PHYTOREMEDIATION POTENTIAL OF VETIVER GRASS (*Vetiveria zizanioides*) FOR WATER CONTAMINATED WITH SELECTED HEAVY METAL

By

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A project report submitted to the Faculty of Environmental Studies, Universiti Putra Malaysia, in partial fulfilment of the requirement for the degree of Bachelor of Environmental Science and Technology

DEPARTMENT OF ENVIRONMENTAL SCIENCE
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ABSTRACT

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December 2015

Dr. Faradiella binti Mohd. Kusin
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Malaysia is subjected to rapid urban development, in which is further exacerbated by growing human population, has resulted in surface water contamination. Phytoremediation technique by using Vetiver grass (VG) has been introduced since the past few decades worldwide but the study on its efficiency of uptake mechanism in water is yet to be explored. Hence, this study aimed to assess and evaluate the heavy metal removal efficiency (Cu, Fe, Mn, Pb, Zn) based on the root length, varying concentration and the Vetiver density – Concentration and root lengths (Experiment 1), and Concentration and Vetiver density (Experiment 2), whereby the synthetic mixture were set based on the river concentration found in Malaysia – Low Concentration Treatment (LCT) and High Concentration Treatment (HCT), whereby water sampling and plant harvesting (only Experiment 1) were done at interval of 0, 24, 72, 120, 168 and 240 hours. Throughout the experiment, there were no major toxicity symptoms shown by the plants like necrosis, except for chlorosis, browning, and slight wilting due to malnutrition of macronutrients or over-excessive of heavy metal. The results have shown that there were statistically significant difference between heavy metal removal from water in Experiment 1 ($p<0.005$) and Experiment 2 ($p = 0.018$), but no significance in different root lengths and densities for both experiments ($p<0.05$). However, there were significant difference in heavy metal removal between treatments ($p<0.005$), except for Fe. There were also significant in heavy metal accumulation in different plant part ($p<0.0005$) in both LCT and HCT, except Mn. The order of heavy metal removal efficiency from water for both experiments was Fe>Pb>Cu>Mn>Zn, same as heavy metal accumulation in roots for LCT but not HCT (Fe>Pb>Mn>Cu>Zn). For accumulation in shoot, the order was Pb>Fe>Mn>Cu> Zn. It is suspected to be due to antagonistic or synergistic effect of between Fe and Zn, Mn and Pb and Mn and Zn, hence there was a low uptake in Mn and Zn. Plant age, seasonal variation and climatic condition would be the factors that control the plant uptake. All the plants had BCF>1, which means that VG has tendency to accumulate heavy metal in the shoot, but most of the plants had TF<1 showing that VG is more to a rhizofiltrator than phytoextractor. However, in this study, it has been found that VG is a Pb hyperaccumulator and accumulators for other heavy metal elements. From this study, it could improve the knowledge on pollutant uptake mechanism, accumulation and tolerance towards heavy metal under different analytical conditions.

Keywords: vetiver grass (VG), heavy metal, removal efficiency, accumulation

ABSTRAK

POTENSI FITOPEMULIHAN RUMPUT VETIVER (*Vetiveria zizanioides*) UNTUK AIR YANG TERCEMAR DENGAN LOGAM BERAT TERPILIH

Oleh

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Malaysia adalah tertakluk kepada pembangunan bandar yang pesat, di mana diburukkan lagi oleh pertumbuhan populasi manusia, telah menyebabkan pencemaran air permukaan. Teknik Fitopemulihan dengan menggunakan rumput Vetiver (VG) telah diperkenalkan sejak beberapa dekad yang lalu di seluruh dunia tetapi kajian mengenai kecekapan mekanisme pengambilan dalam air masih belum diterokai sepenuhnya. Oleh itu, kajian ini bertujuan untuk menilai kecekapan penyingkiran logam berat (Cu, Fe, Mn, Pb, Zn) berdasarkan kepanjangan akar, kepekatan yang berbeza dan ketumpatan Vetiver - kepekatan dan akar panjang (Eksperimen 1), dan kepekatan dan ketumpatan Vetiver (Eksperimen 2), di mana campuran sintetik telah ditetapkan berdasarkan kepekatan sungai yang terdapat di Malaysia - Rawatan Kepekatan rendah (LCT) dan Rawatan Kepekatan Tinggi (HCT), di mana persampelan air dan penuaian tumbuhan (hanya Eksperimen 1) telah dilakukan pada selang 0, 24, 72, 120, 168 dan 240 jam. Sepanjang eksperimen, tiada tanda-tanda keracunan utama yang ditunjukkan oleh tumbuh-tumbuhan seperti nekrosis, kecuali kekuningan daun, pemerangan dan layu disebabkan oleh kekurangan zat makanan makronutrien atau berlebihan logam berat. Keputusan telah menunjukkan bahawa terdapat perbezaan statistik yang signifikan antara penyingkiran logam berat daripada air dalam Eksperimen 1 ($p < 0.005$) dan Eksperimen 2 ($p = 0.018$), tetapi tidak penting dalam panjang akar yang berbeza dan kepadatan untuk kedua-dua eksperimen ($p < 0.05$). Walau bagaimanapun, terdapat perbezaan yang signifikan dalam penyingkiran logam berat antara rawatan ($p < 0.005$), kecuali Fe. Terdapat juga penting dalam pengumpulan logam berat dalam bahagian tumbuhan yang berbeza ($p < 0.0005$) dalam kedua-dua LCT dan HCT, kecuali Mn. Susunan logam berat kecekapan penyingkiran dari air untuk kedua-dua eksperimen adalah Fe> Pb> Cu> Mn> Zn, sama seperti pengumpulan logam berat dalam akar untuk LCT tetapi berbeza untuk HCT (Fe> Pb> Mn> Cu> Zn). Bagi pengumpulan dalam batang, susunan adalah Pb> Fe> Mn> Cu> Zn. Ia disyaki disebabkan oleh kesan bermusuhan atau sinergi antara Fe dan Zn, Mn dan Pb dan Mn dan Zn, oleh itu terdapat pengambilan yang rendah pada Mn dan Zn. Umur tumbuhan, variasi bermusim dan keadaan iklim yang akan menjadi faktor yang mengawal pengambilan logam berat. Semua tumbuh-tumbuhan mempunyai BCF> 1, yang bermaksud bahawa VG mempunyai kecenderungan untuk mengumpul logam berat dalam batang, tetapi kebanyakan tumbuh-tumbuhan mempunyai TF <1 menunjukkan bahawa VG lebih kepada rizopenurasan daripada fitopengekstrakan. Walau bagaimanapun, dalam kajian ini, ia telah mendapati bahawa VG ialah hiperakumulaot Pb dan akumulator untuk lain-lain unsur-unsur logam berat. Daripada kajian ini, ia boleh meningkatkan pengetahuan mengenai mekanisme pengambilan pencemar, pengumpulan dan toleransi terhadap logam berat di bawah keadaan analisis yang berbeza.

Kata kunci: rumput vetiver (VG), logam berat, kecekapan penyingkiran, pengumpulan

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APPROVAL SHEET

This project report was submitted to and approved by the supervisor and has been accepted as partial fulfilment of the requirement for the degree of Bachelor of Environmental Science and Technology.

Title : Phytoremediation Potential of Vetiver Grass (*Vetiveria Zizanioides*) for Water Contaminated with Selected Heavy Metal

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Approved by,

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DECLARATION FORM

Declaration by student

I hereby confirm that:

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Name and Matric No.: Ashton Lim Suelee (166651)

Declaration by supervisor

This is to confirm that:

- the research conducted and the writing of this project report was under our supervision.

Signature:

Name of Supervisor: Dr. Faradiella binti Mohd. Kusin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVATIONS

%	percent
°C	degree Celcius
μS	microSiemens
AAS	Atomic Absorption Spectrometry
AMD	Acid Mine Drainage
APHA	American Public Health Association
As	Arsenic
ATSDR	Agency for Toxic Substances & Disease Registry
BCF	Bioconcentration factor
BDL	Below Detection Limit
Cd	Cadmium
cm	centimeter
conc.	concentration
Cr	Chromium
Cu	Copper
DW	dry-weight
F-AAS	Flame Atomic Absorption Spectrometry
Fe	Iron
FES	Flame Emission Spectrometry
g	gram
HCT	High Concentration Treatment
Hg	Mercury
H_2O	Water
HM	Heavy metal
HNO_3	Nitric Acid
IJSRSET	International Journal of Scientific Research in Science, Engineering and Technology
K	Potassium
LCT	Low Concentration Treatment
mg	milligram
mg/kg	milligram per kilogram
mg/L	milligram per litre

ml	millilitre
Mn	Manganese
N	Nitrogen
Ni	Nickel
NRMNL	National Risk Management Research Laboratory
P	Phosphorus
Pb	Lead
r	correlation coefficient
Se	Selenium
SPSS	Statistical Program for Social Sciences
UPM	Universiti Putra Malaysia
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
VG	Vetiver grass (Vetiveria zizanioides)
Zn	Zinc

