ANALYSIS OF HEAVY METALS (Cr, Cd, Hg) UPTAKE IN *Allium Cepa L.* PLANTS FROM SOILS BY USING ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

by

SHARIFAH NOR AZREEN SUHAILA BINTI SYED

MOHD ANUAR

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

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THESIS DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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Student

Name : SHARIFAH NOR AZREEN SUHAILA BINTI SYED MOHD ANUAR

Date : 10th JANUARY 2019

I certify that the report of this final year project entitled "Analysis of Heavy Metals (Cr, Cd, Hg) Uptake in *Allium cepa L*. Plants from Soils by Using Atomic Absorption Spectrophotometer (AAS)" by SHARIFAH NOR AZREEN SUHAILA BINTI SYED MOHD ANUAR matric number E15A0259 has been examined and all the corrections recommended by examiners have been done for the degree of Bachelor of Applied Science (Sustainable Science) with Honours, Faculty of Earth Science, University Malaysia Kelantan.

Approved by:

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Supervisor

Name : DR. MUSFIROH BINTI JANI

Date : 10th JANUARY 2019

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ANALYSIS OF HEAVY METALS (Cr, Cd, Hg) DISTRIBUTION IN *ALLIUM CEPA L.* PLANTS FROM SOILS BY USING ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

ABSTRACT

Vegetables are essential in eating habit for human due to their richness contents such as protein, carbohydrate, fibres, vitamin and minerals but they are easily to be affect by the concentration of heavy metals accumulation from soil. Therefore, Allium cepa L. (onion plant) are chosen to study the ability to uptake some concentration of heavy metals from different soil treatment levels and the distribution to roots, bulbs and leaves. The concentration of heavy metals in parts of plant were measured based on the ability to translocate the heavy metals concentration overall and the amount of heavy metals concentration in soil samples. The plant samples were separated into three different parts such as roots, bulbs and leaves. By using Atomic Absorption Spectrophotometer (AAS), the part of plants was analysed. Through analysis, the distribution of heavy metals (Cd, Cr, Hg) accumulated at different part of plants was identified. The distribution of heavy metals was based on bioconcentration factor (BCF) and translocation factor (TF). Furthermore, soil samples were collected to analyse the amount of remaining heavy metals concentration in it by using EDS. Water samples were also collected to analyse the amount of heavy metals deposited from the plants. Thus, that the amount of heavy metals uptake by the plant can be determine and observe either it has bad consequences to human health or not. Heavy metals such as Cd accumulated higher in plant for most of the treatment compared to Cr and Hg. The bioconcentration factor of onion was ranging between 0.08 to 0.20 respectively. Most of the mean concentration detected were higher than the permissible limit which regulated by Malaysian Food Regulation 1985. Therefore, onion can be one of the accumulator plants but the concentration of heavy metals accumulation might increase to higher amount if the soils used to plant onion have higher concentration of heavy metals contaminated.

ANALISA TERHADAP PROSES DISTRIBUSI LOGAM BERAT (Cr, Cd, Hg) DI DALAM POKOK *ALLIUM CEPA L.* DARIPADA TANAH DENGAN MENGGUNAKAN ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

ABSTRAK

Sayur-sayuran adalah penting dalam rutin pemakanan untuk manusia kerana kaya dengan kandungan seperti protein, karbohdrat, serat, vitamin dan mineral tetapi sayuran tersebut mudah terjejas oleh proses akumulasi logam berat dari tanah. Oleh itu, Allium cepa L. (pokok bawang) telah dipilih utuk dikaji keupayaannya untuk menyerap kepekatan yang berbeza untuk logam berat dari tanah rawatan yang berbeza kandungan seterusnya proses distribusi ke akar, mentol dan daun, Kepekatan logam berat didalam bahagian tumbuhan diukur berdasarkan keupayaan untuk memindahkan kepekatan logam berat secara keseluruhan dan jumlah kepekata logam berat dalam sampel tanah. Sampel tumbuhan telah dibahagikan kepada tiga bahagian yang berlainan iaitu akar, mentol dan daun. Analisis seterusnya menggunakan Atomic Absoprtion Spectrophotometer (AAS) pada awal kajian untuk mengenalpasti dan mengkaji kadar pengagihan oleh logam berat (Cd, Cr, Hg) yang terkumpul didalam bahagian tumbuhan yang berbeza. Berdasarkan faktor biokonsentrasi (BCF) dan faktor pemindahan (TF), kandungan logam berat tersebut dikenalpasti. Selain itu, sampel tanah dikumpulkan untuk menganalisis jumlah kepekatan logam berat yang terdapat didalamnya dengan menggunakan EDS. Sampel air juga telah dikumpulkan untuk dianalisis jumlah logam berat yang terkumpul daripada tanaman. Tambahan pula, jumlah pengambilan logam berat didalam tumbuhan dapat menentukan dan memastikan sama ada ianya mempunyai akibat yang buruk kepada kesihatan manusia atau sebaliknya. Logam berat seperti Cd telah terkumpul lebih tinggi jumlahnya dalam tumbuhan untuk hamper kesemua peringkat tanah rawatan berbanding logam berat Cr dab Hg. Faktor-faktor biokonsentrasi untuk pokok bawang adalah 0.08 hingga 0.20. Kebanyakan purata kadar kepekatan yang dikesan lebih tinggi daripada had kawalan yang dibenarkan oleh Peraturan Makanan Malaysia 1985. Oleh itu, pokok bawang boleh menjadi salah satu tumbuhan yang boleh menyerap tetapi kepekatan logam berat yang terkumpul mungkin meningkat kepada jumlah yang lebih tinggi jika tanah yang digunakan untuk menanam pokok bawang telah dicemari dengan kepekatan logam berat yang lebih tinggi.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AAS	Atomic Absorption Spectrophotometer
SEM	Scanning Electron Microscope
EDS	Energy-Dispersive X-Ray Spectroscopy
ROS	Reactive Oxygen Species
HCl	Hydrochloric Acid
Cr	Chromium
Cd	Cadmium
Hg	Mercury
Ca	Calcium
Cu	Copper
Zn	Zinc
HNO_3	Nitric Acid
H_2O_2	Hydrogen Peroxide
HCl	Hydrochloric Acid
MFR1985	Malaysian Food Regulations 1985
BCF	Bioconcentration Factor
TF	Translocation Factor
QC	Quality Control

LIST OF SYMBOLS

=	Equal to
^{o}C	Temperature (degree celcius)
%	Percentage
>	Greater than
<	Less than
M_1	The concentration in molarity of the concentrated solution
M_2	The concentration in molarity of the dilute solution
V_1	The volume of the concentrated solution
V_2	The volume of the dilute solution
mg/L	Miligram per litre

CHAPTER 1

INTRODUCTION

1.1 Background of study

A green plant is classified when abundantly amount of plant are detected on Earth. Since the start of anthropological activities on Earth by human, plants have been consumed by other living organisms such as human and animals for own purposes and they tend to produce by-product from the natural resources (Karayil, Veeraiah, & Sambasiva Rao, 2011). Thus, the natural resources on Earth become limited. Metabolism functions are important for human being that contains a few elements that can be traced by plants as a main source. Moreover, all necessary nutrients which contain in plants itself will be contributed to human health and nutrition significantly (Subramaniam *et al*., 2012). In order to maintain good, healthy and productive life, human body needs large number of essential minerals such as calcium (Ca), potassium (K), magnesium (Mg) and trace minerals. Plants are the main contributors of the essential minerals to human diet.

Heavy metals give harmful and poisonous to human health thus it can cause a lot of bad consequences. The bad consequences are the density of metal are at five times compare to water and the metals are very poisonous and high toxicity levels at low concentrations (Gebregziabher & Shiferaw, 2014). Heavy metals include iron (Fe), silver (Ag), chromium (Cr), mercury (Hg), lead (Pb), copper (Cu), zinc (Zn), cadmium (Cd) and platinum group elements. Besides, heavy metals are also one of the contaminant agents of food supply and can cause harmful effect to the environment

(Wuana & Okieimen, 2011). In agricultural production system, obviously they are toxic in nature, have long biological half-lives, not biodegradable and have potential to accumulate at every different parts of body organs which can lead to unwanted or harmful side effects either in soil or into the body (Gebregziabher & Shiferaw, 2014). Nutritional exposure towards heavy metals and other has been recognized as a risk to human health through the consumption of vegetables crops (Birhanu & Chandravanshi, 2012).

Allium cepa L. is also known as onion plant in English and "Pokok Bawang" in Malay. Onion plant have been discovered in the past 5000 years ago. It can be traced in Asian countries compared to the others. The Onion *(Allium cepa L.)* which is also called as bulb onion can be divided into two types which are garden onion and common onion (Mehta, 2017). Throughout years, onion have been used for variety of purposes especially in cooking. *Allium cepa L.* plants are also used as dietary foods among the communities in Malaysia because abundance amount of this plant can be found at the market. There are varieties of onion with each own unique flavour and taste from sweet to very strong spicy such as green, red and yellow. Onions can be eaten in many ways such as dried, fried, raw, roasted or cooked. Usually onions are used in salads as it is one of the main ingredients (Wyk, 2014).

There are a lot of factors that can be related and affect the distribution pattern in soil and the content in soil. The factors are aerosol deposition, amount of plants needed, types of plants, particle size distribution, drainage system, organic matter and last the parent material. Increases number of heavy metals uptake by plants can cause harmful effects such as lower uptake of nutrient, prevent plant's progress in growth and lower the metabolic processes. Fluctuate phenomena around the world is when the increasing amounts of heavy metals in food, soil and water. Due to the

anthropogenic activities such as mining, poor solid waste management and fertilizers, the quality of sources availability for agriculture is decreasing. Anthropogenic activities also are the main reason of pollution. The cross-contamination of food chain through irrigation is directly linked with the pollution of water and soil. Food tubers such as onions, potatoes and carrots were tainted by excessive amount of heavy metals accumulation (Sotiris *et al.,* 2014). This also gives harm to root tips, affect chlorosis, and minimize the nutrient absorption. Thus, this study will be conducted to determine the distribution of heavy metals into the different parts of plant by using Atomic Absorption Spectrophotometer (AAS).

1.2 Problem Statement

Allium cepa L. plant is one of the essential ingredients in cooking preparation processes. Thus, accumulation of heavy metals that can be detected in the plants give bad impacts to human health which leads to health risk problem. Moreover, soil also one of the factors that trigger the absorption of heavy metals into plants. The distinctive advantages found in heavy metals can also accelerate the absorption into plants. Heavy metals especially such as Cd, Hg, Pb, Zn, Cu, Al, Ni, Co, Cr, Fe, Cr and Pb, cause a great risk to human health and induce cancer formation and developmental disorders for children (Meltem, Aysel, & Arslan, 2011).

Plants take heavy metals from soil into their roots. After entry into roots, heavy metal ions can be either be stored in the roots or translocated to the shoots significantly through xylem vessels where they are mostly deposited in vacuoles (Ali, Khan, & Anwar, 2013). In *Allium cepa L.* plants, the metals ions can be either stored or translocated in the roots, bulbs and the leaves. The accumulation of heavy metals in different parts of *Allium cepa L.* plants which are the roots, the bulbs and the leaves

are the main problems because of people consuming onion in dietary. So, people will eventually be consuming the heavy metals in indirectly ways. Heavy metals such as Cd, Cr and Hg can cause serious health problem to human which can lead to fatal. Transport of bioavailable metal ions across the plasma membrane is the first step in metal uptake and accumulation (Palmgren *et al.*, 2008).

1.3 Objectives

The objectives of this study are:

1. To analyse the heavy metals distribution in different part of plants (root, bulb, leaf) by using Atomic Absorption Spectrophotometer (AAS).
2. To compare the distribution of heavy metals (Cr, Cd, Hg) in different part of *Allium cepa L.* plants (bulb, root, leaves) from synthetic contaminated soil.

1.4 Significance of Study

This study is important to human society because *Allium cepa L.* plants also known as onion plant is widely used as food for human especially in cooking preparation process. Therefore, this study is significantly focus about how the uptake of contaminants from soil by plant roots and their distribution and accumulation to different parts in plants by determining their distribution. The research focus on three types of heavy metals which are chromium (Cr), Cadmium (Cd) and mercury (Hg). Furthermore, the results of this study could help researchers to find solution when problems related to heavy metals contamination in onion plant occurs and it helps future researchers by providing a proper baseline for future investigation.

CHAPTER 2

LITERATURE REVIEW

2.1 Plant

Plants are known as multicellular organisms because it's consists of more than one cell and it's operates between two or more cells to produce by-product such as glucose and oxygen. They are the by-product of photosynthesis. It provides shelter, foods and other basic uses for living things. Indeed, to be healthy, plants need nutrients, water, warmth, air and light. They continue to make their own food through the process of photosynthesis if they grow well. Usually plants consist of roots, stem and the leaves which have different functions to help they keep healthy. The plant's roots take up nutrients and water from the soil. The called as "anchor" in plants because the roots always keep plant upright and steady in the soil. The stem carries water and nutrients to different parts of the plant. Photosynthesis is carried out when the leaves use the light source from the Sun together with carbon dioxide from the air and water to make their own food. Food are transported to the other part of plants such as roots and stems once it is made in the leaves.

Plants also maintaining ecosystem balance for human population. Firstly, as photosynthesizers, they absorbed carbon dioxide and produced oxygen for living thing. Through this processes, organic molecules such as food is provided. Certain contaminants are removed as they filtered water and air which entered into plants by the roots. Moreover, plants are enriched with high nutritional values. There are a lot of mineral component contain in plants. Minerals such as Potassium, Calcium,

Nitrogen and Magnesium are the most needed components for solid bones, repair of damaged cells and make teeth in people become stronger.

2.1.1 *Allium cepa L.*

The onion (*Allium cepa L.*) which is also called as bulb onion can be divided into two types which are garden onion and common onion (Mehta, 2017). Bulb of the onion also has this property such as anti-flammatory, anthelmintic, antispasmodic, antiseptic, diuretic, febrifuge, expectorant and carminative, hypotensive, hypoglycaemic, tonic and stomachic (Kumar *et al.*, 2015). The plant is classified under Liliaceae family which is also known as "Lily family". The most important parts in onion plant are the bulb, leaf and root. Different types of onion species have varieties of size, colour and taste. It contains sulphur compounds situated inside the bulb which give onion its strong smell and attractive flavour. Moreover, a green shoot grows when onion become matured (when the bulb aged) and grows upward the ground. Usually after a few periods of time, the shoot grows slowly produce leaves and stem. Onion consist of several notably quercetin and flavonoids, which has many biochemical characteristics such as antiviral and antibiotic, cell signalling and anti-inflammatory (Slimestad, Fossen, & Vågen., 2007). Through studies, it said was quercetin undergo extensive metabolism in the liver and gut following absorption and the result will remain in some biological activity (Zheng *et al.*, 2005). Besides some researcher found that dried bulb is good when crushed into powder form but eating raw onion also good for health (Eldin, 2011).

