Cd and Pb. Pot 3 is act as a main 10 ppm and its pair is act as duplicate.

After that, pot 4 was contained 15 ppm of Cr, Cd and Pb and same goes to pot 9 but pot 4 is a main for this concentration and pot 9 is a duplicate for this concentration. Next is pot 5 and pot 10. They were contained with 20 ppm of Cr, Cd and Pb and pot 5 is the main concentration while pot 10 is a duplicate. last concentration is 25 ppm. This concentration was contained in pot 6 as main pot and pot 11 as duplicate pot for this concentration. Pot 12 is a control pot which contained heavy metals, soils in but without any plants.

pH measurement is essential for this plants and other plants too. This is because any type of soils will directly or indirectly affect the crops. It means from soil, it can

affect the agriculture activities, water quality and it is also function as a medium for plant growth. It means it is important to monitor the pH of water (Patra, Pradhan, & Patra, 2018a). The graph in Figure 4.1 shows that for week 2, mostly all of concentrations have increasing pH compared to original pot which is pot 1. However, all pot shows increasing pH except for pot 3 at week 2 which is containing 10 ppm and pH 6.69 but it is only a little different with the original pot 1 which is pH 6.83. For the main row, the highest pH is at pot 6 pH 7.78 which is containing 25 ppm. While the duplicate 25 ppm which is pot 11, it is has almost same pH with the 25 ppm at main row. Last is comparison of original pot which is pot 1 that only containing plant and pot 12 that containing heavy metals but no plant in it. Pot 1 has almost equally of pH for week 2 and 4. Same goes with pot 12 which is has almost equally pH for week 2 and week 4. World Health Organization (WHO) normal ranges for pH is 6.5 – 8.5 (Nazir, Khan, Masab, Rehman, Rauf, Shahab, Ameer, Sajed, Ullah, Rafeeq, et al., 2015). pH from the water sample that had been collected in week 2 and 4 was recorded within normal ranges based on WHO normal ranges.

4.2.2 pH of Soil

Soil for each of pots was collected every two weeks for one month. The average of soil pH for week 2 and week 4 has been recorded in Figure 4.2;

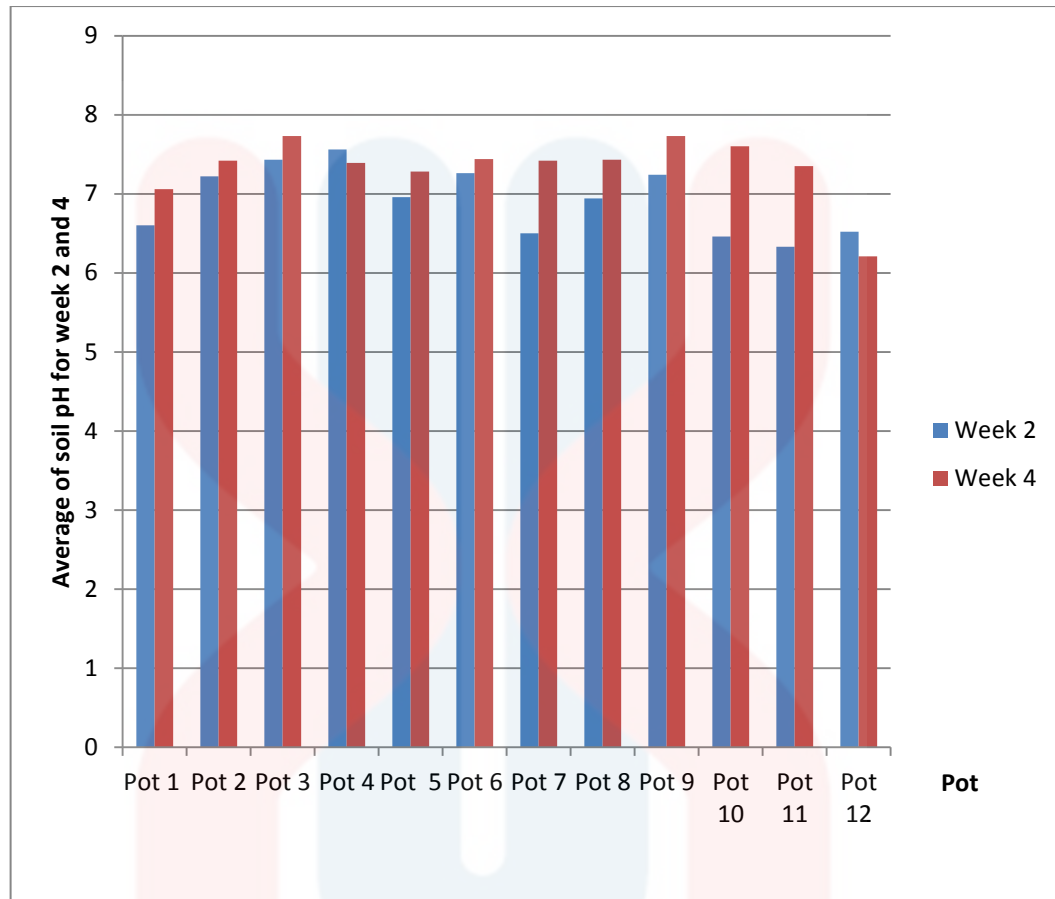


Figure 4.2 : The average of soil pH for week 2 and week 4

Each of pot has their own duplicate. Duplicate was done to get more observation and consistency. Pot 2 is a main row for 5 ppm while its partner which is act as duplicate is pot 7. Pot 3 is paired with pot 8 and contained with 10 ppm of Cr, Cd and Pb. After that, pot 4 has 15 ppm of Cr, Cd and Pb and same goes with pot 9. For 20 ppm, same like others pot which is paired with duplicate. Pot 5 represents main 20 ppm and pot 10 as duplicate. Last concentration is 25 ppm and was represented by pot 6 as main and pot 11 as duplicate. Pot 1 is the original pot that only plant and soil but no heavy metals poured into it. Last pot is pot 12 that containing heavy metals and soil only but no plant.

Besides that, pH measurement is important because the water sample for this research was collected directly under the soil. According to (Singh, 2008), soil is a medium for plant growth and act as sponge. Soil is called sponge because it must store and supply water and nutrients for the plants (Singh, 2008). This is the reason why pH of soil is lower than pH of water because soil act as sponge that absorb heavy metals that been poured into the pot before it going down to the container. Container refers to a container that used for water collection. Based on Figure 4.2, there are increasing pH for every pots that containing different concentrations for the main row which are pot 2 until pot 6 compared to original control pot 1. The original pot which is containing plant only and without any heavy metals poured into it has pH 6.6. For week 2, the main row shows increasing pH of soil. The highest pH for week 2 is pot 4 and it's duplicate also shows high pH of soil. While for week 4 is pot 3 and the duplicate also has almost same pH. pH from the soil sample that had been collected in week 2 and 4 was recorded within normal ranges based on WHO normal ranges which is 6.5 – 8.5 (Nazir, Khan, Masab, Rehman, Rauf, Shahab, Ameer, Sajed, Ullah, Rafeeq, et al., 2015).

4.3 Growth Observation

During the experimental period of 30 days, the growth of *Cymbopogon citratus* plants was observed at different concentration levels. Each pot has different concentration of Cr, Cd and Pb; 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25. The plants were planted into a pot and put into the nursery at Universiti Malaysia Kelantan, Kampus Jeli for 30 days. Half matured of *Cymbopogon citratus* was used and planted into each

of pot. Half matured *Cymbopogon citratus* was used because to observe the growth of the plant after put these selected of heavy metals; Cr, Cd and Pb into the pot.

Length of leaves was recorded weekly in order to know the growth of *Cymbopogon citratus* in Figure 4.3 by using ruler. In Figure 4.3, from week 1 until week 4, the measurement of leaves is increasing even though there is presence of heavy metals in it. From the growth of plants, the plant was seen to be able to adapt the presence of heavy metals. The higher length of leaves is at the higher concentrations which are 20 ppm and 25 ppm. The length of leaves is higher at higher concentration of heavy metals compared to lower concentration is because the plants can adapt or stand or and has high availability to accumulate higher concentrations of heavy metals in it (Patra, Pradhan, & Patra, 2018a).

This result is same with the duplicate which is the higher length of leaves also at high concentration. The possible reason for plant growth even though at low concentrations due to the fact that heavy metals that can affect Fe availability in soil or cellular. From the journal written by (Patra, Pradhan, & Patra, 2018b), they stated *Cymbopogon flexuosus* which is in same class with *Cymbopogon citratus* has high growth of plant even though at low concentration 10 ppm. In previous study, *Cymbopogon flexuosus* has high ability towards plant growth like *Cymbopogon citratus* because Cr in this plant inhibited root to deliver nutrients to the plant. This is leads to plant growth and development of the plant (Patra, Pradhan, & Patra, 2018a).

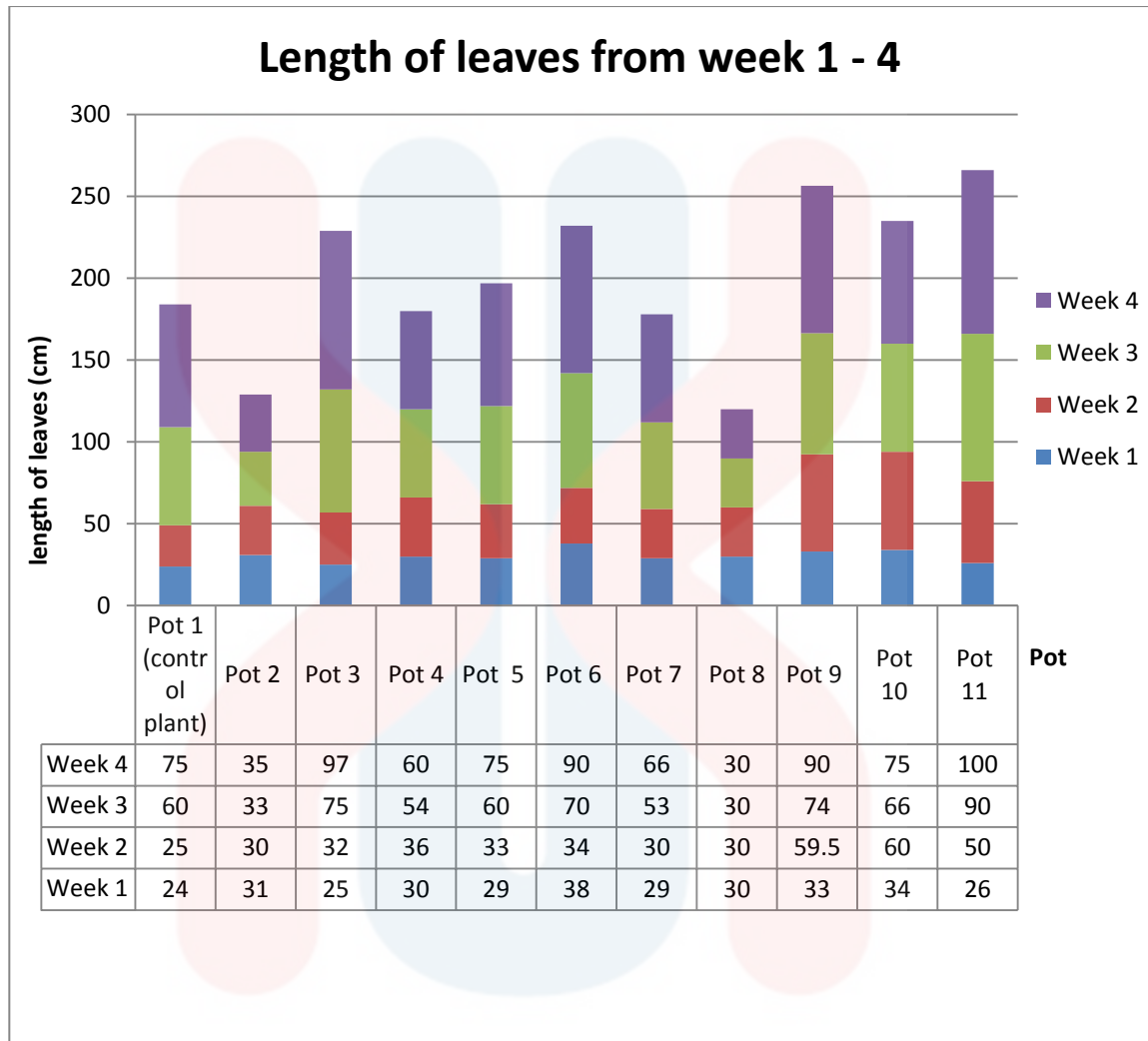


Figure 4.3 : The length of leaves of *Cymbopogon citratus* from week 1 until week 4

Based on Figure 4.3, pot 1 represents the control pot which is only containing plant and soil only. There were no any selected heavy metals poured into it. It also acts as a reference to the other pot due to check the difference between the original pot and other pots. Pot 2 to pot 6 acts as the main row for different concentrations for this project. Pot 2 to pot 6 was contained with 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm respectively. While the other pots (pot 7 to pot 11), they act as duplicates due to get a consistent observation for the main row.