LIST OF CHEMICAL USED

Chemical	Molecular formula	Brand, grade
Concentrated nitric acid	65% HNO_3	*R & M*
	70% HNO_3	
Copper sulphate	$CuSO_4.5H_2O$	*Bendosen, AR*
Iron sulphate	$FeSO_4.7H_2O$	*Bendosen, AR*
Manganese sulphate	$MnSO_4.H_2O$	*Bendosen, AR*
Lead(II) nitrate	$Pb(NO_3)_2$	*R & M*
Zinc sulphate	$ZnSO_4.7H_2O$	*Bendosen, AR*
Standard stock solution	Cu	*Fluka Analytical*
	Fe	
	Mn	
	Pb	
	Zn	

LIST OF APPARATUS AND MATERIAL USED

Apparatus/ Glassware	Materials
Beaker	0.45µm cellulose acetate filter paper
Centrifuge tube	0.4mm Plastic sheet
Pipette	Distilled water
100 mL volumetric flask	Millipore water (Milli O; 18 MΩ cm)
Mortar and pestle	Lab towel
Big containers	Parafilm
Measuring cylinders	Polystyrene
Plastic plates	Sponge
Spatula (plastic)	Tagging tape
Syringe filter	Fertilizer
Aerator pump	
Desiccator	

LIST OF EQUIPMENT USED

	Parameter	Unit	Instrument
Physicochemical parameters	pH	-	Orion 3 Star AQ4500 pH meter
	Electrical conductivity	µS/cm	YSI SCT probe
	Salinity	ppt	
	Temperature,	°C	
	Mass	g	Shimadzu analytical balance
Chemical preparation/ analysis	Sample preparation	°C	Jenway 1000 Hot plate Jeio Tech Oven
	FAAS	ppm	AA-Shimadzu & Perkin Elemer

CHAPTER 1

INTRODUCTION

1.1 Background of study

In a global basis, water stress has become one of the major issues faced by the world – no exception for Malaysia as well. No doubt that people have thought that rapid urbanization and accelerated pace of industrial development, which is further exacerbated by growing human population has resulted in water scarcity due to high water demand (Al-Badaii et al, 2013). After all, experts have concluded that global water crisis is more crucial at this point instead of water scarcity mainly due to poor water management (Chan, 2012; Biswas & Tortajada, 2011; Fulazzaky et al., 2010).

The water source in Malaysia is mainly from surface water (Chan, 2012; Othman et al., 2012), especially river water or dam. Groundwater only acts as the alternative water source. Therefore, the quality of surface water in Malaysia is very important because it is used as water resources for agriculture purposes like irrigation, domestic purposes, a mode of transportation, food sources in fisheries, hydro-electric power, industrial uses, and other purposes in a watershed (Al-Badaii et al., 2013; Cleophas et al, 2013; Chan, 2012). Most of the river water quality is believed to have deteriorated greatly over these years (Kusin et al, 2014; Chan, 2012). The water availability in Malaysia is becoming scarce due to anthropogenic activities resulting from rapid urban development with improper management practices. These activities, in turn, have contaminated the river with various types of pollutants, especially heavy metals and nutrients.

The presence of heavy metals in aquatic environments is of particular interest and concern due to its persistence and toxicity in the environment. The release and disposal of such waste could bring forth adverse hazardous effects towards human health and the environment. They could either be carcinogenic in the long run (chronic effect) or toxic to living organisms (acute effect), and it is possible that the pollutants could further contaminate the environment, especially soil and groundwater. Because of that, feasible measures are demanded in order to prevent or control the pollution. A number of investigations have reported significant levels of heavy metals (such as Cu, Zn, Pb, Mn, Fe, etc.) in surface water coming from settlements and industrial area, especially mining industry (Kusin et al, 2014; Hatar et al., 2013; Hadibarata et al., 2012; Othman et al., 2012; Jopony and Tongkul, 2009; Ali et al., 2004). In additional to that, many researchers have discovered that there are many species of plants that could remove those pollutants from the river (Harguinteguy *et al.*, 2015; Darajeh et al., 2014; Ashraf *et al.*, 2013; Roongtanakiat et al., 2007; Shu & Xia, 2003; Shu et al., 2002).

Phytoremediation is the removal or controlling of various types of pollutants from the environment by using green plants (Salt et al., 1998; Valderrama et al., 2013). Numerous studies in recent decades have paid attention to phytoremediation to treat aquatic environment by using plants for it is a cheap and environmental-friendly technique. In spite of its effective removal of pollutant from the environment, there are several problems surfaced in this application whereby it is limited by slow growth, low adaptability, short root systems and low yield of plants. On top of it all, it would not be a problem with the use of Vetiver grass as it is a highly productive plant by means of yield and that it could adapt with any conditions, either in terms of pollutants or environment.

Vetiveria zizanioides, normally known as Vetiver Grass (VG) originates from India in the Graminae family (Darajeh *et al.*, 2014), is one of a few plant species meeting all the criteria required for phytoremediation. VG is a new and innovative phytoremedial technology for environmental protection due to its effectiveness and low cost natural methods. VG has been used in many countries worldwide such as Australia, Brazil, Philippines, Thailand and Vietnam as slope stability and phytoremediation techniques due to its high tolerance to adverse climatic conditions, elevated levels of heavy metals, and submergence. Its phytoremediation application has been actively used for treating and disposing polluted wastewater, mining wastes and contaminated lands in Australia, Asia, Africa and Latin America (Truong, n.d.).

There are many studies which have been done on VG phytoremediation over several decades. However, most of the studies only focused mainly on standing water environment especially wetlands and on-site drainage as well as acid mine drainage (AMD). Based on the results of river deterioration due to the effluents released from industries, agricultural activities, municipal sewage, livestock wastewater, and urban runoff in Malaysia, it is important to study river water pollution to sustain continuous water supply (Al-Badaii et al, 2013; Cleophas et al., 2013). Hence, phytoremediation in water is being introduced in water due to its low cost and maintenance. Although it has already been used in many countries, it is not well-explored in terms of VG mechanisms in removing heavy metal pollutants in water. Therefore, this study is to truly explore and understand the use of phytoremediation potential of Vetiver grass in water before applying them to running water body treatment.

1.2 Problem Statement

Malaysia is subjected to rapid urban development as the country is progressing towards Vision 2020 to become a developed nation. Consequently, due to improper management and public apathy, it has leads to contamination of surface water (Chan, 2012; Biswas & Tortajada, 2011; Fulazzaky et al., 2010). According to Othman *et al.* (2012), agro-based pollution was known as the largest water pollution sources in Malaysia, whereby it has accounted for approximately 90% of the industrial pollution. The presence of pollutants in the river has become such a concern in our country as clean water is becoming scarce and limited. In the recent years, Selangor, which is known for its high density population in the country, has been facing water crisis due to immensely polluted river, especially Semenyih river (Bernama, 2015; Al-Badaii et al., 2013; Lee, 2013; Fulazzaky et al., 2010).

Several heavy metals such as Mn, Cu, Zn and Fe are micronutrients needed by living organisms. However, these heavy metals could be toxic if taken up in large amount. As the river quality in Malaysia deteriorates, these heavy metals have been found be in increasing trend due to heavy loadings of pollutant discharge into the river, especially areas with heavy industries like acid mine drainage (AMD). Consequently, it has caused the river to be polluted, which has affected its quality by means of biogeochemistry, ecology, living organisms and ecosystem.

The declining trend of river water quality has urged the Malaysian government and the authority to take immediate action to treat the river, especially rivers in Selangor, so as to meet the basic requirements of water needs and its uses by maintaining its sustainability (Kusin et al, 2014). The One State One River programme and Klang River Cleanup Programme are launched in 2005 and 1992 respectively by Department of Drainage and Irrigation Malaysia (DID), a federal agency responsible for managing rivers (Chan, 2012). In additional to that, this project is also part of their project development – a stepping stone in order to apply phytoremediation technique for treating the river.

Numerous research studies in recent years have paid attention to phytoremediation in order to treat many aquatic environment as well as soil for it is a cheap and environmentally-friendly technique by using plants for the treatment. Vetiver grass has been used in Australia, Brazil, Philippines, Thailand and Vietnam as slope stability and phytoremediation techniques due to its high tolerance to adverse climatic conditions, elevated levels of heavy metals, and submergence. However, the research on VG phytoremediation potential in aquatic system is yet to be explored in terms of uptake rate, its other aspects and the development of research work on the use of plants for chemical contamination treatment in environment. Besides that, most of the studies have focused mainly on standing water environment especially wetlands, on-site drainage and soil. Therefore, this study is being conducted to explore and understand more in-depth the use of phytoremediation technique in water by using Vetiver grass.