Onion are widely used in the traditional medicine. It is because onion give positive effects to the circulatory system. Moreover, onion also have natural occurring chemicals known as compounds of organosulfur. Organosulfur compound is

functional to lower the cholesterol and good in lowering blood pressure levels as sulphur is necessary in life. (Kumar *et al.,* 2015). It is abundant in nature which can be derived from animal and plant sources. Humans obtained source of sulphur through a diet which are composed of onion, garlic, broccoli, cabbage, cauliflower, fish, eggs and meat (Batchu *et al.,* 2013). Various organosulfur compounds are claimed to have powerful antioxidant activity. Reactive oxygen species, ROS levels can be decreases with a compound of organosulfur by inhibiting the ROS generating system or by preventing the antioxidant enzymes degradation (Oyagbemi *et al.,* 2009). Onion crops is increasing due to its importance and become most medicinal crop after tomatoes (Arshad *et al.,* 2017).

Onion is very useful to prevent the cardiovascular diseases, especially when it diminishes the risk of blood clotting. Onion also protects against some infections and cancer of stomach. Lung function especially for asthmatics can be improve by taking onions in diet. The strong smell of onion appears to possess the great concentration of health. Moreover, onion is also one of the cancer preventions and can lower the risk of heart disease as it consists of diallyl sulphide compound which can block the effect of carcinogen (cancer causing particles) in human body. Allium and allyl disulphide, two phytochemical compounds in onions have been found to lower the risk of several types of cancer, assist in regulating blood sugar, reduce inflammation and heal infections (Hedges & Lister, 2007). Through dietary of an onion a day can help to protect teeth from numerous of dental diseases. Mixed the juice with honey and ginger juice is very good for asthma, pulmonary diseases and can correct the menstrual cycle flow. Flavonoid compounds such as quercetin which can be obtained from the onion also help to prevent blood clots and fight against heart disease. In addition, onions are

very rich in chromium, a trace mineral that helps cells respond to insulin, and are a good source of vitamin C and other trace elements (Siyanna, 2017).

The chemical composition of onions for carbohydrates, moisture, vitamin-C, total sugar, P, Ca and K ranged from (14.146 and 14.772%), (82.99 and 82.77%), (6.5 and 5.7%), (4.74 and 2.32%), (50.6 ad 30.3mg), (46.9 and 25.7mg) and (140 and 129mg) respectively. The level of protein is (2.62 and 1.489% and the level of fat is (0.4%) including other trace elements (Bhattacharjee *et al.*, 2013). Onion bulb quality strongly depends on the dry matter content. Carbohydrates compound is the main portion of dry matter content in onion bulbs such as sucrose, fructose and glucose with a degree of polymerization of 3 to 12 (Sharma *et al.*, 2014). Figure 2.1 showed the *Allium cepa L. plants* (Kumar, 2010). The type of onion plants used were determine through the colour, odour and the physical appearances.

Figure 2.1: *Allium cepa L.* plants (Source: Seed Savers Exchange, 2018)

2.2 Heavy Metals

The possible basis to classify the heavy metals are the element's specific weight, the atomic number, the atomic weight, the toxicity and specific chemical properties. Examples of heavy metals include chromium (Cr), cadmium (Cd), mercury (Hg), arsenic (As), lead (Pb) and thallium (Tl). Heavy metals such as iron, copper and zinc is very important in our bodies. Others metal doesn't have benefit for human health. Even in low concentration, heavy metals still can be the main factor of the harmful effects to human and animals because there is no good mechanism in human and animal body to eliminate it significantly. Nowadays, heavy metals are easier to detect because of the excessive use in the industrial sector (Arora *et al.,* 2008). Metals pollution are very dangerous to biological systems as they do not undergo biodegradation. Heavy metals toxin such as cadmium and lead can be differentiated from other pollutants since they do not undergo biodegradation but they can accumulate in human or other living organisms. Furthermore, risky disorders and diseases can occur even in low concentrations (Pehlivan *et al.,* 2009).

Heavy metals can be classified as essential (iron, zinc, copper, manganese), probably essential (vanadium, nickel, cobalt) and lastly potentially toxic (mercury, cadmium, arsenic, lead). These essential metals can also produce toxic effect when their uptake is excessive. Heavy metals are very dangerous and harmful to human body because of its presence can interface the metabolism process (Uluozlu *et al.,* 2007). There are two paths which are accumulation and uptake of heavy metal elements through the foliar surface, leaf and roots. Thus, heavy metals may be consumed by absorption in plants through a few processes and lastly enter the food chain. Through absorption, uptake of heavy metals by plants from the contaminated soil may be increases. Absorption through roots can be increases or decreases due to the a few

factors such as soil pH, the solubility heavy metals content in soil, types of soils and fertilizers (Sharma, Agrawal, & Marshall, 2006). Table 2.1 showed the effects of heavy metals on human health. Heavy metals can cause serious health effects to human and other living things which is lead to fatal.

Table 2.1: Effects of heavy metals on human health

Heavy metals	Uses	Health effects
Cadmium	Fertilizers, electroplating, battery manufacturing and mineral processing.	Kidney damage, lung damage and cancer.
Chromium	Mining galvanometric, metal plating electroplating leather and dye pollution.	Kidney damage, ulcer and skin irritation of liver (Kumar *et al.*, 2006).
Mercury	Chemical manufacturing, metal finishing and metallurgy industries.	Heart rate increasing, kidney damage, brain damage, tremor and short-term memory problem.

(Source: Moges, 2014)

2.2.1 Heavy Metals Content in Plants

According to Sotiris Stasinos et al., (2014), onions and other source of food samples are analysed and examined by using AAS and ICP-MS and it traced the presence of fifteen trace elements such as As, Ag, Ba, Zn, Cd, Cr, Co, Mn, Cu, Pb, Ni, Se, Rb, V and Sr. All the samples are grown at allotment gardens or farmlands. Zn,

Cu, As and Ni are the example of heavy metals known as the basic of follow components especially for plants. Mn, Fe, Co, Ni, Mo, Cu and Zn are the examples of various heavy metals that recognize as the key to micronutrients and significantly needed for development, electrons exchange and metabolism of mechanism in plants (Ghani *et al.,* 2012). However, heavy metals such as Pb, Hg and Cr are considered as non-essential but they have high potential of toxicity to plants if abundantly present and cause the inhibits the growth rate of the plants. Besides that, various diseases can affect human if metal contamination occur in plants. Heavy metals are among the contaminant in the environment. Plants absorb either naturally or from pollution in order to obtain heavy metals. Heavy metals commonly occur in the system of biological with higher fixation (Oves *et al.,* 2016). Anthropogenic activities are one of the main activities that can cause contamination in soils. Moreover, heavy metals usually combine with other elements in surrounding environment such as sulphur or chlorine, oxygen and inorganic substances. Moreover, heavy metals usually combine with other elements in surrounding environment such as sulphur or chlorine, oxygen and inorganic substances.

2.2.2 Chromium Uptake in Plants

Chromium, Cr is the example of heavy metals which have less attraction for plant uptake. But Cr is dangerous to environment and to human health. Cr give harmful effects to plant photosynthesis, germination, nutrition and cause stress in oxidation process (Gomes *et al.,* 2017). Toxicity of chromium depends on its metal speciation, which is determinant for translocation, accumulation process and its uptake. The toxic of Cr for plants can be consider from 0.5 until 5.0 mgm L^{-1} in the nutrient solution and

from 5 until 100 mgm L^{-1} in soils. However, the normal conditions for chromium concentration is lower than 1 μg g^{-1} (Saha, Shinde, & Sarkar, 2017).

2.2.3 Cadmium Uptake in Plants

Cadmium, Cd is another type of heavy metals which act as micronutrient to plant due to its transport in soil-plant system. Cd toxicity towards plants system will be affected the biochemical, ecological and physiological aspects. Thus, toxic symptoms of Cd in plants can be seen through plant development and growth, enzymatic reactions, membrane functioning, stomata regulation and protein metabolism (Tuan *et al.,* 2013). The amount of cadmium absorption towards plants depend by the soil factors.

2.2.4 Mercury Uptake in Plants

Mercury, Hg is a poisonous substance which has no recognized feature in human biochemistry or body structure and does no longer occur obviously in living organisms. Hg is a global pollutant with complicated and unusual chemical and bodily residences. The important herbal supply of mercury is the degassing of the Earth's crust, emissions from volcanoes and evaporation from the nature of our water bodies. Hg entered human body through the food chain which means that also through foods that we ate daily. Although the roots of plants can absorb certain amount of mercury, Hg from the soil, however the mercury, Hg does not move from the roots because it is not been release to the environment (Tomiyasu *et al.,* 2005). A metal such as Hg have singular and different properties compare to others transition metals. But when it exists in room condition, it exists in liquid form. Mercury, Hg often being applied in many technologies such as light bulbs and informatics areas as it is very good conductor of

electricity. These proprieties explain the ability and effectiveness of Hg to move in several ecosystems and remain in the atmosphere for long periods, being later on deposited in the soil or water bodies (Pheng *et al.*, 2003). Due to the additional amount of heavy metals in fertilizers, sludge, manures and lime, contamination of soils by Hg is occurred. The existence of Hg in soils is dynamic with amount of Hg uptake in plants is not linear and depends at several variables. The variables are soil pH, plant species, cation exchange capacity and soil aeration. When the pH of soil is high it will reduce the Hg uptake or there is an abundance of salts and lime (Patra, 2004).

Type of species and the variety of plants is one of the factors affecting the accumulation of Hg level in plants (McGrath, Zhao, & Lombi, 2001). All of them have 45 plant species including the metal-accumulating species (Reeves & Baker, 2000). Many plants which uptake amount of Hg tend to accumulate in the shoots but in moderate amounts and also tend to accumulate on the roots due to direct absorption and translocation (Dushenkov *et al.*, 2005). Highly employment in seed disinfectant, herbicides and fertilizers are the factors of why the interaction between plant systems and mercury, Hg is significantly important (Cavallini *et al.*, 2009). Hg is known to affect the antioxidant defense system, by interfering with the modulation of the non-enzymatic antioxidants glutathione (GSH) and non-protein thiols (NPSH) and the enzymatic antioxidants superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Villasante *et al.*, 2005). By taking the same process as micronutrients, the toxic metal ions tend to enter plant cells and compete with these elements for absorption. Mercury, Hg can be classified as class B metal significantly binds with nitrogen ligands and sulphur. Through the ionic channel, the Hg compounds enter the cell while competing with other heavy metals such as zinc, cadmium, iron and copper (Blazka & Shaikh, 2012). When Hg attached with

sulfhydryl and selenohydryl groups, it damaged the tertiary and quaternary protein structure along with altered the function of cellular respectively. Thus, mercury blocked the cellular structure (Bernard, 2008).

2.3 Effect of Heavy Metals on Human Health

Mercury is labelled as the most dangerous heavy metal in the environment. Pink diseases also known acrodynia can be prefer to mercury poisoning. Mercury is released into the environment by the industry activities such as paper and pulp preservatives, pharmaceuticals by-product, mining and agriculture industry. Mercury is labelled as the most dangerous heavy metal in the environment. Pink diseases also known acrodynia can be prefer to mercury poisoning. Mercury is released into the environment by the industry activities such as paper and pulp preservatives, pharmaceuticals by-product, mining and agriculture industry (Morais, Costa, & Pereira, 2012). With the combination with other elements, Hg formed organic and inorganic Hg. Exposure to huge amount of metallic, organic and inorganic mercury can damage the kidneys, brains and the early development of fetus (Alina et al., 2012). Mercury is present in most foods and beverages in the range <1 to 50 μg/kg. Micro-organisms convert the mercury present in soil and water into methyl mercury. EPA has declared mercuric chloride and methyl mercury to be highly carcinogenic. The nervous system is very sensitive to all types of mercury. The malfunction of brain system may be caused by increased exposer to mercury. This action leads to shyness, memory problems, tremors, change in hearing or vision and irritability. Furthermore, metallic mercury exposure towards human in short period of time can lead to vomiting, lung damage, increased the blood pressure, nausea and diarrhea. When a person exposed to organic mercury, there are so many symptoms can be occurred such as hair

loss, headache, memory problems, depressions and tremors. These symptoms are difficult to diagnose because it became common in other conditions nowadays (Martin & Griswold, 2009).

The applications that related to cadmium are plastic and metal coatings, electroplating and batteries. Cadmium and its compounds are classified as Group 1 carcinogens for humans by the International Agency for Research on Cancer. Cadmium is released into the environment through natural activities such as weathering, eruptions, river transport and human activities such as tobacco smoking, smelting, mining and incineration of municipal waste. People who lived nearby areas which being polluted by cadmium are living in fear. Both chronic and acute intoxications occurred from the excessive exposure from the cadmium (Chakraborty *et al.*, 2013). Consumption of cadmium can cause bone mineralization either through renal dysfunction or bone damage. Recent studies which related to humans and animals have exposed osteoporosis also known as skeletal damage is the most dangerous effect of cadmium exposure that disturbs the metabolism of calcium, hypercalciuria and renal stones formations. Huge damage to the lungs can occurred if inhalation of higher levels of cadmium. On very long exposure time at lower concentrations, it can become deposited in the kidney and finally lead to kidney disease, fragile bones and lung damage (Bernard, 2018).

2.4 Phytoremediation

Heavy metals are considered as non-biodegradable compound. They cannot decay either in soil or above the soil. They tend to accumulated in the environment and eventually causing the food chain contamination. The contamination occurred poses hazardous to human health and to the environment. Compound of heavy metals

caused neurological and behavioural changes especially for children. For pregnant women, it caused the birth of pre-mature baby. Some of the heavy metals are also mutagenic, carcinogenic and endocrine disruptors. Due to the heavy metal's pollution, there are many remediation methods which can be divided to chemical and physical methods (Zhitong, *et al.,* 2012) High cost is the main issue. Therefore, phytoremediation is a better solution to the problem.

Phytoremediation is the direct use of living green plants for in-situ, or in place for removal degradation or containment of contaminants in soils, sediments, sludge, groundwater and surface water (Cristaldi *et al.,* 2017). This technique has many benefits to the environment and also to the consumer such as it is very useful for treating a wide variety of contaminants especially occurred in the environment. It is also a low cost, solar energy that used when in clean-up technique. Lastly, it is very useful a shallow site for low levels of contamination. To remove pollutants from soil, sediment and/or water, plants can break down, or degrade, organic pollutants or contain and stabilise metal contaminants by acting as filters or traps (Marmiroli, Marmiroli, & Maestri, 2006).

2.4.1 Phytoextraction

There are different categories of phytoremediation, such as phytoextraction, phytodegradation, rhizosphere filtration, phytovolatilization, phytostabilization and phytostimulation. Plants are the most important elements in components of ecosystem as they converted elements from abiotic into biotic surroundings. Phytoextraction also known as phytoaccumulation. Both techniques have their own definition. Phytoextraction can be defined as removal of contaminant from soil, groundwater or surface water by plants while phytoaccumulation can be defined as the contaminant

taken up by the plant is not degraded rapidly or completely. Both processes involve to remove contaminant from the contaminated soil. Plants which are involved will absorb, concentrate and precipitate the metal toxins from the contaminated soils into above ground biomass such as shoots, leaves, fruits and stems. Some plants can tolerate hyper-accumulation such as perchlorate and can grow in contaminated areas. Other plants that cannot tolerate may die, some can still be used in contamination area then been harvested and disposed (Steven *et al.*, 2014). Replant if necessary, to complete remediation. If the goal is to harvest, select plant that able to translocate contaminant from the root into above ground tissue such as shoot and leaves. If the contaminant remains in the root, the harvesting process may be difficult.