From week 1 until week 2, the length of leaves was not too increased. This is happened due to because of the plants still in adaptation process which is in each of pot have different concentration of three types of heavy metals and the new place. Usually *Cymbopogon citratus* plants were planted on the open surface ground but for this project, the plants was planted in a pot. It can give an effect on the plants which is because of limited space for growing *Cymbopogon citratus* plants. While in week 3 and week 4, the growth or length of leaves for *Cymbopogon citratus* were growing with quite rapidly. Due to sufficient of water which is also plays a role in the growth of plants and 250 mL of water was used to get sufficient and consistent water. In plant, there is a process called transpiration which is water moves up to the stem and leaves from root when the water is lost because of evaporation. This continuous flow of water keeps the plant strong and get sufficient water and nutrients (Vanstone, 2014).

4.4 Moisture Content

4.4.1 Soil Moisture Content

Moisture content was calculated to know the amount of water content in the soils. In general, if the moisture content of soil is optimum, so the plants can readily adsorb the water. Logically, not all of the water will be adsorbed in a soil but sometimes plant will absorb the water (Walt, 2015). The soils was being contaminated by using five concentration which are 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm so it is important to calculate moisture content. In order to know the distribution of heavy metals because water that used from watering steps helped the heavy metals distributed.

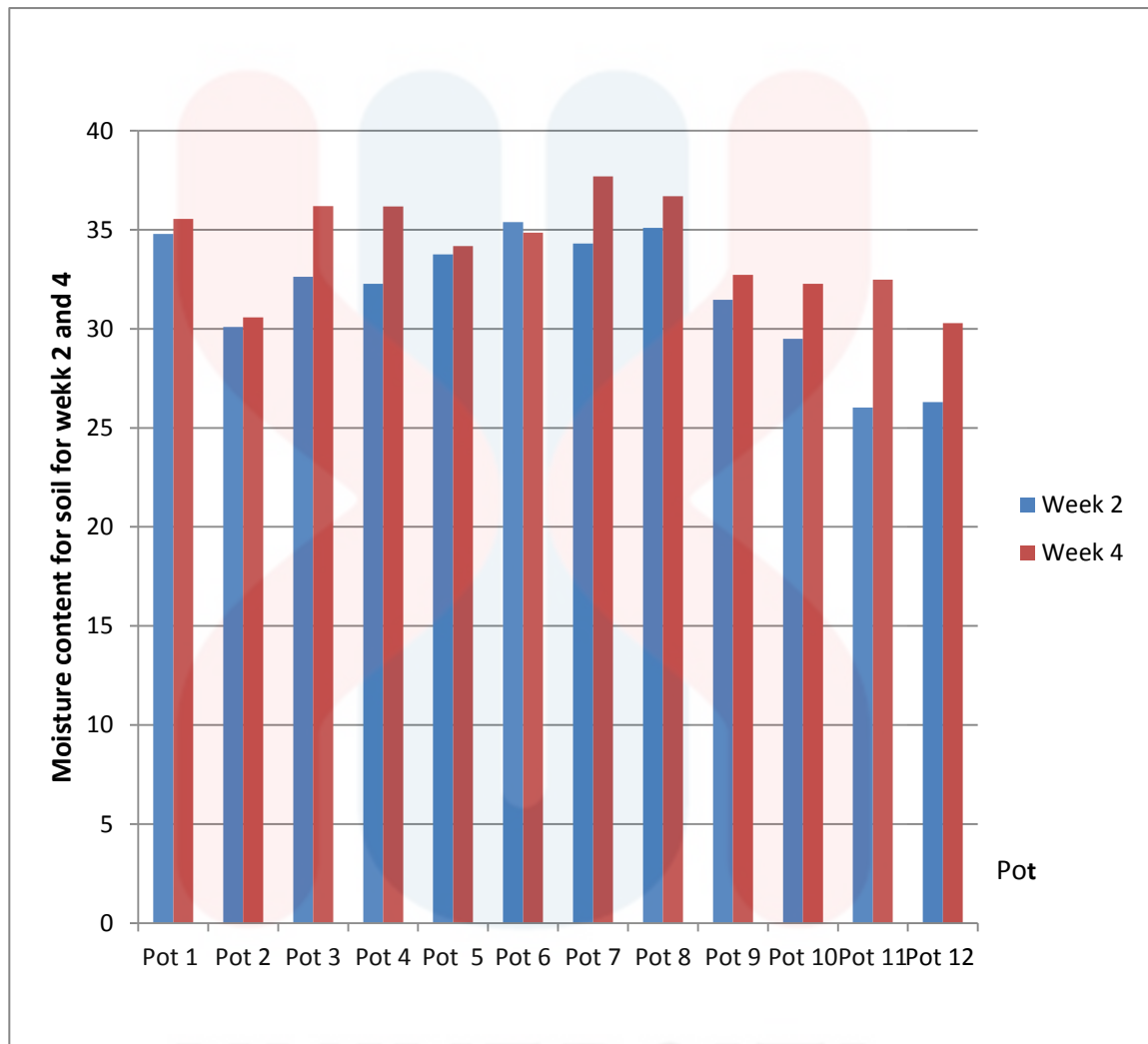


Figure 4.4 : The moisture content for soil at week 2 and week 4

Based on the result of moisture content Figure 4.4, week 4 have higher amount of water in soil compared to week 2. The highest amount of moisture content is pot 7 in week 4 which is containing 5 ppm of Cd, Cr and Pb in it. Pot 7 is also act as a duplicate. It may have a relationship between the growth and the moisture. When amount of water in soil is high so the water in it, it was distributed to the leaves and act as nutrients.

When the moisture content of water is high, it means the plant have many nutrient as it can acts as nutrient itself based on journal from (Walt, 2015).

4.4.2 Plant Moisture Content

Moisture content of *Cymbopogon citratus* plant was calculated in order to know the amount of water available in the plant. The plants were divided to three parts which are leaves, stems and roots. This is because not all of the water will be absorbed in roots only but it may be absorbed into leaves and bulbs. Water is very important for plant growth. If the plant don't get sufficient amount of water, it can reduce plant growth. In other words, it can disturb plant functions and food chain and can cause the plant die. Like a statement above, most of water that enters through the plants roots does not stay in that part only. Some of it will be withdrawn by the plant is actually used in photosynthesis (Thomas F. Scherer, 2017). Too much of water also can contribute to negative effect to the plants. It means when the plant have too much water, the plant cannot get enough oxygen in the soil but when the plant have insufficient water, the food or nutrients cannot be distributed to the whole part of plants. So, the plants need sufficient water only. Moisture content for three part of *Cymbopogon citratus* plant was recorded in Figure 4.5;

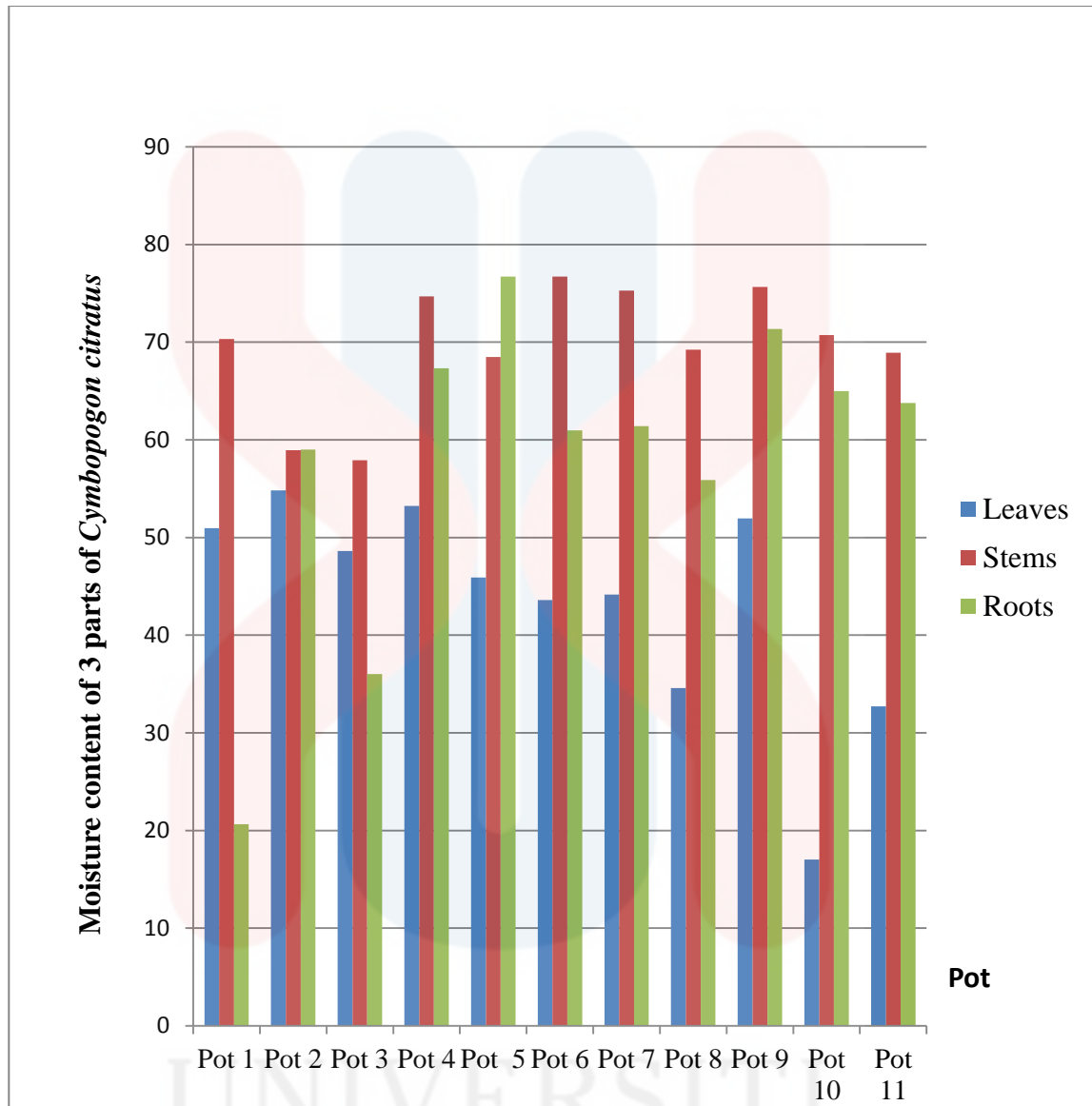


Figure 4.5 : The combination of moisture content of three parts (leaves, stems and roots) of *Cymbopogon citratus*

According to Figure 4.5, mostly of water is in the stems and then in roots and leaves. Leaves have low amount of water is because of mostly of water at the leaves will loss via transpiration. Constant of water that loss via transpiration causes a negative water pressure in the leave's parts. Based on Figure 4.5, pot 1 has high moisture content at leaves compared to roots. It shows at pot 1 has high amount of water because not all

water has loss via transpiration Roots will push the water content towards to leave's parts (Oztas & Bozkurt, 2011). This negative pressure of water is works like a suction force which is pulling up the water from the stem. Water will be moved up to the stem due to response of suction that caused by transpiration. There are two forces that involved which are adhesion and cohesion. Adhesion means the tendency of water to stick to another places or surfaces while cohesion means the tendency for water to stick together (Oztas & Bozkurt, 2011).