1.3 Objectives

1. To assess the efficiency of phytoremediation technique using Vetiver grass (VG) in water by means of visual changes

2. To determine the rate of heavy metal uptake by VG in varying pollutant concentrations, root length and density of Vetiver grass

3. To evaluate on the removal efficiency of heavy metal uptake by VG

1.4 Research Questions

1. What is the potential pollutant uptake of heavy metals by Vetiver grass in varying concentration and root length over time?
2. Would different concentration of synthetic mixture affect the Vetiver grass growth?
3. What is the bioconcentration factor (BCF) and translocation factor (TF) by Vetiver grass in the synthetic mixture?
4. Does the density of Vetiver grass affect the amount of heavy metal uptake in the water?

1.5 Significance of Study

Vetiver grass is mostly used for slope stability in Malaysia, hence phytoremediation is yet to be ventured. With the outputs of this study, it would provide the knowledge on the amount of pollutants uptake mechanism, in terms of physicochemical parameters and heavy metals, by vetiver grass. In this study, the main highlight was to indicate whether different root length or vetiver density would affect the heavy metal uptake from water. It is hypothesized that longer root length and denser plant would remove higher amount of heavy metal. From this study, it will improve the knowledge about vetiver grass phytoremediation in the water under different analytical conditions, in which the uptake mechanism, toxicity, accumulation and tolerance to heavy metals can be updated. Up to certain extent, it could contribute as the baseline data for the development of vetiver system in Malaysia. In additional to that, this study could contribute in developing guidelines related to river water quality improvement by providing guidelines in building pontoons to treat river water.

1.6 Project Challenges

There are several challenges that could be faced by the researchers in carrying out this project include:

- The time allocated to complete the entire research project to be carried out is only 8 months (March to October), whereby two experiments have to be carried out by the researcher.
- The plants have to be classified into groups with different root lengths and densities.
- The researcher has to grow and cultivate the plants in the water to reach the specification for the experiments.
- The plants have to be cultivated in water as part of plant acclimatization and due to absence of suitable land for cultivation
- The budget allotted for the overall project is RM 1000 to purchase equipment and materials required for the experiment as well as the plant cultivation. The funds available do not allow for higher-end plant cultivation and experimental set-up.
- There is a need to find a location to cultivate the plants as well as to carry out experiment.
- There were not much guidance that could be provided as it is a new field in the faculty. It could be a good opportunity for the researcher to be independent in doing this project.

CHAPTER 2

LITERATURE REVIEW

2.1 Water pollution

Water is vital for living organisms as it is the basic unit of life. Although water is seemingly abundant, only 2.5% of water found on Earth is fresh water. Even then, the world's fresh water only covers approximately 1%, that is only 0.007% of the planet's water, can be accessed directly for human use. Water can be found in lakes, ponds, reservoirs, rivers, and underground sources. Globally, the world appears besieged by water stress but the main issue is poor management, which results in global water crisis, instead of water scarcity (Chan, 2012; Biswas & Tortajada, 2011; Fulazzaky *et al.*, 2010).

Off-site pollution from industrial areas and chemicals use for agricultural purposes are suggested to be main sources of toxic contamination in the environment (Al-Badaii *et al.*, 2013; Othman *et al.*, 2012; Fulazzaky *et al.*, 2010) due to improper management (Chan, 2012; Biswas & Tortajada, 2011; Roongtanakiat & Chairoj, 2001). During rainy season, these toxic substances found in the soil may leach out and carried away by the rain as surface runoff, hence ending up in the water body such as streams, river and ponds. These harmful substances could bring about adverse yet significant health effects to both human and flora and fauna at elevated levels.

2.1.1 Water quality in Malaysia

In Malaysia, rivers are vital for nature and human society as 97% of Malaysia's water supply comes from rivers (Othman *et al.*, 2012; Chan, 2012). However, many Malaysian rivers, especially in urban areas, are in an appalling state for major cities have been established along rivers. This is due to rapid urbanization, population growth, and increasing water demand from agriculture, industry, navigation, recreation, tourism and hydroelectric generation, resulting in floods and pollution. According to DOE (2003), it is estimated that the water demands in Malaysia intend to increase 60% from 1995 to

2010 and increase to 113% in 2020 within these 25 years. Furthermore, a combination of improper management, low supremacy on government agendas which results in lack of funds and enforcement, and low public participation has led to severe deterioration of river water quality.

River pollution has become such a concern in Malaysia as the most rivers have already been contaminated. (Kusin *et al.*, 2014; Chan, 2012). The percentage of polluted rivers has increased significantly between 1987 and 2009, resulting in poor water quality that has affected water supply (Chan, 2012). Rapid industrialization has raised the pressure in the urban regions, especially in the Klang River basin – the area with highest human population in the country. It is believed that water quality degradation will always be a problem in the Selangor River due to improper and ineffective handling of pollutant loads from poultry farms, industrial and municipal wastewaters (Fulazzaky *et al.*, 2010). Coupled with the agricultural development ever since the 1960's and 1970's, agro-based pollution was known as the largest water pollution sources in Malaysia, whereby it has accounted for about 90% of the industrial pollution due to lack of provisions in regulating the effluent discharge (Othman *et al.*, 2012).

Non-point source pollution, pollution contributed by storm runoff over land surfaces, has become a growing concern in Malaysia. The pollutants emitted from the diffused sources include toxic pollutants such as heavy metals (As, Cd, Cr, Cu, Fe, Pb, Zn, etc) and organic materials, especially nutrients (nitrogen-based compounds, N; phosphorus-based compounds, P; and ammoniacal nitrogen, AN). In general, both point and nonpoint sources water pollution poses environmental problems and human health problems in term of acute and chronic effects. Moreover, it has detrimentally affected the environment by means of ecological and biological factors. Due to these problems, the government has started to take action in order to conserve and preserve the rivers so as to sustain human needs for water and their beneficial uses.

2.2 Passive Water Treatment System

According to Younger (2000) and Kusin (2013), passive treatment technology, an engineered facilities with a series of treatment that require minimal or no maintenance once constructed and operational (US EPA, 2002), is introduced to the United Kingdom since the early 1990s. The function of the systems is normally run due to water pressure created by differences in elevation or water flow by gravity. Nonetheless, this technology is mostly applied to mine-impacted water such as acid mine drainage (AMD) (Muhammad *et al.*, 2015; Kusin *et al.*, 2014) and other open systems such as constructed wetlands and lagoons. AMD normally has a pH value lower than 4 with an elevated concentration of heavy metals such as Al, Pb, Zn and Fe, in which could bring forth significant effect on the water quality (Muhammad *et al.*, 2015; Shu, 1997, as cited in Shu, 2003).

Vetiver grass phytoremediation has been successfully applied for land rehabilitation and phytoremediation of highly contaminated land and solid industrial wastes in Australia, Asian countries (China & Thailand), Southern America (Chile & Venezuela) and South Africa (Truong, n.d.). In Malaysia, it is only used for slope stability at highway area. Phytoremediation using passive treatment technology on running water bodies, especially river, is yet to be well-developed, but it is currently being intensively studied by researchers.

Table 2.1 Previous studies on AMD pollution

Mining water / AMD	Elements analysed	References
Kuala Lipis gold mine, Pahang	As, Al, Cd, Cu, Fe, Mn, Pb, Zn	Bakar *et al.*, 2015
Synthetic AMD based on Mamut former mining area	Al, Mn, Fe, Cu, Pb, Zn	Muhammad *et al.*, 2015
Water sources of Bekok Intake and Sembrong Lagoon, Pahang	Al, Fe	Kusin *et al.*, 2014
AMD Mamut water samples	Al, Cu, Fe, Zn	Payus *et al.*, 2014
AMD at abandoned and active mining area in Pahang & Terengganuu	As, Cd, Co, Cu, Fe, Mn, Zn	Hatar *et al.*, 2013
Lechang Pb/Zn mine tailings, China	Cd, Cu, Mn, Pb & Zn	Shu, 2003

2.3 Phytoremediation

Phytoremediation consists of two terminologies, whereby ‘phyto’ means plants and ‘remedium’ means to clean or keep (Sola, 2011). Phytoremediation is the application of removing or controlling various types of pollutants from the environment with green plants (Salt *et al.,* 1998; Valderrama *et al.,* 2013), in terms of water, soil or sediments. It is classified into several respective areas such as phytoextraction, phytodegradation, rhizofiltration, phytostabilization, and phytovolatilization. It is an effective cleanup technology for it can removes various types of pollutants which are organic or inorganic. Organics can be degraded or broken down in the root region or uptake by the plant, followed by degradation, sequestration or volatilization. Inorganics cannot be broken down but can either be neutralized or concentrated in the harvestable plant area (Pilon-Smits, 2005).

2.3.1 Phytoextraction

Phytoextraction is the extraction of heavy metal in the soil involving translocation process to uptake the contaminant by using plants roots (Salt *et al.,* 1995). The root plant is responsible to accumulate the heavy metal with the application of plant translocation. The accumulation of heavy metal or contaminant will be removed by plant harvesting (National Risk Management Research Laboratory, 2000). The rate of phytoextraction depends on the root depth in soil or medium because root acts as the metal accumulator (Kidney, 1997).