2.5 Contaminated Soil

Soil may become contaminated because of the heavy metal's accumulation through the rapidly production from the mine tailings, industrial areas, wastewater irrigation, disposal of heavy metal waste, pesticides, sewage sludge and animal manures (Khan *et al.*, 2007). The most element that can be found in contaminated soil are lead (Pb), mercury (Hg), chromium (Cr), zinc (Zn), copper (Cu) and arsenic (As) that also can give effects to human health as well (Wuana & Okieimen, 2011). When the toxic metals present in the soil, it can severely cause the inhibition of the organic contaminant's biodegradation. Contamination soil cause by heavy metals are dangerous to human health and to the ecosystem either through contact or direct ingestion with contaminated soil. Through phytoremediation, there is a need to decrease the amount of heavy metals that could transfer from soil to plant (Rollon *et al.*, 2017). Anthropogenic activity is one of the main affects to contaminated soil.

2.5.1 Synthetic Soil

Synthetic can be defined as noting or pertaining to compounds formed through a chemical process by human, as opposed to those of natural origin. In other words, there are any substances that are made by mixing chemically, especially to imitate a natural product. Creating a synthetic soil has been an agrarian practice which uses various techniques such as the addition of substances or organisms (Armstrong, 2014).

2.6 Atomic Absorption Spectrophotometer Analysis (AAS)

Atomic Absorption Spectroscopic Analysis (AAS) is a spectro-analytical procedure for the quantitative determination of elements in chemical by using the absorption of optical radiation which is light by freeing the atoms in the state of gaseous (Santos *et al.,* 2014).

2.7 Electron Dispersive X-Ray Spectroscopy (EDS)

Electron Dispersive X-Ray Spectroscopy (EDS) is an analysis by using microanalysis chemically technique which correlates with the using of SEM. The EDS have an x-ray that detects the relative abundance of x-rays emitted versus energy. The range of x-ray energy spectrum counts is measured to determine the composition in element of the sample (Severin & Kenneth, 2004).

CHAPTER 3

MATERIALS AND METHODS

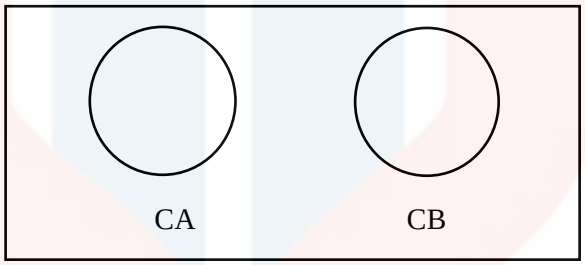
3.1 Materials

The materials used in this research are the *Allium cepa L*. plants, soils, pots, syringe filter, filter paper (Whatman 102). The chemicals used are 3 % hydrochloric acid (HCl), 65 % nitric acid (HNO_3) and 30 % hydrogen peroxide (H_2O_2). The apparatus that were used including pestle and mortar, blender, volumetric flasks, beakers, conical flasks, 50 mL and 15 mL of Falcon tubes, measuring cylinders, filter funnel, 60 mL syringe, porcelain crucible and reagent bottles.

3.2 Preparation of Pots

The amount of pots that were used for this research consists of twelve medium size pots with same height, width and volume. All the pots were rinsed with distilled water in order to remove any unwanted substances and let it dry with room temperature. Two pots were used as control pot. First pot was labelled with CA which contain only soil sample with *Allium cepa L.* plant and second pot was labelled with CB which contain only soil sample. The soil sample were filled with homogenous solution of same concentration heavy metals (Cr, Cd, Hg) which was 20 mg/L each. Another five pots were filled with the synthetic soil and labelled as T1, T2, T3, T4 and T5 each. Those pot were filled with different concentrations of heavy metals (Cr, Cd, Hg) starting from 5 mg/L, 10 mg/L, 15 mg/L, 20mg/L and 25 mg/L each. This was the

main line to observe. Next, pots were duplicated into second line by using another five pots. Figure 3.1 showed the illustration arrangement of ten pots including two controls pots.



5ppm Chromium Cadmium Mercury	10ppm Chromium Cadmium Mercury	15ppm Chromium Cadmium Mercury	20ppm Chromium Cadmium Mercury	25ppm Chromium Cadmium Mercury
T1	T2	T3	T4	T5
D1	D2	D3	D4	D5

Figure 3.1: Illustration arrangement of ten pots including two control pots

3.3 Preparation of Synthetic Mixture of Soil Samples

Synthetic can be defined as noting or pertaining to compounds formed through a chemical process by human, as opposed to those of natural origin. In other words,

are any substances that are made by mixing chemically, especially to imitate a natural product (Nortcliff *et al*., 2006). The techniques used in this research were by mixing the soil samples which were took from the Agropark with selected heavy metals such as cadmium, chromium and mercury. The initial soil sample was measured and analyzed by using Energy-dispersive X-ray spectroscopy (EDS) in order to determine the original composition in the soils before mixed them with new concentration of heavy metals (Cd, Cr, Hg). The soil samples weighted 20 g were kept into sample bag and ready to be analyzed.

Different concentration of heavy metals was used which are 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L. Three heavy metals solution were mixed in 50 mL Falcon tube with the same concentration and made-up to 45 mL. Next, weighed the soil for 1000 g and put into the sample bag. After the concentration have been determined, took 45 mL of homogenous 5 mg/L of heavy metals (Cr, Cd, Hg) and poured into each sample bag. Mixed thoroughly with the soil. This is very important step because each pot only can hold with 1000g of soil and 45 mL of homogenous solution of heavy metals. The mixtures becoming the synthetic soil which mimicking the actual situation when soils are contaminated with heavy metals.

3.4 Nursery Description and Plant Monitoring

The experiment was conducted in a nursery nearby the aqua-culture lab in University Malaysia Kelantan of Jeli Campus within 28 days approximately 4 weeks. The plants were chosen and identified by using its common characteristics such as features, colors and odor. Eleven pots of medium sizes are used for cultivation and onion monitoring process. This study was initiated by planting bulb of onions into the synthetic soil with 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L concentration of

heavy metals respectively. Firstly, each bulb of onion is rinsed with distilled water to remove any impurities. Each pot was planted with one bulb of onion in the synthetic soil. The remaining five pots were used as the duplicate from the first five pots. Meanwhile, two pots were separated and labelled as control pot A (CA) and control pot B (CB). CA consist the mixture of soil sample and heavy metals and CB consist only soil sample with *Allium cepa L.* plant. An uncontaminated soil sample was served as control soil in this study. The pots were arranged from T1 until D5 based on Figure B.1 (Appendix B).

The monitoring process of onion plants begins by putting all the plants in same controlled temperature between 27 °C – 29 °C in the nursery. The nursery had transparent roof that made-up of strong synthetic plastic which allowed enough amount of sunlight to enter and prevented the plant from rainfall. The plants were watered every day. The excess water that flowed at the bottom of the pots were re-used to water the plant for 4 weeks. The changes of onion plants were observed according to their parameters such as color, height and width. Each week, pH and moisture content of soil were measured and recorded. 20 g of soil samples from each pot was collected for tested. Moreover, 10 mL of water samples were collected to measure the pH value for each week.

3.5 Preparation to Identify Soil Physical

3.5.1 Soil pH

The pH of the soil was tested by using pH meter. Every one week, the soil samples were collected from each pot. The collected soil samples were put into the oven at 60 °C for three days until the drying process had completed. After three days,

the soil samples were crushed by using blender and further grinded by using pestle and mortar in order to obtain smaller particles. Mixed thoroughly the soil samples for better results and sieved through 250 μm receiver to obtain fine powder form. 8 g of soil sample was placed in 100 mL conical flask. Next, the soil sample was filled-up with 30 mL of distilled water and shaken the mixture by using orbital shaker at 135 rpm for 10 minutes. The mixture was kept to shake for 10 minutes to make sure that the mixture mixed properly. The pH meter was calibrated with pH 7 buffer solution before performed each readings of soil samples. Dipped the sensitive delicate pH scan glass electrode bulb properly into distilled water until the meter changed between pH 6 – pH 7. The pH scan electrode was dipped inside the conical flask. The pH reading was taken when it stabilizes. The steps were repeated for three times in order to calculate the average of pH and the data was recorded. Figure 3.2 showed an experiment conducted to test soil's pH by using the pH meter.

Figure 3.2: Tested soil's pH by using pH meter.

3.5.2 Soil Texture

Soil texture is one of the most important properties of a soil (Martens, 2005). Based on USDA system, soil particles can be separated into four groups such as sand, gravel, clay and silt. Besides, soil textural class names were determined by their relative mass percentages of sand, clay-sized and silt particles in the soil (Yolcubal *et al.*, 2004). Soil texture was determined on the drying process and 250 μm sieved soil sample by combination of wet sieving and hydrometer methods. Figure 3.3 showed the standard soil texture triangle which been used to identify the soil texture of the soil sample. Table 3.1 showed soil particle size according to USDA classification.

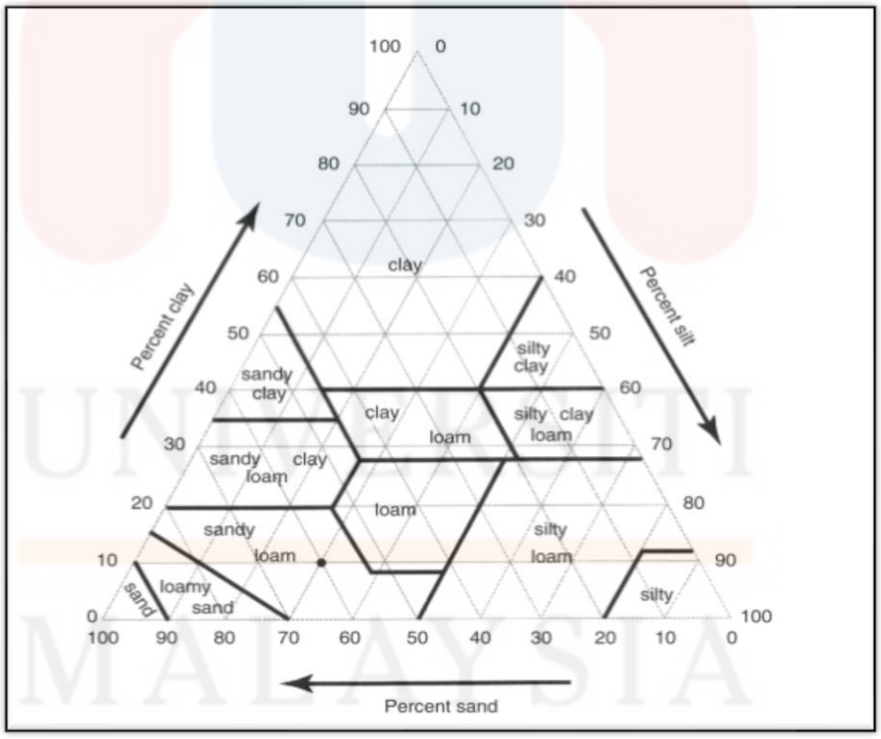


Figure 3.3: The Soil Texture Triangle

Table 3.1: USDA classification of soil particle size.

Type	Diameter (mm)
Gravel	>2
Sand	0.05 - 2
Very coarse sand	1 - 2
Coarse sand	0.5 - 1
Medium sand	0.25 - 0.5
Fine sand	0.10 – 0.25
Very fine sand	0.05 – 0.10
Silt	0.002 – 0.05
Clay	<0:002

(Source: Yolcubal *et al.*, 2004)

3.5.3 Soil Moisture Content

Gravimetric method was the most reliable, accurate and cost-effective method to determine the moisture content in soil (Lunt *et al.*, 2005). The soil moisture content was used to show the amount of water present in the soil. Using the gravimetric method involved weighing the wet sample of soil to obtain the wet mass and put into an oven for removing the water by drying it at 60 °C for three days. Drying soil samples were reweighing to determine the dry mass. Dry mass indicates that how much the amount of moisture had been removed.

Soil moisture content was obtained by dividing the difference between wet and dry masses by the mass of the dry soil sample to obtain the ratio of the mass of water

to the mass of dry soil. When the ratio multiplied by 100, the percentage of water in the soil was obtained. The gravimetric soil moisture content was calculated by using the formula below (Equation 3.1):

$$\text{Moisture content \%} = (Mw / Ms) \times 100 \quad (3.1)$$

Where Mw is the mass of water and Ms is the mass of dry soil.

3.6 Plant and Soil Sample Preparation for Analysis

3.6.1 Preparation of Plant Sample by Improved HNO_3 / H_2O_2 Digestion

The collected plants sample were dissected into three different parts such as bulbs, roots and leaves. All the parts were rinsed with distilled water to remove any impurities in the sample. Weighed all the plant sample to obtained wet mass. The different parts of the plants (roots, bulbs and leaves) were let dry in the oven dry at 105 ºC for 24 hours. The wet and dry mass of plant samples were recorded precisely. The moisture content of plant samples was calculated. The dried parts of plant samples were crushed by using blender and further grinded by using pestle and mortar into a fine powder. Let the samples dried again in oven for 3 hours at 37 ºC temperature to obtain their constant mass. Kept the powder sample in desiccator in order to maintain it in dry condition (Salem, 2011).

The plant samples were digested by using improved HNO_3 / H_2O_2 digestion before analyzed by using atomic absorption spectrophotometer (AAS). 5 mL of 65 % HNO_3 were added into 0.5 g fine powder of plant samples in 50 mL crucible and stirred it vigorously with glass rod. Next, slowly added 4 mL of 30 % H_2O_2 into the mixture and slightly stirred until bubbles can be seen. Heat on the hot plate until strong bubbles

were produced. Heat it on the hot plate for 8 minutes with 180 °C until the brown fumes can be seen less dense in the fume cupboard. The solution was allowed to cool beside the hot plate until slightly yellow solution with a small white solid quantity in suspension can be seen at the bottom of the crucible. Figure 3.4 showed the yellowish color solution with a small white solid in suspension at the bottom of the crucible.

Figure 3.4: Yellowish solution with small white deposited at the bottom in crucible

The solution was filtered by using filter paper (Smith 102 Qualitative) into 50 mL volumetric flask and washed with 5 mL of 3 % HCl. The solution was diluted with 3 % HCl until reached the calibration mark (Hunt, 2012). Lastly, the solution was filtered again by using syringe and 0.45 μm syringe filter. The solution was transferred into 50 mL Falcon tube and ready to analyze by Perkin Elmer Atomic Absorption Spectrophotometer (AAS).

3.6.2 Preparation of Soil Samples

Weighed all the soil samples to obtained wet mass. The sample of the soils were let dry in the oven dry at 105 °C for three days. The wet and dry mass of soil samples were recorded precisely. The moisture content of soil was calculated. After

three days, the soil samples were crushed by using blender and further grinded by using pestle and mortar in order to obtain smaller particles. Mixed thoroughly the soil samples for better results and sieved through 250 μm receiver to obtain fine powder form. The powder sample was kept in sample bag until used for analyzed by using Energy-dispersive X-ray spectroscopy (EDS).