In the stem, there is a process called capillary action. Stem is generally known to structurally adapted to take advantage of capillarity. This is because they have long narrow diameter. In roots, the water can move up to the stem via a push force from the roots zone. Usually, roots will absorb most of water because in order to distribute the water along the plants, it must through root zone first. Due to the negative water pressure in the roots, the water can be absorbed by the roots (Oztas & Bozkurt, 2011). This movement will make the water move up to the stem. This is the reason why the stem have a lot amount of water compared to the roots and leaves.

4.5 Heavy Metals Accumulations in Three Parts of Plant

Translocation factor and bioaccumulation factor was done to determine the effectiveness of heavy metals movement from roots to leaves and for uptake of heavy metals (Deng, Ye, & Wong, 2004). The result of translocation factor and bioaccumulation factor was recorded in Figure 4.6 and Figure 4.7;

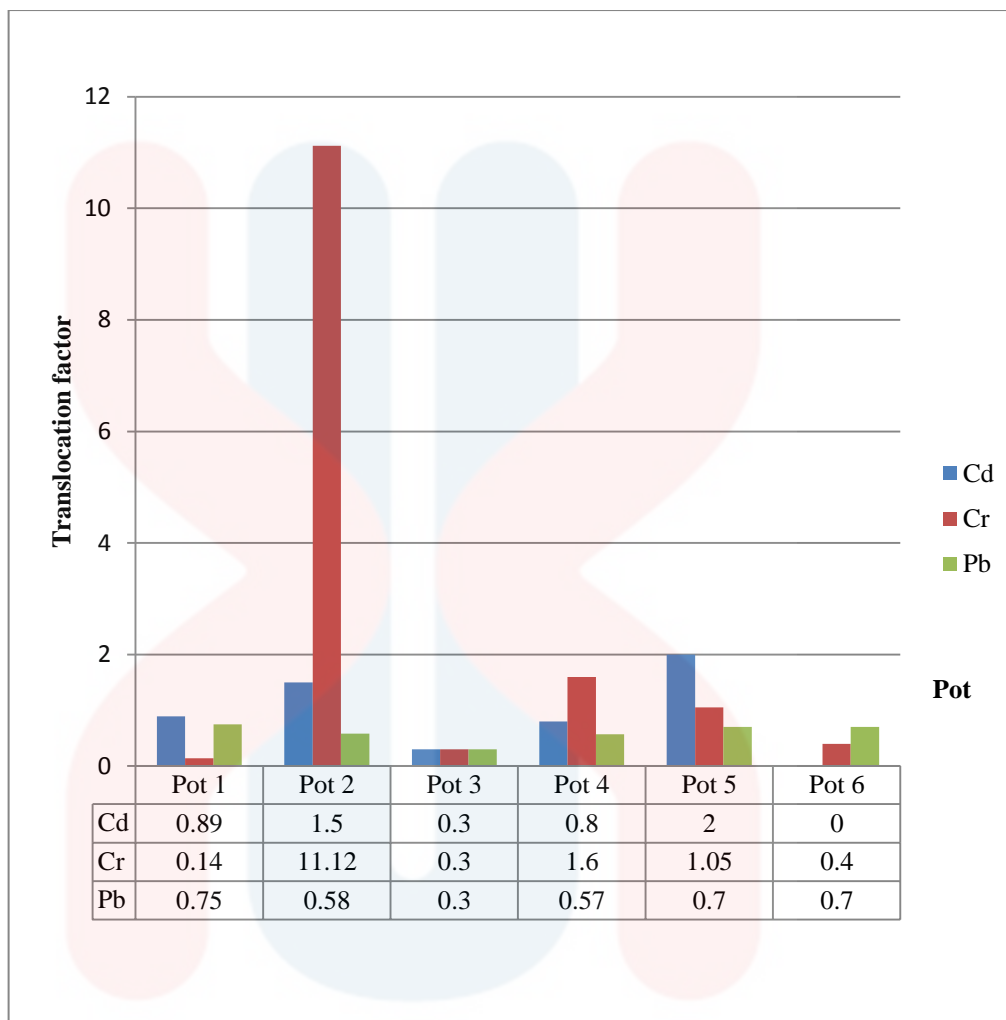


Figure 4.6 : The translocation factor for heavy accumulation

\ According to Figure 4.6, translocation factor for Cr in every concentrations of *Cymbopogon citratus* are greater than 1. Some of Cd concentrations at 5 ppm and 20 ppm has value of translocation factor greater than 1. Due to the translocation factor of Cd and Cr are greater than 1, it shows that *Cymbopogon citratus* can phytostabilize metals in roots. If the translocation factor is less than 1, it means the controlled movement of heavy metals from root (Gołda & Korzeniowska, 2016). Based on Figure 4.6, Pb has all translocation values that less than 1.

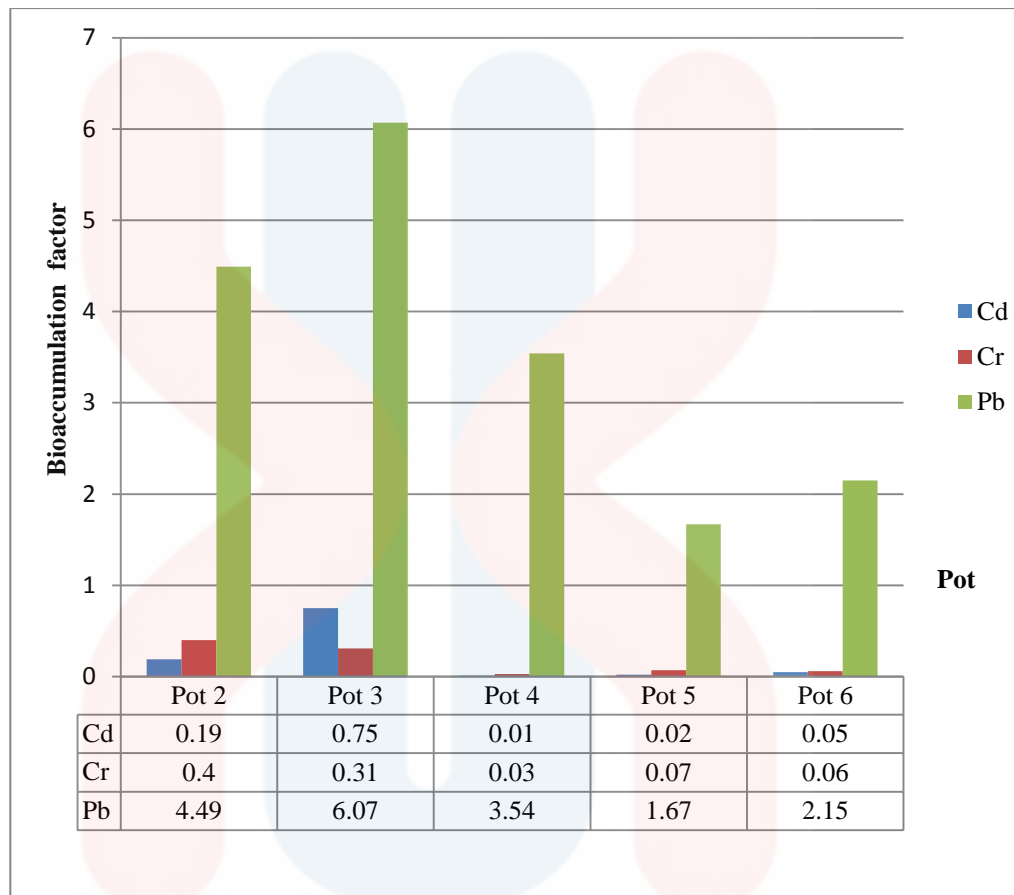


Figure 4.7 : The bioaccumulation factor for heavy accumulation

Based on Figure 4.7, for bioaccumulation factor, Pb at every concentration has values that are greater than 1. The bioaccumulation factor values for Pb at every different concentrations are 4.49, 6.07, 3.54, 1.67, and 2.15 respectively. From previous study that also used *Cymbopogon citratus*, the result shown that Cr and Cd values for translocation factor are greater than 1 that represents effectiveness of this plant to transport heavy metals from root. From the similar study, it shown that if the heavy metals has translocation factor less than 1, it means that the heavy metals indicates the controlled movement of heavy metals from root (Gautam, Pandey, & Agrawal, 2017).

Based on the result obtained and calculation for translocation factor, Pb has value that less than 1. It is proved when the amount of Pb is higher in root. At pot 2 which is containing 5 ppm concentration and for leave's parts, Pb has high mean values compared to Cr and Cd. Pb has mean values which is $0.150 \text{ mg/L} \pm 0.0055 \text{ mg/L}$ while Cr has $0.035 \text{ mg/L} \pm 0.0445 \text{ mg/L}$ and last is Cd has $0.006 \text{ mg/L} \pm 0.0000 \text{ mg/L}$.

While for stem's parts, Pb also has high reading of mean values among the other heavy metals. The readings are Pb = $0.141 \text{ mg/L} \pm 0.0210 \text{ mg/L}$, Cr = $0.013 \text{ mg/L} \pm 0.0081 \text{ mg/L}$ and last is Cd = $0.002 \text{ mg/L} \pm 0.0006 \text{ mg/L}$. Last part is root. For roots, Pb has high mean values which is $0.258 \text{ mg/L} \pm 0.0273 \text{ mg/L}$ while Cr = $0.013 \text{ mg/L} \pm 0.0081 \text{ mg/L}$ and Cd = $0.004 \text{ mg/L} \pm 0.0007 \text{ mg/L}$. So, for 5 ppm concentration, among these three heavy metals, Pb is the most absorbed heavy metals in each part of plants and the lowest absorbed heavy metals is Cd.

After that, leave's part for pot 3 which is containing 10 ppm, Pb has the high accumulation compared to Cr and Cd. In leave's part, Pb = $0.121 \text{ mg/L} \pm 0.0256 \text{ mg/L}$ while Cr = $0.017 \text{ mg/L} \pm 0.0213 \text{ mg/L}$ and Cd = $0.003 \text{ mg/L} \pm 0.0006 \text{ mg/L}$. For stem's part, Pb also have highest amount of accumulation which is $0.113 \text{ mg/L} \pm 0.0358 \text{ mg/L}$ while Cr is $0.006 \pm 0.0225 \text{ mg/L}$ and Cd is $0.08 \text{ mg/L} \pm 0.0020 \text{ mg/L}$. Last part is root. As like above, Pb has highest accumulation which is $0.392 \text{ mg/L} \pm 0.0307 \text{ mg/L}$ while Cr has $0.061 \text{ mg/L} \pm 0.0179 \text{ mg/L}$ and Cd has $0.009 \text{ mg/L} \pm 0.0003 \text{ mg/L}$. So, same like 5 ppm concentration, at 10 ppm, Pb also is most absorbed heavy metals.