2.3.2 Phytostabilization

Phytostabilization technique is normally applicable to processes such as leaching and soil dipersion, in order to avoid the migration or dispersion of contaminant through wind and water erosion. The plant roots take an important role to absorb the contaminant from the environment (NRMRL, 2000), as a result, the soluble metal ions would become insoluble metal ions (Salt *et al*., 1995). This technique is very suitable for soil contaminated with high organic matter and heavy textured soil (Cunningham *et al.,* 1995).

2.3.3 Phytodegradation (Phytotransformation)

Phytodegradation is the process in which uses the plant metabolism process, either internal or external metabolism, by breaking down the contaminant for uptake. It is quite different from rhizodegradation that uses microorganisms as the main medium, instead it depends on the solubility, polarity and hydrophobicity of the medium. Thus, it can not only be used in soil, sludge and sediment but also in groundwater and surface water (NRMRL, 2000). Moreover, the compounds with high solubility will not be infiltrated into the root (Schnoor *et al.*, 1995).

The organic contaminant in the soil can be easily broken down by microbial activities, yet it would be more effective by using plant roots – a technique known as rhizodegradation (NRMRL, 2000). The microbial activities and population are influenced by existence of organic acid, fatty acids, sterols, growth factor, nucleotides, sugar, amino acid, enzymes and other compounds in the root zone. All the matter or substances come from product of the root plant (Schnoor *et al.,* 1995; Shrimp *et al.,* 1993).

2.3.4 Rhizofiltration

Rhizofiltration is the effective treatment by adsorbing contaminant contained in the surrounding root zone in abiotic or biotic process. This application is almost similar to phytoextraction but it is normally used on groundwater, wastewater and surface water as hydroponic treatment; because the root can come in contact with water (NRMNL, 2000). The process occurs during the adsorption of contaminants onto root surface or plant root absorption. In order to carry out this treatment, the plants have to acclimatize to the pollutants first to familiarize with the pollutants to be uptake.

2.3.5 Phytovolatization

Phytovolatization is related to phytodegradation, whereby it also absorbs contaminant into the plant, but the contaminants will be released through the evapo-transpiration process after modification in the plant. Hence, the contaminants will be released into the atmosphere, whereas the groundwater or other medium would be less toxic (NRMRL, 2000).

Heavy metal pollution may bring forth to potential ecological risk. In contrast, phytoremediation is a promising method for cleaning of soil and water via the pollutant uptake by the plant (Mathe-Gaspar and Anton, 2005).

2.4 Vetiver grass (VG)

Vetiver grass (VG), scientifically known as *Chrysopogon* (Vetiveria) *Zizanioides*, originates from India in the Graminae family. It also comes from the same grass family which includes lemon grass, maize, sorghum, and sugarcane (Darajeh *et al.*, 2014). VG has been distributed throughout the equatorial and Mediterranean regions of many countries worldwide which include all the continents of the world except Antartica (Danh *et al.*, 2009). VG has been used for a long time in land conservation by means of soil and water by World Bank (Darajeh *et al.*, 2014), but its advantages of being cheap, effective and easy for water and soil conservation, particularly in wastewater treatment, only emerged in the 1980s (Danh *et al.*, 2009; Truong., 2000), due to its extraordinary and outstanding physiological and morphological characteristics.

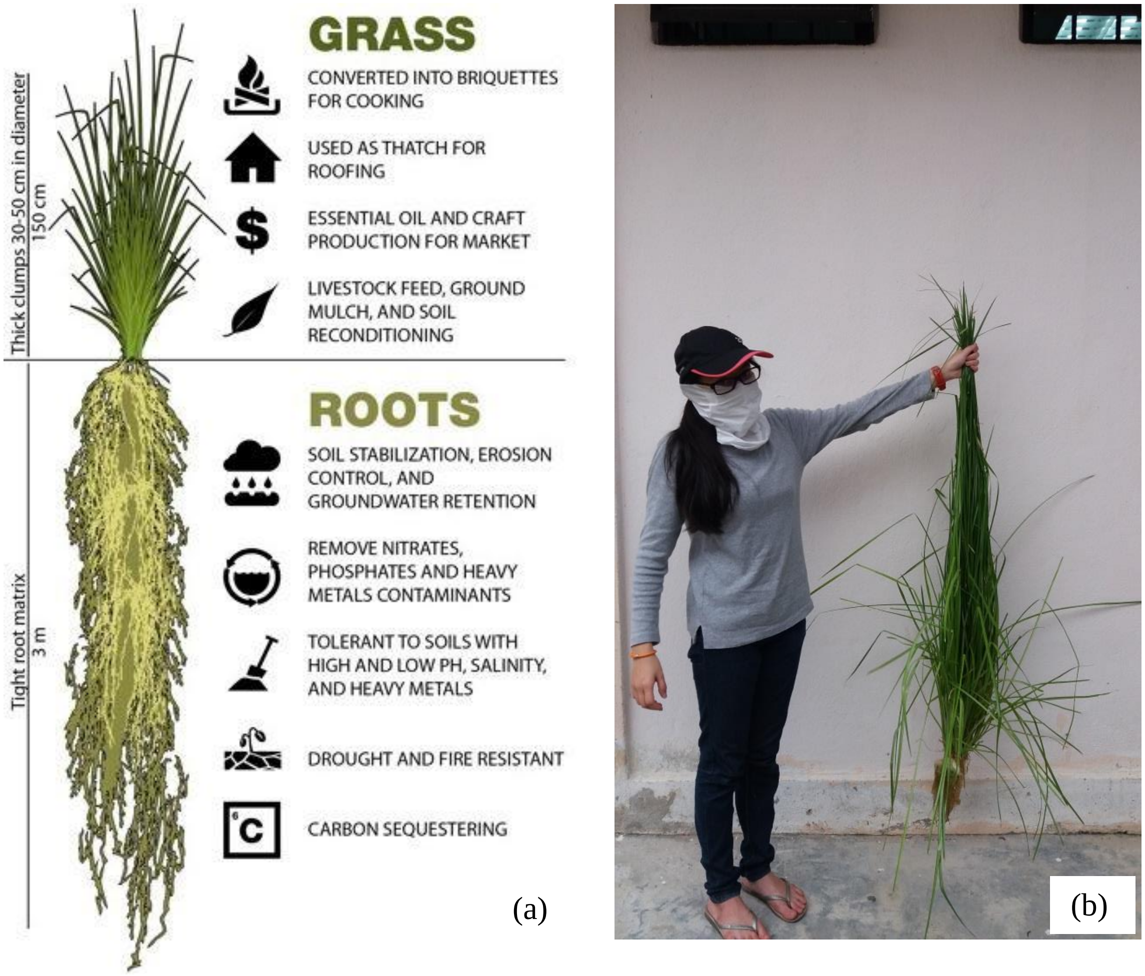

Figure 2.1 (a) Vetiver grass and its uses; (b) Vetiver grass grown hydroponically

Extensive R&D over the last 20 years in Australia, China and Thailand have found out that VG is non-invasive species which could take up water and nutrient efficiently, and thrives under extreme conditions in terms of soil, climate, and toxicity such as heavy metals and agrochemicals (Truong, 2000; Truong, n.d.). Besides, Hence, VGT would be a promising technology, in terms of economic and environment, for exploring the treatment of running water bodies, especially river, in spite of low cost, effective, and environmentally friendly.

VG has many distinctive characteristics which make it a suitable species for phytoremediation of many types of pollutants such as land recovery, soil and water management, and many more. The outstanding characteristics of VG will be discussed below:

2.4.1 Morphological and genetic characteristics

VG is well known for being a perennial and non-invasive grass species. It has a straight and stiff stem in which allows it to withstand high velocity flows of water or air. For example, Truong (2002) and Hengchaovanich (1998) have claimed that VG up to 2-m high can survive in relatively deep-water flow (as cited in Danh *et al.*, 2009, p. 666). As a result, it can increase the detention time. In additional to that, it has long narrow leaves which would produce a thick growth that will form a living barrier that cuts off and spreads runoff water. This type of growth also allows VG to act as a potent filter by trapping sediments and sediment-bound pollutants such as heavy metals and some pesticide residues (Chomchalow, 2003).

Moreover, it has a complex and lacework root system, whereby it can penetrate deep into the soil as well as in water. This extensive root system can reduce and prohibit deep drainage, enhance bed stability of the soil and nutrient uptake. Hengchaovanich (1998) asserts that the root system can grow up to 3 – 4 metres in a year (as cited in Danh *et al.*, 2009, p. 666 & Truong, 2000, p. 47). Furthermore, this dense and finely structured root system could create an environment ideal for microbiological processes in the rhizosphere.

A distinctive characteristic of VG is that it tends to grow into a thick hedge when closely planted together. As VG loves to grow upright then extending laterally, it is good to plant on farms with minimal changes to its existing layout. Not only that, its non-invasive feature ensures that there is no competition with adjacent plants for survival. Due to these characteristics, VG is often used as slope stability by river banks and highways to prevent erosion.