3.7 Preparation of water samples

The tap water used to water the plants were collected and analyzed for the heavy metals concentration by using AAS. The excess water which was deposited at the bottom of the pots was collected in sample bottles. Nitric acid (HNO_3) digestion method was preferred by filtering of the samples (Hseu *et al.*, 2002). 100 mL of mixed water sample was transferred into 150 mL beaker. 3 mL of 65 % HNO_3 were added and slightly stirred. Heat the mixture on the hot plate for thirty minutes at 100 °C on to almost dryness. Then, 2 mL of 33% HCl were added to the sample and allowed it to cool in the fume cupboard. After cooled, transferred the solution into 100 mL of volumetric flask and diluted until reached the calibration mark with 3 % of HCl. The digested water samples were tested by using Perkin Elmer 400F Absorption Spectrophotometer (AAS).

3.8 Dilution of Standard Solution

The standard solutions for this study are chromium (Cr) solution, cadmium (Cd) solution and mercury (Hg) solution. All the solution will be diluted until it reached the calibration mark of 50 mL volumetric flask.

A series of chromium, Cr standard solution is prepared. A dilution is diluted from 1000 mg/L of Cr in order to get 100 mg/L and further successive dilution to get

certain volume for the concentration of 0 mg/L, 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L as the series of chromium, Cr standard solution (Hegazi & Hala, 2013). Another dilution has been conducted in order to get the standard solution in milliliter, mL unit by using as following formula:

$$M_1V_1 = M_2V_2 \quad (3.1)$$

Where,

M_1 = the concentration in molarity of the concentrated solution.

M_2 = the concentration in molarity of the dilute solution.

V_1 = the volume of the concentrated solution.

V_2 = the volume of the dilute solution.

The dilution was diluted by using same volumetric flask which is 50 mL. So, the volume of volumetric flask was considered as V_2 which is the volume of the dilute solution. Thus, with the constant concentration in molarity of the concentrated solution which is 100 mg/L, the volume of concentrated solution was obtained. For this study, the concentration in molarity of the dilute solution were calculated by using different concentration in order to get the exact volume of the diluted solution. Table 3.2 showed the volume of the concentrated solution obtained after the calculations between the concentration in molarity of the concentrated solution (M_1) and the volume of the concentrated solution (V_1).

Pipette the volume of concentrated solution and dilute with distilled water until it reached the calibration mark of volumetric flask. The steps were repeated for

cadmium, Cd and mercury, Hg solution. The reading of prepared standard solution was recorded after analysing by using AAS.

Table 3.2: The volume of concentration obtained after calculation

M_1	M_2	V_2	V_1
100 mg/L	0 mg/L	50 mL	0 mL
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	10.0 mL
100 mg/L	25 mg/L	50 mL	12.5 mL

3.9 Identification of Heavy Metal Concentration Using AAS

The previous Improved HNO_3 / H_2O_2 digestion method of plants sample were continued by obtaining the diluted solution to determine heavy metals content by using AAS. Transferred 50 mL of diluted solution into 50 mL Falcon tube by using syringe filter and act as stock solution. Each four of Falcon tube are labelled with 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} respectively. 15 mL of solution was transferred into other 15 mL Falcon tube and act as analysis solution. 1.5 mL of analysis solution was transferred and mixed with 13.5 mL of 3 % HCl in Falcon tube labelled with 10^{-1}. The solution was shaking until it become homogenous. Next, transferred 1.5 mL from Falcon tube 10^{-1} and mixed with 13.5 mL of 3 % HCl in Falcon tube labelled with 10^{-2}. The solution was shaking to become homogenous. Repeated these steps for Falcon tube labelled

with 10^{-3} and 10^{-4}. The heavy metal elements in plants sample were analysed by using Atomic Absorption Spectrophotometer (AAS).

3.10 The Distribution of Heavy Metals

The distribution of heavy metals from soil to plant was determined by comparing the initial value of heavy metals concentration in soil with the final value of heavy metals concentration in soil in each pot. The plant's ability to absorb the heavy metals and translocated to all part of the plant such as roots, bulbs and leaves were measured by using Bioconcentration Factor (BCF) (Equation 3.2) (Melgar, Alonso, & García, 2009) and the Translocation Factor (TF) (Equation 3.3) (Nouri *et al.*, 2009). However, if the translocation factor was less than one, this could be a desirable property that can avoid potential risk to the environment by overcoming the transmission of excess heavy metals concentration into food chain through the herbivores. The efficiency of heavy metals translocation at different parts of plant was calculated by using the equation below:

$$\textbf{Bioconcentration Factor (BCF)} = \frac{\text{Heavy metals concentration in plants (mg/L)}}{\text{Heavy metals concentration in soil (mg/L)}} \quad (3.2)$$

$$\textbf{Translocation Factor (TF)} = \frac{\text{Heavy metals concentration in shoots (mg/L)}}{\text{Heavy metal concentration in roots (mg/L)}} \quad (3.3)$$

3.11 Statistical Analysis

The heavy metals distribution and translocation in plants was analysed using one-way ANOVA at 95 % significant level. All comparisons were subjected to One-Way analysis of variance (ANOVA) analysis by using the SPSS Statistics version 22, the different between the mean were compared by analysed through Duncan's post Hoc tests at the significant level of $p < 0.05$. The data of distribution between the soil and plant were calculated and recorded using statistical data for better interpretation and understanding the ability of the *Allium cepa L.*to translocate heavy metals to all part of plant respectively. The comparison of interpretation data was applied into graph form. All the results obtained were expresses on concentration (mg/L).

3.12 Quality Control (QC)

Procedures that lead to control different step in measurement process is quality control (QC). QC done to prove the reliability of the results included duplication of sample and reagent blank analyses. To improve the accurateness, steps were taken to control the sample exposure to outside contamination such as contaminated acids and reagent, airborne contaminated and personal contamination.

CHAPTER 4

RESULTS AND DISCUSSIONS

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4.1 Soil

4.1.1 Type of Soil

The taxonomic classification of the experimental soil texture in this study was silt clay soil where 30 % silt, 50 % clay and 20 % sand. This type of soil has many characteristics that can be seen with naked eyes. Firstly, the particles of the sand itself can be seen clearly when mixed into soil. Secondly, easily formed into a string when it had bigger surface area. Bigger surface area of soil can cause higher rate of evaporations. Thirdly, the size of the particles. The diameter of the soil particles defined type of soil. Based to USDA classification of soil particles size, the diameter of silt and very fine sand were between the range of 0.002 – 0.05 and 0.05 – 0.10 in mm unit. Lastly, the silt particles consist of low ability to reserve plant nutrients. According to Figure 4.1, SEM was used to determine the diameter of the soil particles. Electron microscope in the SEM produced a zoomed image with x1000 magnification that showed the diameter of soil particles was 0.01 mm. Table 4.1 shows its an elemental distribution of soil sample took from the Agropark.

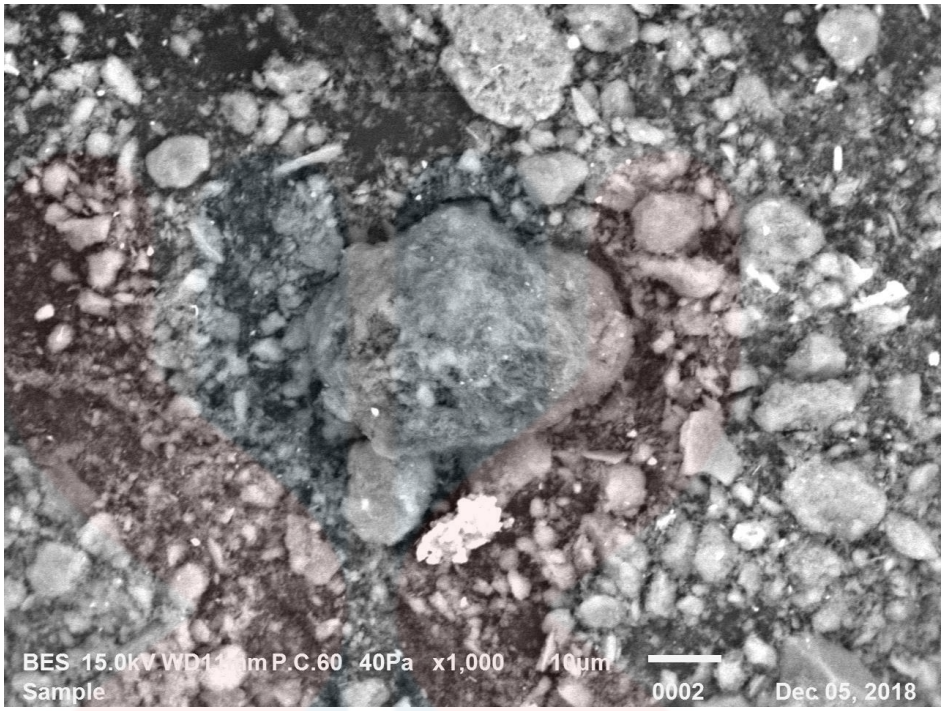


Figure 4.1: The image represents soil particles analysed by using SEM machine with x1000 magnification.

Table 4.1: Elemental distribution of soil sample took from Agropark

Type of nutrient	(%)	Concentration (Mg/L)
Carbon, C	14.96	0.0015
Oxygen, O	46.79	0.0047
Bromium, Br	10.20	0.0010
Aluminium, Al	7.63	0.0008
Silicon, Si	15.68	0.0016

4.1.2 Initial Soil pH Value

The pH and moisture content of original soil sample also were measured. The pH value of original soil was 6.26 pH. Soil pH that suitable for growth of *Allium cepa L.* plants usually slightly acidic (Karim, Rezaul & Ibrahim, 2013). The range of pH is between pH 5.50 – 6.50. Soil pH can be defined as the measurement of acidity range and alkalinity range in a soil. It is very important because it affect some of soil factors for plant growth such as nutrient availability, nutrient leaching, structure of soil and soil bacteria. Nitrogen from fertilizers and organic properties was released because of bacterial activity is affected by soil pH. This is because bacteria work the best in the pH 5.5 to 7.0. The structure of soil especially clay type of soil was easily affected by pH value. When the pH soil was below pH 5.0, plant nutrients leach out more rapidly from the soil compared to soils that had pH range between pH 5.0 and 7.5.

Soil pH plays an important role in the absorption and retention of heavy metals in soil. It controls the hydrolysis and solubility of metal carbonates, phosphate group and hydroxides and also effect the solubility of organic matter, iron pairing information, clay edges and organic properties, aluminium oxides and manganese (Violante *et al.*, 2010). The attenuation of heavy metals in the soil were depends on the properties of soil. Through the reactions with solid phases, it controlled the solubility of heavy metals in soil structure. Moreover, clay fraction which was the composition of clay minerals caused the soil structure to have high potential to combine with heavy metals. Soil also had composition in granulometric form for silt, clay and dust. Higher content of organic matter caused a stronger ability and a higher absorption capacity to bind with metallic elements (Sherene, 2010). However, sandy type of soil had a low absorption capacity and acidity weakly properties.

The pH value of soil samples after added the heavy metals with different concentrations was also measured. The soil pH was within the range between 6.10 – 6.40 pH which is the suitable pH condition for most vegetable growth and ensure the bioavailability of most essential nutrient which affected the plant's height, growth and development (Violate *et al.,* 2010). Figure 4.2 shows the pH value of each pot after added heavy metals with different concentrations into the soil. The pH value was the initial reading of the soil after added heavy metals to ensure that the soil's pH suitable for plant's growth.

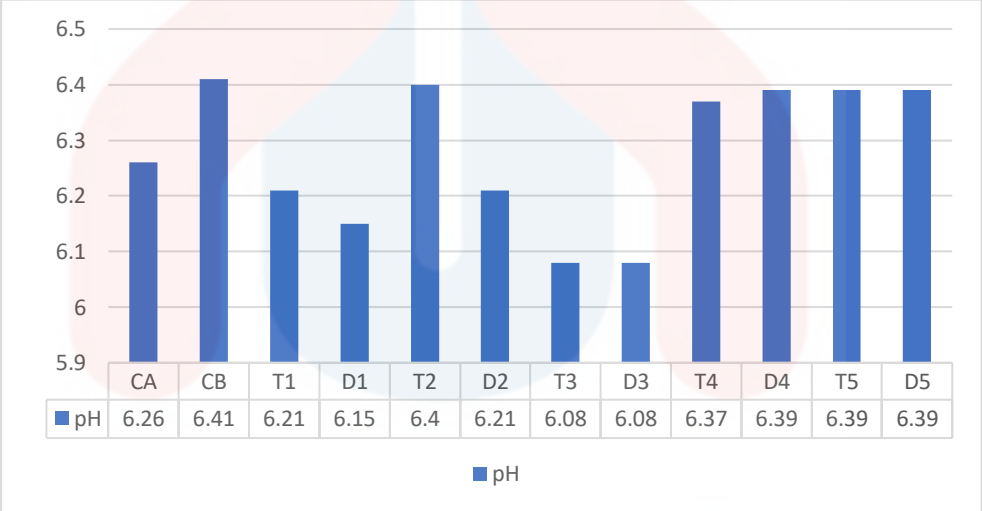


Figure 4.2: The pH values that represent each treatment for a pot

Besides, there were twelve pots used for this experiment and three type of heavy metals (Cr, Cd, Hg) with different treatment in each pot. Two pots were labelled as control, Control A (CA) and Control B (CB). Control A consisted of one plant with no additional of heavy metals in the soils. The growth performances were measured every week. Otherwise, Control B consisted of no plant but only had synthetic soil. The synthetic soil contained heavy metals (Cr, Cd, Hg) with same concentration (20 mg/L).

There were another five pots that consisted of different concentration of heavy metals. The pots named as Treatment 1 (T1), Treatment 2 (T2), Treatment 3 (T3), Treatment 4 (T4) and Treatment 5 (T5). Each treatment represented one same concentration with three different heavy metals in it. The five pots were duplicated into another five pots that were used for growth observations and performances of *Allium cepa L.* plant such as colours, length of leaves and its moisture content. Table 4.2 shows the pot description with the reading of pH values for each treatment that were tested on first day after planted to obtain the initial value and can be compared with final value on the last day (harvesting day).

Table 4.2: The reading of soil's pH value for each treatment in a pot

Pot	Type of Heavy Metals	Initial pH	Initial MC
CA	Plant + soil (no heavy metal)	6.26 pH	44.76 %
CB	Soil only with 20 mg/L of Cr, Cd, Hg	6.41 pH	42.85 %
T1	Plant + 5 mg/L of Cr, Cd, Hg	6.21 pH	41.55 %
D1	Plant + 5 mg/L of Cr, Cd, Hg	6.15 pH	42.67 %
T2	Plant + 10 mg/L of Cr, Cd, Hg	6.40 pH	41.16 %
D2	Plant + 10 mg/L of Cr, Cd, Hg	6.21 pH	34.02 %
T3	Plant + 15 mg/L of Cr, Cd, Hg	6.08 pH	44.1 %
D3	Plant + 15 mg/L of Cr, Cd, Hg	6.08 pH	41.79 %
T4	Plant + 20 mg/L of Cr, Cd, Hg	6.37 pH	43.06 %
D4	Plant + 20 mg/L of Cr, Cd, Hg	6.39 pH	41.32 %
T5	Plant + 25 mg/L of Cr, Cd, Hg	6.39 pH	43.03 %
D5	Plant + 25 mg/L of Cr, Cd, Hg	6.39 pH	44.34 %

4.1.3 Initial Soil Moisture Content (MC)

The moisture content (MC) value of original soil was 40.2 %. Onions were very sensitive to amount of water or water stress (Feibert *et al.*, 2018). Figure 4.3 shows the moisture content of soil samples for each pot after added heavy metals into the soil. The MC of each soil sample is shown in Table 4.2**.** The highest MC was 44.76 % for CA and the lowest MC was 34.02 % for D2. There were different MC in each synthetic soil treatment. MC also known as moisture content was the important element in soil because it acted as a medium for supplying nutrients for plant growth (Magdalena, Andrzej & Polakowski, 2015). The MC of pot T2 is lower among the others because the texture of the soil was dry.