Next, 15 ppm of three selected heavy metals has been put into pot 4. Pb has high accumulation of heavy metals in leave, stem and root which are $0.130 \text{ mg/L} \pm 0.0078$

mg/L, 0.139 mg/L \pm 0.0253 mg/L and 0.227 mg/L \pm 0.0235 mg/L respectively. While Cr has second highest accumulation after Pb which are 0.017 mg/L \pm 0.0341, 0.003 mg/L \pm 0.0404 mg/L and 0.026 mg/L \pm 0.0203 mg/L respectively. Cd has lowest accumulation of heavy metals among the others which are 0.002 mg/L \pm 0.0016 mg/L, 0.00 mg/L \pm 0.0005 mg/L and 0.002 mg/L \pm 0.0020 mg/L respectively. Besides that, for 20 ppm, at leave's part, the highest accumulation is Pb and same goes to stem and root. At leave's part, Pb has 0.140 mg/L \pm 0.0090 mg/L, while at stem's part is 0.130 mg/L \pm 0.0173 mg/L and among of these part of plant, root has the highest accumulation of Pb which is 0.193 mg/L \pm 0.0090 mg/L. Second highest accumulation in 10 ppm concentration is Cr and then followed by Cd.

Last concentration is 25 ppm in pot 6. Like other concentrations, Pb has greater number of accumulation compared to other two heavy metals. It is also has high number of accumulation in part of leave, stem and root which are 0.151 mg/L \pm 0.024 mg/L, Figure 4.9 represents root accumulation for three heavy metals in five different 0.112 mg/L \pm 0.0264 mg/L and 0.211 mg/L \pm 0.0069 mg/L respectively. Cr has second highest number of accumulation and followed by Cd. All of this number of accumulation or data of heavy metals accumulation have been analyzed in graph by using one way ANOVA as shown in Figure 4.8 to 4.10;

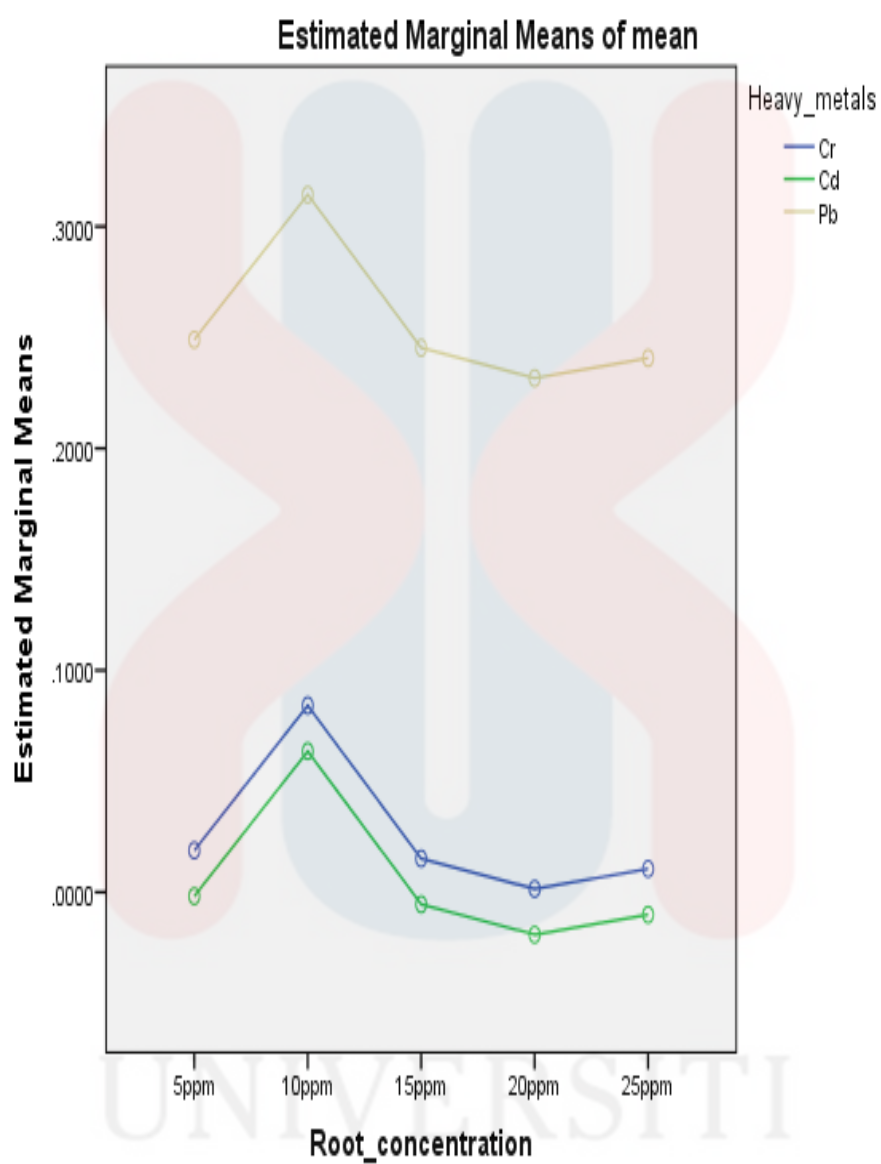


Figure 4.8 : Root accumulation for three heavy metals in five different concentrations

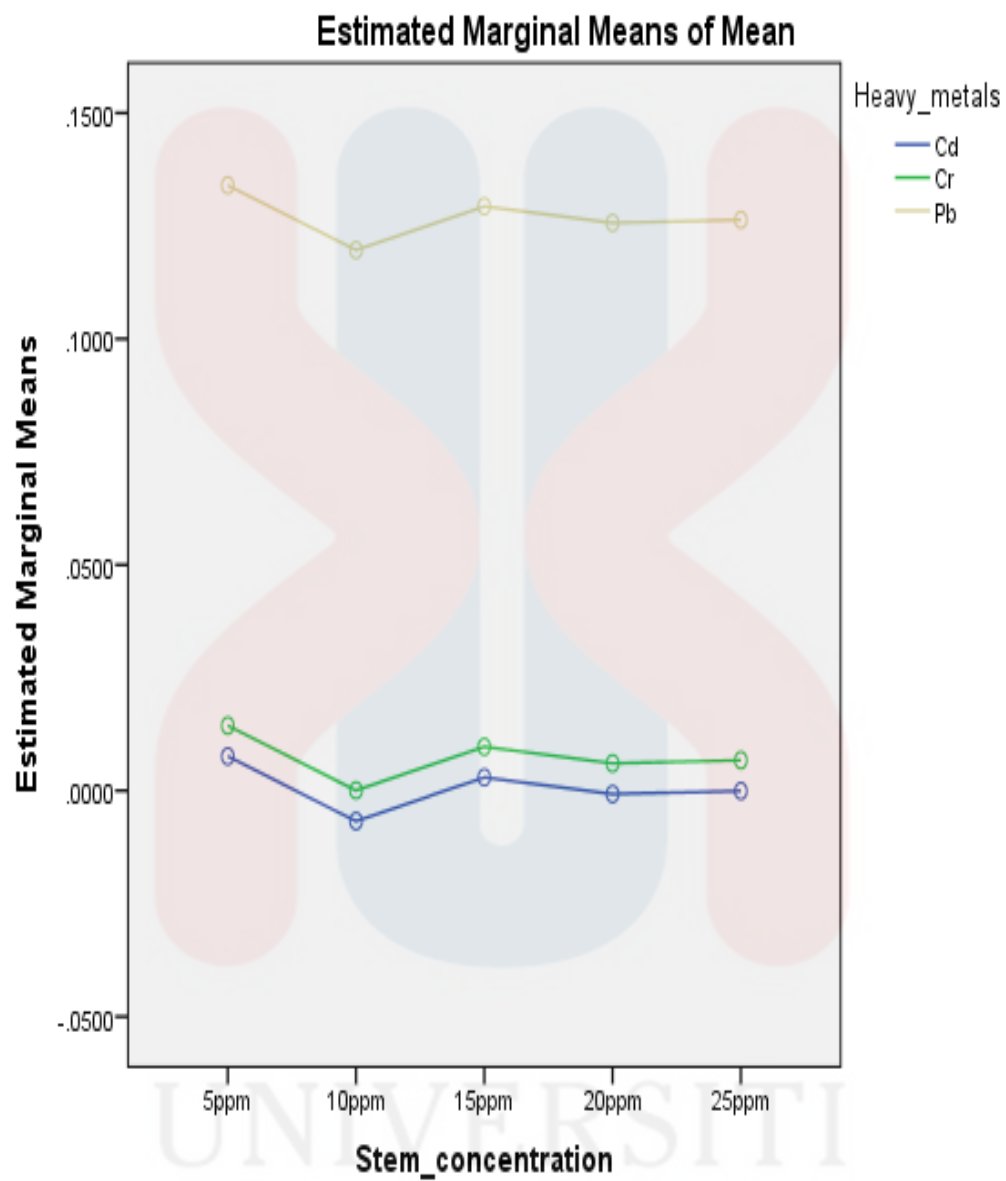


Figure 4.9 : Stem accumulation for three heavy metals in five different concentrations

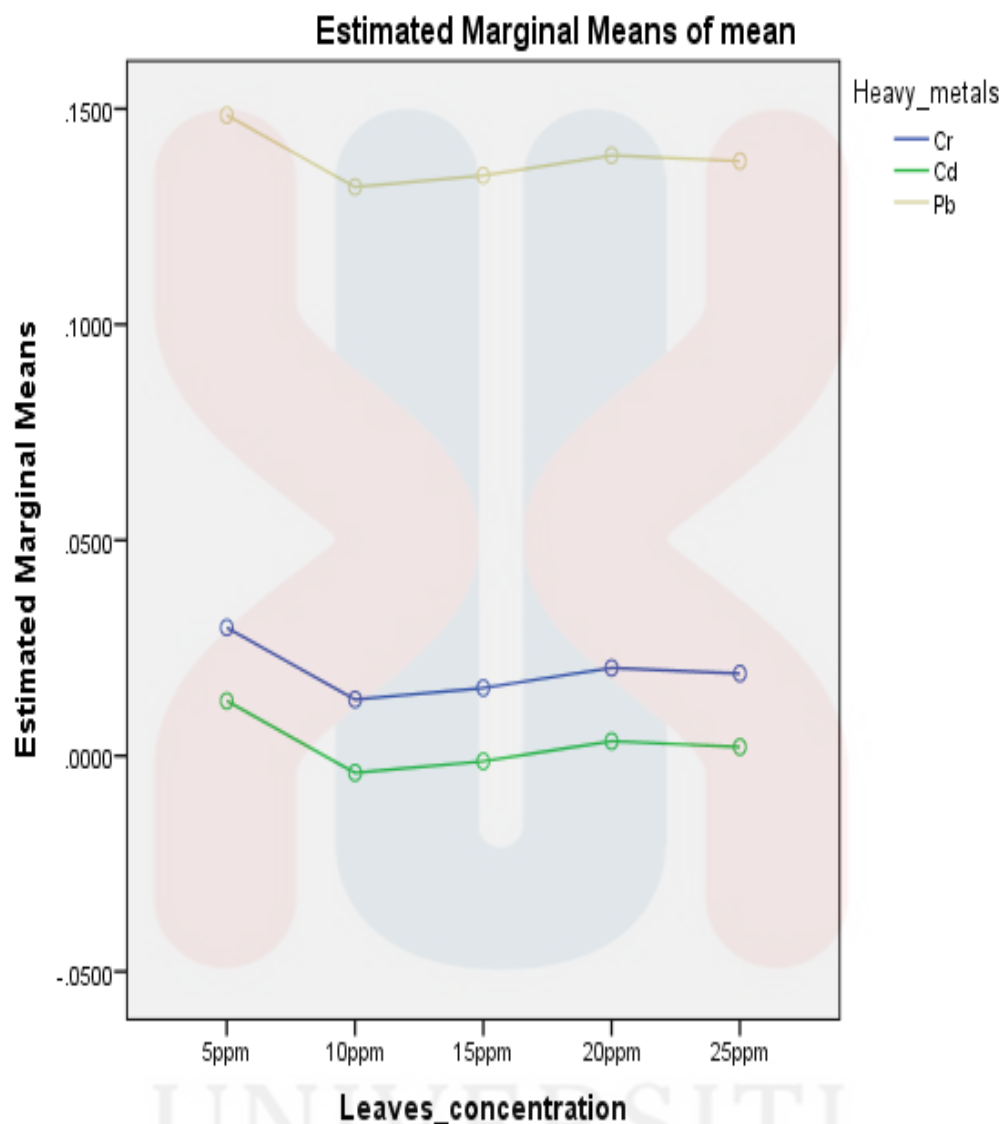


Figure 4.10 : Leave accumulation for three heavy metals in five different concentrations