2.4.2 Physiological characteristics

VG can adapt extreme climatic and weather conditions such as drought, flood, frost, heat wave and inundation (Chomchalow, 2003). In fact, Truong (1999a) has claimed that VG can survive up to 6 months in drought. It is able to tolerate flood and areas where with too much water, for instance submergence for more than 120 days (Xia *et al.,* 2003), making it perfect for wetlands for it can consume high amount of water.

Although VG is a perennial and tropical grass, it can tolerate and survive under extreme temperature ranging from -15°C to 55°C, which means it can survive in extreme cold and hot climatic condition. A study by Dudai (2006) has also claimed that VG can be used in Mediterranean conditions. VG growth is limited by frost, in which the shoot died but the root survived (Danh *et al.*, 2009).

VG has high tolerance and adaptability to extreme edaphic conditions such as soil with high acidity and alkalinity. It can thrive under a wide pH range and adapt to saline, sodic and magnesic conditions, and aluminium and manganese toxicities (Chomchalow, 2003; Truong and Baker, 1998 and 1997, as cited in Danh *et al.*, 2009). VG can survive between pH 3.3 to 9.5 at nutrient-adequate condition and saline soil with high electrical conductivity up to 4.0 (Danh *et al.*, 2009; Webb, 2009).

Another unique characteristics of VG is that it has high tolerance to elevated concentrations of heavy metals such as As, Cd, Cu, Cr, Pb, Hg, Ni, Se and Zn in the soil (Danh *et al.*, 2009: Chomchalow, 2003; Shu, 2003). Furthermore, it has great potential in dissolved nutrient uptake and other organic constituents, especially N and P, and BOD and COD respectively (Darajeh *et al.*, 2014; Shu, 2003; Xia *et al.*, 2000; Pinthong *et al.*, 1998 as cited in Truong, 2000, p. 47). Another finding about VG is its high resistance towards herbicide and pesticide (Chomchalow, 2003; West *et al.,* 1996 as cited in Truong, 2000, p. 47).

Table 2.2 Limited potential uptake of Heavy metal for Vetiver growth

Heavy metals	Potential uptake for plant (mg/kg)		Potential uptake for VG growth (mg/kg)	
	Hydroponic	**Soil**	**Soil**	**Shoot**
As	0.02 – 7.5	2.0	100 – 250	21 - 72
Cd	0.2 – 9.0	1.5	20 – 60	45 - 48
Cu	0.5 – 10.0	NA	50 – 100	13 – 15
Ni	0.5 -2.0	7 – 10	100	347
Pb	NA	NA	>1500	>78
Zn	NA	NA	>750	880

NA not available\
[Source: Danh *et al.*, 2009; Truong, 2003; Bowen (1979) and Baker & Eldershaw (1993), as cited in Truong *et al.* (2010)]

2.4.3 Economic Characteristics

As VG is well known for its phytoremediation purposes nowadays, it is crucial to cultivate them in a very large amount. In spite of it all, VG has another special feature that most of VG species are naturally sterile hybrids. This allows VG to be propagated easily for it has neither seeds nor stolons. Hence, it is a non-invasive species, whereby it can be propagated from nodal cuttings. Due to its high growth yield, VG could contribute in the business industry, especially those companies which apply VG as phytoremediation purposes. Besides that, it has a natural recycling method which its end-product can be used as animal fodder, roof thatching, soil mulching, handicraft and material for organic farming (Truong, n.d.; Darajeh *et al.*, 2014; Roongtanakiat & Chairoj, 2001), and fire breaks (Webb, 2009), whereby during the treatment process, VG would absorb and take up essential plant nutrients such as nitrogen (N), phosporus (P), and other cations to be stored for other purposes (Darajeh *et al.*, 2014). Not only that, the scented oil comes from VG root has been found to be a deterrent to burrowing rats. (Webb, 2009; Dudai *et al.*, 2006).

2.5 Phytoremediation potential of Vetiver grass

For the past decades, vetiver grass (VG) has undergone a lot of studies in order to determine its phytoremediation potential towards the environment. Based on the previous studies, VG has the high ability for pollutant removal in terms of organic or inorganic materials from the environment. In fact, VG has been applied in many type of environment which includes soil (mine tailings, landfill, etc.) and water (wastewater, river, pond, wetland, AMD, etc.). The table below shows the previous studies that have been carried out for the past decades.

Table 2.3 Previous studies on phytoremediation by using Vetiver grass

Type of sources	Type of pollutant	Findings/Highlights	References
Palm Oil Mill Effluent (POME)	BOD, COD,	BOD in low conc. (90%), high conc. (60%), & control (no plant) is 15%. COD reduction was 94% (low conc.), 39% (high conc.) & 12% (control) Treatment : low concentration >high concentration	Darajeh *et al.*, 2014
Hydroponic and pot study	Pb	VG can be used to enhance Pb phytoextraction from contaminated soil in association with AM fungi	Punamiya *et al.*, 2010
Industrial wastewater	Cu, Fe, Mn, Pb, Zn	Fe>Mn>Zn>Cu>Pb, concentrate in root>shoot	Roongtanakiat *et al.*, 2007
Lechang Pb/Zn mine tailings	Cu, Pb, Zn	VG is an excluder of heavy metals	Shu & Xia, 2003
Mine tailing ponds		VG has highest tolerance to metal toxicities & accumulate at root>shoot	
Mining wastewater		On-going at that time	
Lechang Pb/Zn mine tailings, China	Cu, Cd, Pb, Zn, Mn, SO_4^{2-}	*V. zizanioides* has a high tolerance to AMD	Shu, 2003
Pb/Zn Mine Tailings: Field Experiment (soil)	Cd, Cu, Pb, Zn	*V. zizanioides* > *P. notatum* > *C. dactylon* > *I. cylindrica var. major*	Shu *et al.*, 2002
Batch test (soil)	Cd, Cu, Mn, Pb & Zn	HM uptake was inversely proportional to the HM concentration	Roongtanakiat & Chairoj, 2001
Garbage leachate	N, P, Cl^-, AN, BOD, COD	Removal efficiency : vetiver > alligator weed > Bahia grass > water hyacinth	Xia *et al.*, 2000

2.6 Other plant species for phytoremediation

Apart from vetiver grass, Jiji grass (*Achnatherum splendens*), a perennial grass in north China, is noted to have similar characteristics to vetiver. However, it is extremely drought and cold tolerant although it has a less dense and weaker leaf system (Xu, 2002). According to Xia et al. (2003), Bermuda grass also has high tolerance towards submergence but it is native to Africa and widely spread throughout the southwest and southern United States, which is not common in Malaysia. Moreover, there are many different plant species that are used for phytoremediation like bahia grass (*Paspalum notatum* Flugge), Bulrush (Typha), Common Reed (*Phragmites Australis*), Water Hyacinth (*Eichhornia crassipes*), and Water Lettuce (*Pistia stratiotes L.*), other than Vetiver Grass (*Chrysopogon zizanioides*) and for the treatment of water, and it turned out that water hyacinth, water lettuce and VG were selected for review due to their efficiency of HM removal and other pollutants with high biomass yield and adaptability of ecological factors (Gupta *et al.*, 2012). These three plants have their distinct pollutant removal capabilities depending on both abiotic or biotic environmental factors, level of contamination, and others. In short, VG is used in this study in order to increase the knowledge of VG technology in treating river water.

Table 2.4 Previous studies of other plant species for phytoremediation of water

Type of water	Species	Uptake of HM	References
Ctalamochita river water	*Potamogeton pusillus L.* *Myriophyllum aquaticum (Vell.) Verdc.*	Co, Cu, Fe, Mn, Ni, Pb & Zn	Harguinteguy *et al.*, 2015
Composting wastewater	*Eichhornia crassipes*	Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Rezania *et al.*, 2015
Swartkops Estuary	*Phragmites australis* *Typha capensis* *Spartina maritima*	Cd, Cu, Pb, Zn	Phillips *et al.*, 2015
Highway stormwater ponds	*Carex riparia* *Juncus effusus*	Cd, Ni, Zn	Ladislas *et al.*, 2015
Watercourses of Egypt	*Myriophyllum spicatum L*	Mn>Fe>Zn>Cu>Ni>Pb>Cd	Galal & Shehata, 2014
Synthetic water (Batch study)	*Eichhornia Crassipes*	Cd & Cu	Swain *et al.*, 2014
Urban stormwater runoff	*Carex riparia* *Juncus effusus*	Cd, Ni, Zn	Ladislas *et al.*, 2013
Gold mine wastewater	*Cabomba piauhyensis***,** *Egeria densa* *Hydrilla verticillata*	As, Zn, Al	Bakar *et al.*, 2013
Ex - tin mining catchment	*Cyperus rotundus L.* *Imperata cylindrica* *Lycopodium cernuum,* *Melastoma malabathricum,* *Mimosa pudica Linn,* *Nelumbo nucifera,* *Phragmites australis L.,* *Pteris vittata L.* *Salvinia molesta*	As, Cu, Pb, Sn, Zn	Ashraf *et al.*, 2011; Ashraf *et al.*, 2013
Synthetic wáter	*Pistia stratiotes*	Co, Cr	Prajapati *et al.*, 2012
Synthetic water	*Phylidrum lanuginosum*	Cu, Pb, Zn	Hanidza *et al.*, 2011
Metal-contaminated coastal water	*Eichornia crassipes*	As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, V and Zn	Agunbiade *et al.*, 2009
Mine tailing ponds	*Rumex acetosa* *Z. mays*	Cu, Pb, Zn	Shu & Xia, 2003
Mining wastewater	*Alocasia macrorrhiza* *Chrysopogon aciculatus*	Cd. Cu, Pb, Zn	
AMD	*Cyperus alternifolius* *Gynura crepidiodes* *Panicum repens* *Phragmites australis*	Cd, Cu, Mn, Pb, Zn	