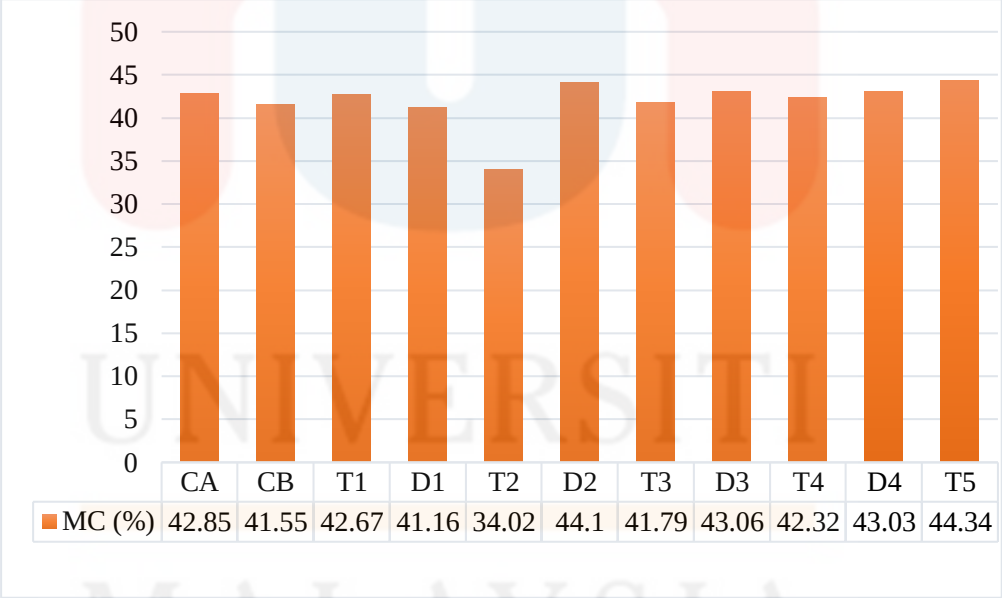


Figure 4.3: The MC of initial soil in each pot.

4.1.4 Soil pH Value at Week 1, Week 2, Week 3 and Week 4

The pH values were tested by using pH meter after crushing into fine powder form. For week 1 the highest reading of pH value was 6.45 pH and week 2 was 6.44 pH. There were slightly different of pH values between week one and week two. However, the range of pH values between that two weeks were at the suitable pH condition for onion plants to grow. The highest pH values for week three and week four were 6.45 pH and 6.47 pH. There were also slightly different between both readings which were only 0.002 pH. The different pH values do not affect the growing of the onion such as changes in height and changes in colour of the plants. Reading of pH values in each pot for week 1, week 2, week 3 and week 4 is shown in Figure 4.4 and all the numeric data can be referred table in Table 4.3. The pH is important because it influences the availability of essential nutrients.

Table 4.3: The pH Reading of Soil at Week 1, Week 2, Week 3 and Week 4

Pot	**pH Reading of Soil (%)**			
	Week 1	Week 2	Week 3	Week 4
CA	6.43	6.19	6.26	6.31
CB	6.41	6.55	6.36	6.45
T1	6.45	6.29	6.52	6.40
T2	6.43	6.33	6.36	6.44
T3	6.51	6.14	6.88	6.45
T4	6.18	6.69	6.89	6.64
T5	6.18	6.80	6.26	6.49
D1	6.39	6.55	6.17	6.38
D2	6.34	6.53	6.59	6.61
D3	6.49	6.60	6.58	6.52
D4	6.31	6.55	6.47	6.60
D5	6.37	6.67	6.73	6.62

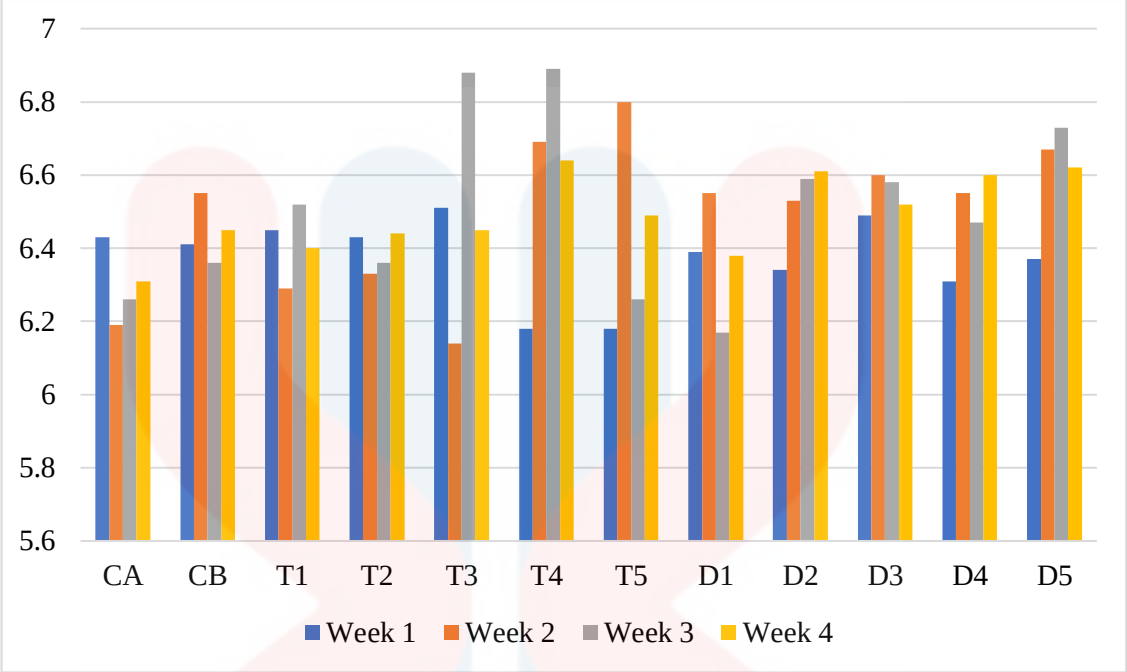


Figure 4.4: The reading of increases and decreases pH values for soil in 4 weeks

4.2 Growth Observation of *Allium cepa L.*

The experiment was conducted for 28 days in a nursery nearby the Aqualab. With the sufficient enough of sunlight and the suitable temperature, all the plant grew well with different concentration of heavy metals (Cr, Cd, Hg). Growth observations process included the plant's growth rate, plant's height and plant's colour were observed every week. The growth rate of the plant depends on the volume of heavy metals added into soil, the changing seasonal (hot, sunny, rainy) and the temperature surrounding. Table 4.4 shows the growth observation for *Allium cepa L.* plant included the height of leaf (measured every week), the colour of leaf and the growth rate of the plant respectively. The growth rate data either state survived or died for plants at week four.

Table 4.4: A table of growth observation for *Allium cepa L.* plant in four weeks

Pot	Length of leaf (cm)				Colour of leaf	Growth rate
	W1	W2	W3	W4		
CA	15.0	28.0	32.5	45.6	Green	Survive
CB	0	0	0	0	-	-
T1	14.6	27.0	33.0	63.8	Green	Survive
D1	17.0	25.0	43.0	63.6	Green	Survive
T2	16.7	29.4	43.0	54.1	Green	Survive
D2	15.0	24.7	8.70	0	-	Died
T3	14.6	26.4	39.5	60.4	Green	Survive
D3	12.3	26.7	35.0	59.8	Green	Survive
T4	13.3	21.2	47.5	35.0	Green	Survive
D4	15.2	32.4	49.0	34.6	Green	Survive
T5	5.6	17.5	35.5	58.7	Green	Survive
D5	15.2	25.0	49.5	68.5	Green	Survive

Plant in pot T2 does not die but crops grew well with the same concentration of heavy metals. Based on Figure 4.5 shows that the duplication of pot T2, D2 was dying slowly at week 3 with the 10 mg/L concentration of heavy metals because the leaf's length become shorter. Another nine pots showing the plant growth was normal and free of diseases symptom. The parameter such as colour and height of plants were measured and recorded every week. The soil was cultivated by using garden towel in order to allow oxygen soluble as well as water dissolved to the root zone easier.

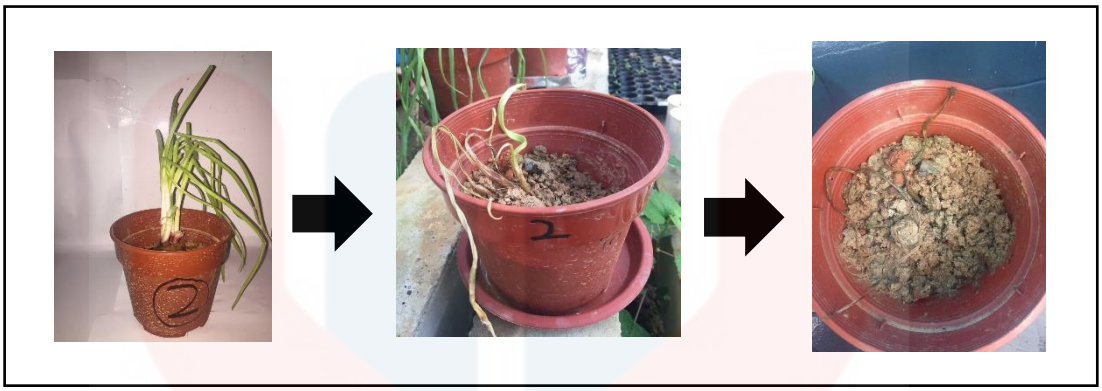


Figure 4.5: The growth of plant in D2 from week two, week three and week four

The length of leaf in pot D5 was the highest at week four which was 68.5 cm while plant in pot CA which contained 0 mg/L of heavy metals had the lowest height. Pot CB contained no plant and acted as control pot which consist only synthetic soil contaminated with 20 mg/L of heavy metals (Cd, Cr, Hg). The height differences between the plants in each week were represented in Figure 4.6.

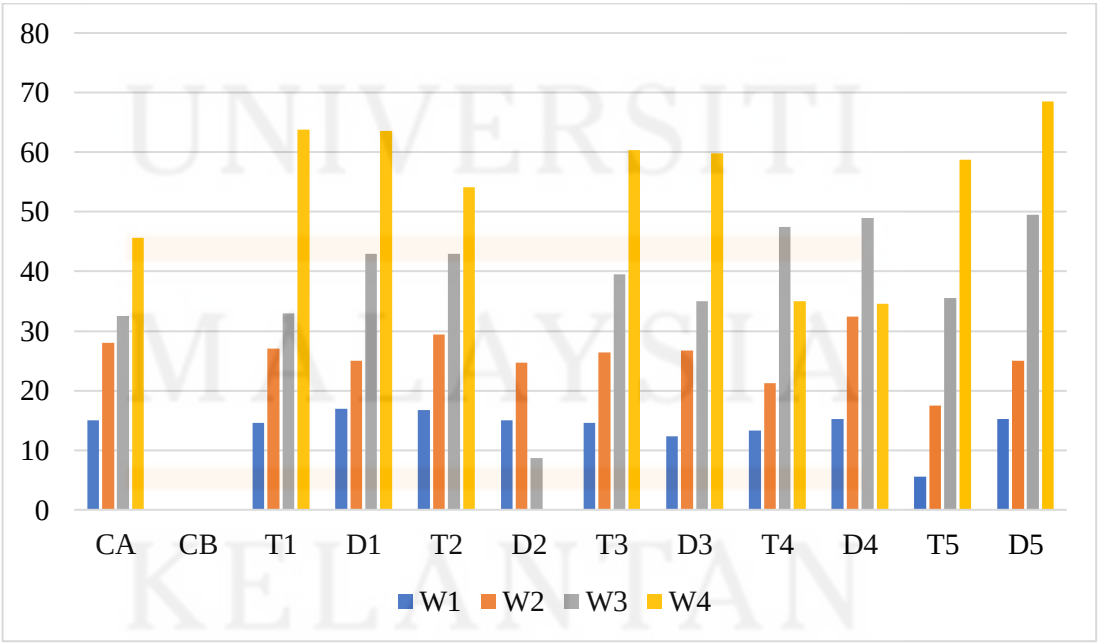


Figure 4.6: The height of leaves in 4 weeks observations

At week four, all the plants were harvested and the weight of plant's part (leaf, bulb, root) were measured. The length leaves decrease for pot T4 and D4 in week 4. Only measured the green colour of the leaf. Thus, leaf that decreases in length shows the yellow colour at the tip of the leaf. Therefore, leaf that turn into yellow colour not consider to measure. All the data were represented in Table 4.5. The date represented the weight of different part of plants (leaf, bulb, root) in gram and their moisture content in percentage after harvested.

Table 4.5: The weight and MC of plant parts

Pot	Weight of Plant Parts (g)			MC of Plant Parts (%)		
	Leaf	**Bulb**	**Root**	**Leaf**	**Bulb**	**Root**
CA	8.68	4.90	5.55	71.43	62.04	63.93
CB	-	-	-	-	-	-
T1	4.10	9.58	6.48	38.29	70.67	62.19
D1	3.33	18.28	2.93	65.46	86.70	62.11
T2	8.68	12.70	8.04	69.70	76.06	51.87
D2	-	-	-	-	-	-
T3	7.84	12.99	4.91	67.86	78.14	56.42
D3	22.38	38.41	9.18	78.24	87.01	52.01
T4	16.68	18.87	11.13	80.93	82.14	76.46
D4	5.39	13.19	3.64	80.70	86.80	62.08
T5	15.71	26.35	6.67	73.77	84.82	67.17
D5	3.34	16.96	5.72	26.65	84.14	37.41

In pot D3 the reading of weight of the leaves were the highest among the others which was 22.38 g compare to the lowest was 3.3 g of leaf in pot D1. The leaves become decreases in their weight because the higher concentration of Cd in plant tissues, the decreases the weight of the leaves.

For root part, the increases the heavy metals concentration in soil treatment levels such as 25 mg/L caused the root to absorb more. The weight of the root become heavier.

4.3 Heavy Metals Accumulation in Plants

After 28 days of heavy metals exposure, *Allium cepa L.* plants sample were harvested and prepared through improved HNO_3 / H_2O_2 digestion for heavy metals concentration in part of plants by using Atomic Absorption Spectrophotometer (AAS). The plant samples were separated into three different parts such roots, bulbs and leaves, originally to identify and study the distribution of heavy metals (Cd, Cr, Hg) accumulated at different part of plants based on Bioconcentration factor (BCF) and Translocation factor (TF). Heavy metals accumulation depends on the ability of different parts of plants (Remon *et al.*, 2005). The amount of heavy metals been absorbed and accumulated in plants affected by the ability of heavy metals plant uptake mechanism and bioavailability of metals in soils samples.