Based on the estimated marginal means for mean for all three heavy metals by using one way ANOVA and explanation above, overall the highest or majority number of accumulation for every concentration and parts of plant is Pb. Second highest accumulation is Cr and followed by least accumulation which is Cd. Pb is a toxic element for environment including plant and human. There is a study has revealed that

Pb is highly mobile metal, and easy being absorbed by plants via root to leave (Pandey, Singh, & Pandey, 2012). For Pb concentration, highest part is root then leave and the lowest part is stem. Highest concentration of Pb that can be absorbed or active is at 10 ppm while the lowest is 20 ppm. For Cr, the highest part of plant that containing Cr is root, then leave and stem. At 10 ppm, Cr has being absorbed a lot at the root. From the previous study that using *Cymbopogon flexuosus* mentioned that Cr also been absorbed a lot in root and medium accumulation in leaves same with *Cymbopogon citratus* even though at 10 ppm (Jena, Pradhan, & Patra, 2016). Last is for Cd. Cd has been absorbed a lot at 5 ppm and 10 ppm. All these three heavy metals have been absorbed a lot at root especially Pb.

Pb accumulation is higher because it can alter the uptake of other essential minerals by plants by through their availability of minerals from soil (Pandey, Singh, & Pandey, 2012). This indicated that Pb concentration in roots are positively connected to the leaves and probably due to passive Pb translocation from root to leaves (Ramos, Hernandez, & Gonzalez, 1994). Based on literature review above, once the lead (Pb) has permeate into the root system, it can accumulate aerial parts of plants (Piechalak et al., 2002). The significant value between concentration for each pot has determined as ($P < 0.05$). Based on Figure 4.8, 4.9 and 4.10, there are no significant level between concentrations which are root is ($P = 0.208$), stem is ($P = 0.536$) and leaves is ($P = 0.257$) respectively.

Highest accumulation of Pb can be found in root because the root cells are young and thin. Once Pb has been absorbed onto the rhizoderm root , Pb can enter the root passively (Tung & Temple, 1996). From the data obtained, the high concentration

of Pb is at root at 10 ppm. Mostly, Pb concentration will be absorbed from soil and remain concentrated in roots and some of it will be move upwards to stem and leaves (Winska-Krysiak, Koropacka, & Gawronski, 2015). It shows that *Cymbopogon citratus* still can stand or tolerate with quite high of concentration of Pb. From previous study by using *Tithonia diversifolia* and *Helianthus annuus*, the result shown the similar result with this result which is roots had the highest values of Pb (Netherlands, 2010) . It means Pb has been absorbed a lot in roots compared to stems and leaves.

The lowest accumulation of heavy metals is Cd. At 10 ppm, 15 ppm and 25 ppm, most of Cd can be found in root. Few of it can be found in leave's part. Symptom that shows there is presence of Cd is chlorosis and leave rolls. From growth of observation, pot 2 which is containing 5 ppm has the lowest of leaves growth. It may be because of presence of Cd in leave's part that leads to chlorosis. Chlorosis may happen because of compacted root, high alkalinity and nutrient deficiencies. It may happen because of high pH and based on pH data obtained, pH for pot 2 is pH 7 and above. Based on literature review above, it stated that Cd also readily absorbed by plant but it is lower than Pb because Pb can alter many essential heavy metals uptake. From previous study, the result is similar, increased concentration Cd has reduced the leaves and root growth due to chlorosis and interfering the uptake of nutrients (Wahid, Ghani, & Javed, 2008).

4.6 Soil Analysis

Soil analysis was observed by using Scanning electron microscopy (SEM)-Energy dispersive energy (EDS). EDS used for to determine or observe the element

analysis of a sample. Seven samples was observed which are include control that containing heavy metals and another one control pot is containing original plant without heavy metals. Others five are pot 2 until pot 6 which are containing 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm respectively. In EDS, there are three location spot was observed due to get an average for each heavy metals in every pots.

For control which is containing plant only without any heavy metals poured into the pot, there are few heavy metals original presence in it such as carbon (C), oxygen (O), aluminium, Iron (Fe) and silicon (Si). Three selected heavy metals which are Cr, Cd, and Pb was presence in this soil. Among these three heavy metals, Cr has high average of weight (%) which is 0.173 and the average have been calculated by using data obtained from three location spot. Other control pot which is containing heavy metals only also has Cr as highest number of average that calculated from addition of weight (%) from three spot locations. Cr has highest number in an average which is 0.697% compared to two another selected heavy metals such as Cd and Pb which are 0.253% and 0.187% respectively.

Besides that, Cr also has greater number in pot 2 which is 0.13% compared to Cd and Pb which are 0.063% and 0.123% respectively. For pot 2, the second highest is Pb then followed by Cd. Pot 3 also has Cr as highest number of average which is 0.267% then Cd at second place which is 0.123% and followed by Pb that hold 0.103% which is the lowest heavy metals among these three heavy metals due to data obtained from EDS in pot 3. While for pot 4, Cr has high number of weight (%) again which is 1.50% compared to others and pot 5 also has Cr as first place holder for average of weight (%) which is 0.593% and same goes to pot 6 which is 0.77%.

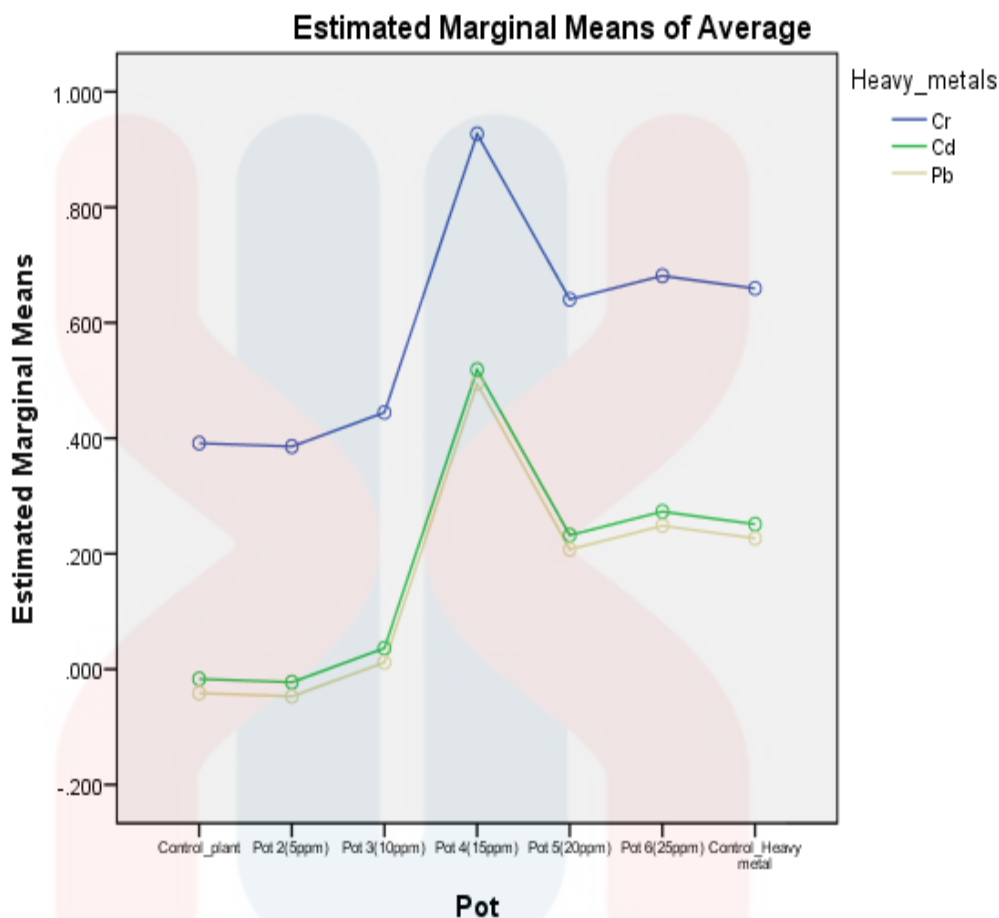


Figure 4.11 : Means of average of three heavy metals presence in each of pot

Based on Figure 4.10, among all of the pots includes two control pot, Cr has the highest number of average compared to other two selected heavy metals. Second is Cd and last id Pb. Furthermore, in pot 4, number of weight (%) of Cr is the highest among the other Cr in other pots. Cr has high of number because elemental decomposition of soils and sediment that been influenced by composition of the rock (Kimbrough et al., 1999). Mostly, Cr was absorbed a lot in pot 4 which is containing 15 ppm. For significance level, it was determined as ($P < 0.05$). Based on figure above, there is significance level of heavy metals which is only has $P = 0.011$ and the significance level is below ($P < 0.05$). However, there is no significant level at pots which is $P = 0.169$.

Due to soil analysis that has been done by using EDS, Pb has lowest number of average in soil compared to Cr. This is because Cr always can be found in soil because of there is rock or other sediment in soil (Kimbrough et al., 1999). High presence of Cr in soil also can give effect to the leaf growth. There is a study stated that they found decrease in plant height and the reason it happened due to presence of Cr compared to the control (Dey, Jena, & Kundu, 2009). According to (Oliveira, 2012), decrease in plant height due to reduce of root growth and decreased of nutrient and water transport. Cr also transport the nutrient to aerial parts that can give directly impact to the plant especially plant height. From data obtained, control pot has 0.173% as an average but much different with pot 4 which is containing 1.50% average. Pot 4 has huge difference with control pot due to presence of rocks or sediment in it.

4.7 Water Analysis

Water analysis was taken to monitor the water come out from the plant. A container was put under a pot in order to get water collection to monitor the heavy metals presence in the water sample. Other reason to put a container under each of pots for this project is to avoid the heavy metals that were put into the pot flow out to the ground. It can lead to soil contamination if the water is coming out from the pot because each of pots was put different of concentrations of Cr, Cd and Pb.