2.7 Heavy metal

Heavy metals are found everywhere in the environment, whereby it could be contributed by natural state (water, air and soil) or anthropogenic activities (surface water, waste, soils, etc.). As a result from the technology development and improvement, they are widely used in the industries such as the application in metal processing, electroplating, electronics and chemical processing (Fingerman & Nagabushanam, 2005). Environmental pollution contributed by toxic heavy metals has becoming such a concern worldwide. This is because it would bring forth adverse effects to the environment, especially agricultural industries in terms of crop yields, biomass and soil fertility, in return it would lead to accumulation and bio-magnification process to occur in the food chain. Due to their persistence, toxicity and ubiquitousness in the environment, heavy metals are of particular interest in stormwater runoff (Ladislas *et al.*, 2013). The effects of heavy metals are undesirable, even in a minute quantity. The toxic effects can be detected in human and animals after a long period of time (Srivastav *et al.*, 1993). The list of heavy metal concentration is tabulated in the Appendix 1.

2.7.1 Copper (Cu)

Copper (Cu) can be naturally found in the environment, however the concentration of Cu in the environment has been rapidly increased due to anthropogenic activities. Some of the natural sources of Cu are such as decaying vegetation, sea spray, and forest fire. On the other hand, the Cu-releasing human activities include activities like mining, wood manufacturing, electric and electronic manufacturing, phosphate fertilizer manufacturing, metal manufacturing, and many others. Cu is one of the minerals required by humans as well as plants. Cu is responsible for energy production during biochemical reaction in human. Not only that, it can transform melanin, which is good for the heart and arteries to maintain and repair connective tissues. However, over-nutrition of Cu can be a problem to living things, which is known as trace element – element that could pose harm to living things even though the concentration is very low. Cu is commonly found in food and water, instead of air because it has low concentration in the atmosphere.

2.7.2 Iron (Fe)

Iron (Fe) is one of the major mineral required in the human and plants. In human, it is crucial as it can be found in every cells in the body, most importantly is that it associates with the red blood cells – oxygen carrier. Malnutrition of Fe would cause body weakness, but excess nutrition of Fe can cause adverse health effect instead. Basically, Fe is highly demanded by the development of any aerobic life on Earth due to its necessity in most biological system but it can be a toxic if over-nutrition.

2.7.3 Lead (Pb)

Lead (Pb) can be found naturally in the Earth's crust in terms of bluish-grey metal. It contains in the environment at a minute quantity. Since it is a toxic substance even at relatively low concentration, people are concerned about the health. Lead can enter into human body via inhalation and ingestion, but mostly via oral ingestion. It tends to bio-accumulate in the blood stream. Lead poisoning is the most vulnerable symptoms of excessive intake of Pb for it can be fatal to human up to certain extent, especially children. Pb can associate with other toxic elements at a relatively low level, especially Cd and Hg, in terms of synergistic toxicity.

2.7.4 Manganese (Mn)

Manganese (Mn) occurs naturally not only in the environment (rocks) but also in foods, in which it could be either natural or synthetic form. Mn is one of the trace elements which is essential for good health. There are several types of food that consists of Mn, such as whole grains, beans, cereals and tea. Therefore, Mn normally enters the body via ingestion. Mn is important to human because it would maintain healthy bone structure and metabolism, and it aids in production of collagen which maintains skin health.

2.7.5 Zinc (Zn)

Zinc (Zn) is an essential trace mineral for living organisms, especially human because it exists in most cells for human metabolism. Zn is found in all medium on Earth – air, water, and soil. Sphalente (ZnS) is often found on Earth's surface and underground. In fact, Zn is mostly found in water due to deposition into sediment, in which it binds with inorganic and organic matter. The importance of Zn is immune system, growth, vision, taste, smell, and reproduction.

Table 2.5 Effects of each heavy metal element towards human health

Element	Source	Effects to human	
		Acute	Chronic
Cu	Manufacturing (wire, plumbing pipes & sheet metal), preservative (wood, leather, fabrics)	Irritation in nose, eye, and mouth; headache, stomachache, vomiting, diarrhoea	Kidney failure, liver failure, death
Fe	Metal production (automobile, machinery, ships, buildings), manufacturing (steel)	Dizziness, iron poisoning	Liver and heart damage, coma, shock, death
Pb	Mining, Manufacturing (Paint, battery, solders & pipes), Fossil fuel (incinerator, automobile exhaust)	Blood lead poisoning in children	Kidney failure, Low chance of conceiving, damage to nervous system, development, digestive, cardiovascular and hematological system, cancer
Mn	Manufacturing (steel, batteries, glass, ceramic, fertilizer, fungicide,), fuel additive in gasoline, pesticides	Lung inflammation/irritation	Liver failure, damage to cardiovascular, nervous (behavioral change) and respiratory system
Zn	Manufacturing (paint, rubber, ointments, dyes, wood preservatives, etc.),	Diarrhoea, eye and skin lesions, nausea, vomiting, abdominal cramps, headache	Immune system, delayed sexual maturation (infertility), hair loss

Source: ASTDR, 2011

2.8 Nutrients essential for plant growth

The nutrients essential for plant growth are classified into two categories which are macronutrients and micronutrients. Macronutrients are the most important class of nutrients as it requires a relatively large amount for the plant growth. On the other hand, micronutrients are required only a small amount in which they are used in aid of the plant growth. As Weast (1984) asserts, "there are 17 heavy metals indicated to be bio-available for living cells and importance for organism and ecosystems based on the solubility under the physiological condition (as cited in Kamaruzaman, 2011, p.16). Among these heavy metals, Fe, Mo, Mn and Cu are some important micronutrients in order to carry out normal physiological regulatory functions for plants. Meanwhile, Zn, Ni, Cu, V, Co, Pb and Cr are trace elements that could be toxic to plants at certain toxic level. As Mengel and Kirkby (2001) asserts, the cell organelle which is most sensitive to Mn deficiency is chloroplast, a plant organelle responsible for photosynthesis process (McCauley, 2011, p. 12). The amount of nutrients absorbed by the plant depends on the plant function, plant mobility and plant deficiency (Khalil, 2011). Table 2.8a shows the essential nutrients required for plant growth, while Table 2.8b shows overall symptoms of malnutrition or over-nutrition.

Table 2.6 Essential nutrients required for plant growth

Category	Element	Chemical symbol	Relative (%) in plant	Function
Primary macronutrient	Nitrogen	N	100	Proteins, nucleic acids and chlorophyll production
	Phosphorus	P	6	Nucleic acids, sugars and ATP (energy) development
	Potassium	K	25	Enzyme activation, photosynthesis, sugar transport, and protein formation
Secondary macronutrient	Calcium	Ca	12.5	Cell wall component
	Magnesium	Mg	8	Chlorophyll content & co-factor for ATP production
	Sulphur	S	3	Amino acids & protein constituent
Micronutrients	Chlorine	Cl	0.3	Photosynthesis & leaf turgor
	Iron	Fe	0.2	Respiratory & photosynthesis reactions
	Boron	B	0.2	Cell wall component & reproductive tissue

	Manganese	Mn	0.1	Enzyme activation for photosynthesis
	Zinc	Zn	0.03	Growth hormone production & internode elongation (Enzyme activation)
	Copper	Cu	0.01	Enzyme component(chlorophyll production, respiration and protein synthesis)
	Molybdenum	Mo	0.0001	Nitrogen fixation process

Sources: McCauley (2011); Bennett (1993), as cited in Khalil (2011)

Table 2.7 Overall symptoms of malnutrition or over-nutrition

Nutrient	Visual symptoms	
	Deficiency	Excess
N	Light green to yellow appearance of leaves, esp. older leaves; stunted growth	Dark green foliage – susceptible to lodging, drought, disease and insect invasion
P	Purple coloration; stunted plant growth; delay in plant development	Micronutrient deficiencies, esp. Fe or Zn
K	Older leaves turn yellow initially around margin then die	Deficiencies in Mg and possibly Ca
Fe	Initial distinct yellow or white areas between veins of young leaves, leading to spots of dead leaf tissue	Possible bronzing of leaves with tiny brown spots
Mn	Interveinal chlorosis or mottling of young leaves	Older leaves have brown spots surrounded by chlorotic circle or zone
Zn	Interveinal chlorosis on young leaves; reduced leaf size	Iron deficiency in some plants
Cu	Leaves are dark green; stunted plant growth	Fe displacement and other metals from important areas in plant, causing chlorosis and Fe deficiency symptoms

Source: McCauley (2011); Hosier & Bradley (1999); Bennett (1993) as cited in Khalil (2011)

* Magnesium deficiency symptom in leaf evident in yellow parts of leaf.