There were 5 treatment of plant with different concentration were analysed by using Perkin Elmer 400 AAS. The experimental pots were all arranged in a straight 2 lines with each had 5 pots. Altogether 12 experimentally pots were arranged and labelled respectively. First line pots were the main focused and the second line pots were the duplication from the first line. It has same type of heavy metals but different concentration, same weight of synthetic soil and same number of plants. The concentration of heavy metals does not cause adverse effect to the environment as the concentration was very low. Heavy metals were all transported from the synthetic soil and translocated to the different part of plants.

Through this experimental duration, soil type and plant were kept as constant. Thus, the heavy metals accumulation in plant can be measured and observed by the length of leaves. The dependent variable in this experiment was the mean (mean and SD) heavy metals accumulated in different parts of plant (roots, bulbs, leaves). Thus, the independent variable was the treatment of heavy metals (5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L).

4.3.1 Distribution of Cadmium in Plants

This study on Cd accumulation in different part of the plant (leaf, bulb, root) was determined the mean. Therefore, distribution of Cd into different parts were calculated. Table 4.6 shows the Cd accumulation at leaf, bulb and root of *Allium cepa* L. which correlates to the different of Cd concentration levels of soil treatment. Therefore, T1, T2, T3, T4 and T5 (5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25mg/L) represented the value of Cd concentration in each pot.

Table 4.6: The Cd accumulations in parts of the plant's samples in mg/L

Pot	Part of Plants (mg/L)			Total Accumulation
	Leaf	Bulb	Root	
T1	0.017 ± 00006	0.024 ± 0.0006	0.017 ± 0.0017	0.058 ± 0.0388
T2	0.022 ± 0.0009	0.028 ± 0.0017	0.038 ± 0.0013	0.088 ± 0.0114
T3	0.025 ± 0.0018	0.030 ± 0.0007	0.041 ± 0.0010	0.097 ± 0.0116
T4	0.027 ± 0.0010	0.067 ± 0.0011	0.063 ± 0.0008	0.157 ± 0.0311
T5	0.054 ± 0.0006	0.022 ± 0.0002	0.027 ± 0.0008	0.103 ± 0.0243

Treatment 1 showed that the highest mean accumulation of Cd was 0.024 ± 0.0006 mg/L in the bulb part. This accumulation at different parts of plant correlate to the 5 mg/L of soil treatment. The total accumulation of Cd in *Allium cepa L*. was 0.058 ± 0.0388 mg/L. The translocation factor between leaves and roots was TF < 1. Thus, hyperaccumulation does not occur in this treatment. Not all plants translocate nutrient and inorganic essential from root to leaf part (Roselli *et al.,* 2003).

Plant that treated with 10 mg/L (T2) of Cd had the highest accumulation at root which the mean value was 0.038 ± 0.0013 mg/L. The lowest Cd accumulation was at the leaf part (0.022 ± 0.0009 mg/L). The TF of Cd for this treatment was 0.6 which is less than 1. The hyperaccumulation process also does not significant occur. The mean value of Cd concentration can be found higher in the roots part which was 0.041 ± 0.0010 mg/L compare to the leaves part (0.025 ± 0.0018) mg/L for 15 mg/L of soil treatment. The TF also does not exceed the limit value which was 0.6 (TF < 1). Root showed the highest accumulator. The main justification of this phenomena to be happen was the roots were the main part of plants which keep in contact with soil medium to accumulate nutrients in order to speed up the growth rate of the plants. Furthermore, the Cd particles pass through the roots before it reached to the aerial tissues of the plant (Peer *et al*., 2014).

Moreover, the mean accumulations of Cd in 20 mg/L of soil treatment also have been analysed by using AAS. Through analysis, Cd accumulated more higher at bulb part which was 0.067 ± 0.0011 mg/L and the lower value at the leaves part (0.027 ± 0.0010) mg/L. The TF value was 0.4 which lower than TF < 1. Based on Figure 4.7, the highest mean accumulation of Cd was in the leaf part (0.054 ± 0.0006) mg/L while the lowest was in bulb part which was 0.022 ± 0.0002 mg/L in 25 mg/L soil treatment. The value of translocation factor for this treatment of *Allium cepa L.* was 2 and thus it

exceeds the limit of TF which was TF > 1. The hyperaccumulation process may occur caused the plant to have excess limit of Cd concentration. All analyte detected by AAS were passed the Quality Control (QC) within limit for Cd 288.80 and the recovery rate was 97.49 %.

The alteration uptake of minerals by plant caused by cadmium through the effects on the bioavailability of other minerals from the soil (Mancalak, 2006). Onion which planted on cadmium polluted soil can cause cadmium accumulation above the acceptable limit. In addition, *Allium cepa L. plant* is one of the good phytoremediation plants to treat cadmium contaminated soils but cannot mistakenly consume in dietary as it may cause adverse effects to health (Bakare *et al.*, 2013). Based on Table C.1 (Appendix C), the concentrations of Cd accumulations in different part of onion plant were not directly proportional with the Cd concentration levels of soil treatment, thus the trend proved that there was no significant value ($p < 0.05$) but the highest and the lowest mean concentration were showed.

The translocation factor was calculated for all the five treatment. There was only one treatment that exceed the limit which was TF > 1. Based on the Figure 4.8 shows the trend of TF for Cd concentration of *Allium cepa L.* plants. The translocation factor was decreasing along with the increasing concentration of soil treatments ($R^2 = 0.1966$) as describe in the graph of figure 4.7. In higher Cd concentration levels of soil treatment, *Allium cepa L.* able to translocate more efficiently thus in specifically the translocation factor more than one.

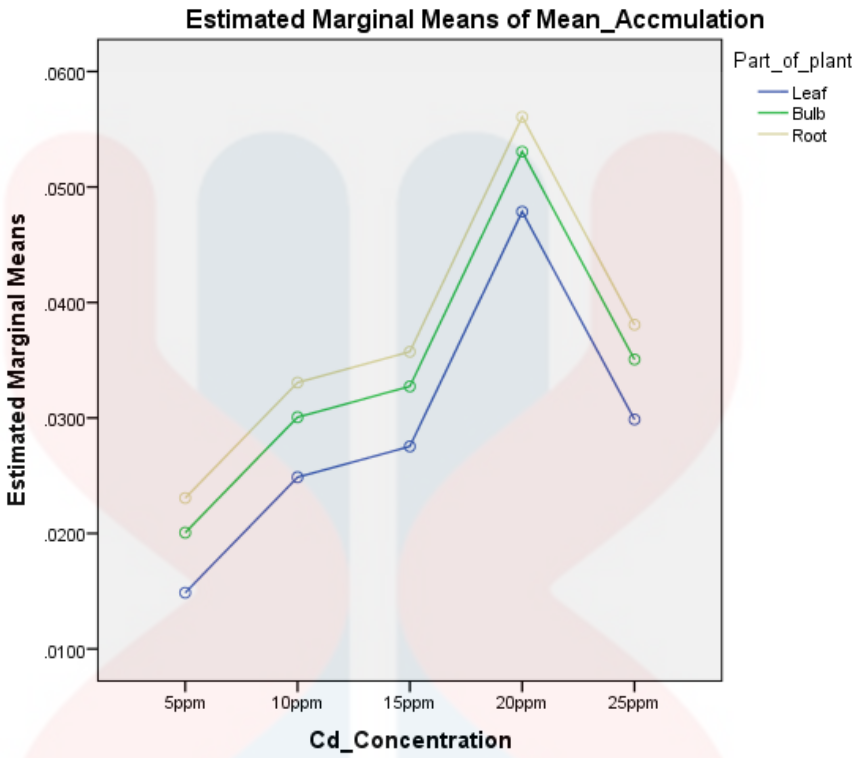


Figure 4.7: The mean accumulation different parts of *Allium cepa L.* plant with five type of soil treatment

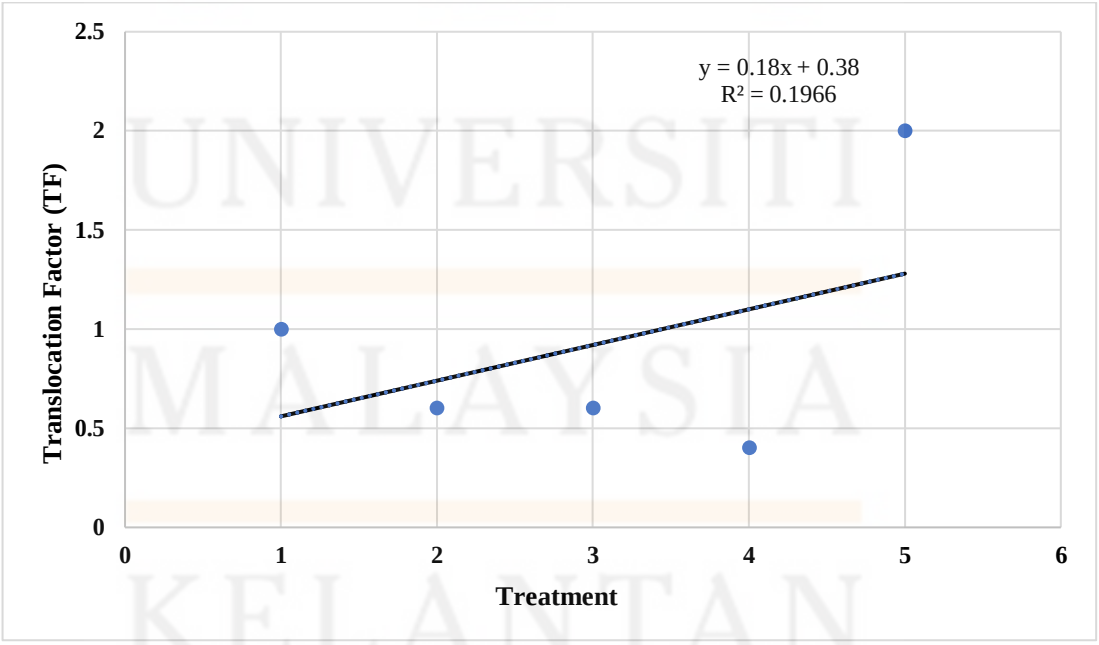


Figure 4.8: The Translocation Factor (TF) of *Allium cepa L.* Plant

4.3.2 Distribution of Chromium in Plants

Plant that treated with 5 mg/L of heavy metals had higher value of chromium accumulated at roots which or in the soil medium which easier to absorb and accumulate the Cd concentration. The lowest was 0.032 ± 0.0102 mg/L. This is because root situated nearby value for mean concentration was at the leaf part which was 0.003 ± 0.0151 mg/L. The total accumulation of Cr in *Allium cepa L.* plant was 0.047 ± 0.0210 mg/L. The translocation factor between leaves and roots was 0.09 which lower than one (TF < 1). Thus, hyperaccumulation of Cr concentration does not occur in this treatment soil level. The main reason of Cr accumulated higher in roots of plants because Cr is attenuated in root cell's vacuoles that may cause Cr toxicity to plant (Shankar, 2014).

Table 4.7: The Cr accumulations in different plant's part at five soil treatment levels

Pot	Part of Plants (mg/L)			Total Accumulation
	Leaf	Bulb	Root	
T1	0.003 ± 0.0151	0.012 ± 0.0134	0.032 ± 0.0102	0.047 ± 0.0210
T2	0.030 ± 0.0256	0.045 ± 0.0196	0.015 ± 0.0021	0.09 ± 0.0212
T3	-0.008 ± 0.0270	-0.012 ± 0.0132	-0.011 ± 0.0211	-0.031 ± 0.036
T4	-0.006 ± 0.0183	0.039 ± 0.0272	-0.007 ± 0.0178	0.026 ± 0.0478
T5	-0.053 ± 0.0265	-0.022 ± 0.0137	0.016 ± 0.0448	-0.059 ± 0.0007

Treatment 2 showed that the highest mean accumulation of Cr was 0.045 ± 0.0196 mg/L in the bulb part. While in leaf and root, the mean accumulation were 0.030 ± 0.0256 mg/L and 0.015 ± 0.0021 mg/L. This accumulation at different parts

of plant correlate to the 10 mg/L of soil treatment. The total accumulation of Cr was 0.09 ± 0.0212 mg/L. The TF of Cr for this treatment was 2 which exceed the translocation factor limit (TF>1). Thus, hyperaccumulation occur in this treatment. Figure 4.9 shows the mean concentration value that were accumulated in different part of plants (leaf, bulb, root).

The mean value for Cr concentration can be found higher in leaf part which was -0.008 ± 0.0270 mg/L compare to the root part for 15 mg/L of soil treatment. The TF value also does not exceed the limit which was 0.7 (TF < 1). Next, the highest mean accumulation of Cr in 20 mg/L of soil treatment was in the bulb part (0.039 ± 0.0272) mg/L and the lowest value was in the root part with only -0.007 ± 0.0178 mg/L. The TF value was 0.9 which lower than TF < 1. The negative mean data also can be considered as 0 because the amount of Cr concentration in part of plants was too little to detect by using AAS. Chromium can cause toxicity effects to the development and growth of plants included the decreasing process of germination, decrease biomass and growth of plant (Nafiseh Nematshahi, 2012). It was noted that chromium accumulated much higher in the root part compare to the leaf and bulb part of the synthetic soil under 15 mg/L treatment.

Lastly, in 25 mg/L of soil treatment levels. The highest value of mean was in root part which indicates 0.016 ± 0.0448 mg/L. Most of Cr was attenuated in vacuoles of roots cells and that's the reason why accumulation in roots was higher (Hayat *et al.*, 2012). The TF also have been calculated. The TF value was 3.3 which exceed the limits. The hyperaccumulation process occur cause the plan tendency to absorb more Cr into the plant. All the Cr mean concentration data have passed the Quality Control (QC) within the limit for Cr 357.87 and the recovery rate was 100.78%.

The increases intake of Cr concentration in the plants because there was an increases tissues in plant structure as *Allium cepa L.* grew larger in biomass. But there were some of Cr concentration that affect the slower growth of plant. Plants that were exposed to high concentration such as 20 mg/L and 25 mg/L of Cr significantly reduce the growth of plant and lead to toxicity environment (Zayed *et al.*, 2003). The concentrations of Cr accumulations in different part of onion plant were not directly proportional with the Cr concentration levels of soil treatment. The trend in graph of mean accumulation showed that there was no significant value because $p = 0.541$ was bigger than $p < 0.05$ but the highest and lowest mean concentration at each treatment were determined. This also proved that *Allium cepa L.* plant was capable to absorb any amount of heavy metals as their nutrient supply but no factor and condition related to it.