For 5 ppm, it has Cd = 0.010mg/L, Cr = 0.002 mg/L and Pb = 0.114 mg/l. Cr is the lowest compared to others concentration which are at 10 ppm, 15 ppm, 20 ppm and 25 ppm. Next is 10 ppm which is has high number of Pb among the others

concentrations. The mean number of Pb in 10 ppm is 0.136 mg/L. it also moderate Cr and Cd which are 0.013 mg/L and 0.011 mg/L respectively. For 15 ppm, it has Cd = 0.013 mg/L, and Pb = 0.134 mg/L. For 20 ppm, Pb has high number of mean among other two heavy metals. Pb has high number of mean which is 0.104 mg/L, while Cr has 0.011 mg/L and Cd has 0.012 mg/L. For the last ppm which is 25 ppm, Pb has 0.104 mg/L, Cr has 0.027 mg/L and Cd has 0.011 mg/L. The mean of each of heavy metals from each of pots was recorded by using EDS as below (Figure 4.12);

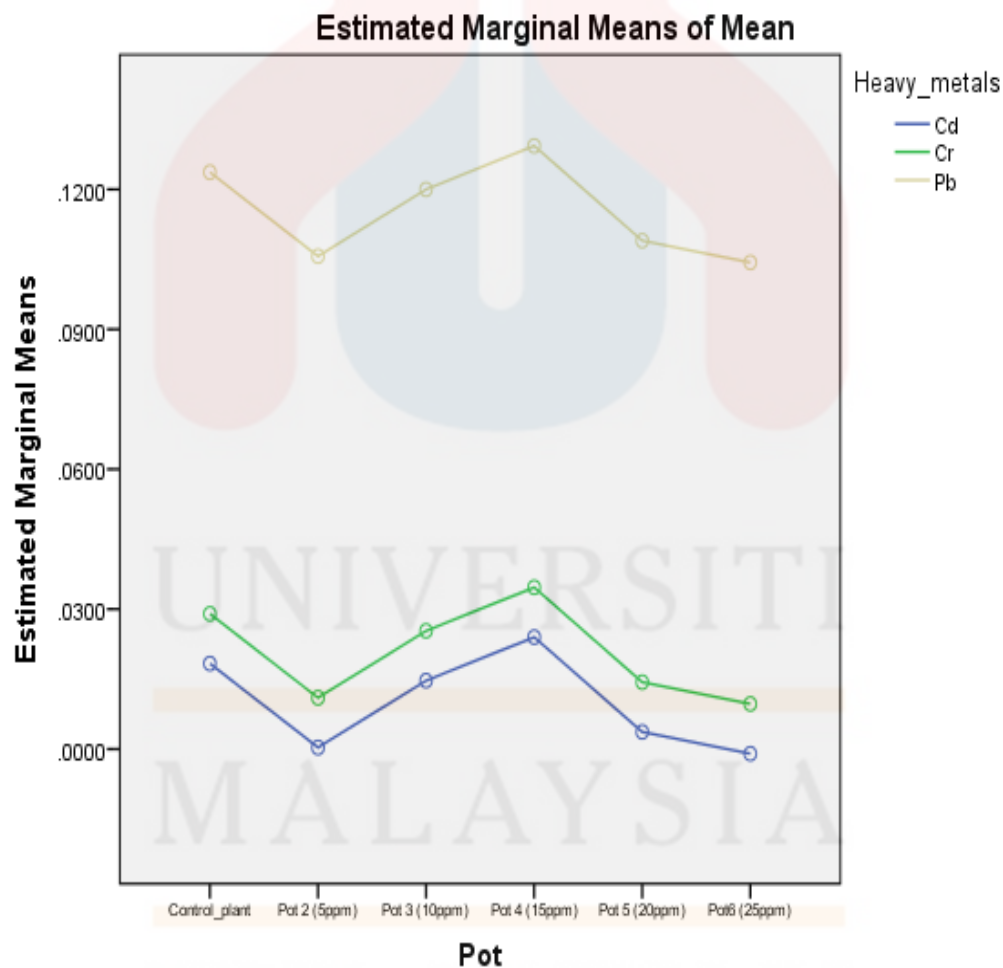


Figure 4.12 : The mean of heavy metals that presence in water for each of pots.

Based on the Figure 4.12, the control pot which is containing plant only without any heavy metals poured into it, it has Cd = 0.0012 mg/L, Cd = 0.030 mg/L and Pb = 0.129 mg/L. It has selected heavy metals presence due to the original soil in pot which is containing original Pb, Cr and Cd in it. All of pots has almost same amount of mean with control pot. It shows that the water collection or samples was not flow out from the container and contaminate the ground. It is depends on the distribution of heavy metals or other nutrients in the plant. Some of them been absorbed in plant, soil and water.

4.8 Comparison of Three Heavy Metals between Plant, Soil and Water

The comparison of three heavy metals between plant, soil and water has done in order to know the distribution of heavy metals among these three elements. Other reason is to know whether which part is containing high amount of heavy metals. The comparison was recorded in Table 4.2 as below;

Table 4.2 : The comparison of heavy metals between plant, soil and water

Concentrations								
		5ppm (mg/L)	10ppm (mg/L)	15ppm (mg/L)	20ppm (mg/L)	25ppm (mg/L)	Control (plant) (mg/L)	Control (Heavy metal) (mg/L)
Cd	Plant	0.012	0.092	0.004	0.003	0.011	0.05	0.253
	Soil	0.063	0.123	0.3	0.21	0.213	0.11	
	Water	0.01	0.011	0.013	0.012	0.011	0.012	
Cr	Plant	0.052	0.084	0.046	0.041	0.048	0.022	0.697
	Soil	0.13	0.267	1.5	0.593	0.77	0.173	
	Water	0.03	0.002	0.013	0.041	0.011	0.027	
Pb	Plant	0.549	0.626	0.496	0.463	0.474	0.426	0.187
	Soil	0.123	0.103	0.14	0.277	0.22	0.05	
	Water	0.129	0.114	0.136	0.134	0.104	0.075	

Based on the Table 4.1, from five different concentrations which are 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm, Cd is mostly found in soils. Cd also can be found from weathering parent rock (Khan et al., 2010). From data obtained, it shows that Cd are been absorbed a lot in soils, then plant and followed by water. The order for Cd for each of different of concentration is soil > plant > water. Cd is one of dangerous metals and non – essential to the plants due to its high mobility and small concentration (Barceló & Poschenrieder, 1990). Even though low concentration was used which is 5 ppm, it shows the effect. The effect is the length of leaves at 5 ppm pot is quite low compared to the others and also has chlorosis. Cd at 15 ppm and 20 ppm has a little different with 5 ppm, 10 ppm and 25 ppm. The different is the sequence for Cd in each of concentrations. The sequence is soil > water > plant. For 15 ppm and 20 ppm still has most of Cd in soils. According to journal, it mentioned that from contaminated soil, Cd is can easily transport or taken up by plants root and then get transport to the ground parts (Di Toppi & Gabbrielli, 1999). This is one of reason why is content if Cd is higher in soil's part.

After that, for Cr, it has equal sequence for every concentrations which is soil > plant > water. It has same sequence for 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm. Cr is normally can be found in soil because there is presence of rocks and sediments that can compose them (Kimbrough et al., 1999). This is the reason why Cr found a lot in soils. It is due to the original presence of rocks in soils and the concentrations of Cr that have been poured into the soil. Some of concentrations that been poured are not totally absorbed in soil only but also plant and water. Plant has second highest amount of Cr.

From data obtained, Cr has high number of Cr in root's part and accumulated a lot in that part but based on Table 4.16, only few of Cr was absorbed into these three part of plants while remaining has been flew out into water collection.

Last selected heavy metals is Pb. From Table 4.16, it shows that mostly Pb was absorbed in plants. Sequence for Pb is plant > water > soil. Pb has high number in plants because it can alter uptake of other essential minerals by plants by through their availability of minerals from soil. Even though, Pb is not essential for growth of plant, but they are readily to be taken by plants and accumulated in plants (Nazir, Khan, Masab, Rehman, Rauf, Shahab, Ameer, Sajed, Ullah, & Rafeeq, 2015). Plant can also take up the minimal of Pb amount from soil (Pandey, Singh, & Pandey, 2012). So, this is the reason why other heavy metals are mostly absorbed in soils not into the plants. This is other reason why Pb is less amount in soil compared to plant.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

In this study, it shows that all three selected heavy metals which are Cr, Cd and Pb can be absorbed by *Cymbopogon citratus* or known as (lemongrass). Three parts of *Cymbopogon citratus* have been observed and analyzed the heavy metals distribution by using AAS. After the result obtained, the fate and transport of Cr, Cd and Pb in *Cymbopogon citratus* had been compared. Due to know which part has absorbed a lot of heavy metals. From the data obtained, Pb has the highest place in three parts of plant which leaves, stems and roots. Mostly Pb has been absorbed a lot in root which is the highest 0.392 mg/L \pm 0.0307. While Cr, it has been absorbed a lot in root but in certain concentration which is 10 ppm 0.061 mg/L \pm 0.0179. Cd also can be found a lot in root but also in certain concentration such as 10 ppm, 15 ppm and 25 ppm which are the values 0.009 mg/L \pm 0.0003, 0.002 mg/L \pm 0.0020, 0.011 mg/L \pm 0.0004. From the heavy metal's fate and transport, it shows that Cr in *Cymbopogon citratus* has translocation factor greater than 1 which is can be used for stabilize other metals. For bioaccumulation factor, Pb at every concentrations has values that are greater than 1.

5.2 RECOMMENDATIONS

This research can be studied further in order to investigate the phytostabilization and phytoremediation by using *Cymbopogon citratus*. This is because the translocation factor is greater than 1 by using this plant but only certain of heavy metals such as Pb. It means further studied can use Pb for phytostabilization process. Last recommendation is using more big concentrations such 100 ppm, 200 ppm and others in order to get more significance level and more clearly the difference of heavy metals accumulation in plants.

REFERENCES

- Alloway, B. J., & Jackson, A. P. (1991). The behaviour of heavy metals in sewage sludge-amended soils. *Science of The Total Environment*, 100, 151-176. doi: [https://doi.org/10.1016/0048-9697\(91\)90377-Q](https://doi.org/10.1016/0048-9697(91)90377-Q)
- Anal, J. M. H. (2014). Trace and Essential Elements Analysis in Cymbopogon citratus (DC.) Stapf Samples by Graphite Furnace-Atomic Absorption Spectroscopy and Its Health Concern. *Journal of Toxicology*, 2014, 5. doi: 10.1155/2014/690758
- Asgari Lajayer, B., Ghorbanpour, M., & Nikabadi, S. (2017). Heavy metals in contaminated environment: Destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. *Ecotoxicology and Environmental Safety*, 145, 377-390. doi: <https://doi.org/10.1016/j.ecoenv.2017.07.035>
- Bachiega, T. F., & Sforcin, J. M. (2011). Lemongrass and citral effect on cytokines production by murine macrophages. *Journal of Ethnopharmacology*, 137(1), 909-913. doi: <https://doi.org/10.1016/j.jep.2011.07.021>
- Barceló, J., & Poschenrieder, C. (1990). Plant water relations as affected by heavy metal stress: a review. *Journal of plant nutrition*, 13(1), 1-37.
- Cecchi, M., Dumat, C., Alric, A., Felix-Faure, B., Pradere, P., & Guisresse, M. (2008). *Multi-metal contamination of a calcic cambisol by fallout from a lead-recycling plant* (Vol. 144).
- Demirezen, D., Aksoy, A., & Uruc, K. (2007). Effect of population density on growth, biomass and nickel accumulation capacity of Lemna gibba (Lemnaceae). *Chemosphere*, 66(3), 553-557. doi: 10.1016/j.chemosphere.2006.05.045
- Deng, H., Ye, Z. H., & Wong, M. H. (2004). Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. *Environ Pollut*, 132(1), 29-40. doi: 10.1016/j.envpol.2004.03.030
- Denmead, O. T., & Shaw, R. H. (1962). Availability of Soil Water to Plants as Affected by Soil Moisture Content and Meteorological Conditions¹. *Agronomy Journal*, 54, 385-390. doi: 10.2134/agronj1962.00021962005400050005x
- Dey, S. K., Jena, P. P., & Kundu, S. (2009). Antioxidative efficiency of Triticum aestivum L. exposed to chromium stress. *Journal of environmental biology*, 30(4).
- Di Toppi, L. S., & Gabbrielli, R. (1999). Response to cadmium in higher plants. *Environmental and experimental botany*, 41(2), 105-130.
- Duran, A., Tuzen, M., & Soylak, M. (2008). Trace element levels in some dried fruit samples from Turkey. *International Journal of Food Sciences and Nutrition*, 59(7-8), 581-589. doi: 10.1080/13561820701507910
- Edelstein, M., & Ben-Hur, M. (2018). Heavy metals and metalloids: Sources, risks and strategies to reduce their accumulation in horticultural crops. *Scientia Horticulturae*, 234, 431-444. doi: <https://doi.org/10.1016/j.scienta.2017.12.039>
- Fernández, V., & Brown, P. H. (2013). From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Frontiers in plant science*, 4, 289.
- Gautam, M., Pandey, D., & Agrawal, M. (2017). Phytoremediation of metals using lemongrass (Cymbopogon citratus (DC) Stapf.) grown under different levels of red mud in soil amended with biowastes. *International journal of phytoremediation*, 19(6), 555-562.
- Gołda, S., & Korzeniowska, J. (2016). Comparison of phytoremediation potential of three grass species in soil contaminated with cadmium. *Ochrona Srodowiska i Zasobów Naturalnych*, 27(1), 8-14.
- Griswold, W., & Martin, S. . (2009). Human health effects of heavy metals. . *Environmental Science and Technology Briefs for Citizens*, 6.