** Interveinal chlorosis, a symptom of iron, zinc and manganese deficiencies, evident in yellow parts of leaf.

Figure 2.2: Deficiency symptoms of the leaves [Source: Hosier & Bradley (1999)]

2.9 Hot Plate Acid Digestion

Acid digestion is a method required to transfer analytes found in the solid sample into liquid form. This is to allow the analytes to be introduced and analysed by spectroscopic technologies such as AAS or ICPMS, which determines the analyte in the sample in terms of liquid phase. Basically, the purpose of carrying out acid digestion is to obtain a complete analyte solution or solid decomposition with minimal loss or contamination in a safe condition and short time. There are two types of acid digestion which includes closed digestion and open digestion. Closed digestion normally involves microwave or "Tölg Bombs", whereas open digestion includes hot plate or digestion block. Closed digestion allows a very high temperature to be achieved (200-260°C) at a shorter time length, and vice versa. (Berghof, n.d.).

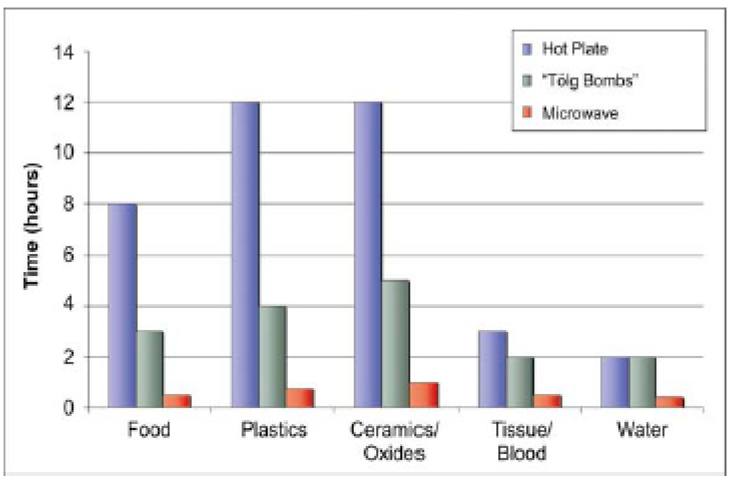

Figure 2.3: Time required for acid digestion of various method [Source: Berghof, n.d.]

Hot plate is often used as an alternative to digestion block or even closed digestion, in which it uses beakers to heat up and digest the samples instead of using digestion tubes. Based on the figure above, the time required for digesting biological tissues is about 3 hours. It is a simple acid digestion method, however, it has a contamination risk or loss of volatile elements, especially Pb salts (Berghof, n.d.). There are many ways to perform acid digestion of heavy metals. The table below shows the general advantages and disadvantage of acid digestion.

Table 2.8 Pros and cons of acid digestion method

Acid digestion method	Advantages	Disadvantages
Hydrofluoric acid-perchloric acid (HF-$HClO_4$) digestion	▪ Most effective extraction ▪ Capable of measuring metals associated with silicates	▪ Acids used are extremely dangerous
Nitric acid (HNO_3) digestion	▪ Measure all metals, except those bounded with silicates	▪ Less effective than HF-$HClO_4$ digestion
Aqua regia digestion	▪ Safer than HF-$HClO_4$ digestion	▪ Longer digestion time required
Nitric acid - hydrogen peroxide (HNO_3-H_2O_2) digestion	▪ Reasonable measurement of metals in samples	▪ Does not measure true total metal concentration ▪ Analytes may loss due to evaporation

The method of digesting plant samples in concentrated HNO_3, with or without $H2O_2$, has been well established and widely used for determination of HM concentrations in plant samples (Huang, 1985, as cited in Huang *et al.*, 2004, p. 428). Zheljazkov and Warman (2002) claimed that HNO_3 and HNO_3-$HClO_4$ provided similar levels and good recoveries of Cd and Pb (as cited in Hseu, 2004, p. 54). Moreover, nitric acid procedure has the highest recoveries of Cd, Mn and Ni of compost samples (Hseu, 2004). Perchloric acid is also advised not to be used for acid digestion due to its risk and problem of $KClO_4$ precipitation. Overnight HNO_3 digestion and HNO_3-H_2O_2 digestion of reed plants have high recoveries of Cd, Cu, Pb, Zn, Fe and Mn (Laing *et al.*, 2003), however, it all depends on the type of plants as well. There are several studies that use HNO_3 destruction of plant tissues for heavy metal analysis (Phillips *et al.*, 2015; Punamiya *et al.*, 2010; Ippolito & Barbarick, 2000; Havlin & Soltanpour, 1980). H_2O_2 is avoided to be used in this research in order to prevent the loss of analytes, rather than undigested. According to Sastre et al. (2002), nitric acid digestion can be used for samples with high organic matter content such as plant material and organic soil as most of the RSD values were lower than 5%. Moreover, the amount of sample used in this experiment is also very small, which is 0.1g. Hence, it is assumed that this method is enough to digest the small amount of sample, whereby the sample is also grinded into very fine powder form.

2.10 Flame Atomic Absorption Spectrometry (F-AAS)

Atomic absorption spectrometry (AAS) is a spectrometric technology that is used to determine inorganic element found in the environmental samples by means of liquid form. There are several types of flame spectrometry which includes F-AAS, FES and Atomic Fluorescent Spectroscopy. The principle of F-AAS is the optical radiation (light) absorption, based on the electromagnetic spectrum of each element (190 – 850 nm), by free atoms into gaseous state, in which the sample is aspirated into a flame and then atomized via a nebulizer due to Venturi effect. Venturi effect is a result of collision from high speed gas with the liquid samples injected into the nebulizer, whereby the liquid would turn into small drops and eventually into aerosol. The flame used normally consists of air and acetylene. It will direct the light beam into a monochromator to measure the concentration of element by using a detector. Each element has its own wavelength for this process to occur, hence only an element can be detected at a time.

CHAPTER 3

METHODOLOGY

3.1 Method Summary

This study consists of two batch experiments, in which both are two factor random block design. The batch test is conducted outside the laboratory in order to provide enough sunlight to the plants, yet sheltered from rain.