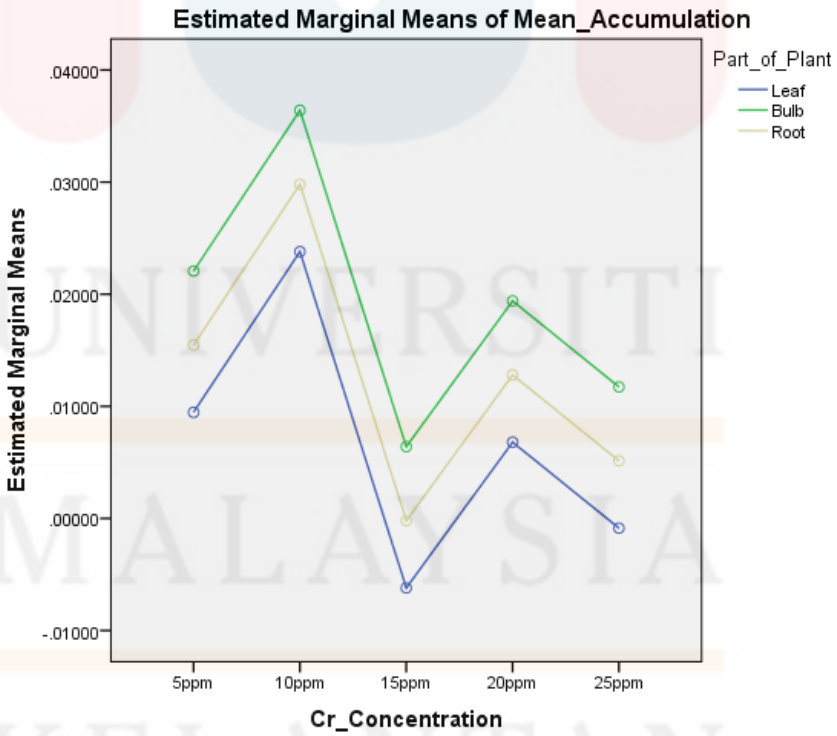


Figure 4.9: The mean accumulation of Cr at different parts of *Allium cepa L.* plant with five type of soil treatment

The TF was calculated by using the formula (refer formula 3.3) for all the five treatment. There were two treatment soil levels (10 mg/L and 25 mg/L) that exceed the permissible limit of translocation factor less than one, TF < 1. Based on Figure 4.10, the trend of TF was not directly proportional to their treatment (R^2 = 0.4404). The highest concentration of soil treatment levels (25 mg/L) caused the plants to accumulate higher value thus the plant can translocate more efficiently at higher levels.

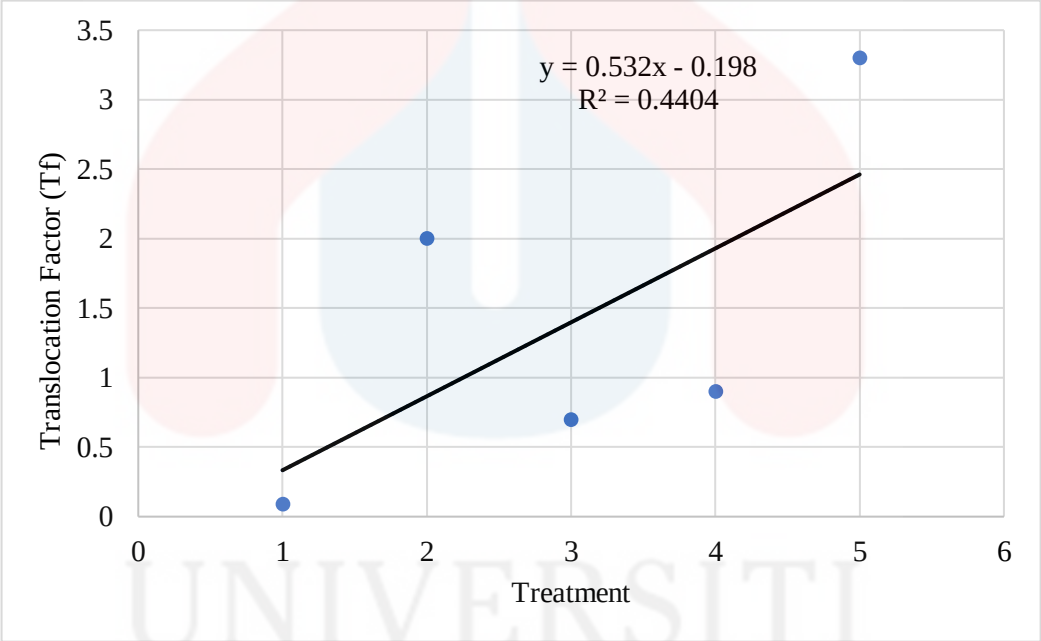


Figure 4.10: The Translocation Factpr (TF) for Cr concentration in *Allium cepa L.* plant

4.3.3 The Distribution of Mercury in Plants

The mean accumulation of Hg concentration at T1 (5mg/L) have higher value at the bulb part which accumulated 0.007 ± 0.2393 mg/L compared to the lowest accumulation was 0.004 ± 1.2055 mg/L at the root part.

Table 4.8: The Hg accumulation in different part of plant at five soil treatment levels

Pot	Part of Plants (mg/L)			Total Accumulation
	Leaf	Bulb	Root	
T1	0.005 ± 0.0485	0.007 ± 0.2393	0.004 ± 1.2055	0.016 ± 0.0022
T2	0.007 ± 1.1620	0.009 ± 0.1035	0.013 ± 0.077	0.029 ± 0.0001
T3	0.008 ± 0.2113	0.003 ± 0.5251	0.013 ± 0.312	0.024 ± 0.0005
T4	0.003 ± 0.3172	0.005 ± 0.0976	0.004 ± 0.0671	0.012 ± 0.0002
T5	0.036 ± 0.1860	0.004 ± 0.2595	0.007 ± 0.1653	0.047 ± 0.0062

The soil pH in this treatment (T1) was the lowest among the others treatment which was 6.40 pH. In environment, there were three type of soluble forms of Hg. Especially in low pH condition, Hg^0 metal is oxidizing with two other ionic elements such as mercurous ion and mercuric ion Hg^{2+}. Hg^+ ion is not stable under environmental conditions since it dissimulated into Hg^0 and Hg^{2+} (Rodriguez *et al.*, 2005). The total accumulation in this treatment was 0.016 ± 0.0022 mg/L. The translocation factor for this treatment was 1.25 which exceed the factor of TF < 1, thus plant at this treatment can be hyperaccumulator.

The mean accumulations of Hg in 10 mg/L in soil treatment also have been analysed by using AAS. The highest value of mean concentration can be detected at the root part, 0.013 ± 0.077 mg/L as it was the nearest to the soil medium that absorbed and translocate more concentration into plant. The TF was 0.54 which less than one. Furthermore, based on Figure 4.11 shows that the mean accumulation of Hg concentration in plant at five of soil treatment levels.

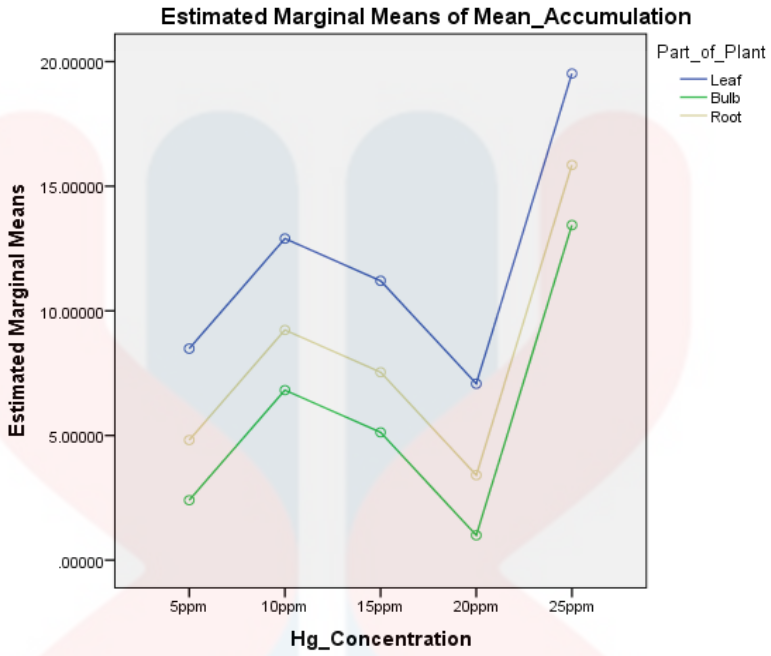


Figure 4.11: The mean accumulation of Hg at *Allium cepa L*. parts with five different of soil treatment level.

Plant that treated with 15 mg/L (T3), the mean value of Hg concentration was detected higher at the root part 0.013 ± 0.312 mg/L. At the bulb, only 0.003 ± 0.5251 mg/L Hg concentration was detected by AAS. Thus, the translocation factor of this treatment was 0.61 only. The value does not pass the threshold where their translocation factor was less than one, TF < 1. Root part was considered as good accumulator for Hg concentration. The total of Hg concentration accumulation was 0.024 ± 0.0005mg/L. Plants easier to absorb the mercury significantly from the soil and the result mercury content higher in roots part.

For soil treatment that consists 20 mg/L, the highest mean of Hg accumulated was at the bulb part (0.005 ± 0.0976) mg/L and the lowest mean Hg accumulated was at the leaves part (0.003 ± 0.3172) mg/L. The root part only accumulates medium value of Hg concentration which was 4.058 ± 0.0671 µg/L. Thus, the total Hg accumulation in this soil treatment level was 0.004 ± 0.0671 mg/L. The TF value was

0.75 which lower than the permissible value TF < 1. Therefore, the plant in this soil treatment can't be the hyperaccumulator plant.

Lastly, in 25 mg/L of soil treatment level, T5. The highest mean value was 0.036 ± 0.1860 mg/L that accumulated at the leaf part. The lowest Hg accumulation was at the bulb part, 0.004 ± 0.2595 mg/L compare to the root part which was 0.007 ± 0.1653 mg/L. Thus, the total Hg accumulation in this soil treatment level was 0.047 ± 0.0062 mg/L. The TF value was 5.14 which higher than one (TF < 1). Therefore, the plant in this treatment can be the hyperaccumulator plant for Hg remediation. All the Hg mean concentration data have passed the Quality Control (QC) within the limit for Hg 253.65 and the recovery rate was 91.25 %. The concentration of Hg accumulations in different part of onion plant were not directly proportional to the Hg concentration levels of soil treatment. There was no significant level between parts of plants (leaf, bulb, root) which the value (0.589) was more than $p < 0.05$ but the highest accumulation was in bulb and root part.

The TF was calculated for all the five treatment and compared significantly between them. Statiscally, there was significant value between the translocation factors. According to Figure 4.12, there were two treatment soil levels (5 mg/L and 25 mg/L) that exceed the permissible limit of TF which should be TF < 1. The trend was increases as first then the value decreases at T2. However, the values were increasing from T3 to T5. The pattern was not directly proportional to each other ($R^2 = 0.4241$).

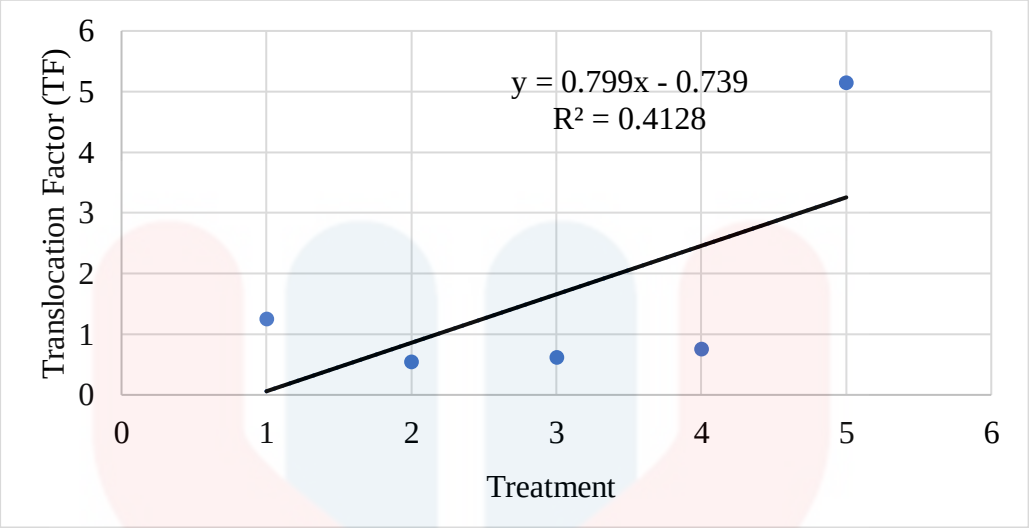


Figure 4.12: The Translocation Factor (TF) for Hg concentration in *Allium cepa L.* plant in 25 mg/L of soil treatment

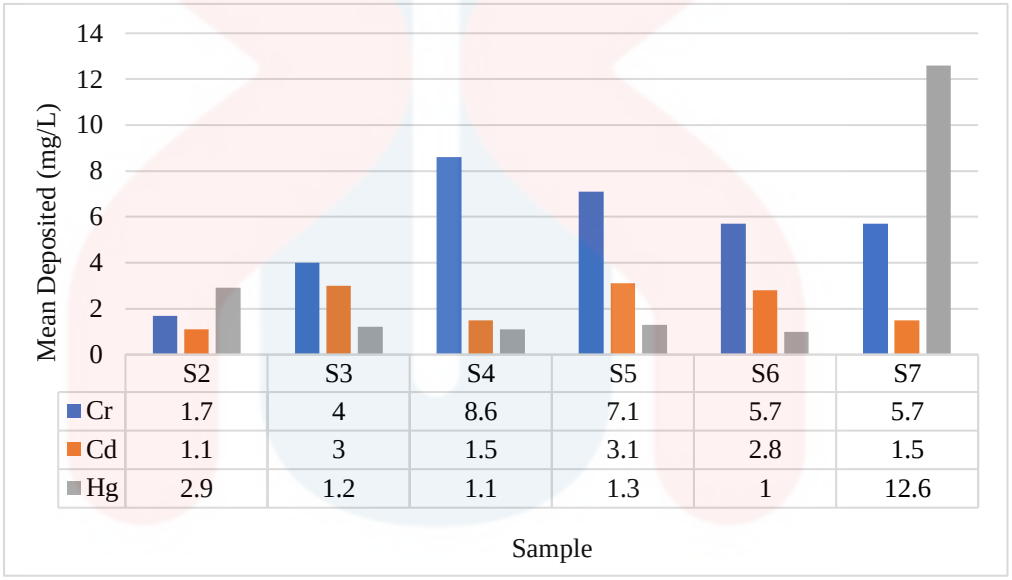
4.4 Heavy Metals in Soil Analysis

Soil is very essential when growth a plant because soil have a lot of micronutrients and macronutrients that needed by a plant to growth. All the 12 synthetic soils were collected at week 4 after harvesting process of the plant. Only 7 soil samples were analysed by using EDS. All the analysis sample were analysed and the three heavy metals (Cr, Cd, Hg) were detected the percentage in soil. The percentage unit have been converted into mg/L. The detected heavy metals were the remaining of heavy metals deposited in soil. Heavy metals also flowed with water particles out from the pot.

Sample 1 was the control pot that does not have any heavy metal concentrations in it. Thus, Cr, Cd and Hg concentration does not exist in the soil sample. Sample 1 was the original soil that were used all in the experimental pot. Sample 2 was also the control pot but it has heavy metals concentrations in it (20 mg/L). The total heavy metals in the soil were Cr = 1.7 ± 0.0332 mg/L, Cd = 1.1 ± 0.0141 mg/L and Hg = 2.9 ± 0.2694 mg/L. Sample 3 represents 5 mg/L of soil

treatment. The remaining heavy metals in the soil were Cr = 4.0 ± 0.2993 mg/L, Cd = 3.0 ± 0.3116 mg/L and Hg = 1.2 ± 0.0640 mg/L.

Based on Figure 4.13, sample 4 have mean deposited of heavy metals in 10 mg/L of soil treatment. The value of Hg mean deposited was 1.1 ± 0.9263 mg/L (lowest) compare to the value of Cd which was 1.5 ± 0.1389 mg/L in this treatment. The Cr mean deposited was 8.6 ± 0.8954 mg/L (highest).