- Gupta, D. K., Nicoloso, F. T., Schetinger, M. R., Rossato, L. V., Pereira, L. B., Castro, G. Y., . . . Tripathi, R. D. (2009). Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. *J Hazard Mater*, 172(1), 479-484. doi: 10.1016/j.jhazmat.2009.06.141
- Helena, L., Chaves, L. H., Alexandra Estrela, M., & Sena De Souza, R. (2018). *Effect on plant growth and heavy metal accumulation by sunflower*.
- Intawongse, M., & Dean, J. R. (2006). Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastrointestinal tract. *Food Additives & Contaminants*, 23(1), 36-48. doi: 10.1080/02652030500387554
- Jena, P., Pradhan, C., & Patra, H. K. (2016). Cr⁺ 6-induced growth, biochemical alterations and Chromium bioaccumulation in *Cassia tora* (L.) Roxb. *Annals of Plant Sciences*, 5(07), 1368-1373.
- Joel G. Burken, & Jerald L. Schnoor. (1996). Phytoremediation: Plant Uptake of Atrazine and Role of Root Exudates. *Journal of Environmental Engineering*, 122(11). doi: [https://doi.org/10.1061/\(ASCE\)0733-9372\(1996\)122:11\(958\)](https://doi.org/10.1061/(ASCE)0733-9372(1996)122:11(958))
- Khan, S., Rehman, S., Khan, A. Z., Khan, M. A., & Shah, M. T. (2010). Soil and vegetables enrichment with heavy metals from geological sources in Gilgit, northern Pakistan. *Ecotoxicol Environ Saf*, 73(7), 1820-1827. doi: 10.1016/j.ecoenv.2010.08.016
- Kimbrough, D. E., Cohen, Y., Winer, A. M., Creelman, L., & Mabuni, C. (1999). A Critical Assessment of Chromium in the Environment. *Critical Reviews in Environmental Science and Technology*, 29(1), 1-46. doi: 10.1080/10643389991259164
- Lake, B. (2000). Understanding LEAFLET NO.2 Soil pH. *Acid Soil Management*.
- Lawal, O. A., Ogundajo, A. L., Avoseh, N. O., & Ogunwande, I. A. (2017). Chapter 18 - *Cymbopogon citratus* A2 - Kuete, Victor *Medicinal Spices and Vegetables from Africa* (pp. 397-423): Academic Press.
- Lunt, I. A. H., Susan & Rubin, Yoram. . (2005). Soil Moisture Content Estimation Using Ground-Penetrating Radar Reflection Data. *Journal of Hydrology*. , 307, 254-269.
- Megateli, S., Semsari, S., & Couderchet, M. (2009). Toxicity and removal of heavy metals (cadmium, copper, and zinc) by *Lemna gibba*. *Ecotoxicology and Environmental Safety*, 72(6), 1774-1780. doi: <https://doi.org/10.1016/j.ecoenv.2009.05.004>
- Mirowski, J., & Paukszt, A. (2018). Determination of the Cadmium, Chromium, Nickel, and Lead Ions Relays in Selected Polish Medicinal Plants and Their Infusion. *Biological Trace Element Research*, 182(1), 147-151. doi: 10.1007/s12011-017-1072-5
- Mohan, D., & Pittman, C. U. (2006). Activated carbons and low cost adsorbents for remediation of tri- and hexavalent chromium from water. *Journal of Hazardous Materials*, 137(2), 762-811. doi: <https://doi.org/10.1016/j.jhazmat.2006.06.060>
- Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvaunya, R., Scrimshaw, M. D., & Lester, J. N. (2006). Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: Implications for human health. *Agriculture, Ecosystems & Environment*, 112(1), 41-48. doi: <https://doi.org/10.1016/j.agee.2005.04.028>
- Mudgal, V., Madaan, N., Mudgal, A., Singh, R., & Mishra, S. (2010). Effect of toxic metals on human health. *The Open Nutraceuticals Journal*, 3(1), 94-99.
- Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters*, 8(3), 199-216. doi: 10.1007/s10311-010-0297-8
- Najeeb, U., Ahmad, W., Zia, M. H., Zaffar, M., & Zhou, W. (2017). Enhancing the lead phytostabilization in wetland plant *Juncus effusus* L. through somaclonal manipulation and EDTA enrichment. *Arabian Journal of Chemistry*, 10, S3310-S3317. doi: <https://doi.org/10.1016/j.arabjc.2014.01.009>
- Nazir, R., Khan, M., Masab, M., Rehman, H., Rauf, N. U., Shahab, S., . . . Shaheen, Z. (2015). *Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants*

- and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat (Vol. 7).
- Nazir, R., Khan, M., Masab, M., Rehman, H. U., Rauf, N. U., Shahab, S., . . . Rafeeq, M. (2015). Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. *Journal of Pharmaceutical Sciences and Research*, 7(3), 89.
- Netherlands, S. (2010). Phytoremediation Potentials of Sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for Metals in Soils Contaminated with Zinc and Lead Nitrates. *Water Air Soil Pollution*, 207(1-4), pp 195–201.
- Oliveira, H. (2012). Chromium as an Environmental Pollutant: Insights on Induced Plant Toxicity. *Journal of Botany*, 2012, 8. doi: 10.1155/2012/375843
- Oztas, F., & Bozkurt, E. (2011). Biology teacher candidates' misconceptions about surface tension, adhesion and cohesion. *Energy Educ Sci Technol Part B*, 3, 75-85.
- Pandey, R., Singh, V. P., & Pandey, S. K. (2012). Lead (Pb) Accumulation Study in Plants and Vegetables Cultivates Around Coal Mines and Power Plant of Singrauli District. *International Journal of Pharmaceutical Sciences and Research*, 3(12), 5079.
- Patra, D. K., Pradhan, C., & Patra, H. K. (2018a). An in situ study of growth of Lemongrass *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson on varying concentration of Chromium (Cr+6) on soil and its bioaccumulation: Perspectives on phytoremediation potential and phytostabilisation of chromium toxicity. *Chemosphere*, 193, 793-799. doi: <https://doi.org/10.1016/j.chemosphere.2017.11.062>
- Patra, D. K., Pradhan, C., & Patra, H. K. (2018b). An in situ study of growth of Lemongrass *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson on varying concentration of Chromium (Cr+ 6) on soil and its bioaccumulation: Perspectives on phytoremediation potential and phytostabilisation of chromium toxicity. *Chemosphere*, 193, 793-799.
- Pequerul, A., Perez, C., Madero, P., Val, J., & Monge, E. (1993). A rapid wet digestion method for plant analysis *Optimization of Plant Nutrition* (pp. 3-6): Springer.
- Philion, C., Ma, D., Ruvinov, I., Mansour, F., Pignanelli, C., Noel, M. Pandey, S. (2017). *Cymbopogon citratus* and *Camellia sinensis* extracts selectively induce apoptosis in cancer cells and reduce growth of lymphoma xenografts in vivo. *Oncotarget*, 8(67), 110756-110773. doi: 10.18632/oncotarget.22502
- Piechalak, A., Tomaszewska, B., Baralkiewicz, D., & Malecka, A. (2002). Accumulation and detoxification of lead ions in legumes. *Phytochemistry*, 60(2), 153-162.
- Qishlaqi, A., & Moore, F. (2007). *Statistical Analysis of Accumulation and Sources of Heavy Metals Occurrence in Agricultural Soils of Khoshk River Banks, Shiraz, Iran* (Vol. 2).
- Ramos, L., Hernandez, L., & Gonzalez, M. (1994). Sequential fractionation of copper, lead, cadmium and zinc in soils from or near Donana National Park. *Journal of environmental Quality*, 23(1), 50-57.
- Singh, A. (2008). Water and Plant Growth.
- Soodan, R. K., Pakade, Y. B., Nagpal, A., & Katnoria, J. K. (2014). Analytical techniques for estimation of heavy metals in soil ecosystem: A tabulated review. *Talanta*, 125, 405-410. doi: <https://doi.org/10.1016/j.talanta.2014.02.033>
- Staessen, J. A., Vyncke, G., Lauwerys, R. R., Roels, H. A., Celis, H. G., Claeys, F., . . . Amery, A. K. (1992). Transfer of cadmium from a sandy acidic soil to man: A population study. *Environmental Research*, 58(1), 25-34. doi: [https://doi.org/10.1016/S0013-9351\(05\)80202-6](https://doi.org/10.1016/S0013-9351(05)80202-6)
- Sumiahadi, A., & Acar, R. (2018). *A review of phytoremediation technology: heavy metals uptake by plants*. Paper presented at the IOP Conference Series: Earth and Environmental Science.
- Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through

- Phytoremediation. *International Journal of Chemical Engineering*, 2011, 31. doi: 10.1155/2011/939161
- Tester, M., & Leigh, R. A. (2001). Partitioning of nutrient transport processes in roots. *Journal of Experimental Botany*, 52(suppl_1), 445-457. doi: 10.1093/jexbot/52.suppl_1.445
- Thomas F. Scherer. (2017). Soil, Water and Plant Characteristics Important to Irrigation (AE1675 Revised).
- Thurmer, K., Williams, E., & Reutt-Robey, J. (2002). Autocatalytic oxidation of lead crystallite surfaces. *Science*, 297(5589), 2033-2035. doi: 10.1126/science.297.5589.2033
- Tung, G., & Temple, P. J. (1996). Uptake and localization of lead in corn (*Zea mays* L.) seedlings, a study by histochemical and electron microscopy. *Science of the total environment*, 188(2-3), 71-85.
- Vanstone, E. (2014). WHY DO PLANTS NEED WATER? *Science Sparks*.
- Velma, V., Vutukuru, S., & Tchounwou, P. B. (2009). Ecotoxicology of hexavalent chromium in freshwater fish: a critical review. *Reviews on environmental health*, 24(2), 129-146.
- Verbruggen, N., Hermans, C., & Schat, H. (2009). Molecular mechanisms of metal hyperaccumulation in plants. *New Phytol*, 181(4), 759-776. doi: 10.1111/j.1469-8137.2008.02748.x
- Verkleij, J. A. C., Golan-Goldhirsh, A., Antosiewicz, D. M., Schwitzguébel, J.-P., & Schröder, P. (2009). Dualities in plant tolerance to pollutants and their uptake and translocation to the upper plant parts. *Environmental and Experimental Botany*, 67(1), 10-22. doi: <https://doi.org/10.1016/j.envexpbot.2009.05.009>
- Wahid, A., Ghani, A., & Javed, F. (2008). Effect of cadmium on photosynthesis, nutrition and growth of mungbean. *Agronomy for sustainable development*, 28(2), 273-280.
- Walt, V. (2015). Why Do We Need To Know The Soil Moisture Content Of Soil
- Wang, H. F., Takematsu, N., & Ambe, S. (2000). Effects of soil acidity on the uptake of trace elements in soybean and tomato plants. *Applied radiation and isotopes : including data, instrumentation and methods for use in agriculture, industry and medicine*, 52(4), 803-811. doi: 10.1016/s0969-8043(99)00153-0
- Winska-Krysiak, M., Koropacka, K., & Gawronski, S. (2015). Determination of the tolerance of sunflower to lead-induced stress. *Journal of Elementology*, 20(2).
- Yamamoto, F., & Kozlowski, T. T. (1987). Effects of flooding, tilting of stems, and ethrel application on growth, stem anatomy, and ethylene production of acer platanoides seedlings. *Scandinavian Journal of Forest Research*, 2(1-4), 141-156. doi: 10.1080/02827588709382453
- Zayed, A., Lytle, C. M., Qian, J. H., & Terry, N. (1998). Chromium accumulation, translocation and chemical speciation in vegetable crops. *Planta*, 206(2), 293-299. doi: 10.1007/s004250050403

APPENDICES

APPENDIXES (A)



Figure A : *Cymbopogon citratus* after been planted and poured by heavy metals Cr, Cd and Pb

APPENDIX B



Figure B : Handling with chemicals (Hydrogen Peroxide (H_2O_2), Nitric Acid (HNO_3))

APPENDIX C



Figure C : Three parts of *Cymbopogon citratus* was cut into 3 parts and was dried by oven

APPENDIX D



Figure D : Plant analysis was digested and filtered before run into AAS

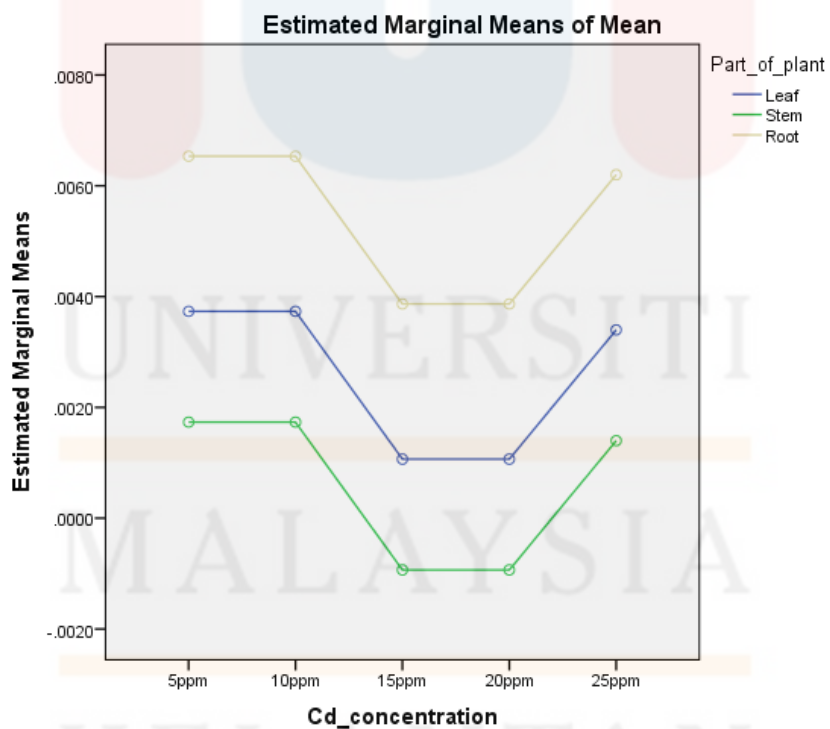
APPENDIX E

One way ANOVA data analysis for Cd concentration

Tests of Between-Subjects Effects

Dependent Variable: Mean

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	.000	1	.000	4.241	.176
	Error	5.813E-005	2	2.907E-005 ^a		
Part_of_plant	Hypothesis	5.813E-005	2	2.907E-005	3.065	.103
	Error	7.587E-005	8	9.483E-006 ^b		
Cd_concentration	Hypothesis	2.373E-005	4	5.933E-006	.626	.658
	Error	7.587E-005	8	9.483E-006 ^b		



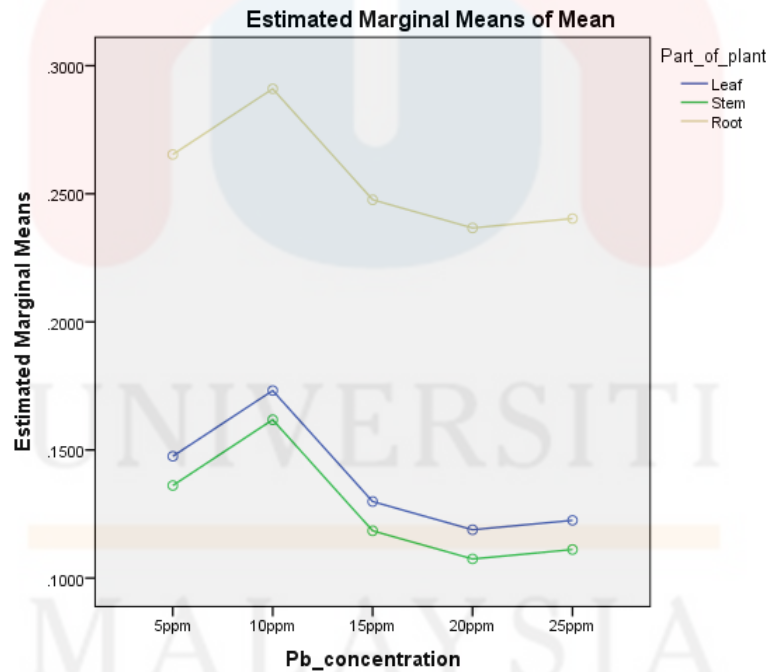
APPENDIX F

One way ANOVA data analysis for Pb concentration

Tests of Between-Subjects Effects

Dependent Variable: Mean

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	.453	1	.453	17.725	.052
	Error	.051	2	.026 ^a		
Pb_concentration	Hypothesis	.006	4	.002	.578	.687
	Error	.021	8	.003 ^b		
Part_of_plant	Hypothesis	.051	2	.026	9.853	.007
	Error	.021	8	.003 ^b		



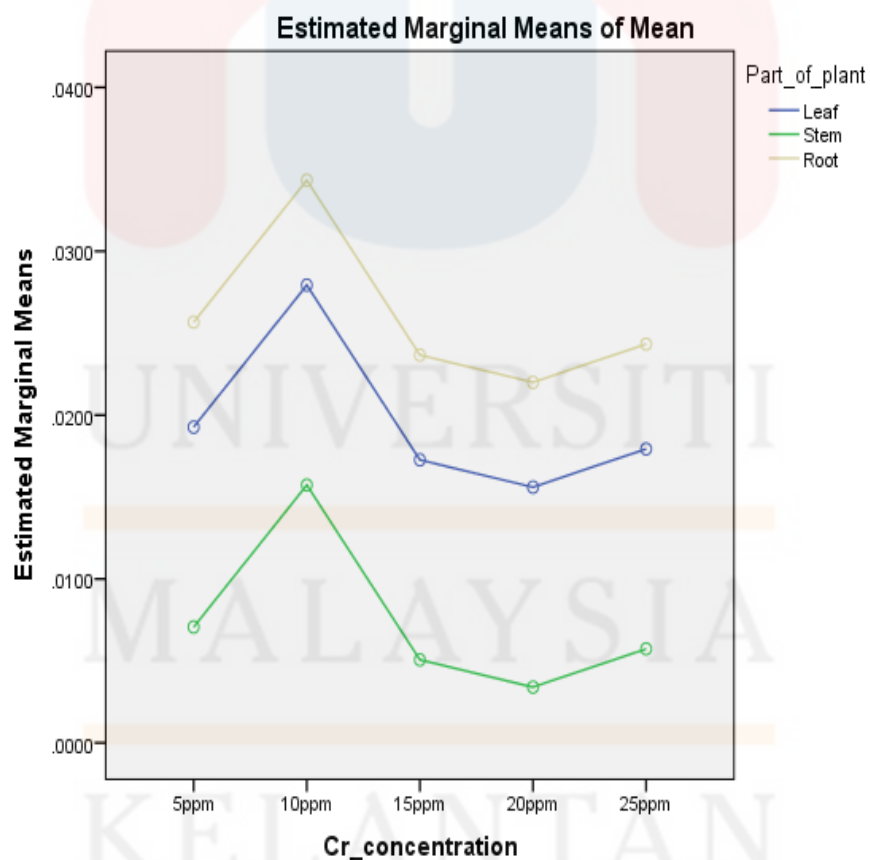
APPENDIX G

One way ANOVA data analysis for Cd concentration

Tests of Between-Subjects Effects

Dependent Variable: Mean

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	.005	1	.005	10.486	.084
	Error	.001	2	.000 ^a		
Cr_concentration	Hypothesis	.000	4	7.033E-005	.251	.901
	Error	.002	8	.000 ^b		
Part_of_plant	Hypothesis	.001	2	.000	1.591	.262
	Error	.002	8	.000 ^b		



APPENDIX H

EDS data analysis for control soil

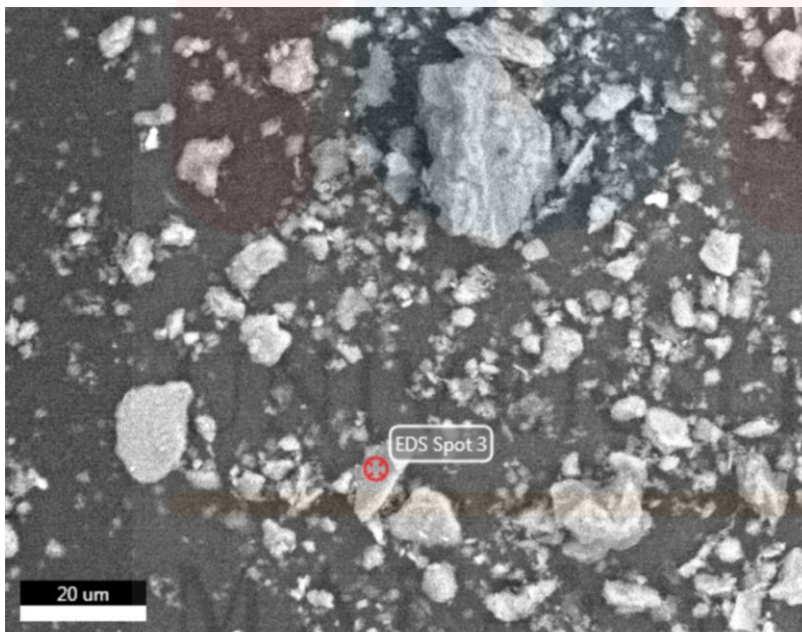
EDAX TEAM

hamizah

Author: umk

Creation: 12/6/2018 11:48:17 AM

Sample Name: soil



APPENDIX I

Moisture content of soil (week 2)

Pot	(wet) / kg + aluminium foil	(dry) / kg + aluminum foil	Moisture content
Pot 2	74.4	57.19	30.09
Pot 3	55.9	42.15	32.62
Pot 4	77.3	58.44	32.27
Pot 5	77.06	57.61	33.76
Pot 6	68.82	50.83	35.39
Pot 7	68.43	50.95	34.31
Pot 8	82.18	60.83	35.1
Pot 9	83.8	63.74	31.47
Pot 10	81.59	63.01	29.5
Pot 11	79.91	63.41	26.02
Pot 12 (control Heavy Metal only)	86.52	68.5	26.3

APPENDIX J**Moisture content of soil (week 4)**

Pot	wet / kg + aluminium foil	dry / kg + aluminum foil	Moisture content
Pot 1 (control plant only)	422.9	311.99	35.55
Pot 2	268.58	205.69	30.58
Pot 3	455.51	334.44	36.2
Pot 4	347.71	255.34	36.18
Pot 5	318.96	237.72	34.17
Pot 6	241.67	179.2	34.86
Pot 7	336.47	244.35	37.7
Pot 8	400.78	293.2	36.69
Pot 9	410.76	309.47	32.73
Pot 10	269.81	203.97	32.28
Pot 11	442.61	334.1	32.48
Pot 12 (control Heavy Metal only)	404.6	310.57	30.28