	S2	S3	S4	S5	S6	S7
Cr	1.7	4	8.6	7.1	5.7	5.7
Cd	1.1	3	1.5	3.1	2.8	1.5
Hg	2.9	1.2	1.1	1.3	1	12.6

Figure 4.13: The comparison of mean deposited between Cr, Cd, Hg in soil

Sample 5 represents 15 mg/L of soil treatment. Compare between three heavy metals, Cr was the highest (7.1 ± 0.1105) mg/L and Hg was the lowest (1.3 ± 0.1338) mg/. Next, sample 6. For 20 mg/L of soil treatment, Cr have the highest value of mean deposited which was 5.7 ± 0.0332 mg/L and Hg have the lowest value which was only 1.0 ± 0.0141 mg/L. Lastly, sample 7 have the highest of mean deposited of heavy metals in 25 mg/L. The highest mean was for Hg, 12.6 ± 0.0310 mg/L. Cd have the lowest mean deposited value which indicates 1.5 ± 0.5063 mg/L in soil sample. Sample 7 have highest of mean deposited because soil treatment in pot T5 have 25

mg/L of heavy metals (Cr, Cd, Hg) which was the highest treatment among others. The highest the concentration of heavy metals in soil, the highest the concentration of heavy metals accumulated into plants. Through all the samples, 5 out 7 samples were dominated by Cr concentration in the remaining soil samples after 4 weeks.

4.5 Heavy Metals in Water Analysis

All the plant samples were watered every day. The excess water that were flow-out from the pot have been collected at the end of the day. Thus, the watering system was a rotation-watering system. It means that 100 mL of tap water was used at the beginning of experiment to water each plant samples. Each pot has their own container to collect the flow-out water from the soil. After 4 weeks of observation and monitoring, the water samples were collected into sample bottles. Type of containers were used can be refer at figure in Appendix B.1 and the type of sample bottles were used also can be refer at figure in Appendix B.3.

Only Cd can detect its mean concentration in all water samples. The highest value was 0.021 ± 0.0016 mg/L detected in pot T5 with 25 mg/L of soil treatment level. The lowest Cd concentration was 0.011 ± 0.0010 mg/L detected in pot T2 with 10 mg/L of soil treatment level. All the analytes passed the Quality Control within the limit for Cd 228.80. For Hg concentration in water samples, only in pot T5 can detect the concentration which was 26.11 ± 0.0220 μg/L. Others pot, Hg were not detectable (ND). The Quality Control was failed because the value less than the lower limit for Hg 253.65. The mean concentration of Cr only can detect in pot T1 which was 0.026 ± 0.0500 mg/L. Others pot, Cr concentration were not detectable (ND) as the concentration were too low to detect. All the data can refer to a table in Appendix C.

All analytes passed the Quality Control as Cr concentration value within limits for Cr 357.87.

4.6 Total Heavy Metals Accumulate in *Allium cepa L.*

There were three main part of *Allium cepa L.* plant such as leaf, bulb and root. The heavy metals (Cr, Cd, Hg) were accumulated from the soil and distributed into three different part respectively. All the plant samples were analysed by using Perkin Elmer 400 AAS and Figure 4.14 illustrates the total mean accumulation of heavy metals in five soil treatment levels (5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L). Table 4.9 shows the mean concentration of heavy metals (Cd, Cr, Hg) in plant's part for five different soil treatment.

Table 4.9: The mean accumulation of heavy metals in plants

Pot	**Mean Concentration in Plant (mg/L)**			**Total Accumulation (mg/L)**
	Cd	Cr	Hg	
T1	0.0580	0.0470	0.0157	0.1207
T2	0.0880	0.0900	0.0290	0.2070
T3	0.0970	-0.0310	0.0239	0.0899
T4	0.1570	0.0260	0.0115	0.1945
T5	0.1030	-0.0590	0.0488	0.0928

Based on the table, Cd accumulated higher in plant for each treatment compared to Cr and Hg. Plant in T2 was showing the good growth rate because the increasing length of leaves but plant in D2 (observation pot) died eventually at week 4. This was because Cd concentration can cause shortening of length of leaves of plant

and reduced the plant growth as well. Moreover, Cd mean concentration was the highest in T4 compare to others. Table 4.10 proved that as the increases of Cd mean concentration, the decreases the height of leaves of plant. The duplicate pot for T4 which was D4 also the leaves of plant become decreases in height.

Table 4.10: The height of leaves in T4 and D4 in four weeks

Pot	**Length of leaf (cm)**				**Colour of leaf**	**Growth Rate**
	W1	W2	W3	W4		
T4	13.3	21.2	47.5	35.0	Green	Survive
D4	15.2	32.4	49.0	34.6	Green	Survive

The highest accumulation of heavy metals (Cr, Cd, Hg) was in T2 (10 mg/L) of soil treatment. The highest accumulation of Hg mean concentration also was in T2. Therefore, heavy metals toxicity occurred in T2 that affected the chlorosis of onion's leaves and eventually caused necrosis. Necrosis can be defined as death of cells in plant's tissues due to the high exposure of heavy metals and lack of nutrient supply (Dang, Chhabra & Verma, 2010).

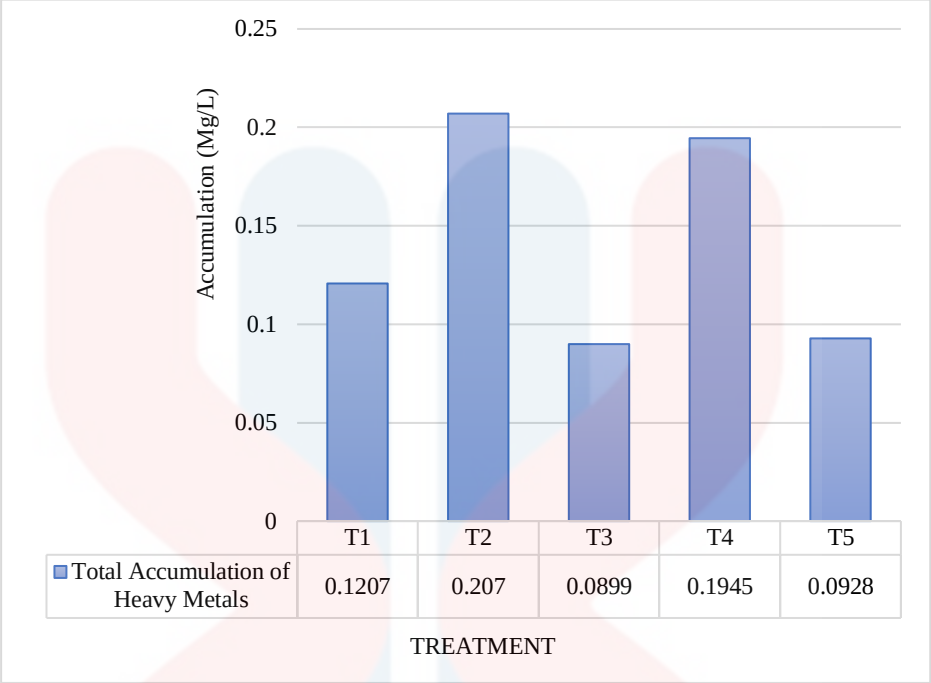


Figure 4.14: The differences of total heavy metals mean accumulation in five soil treatment

The bioconcentration factor (BCF) of *Allium cepa L.* from 5 mg/L to 25 mg/L was ranging between 0.08 to 0.20 respectively. Usually phytoextraction mechanism of heavy metals in treatment soil including the usage of herbs plants to accumulate and transport metal ions from contaminated soils upwards from root to shoot (Tangahu *et al.* 2011) The higher bioconcentration factor was in 20 mg/L (T4) soil treatment. The lowest bioconcentration factor was in 15 mg/L (T3) and 25 mg/L (T5) soil treatment. The bioconcentration factor were decreasing in order with the increasing of heavy metals concentration levels of soil treatments ($R^2 = 0.0147$) as shown in Figure 4.15.

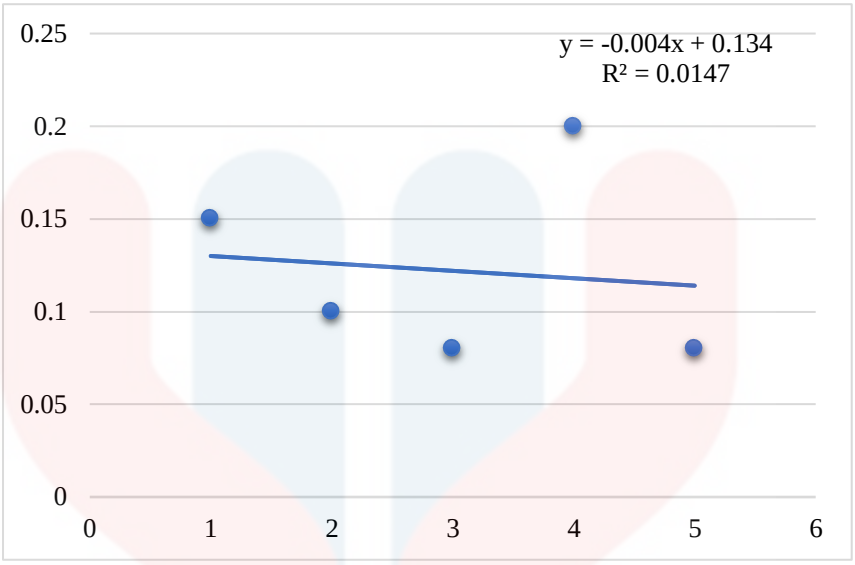


Figure 4.15: The Bioconcentration Factor (BCF) of *Allium cepa L.*

The bioconcentration factor (BCF) increases again at 20 mg/L and decreases at 25 mg/L as shown in figure 4.15. Heavy metals concentration levels of five soil treatment (5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L) have BCF values to be less than one. Therefore, *Allium cepa L.* plant do not suitable to be accumulator plants for absorbing heavy metals.

4.7 Comparison with Malaysian Food Regulation 1985

All heavy metal concentrations in this study were compare with provided permissible limit set by Malaysian Food Regulation 1985. They had set the value for Hg ≤ 0.05 μg/g, Cd ≤ 0.1 μg/g and Cr ≤ 0.05 μg/g. The heavy metal concentrations were converted into wet weight and compared with permissible limit allowed by Malaysia (Fuad *et.*, 2014). Based on the observation, Cd has the highest value of heavy metals concentrations which were exceed the safety limit provided at all soil treatment plant. The heavy metals mean accumulation of Cd were ranging between 0.058 – 0.103 μg/g. Cr and Hg mean accumulation of heavy metals all passed the safety limit

provided by Malaysian Food Regulation 1985 which the values are lower than Hg ≤ 0.05 μg/g and Cr ≤ 0.05 μg/g.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study, the heavy metals distribution in different part of plants (root, bulb, leaf) were analysed by using AAS. At each soil treatment level, Cd has the highest rate accumulation from soil and distributed to different part of plants in volatile form transport from root to shoot. Moreover, when the Cd concentration exceeded the threshold level of the root, the excess Cd ions were pumped into xylem and been started to push up to the shoots with the help of cohesive and adhesive forces. This experimental study shows that the trend of heavy metals accumulation in part of plants was Cd > Hg > Cr. This also proved that *Allium cepa L.* plant was capable to absorb any amount of heavy metals as their nutrient supply but no factor and condition related to it.

Although Hg was the second highest element of heavy metals accumulated in part of plants it is one of the detrimental pollutants which can easily to distribute into most of ecosystem causing high toxicity impacts in process of biological (Raquel & Rodriquez, 2012). Cr has the lowest rate of mean accumulation compare to Cd and Hg. But mostly Cr was accumulated at the root system. Previous researchers have proved that highest amount of Cr that can be absorbed by any plant especially herbs plant only remained in the roots and only certain amount of it transferred to shoot. So, Cr concentration was higher in roots compare in shoots (Data *et al.*, 2011).

The distribution of heavy metals (Cd, Cr, Hg) in *Allium cepa L*. plants from synthetic contaminated soil were measured through BCF and TF. Heavy metals concentration levels of five soil treatment (5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L) have BCF values to be less than one. Therefore, *Allium cepa L.* plant do not suitable to be accumulator plants for absorbing heavy metals.

5.2 Recommendation

The aims of this study were to analysed the heavy metals distribution in different part of plants by using AAS. Type of AAS is Perkin Elmer 400 and to compare the distribution of heavy metals (Cd, Cr, Hg) in *Allium cepa L.* plants from synthetic contaminated soil. There were five levels of soil treatment starting from 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L. Through this study, there are some suggested recommendations related to this research for future researchers to improve next study which related to heavy metals accumulation in plants.

Firstly, do some research on the types of soil that are suitable to be used to plant herbs. Suitable soil causes the plant to grow fertile and shows a good growth performance rate. Moreover, good soil will provide the appropriate medium for translocate plant nutrients from roots to shoots.

Secondly, use wider and bigger range of heavy metals concentrations for soil treatment levels starting from 0 mg/L, 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L and 500 mg/L in order to get accurate result and different significant values for the study.

Thirdly, this research about heavy metals accumulation in *Allium cepa L.* plant can further study to do research which related to phytoextraction in phytoremediation. *Allium cepa L.* plants can act as the hyperaccumulator plant to accumulate much amount of Cd concentration in soil.

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APPENDIX A

Table A: The mean concentration of Cd, Cr, Hg remained in soil samples

Sample	Pot	Type of Heavy Metals			Total
		Cd	Cr	Hg	
1	CA	0	0	0	0
2	CB	1.1	1.7	2.9	5.7
3	T1	3.0	4.0	1.2	8.2
4	T2	1.5	8.6	1.1	11.2
5	T3	3.1	7.1	1.3	11.5
6	T4	2.8	5.7	1.0	9.5
7	T5	1.5	5.7	12.6	19.8

APPENDIX B

Figure B.1: The pot's arrangement from pot labelled with T1 and end with pot labelled D5

Figure B.2: Type of container that used to collect the water samples.

Figure B.3: The container was put below pot to collect the excess water that contain heavy metals.

Figure B.4: Type of sample bottles that were used to collect water samples.

APPENDIX C

Table C.1: Tests of Between Subjects Effects of Mean Accumulation of Cd Concentration between Part of Plant and Cd Concentration

Source	Type III Sum of Squares	df	Mean Square	Sig.
Part_of_plant	0.000	2	0.000	0.678
Cd_Concentration	0.001	4	0.000	0.181
Error	0.002	8	0.000	
Total	0.010	15		
Corrected Total	0.004	14		

Table C.2: Tests of Between Subjects Effects of Mean Accumulation of Cr Concentration between Part of Plant and Cr Concentration

Source	Type III Sum of Squares	df	Mean Square	Sig.
Part of plant	0.000	2	0.000	0.541
Cr Concentration	0.001	4	0.000	0.400
Error	0.002	8	0.000	
Total	0.010	15		
Corrected Total	0.004	14		

Table C.3: Tests of Between Subjects Effects of Mean Accumulation of Hg Concentration between Part of Plant and Cr Concentration

Source	Type III Sum of Squares	df	Mean Square	Sig.
Cr_Concentration	0.000	4	0.000	0.209
Part_of_Plant	0.002	2	0.000	0.428
Error	0.002	8	0.000	
Total	0.020	15		
Corrected Total	0.004	14		