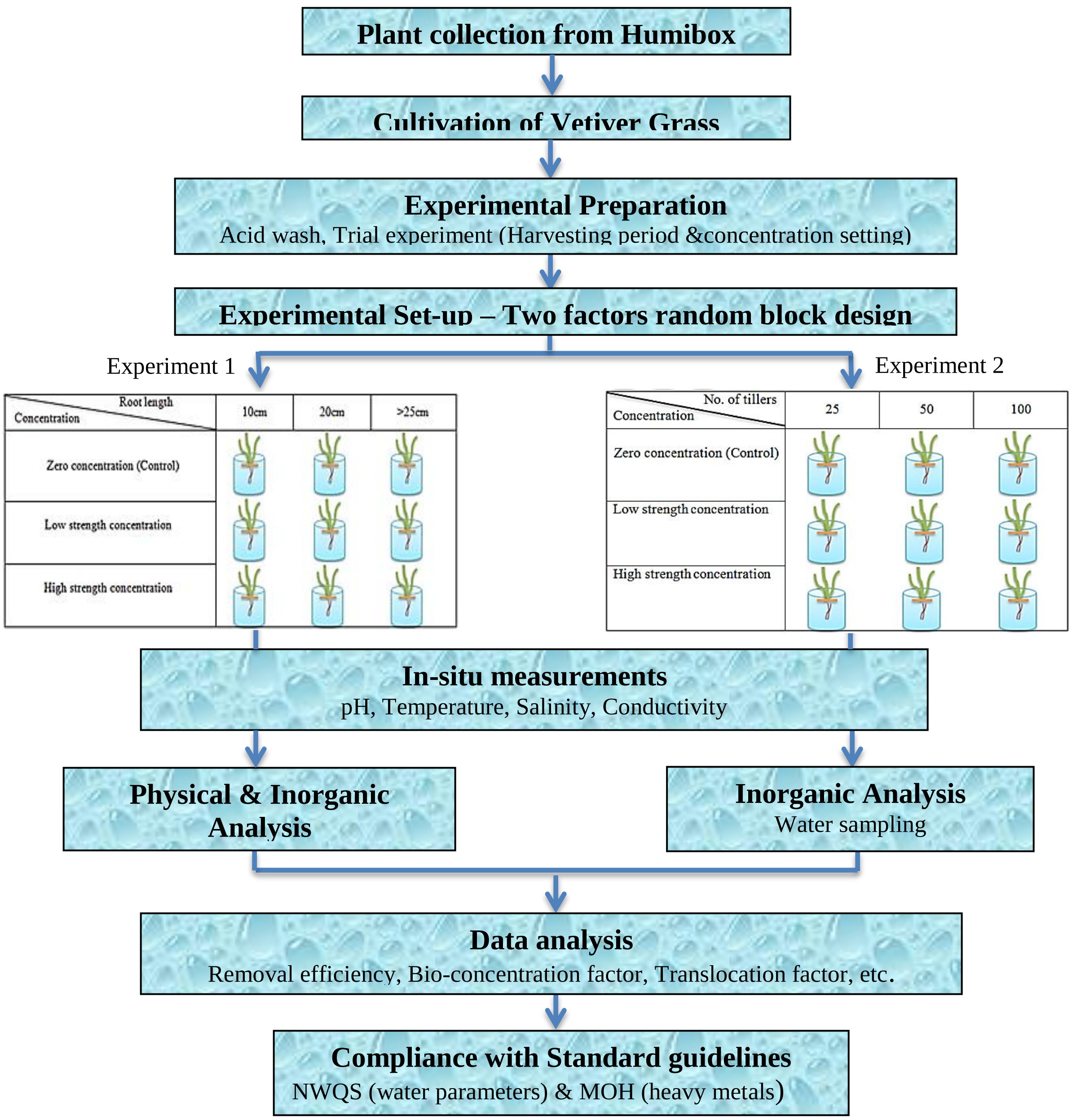

Figure 3.1 Flowchart of the Research Design

3.2 Plant Collection

Vetiver grass, scientifically known as *Vetiveria Zizanioides*, was used in this research. The Vetiver grass (VG) was collected from Humid Tropic Centre (HTC) under the care of Department of Irrigation and Drainage (JPS Malaysia). Around 1050 VG slips were provided by HTC for the two experiments. The VGs were all about the root length and shoot length – root length of about 10 cm d shoot length of 20 cm respectively.

Figure 3.2 200 VGs sent in first batch

Figure 3.3: 850 VGs sent in second batch

3.3 Pre-treatment of Vetiver Grass

The seedlings were placed floating in water containing nutrients with the support of polystyrene and sponge. The purpose was to acclimatize the condition before the experiment as the real experiment is conducted in water without any soil medium. However, due to the small size of the plants, they were grown for 5 months until they have reached the optimum size (root length) designed for the experiment. The plants were provided with nutrients N:P:K with ratio of 7:7:7.

Figure 3.4: VGs grown in water containing nutrients N:P:K with ratio 7:7:7

3.3.1 Trial experiment

The trial experiment was carried out to evaluate the heavy metal uptake by Vetiver grass in two planting densities within a range of heavy metal concentrations. The purpose is to prepare for the big scale experiment so that we could know when to harvest the plants, how many tillers should be used, and which concentration can be set as the low and high strength concentration. In other words, the optimization of the experiment for heavy metal uptake from the water was the main study of this experiment in order to comply with the plant harvesting in the real experiment. The experiment is set up as shown in Figure 3.5, whereby there are two treatments of 4 tillers and 10 tillers exposed to heavy metal concentration of 0.5ppm, 1.0ppm, 2.0ppm, 4.0ppm, 8.0ppm and 10ppm respectively. All the slips were trimmed to a height of 25-cm and root length of 15-cm. The water sample at 0-hour contact time was taken to indicate the initial heavy metal concentration in the synthetic water. The physical parameters such as pH, temperature, salinity and electrical conductivity (EC) were measured daily. 15-mL of water samples were taken at each sampling and filtered through 0.45-μm cellulose acetate filter paper. The samples were then preserved to pH<2 prior to AAS analysis (Appelo and Postma, 1993). The growth and toxicity symptoms were also observed.

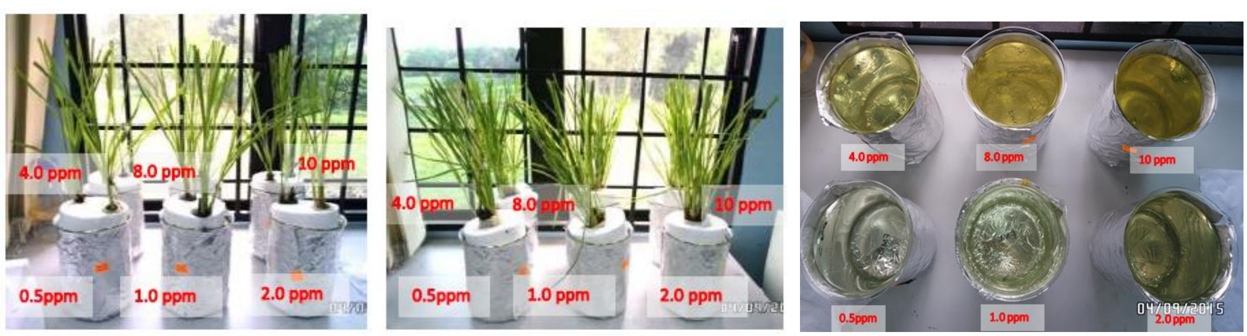

Figure 3.5: Treatment of control and each plant with 4 tillers and 10 tillers respectively

3.4 Experimental Set-up

3.4.1 Preliminary Cleaning

The glassware and apparatus that used for both experiments were first soaked overnight with 5% nitric acid (HNO_3) and rinsed with distilled water and Millipore water in order to eliminate all the heavy metal or other pollutants deposited on the surface from the previous experiments. After that, they were dried at the oven at around 60°C and ready to use. The solution was prepared by diluting 1-L of 70% concentrated HNO_3 into 14-L of mixture with distilled water. The list of apparatus and materials needed for the experiments is listed in page xix.

3.4.2 Synthetic Mixture of Selected Heavy Metals

There were three different treatments of selected heavy metals synthetic mixture used in the experiment which are (1) zero concentration (control), (2) low concentration and (3) high concentration. The selected heavy metals for this experiment are Cu, Fe, Mn, Pb and Zn. There were two treatments used in this experiment which comprises of low concentration and high concentration. The low concentration treatment was selected based on the average river concentration in Malaysia as shown in the Appendix 3, whereas the high concentration treatment corresponded to the one of the worst case scenario of river pollution found in Malaysia. There were two sets of control for both experiments, whereby one set of experimental tank with all the synthetic mixture maintained at its concentration without any plants (Control 1) and another set with plants grown in distilled water without any metals (Control 2). The synthetic mixture was prepared by dissolving salts in distilled water as shown in the table below.

Table 3.1 Concentration of synthetic mixture used in the experiment

Type of element	Salts used	Concentration, ppm			
		Low (Reported)*	Low (Suggested)	High (Reported)	High (Suggested)
Cu	$CuSO_4.5H_2O$	1.7519	2.0	9.19[a]	10
Fe	$FeSO_4.7H_2O$	3.876	2.0**	36.31[a]	40
Mn	$MnSO_4.H_2O$	2.1614	2.5	7.17[a]	8
Pb	$Pb(NO_3)_2$	0.04589	0.5	2.25[b]	2.5
Zn	$ZnSO_4.7H_2O$	0.6889	1.0	6.56[a]	7

* Average of the river concentration from my read-ups

** The average was too high due to an extreme, so the concentration is used from Prasanna *et al*., 2012.

a Hatar et al., 2013

b Hadibarata et al., 2012

3.4.3 Experiment 1

Before starting the experiment, the plants were washed thoroughly without direct contact with running tap water to prevent plant damage, especially to the roots. Then, they were rinsed with distilled water in order to remove any pollutants found on the plant which may affect the experiment. VGs were trimmed accordingly - height of 25-cm and root length for each treatment of 10-cm, 20-cm, and >25-cm respectively. These treatments were carried out in 9 experimental basins, size of 34cm X 29cm X 35cm, filled with 30-L of synthetic mixture of heavy metals, outside the iENFORCE laboratory. Each basin consisted of 9 sets of Vetiver slips with 8 tillers each (based on the tiller size), in which Vetiver grass was supported by a polystyrene (size of 30cm X 30cm, thickness of 48mm) and sponge to wrap around the crown as there is no soil medium to hold the plant in the water.

The experiment was a two factors random block design, whereby the factors are the heavy metal concentration and the root length of VG. The suitable pH would enhance the absorption of heavy metals by plant roots as pH is the main factor that would affect the plant growth based on the 4-months experience in taking care of the Vetiver grass. The plants were harvested and water are sampled at days interval of 0, 1, 3, 5, 7 and 10. In order to prevent the sunrays effects and algae proliferation, the external container was covered with aluminium foil throughout the experiment (Ladislas *et al.*, 2013). The toxicity symptoms of VGs were also observed and recorded.

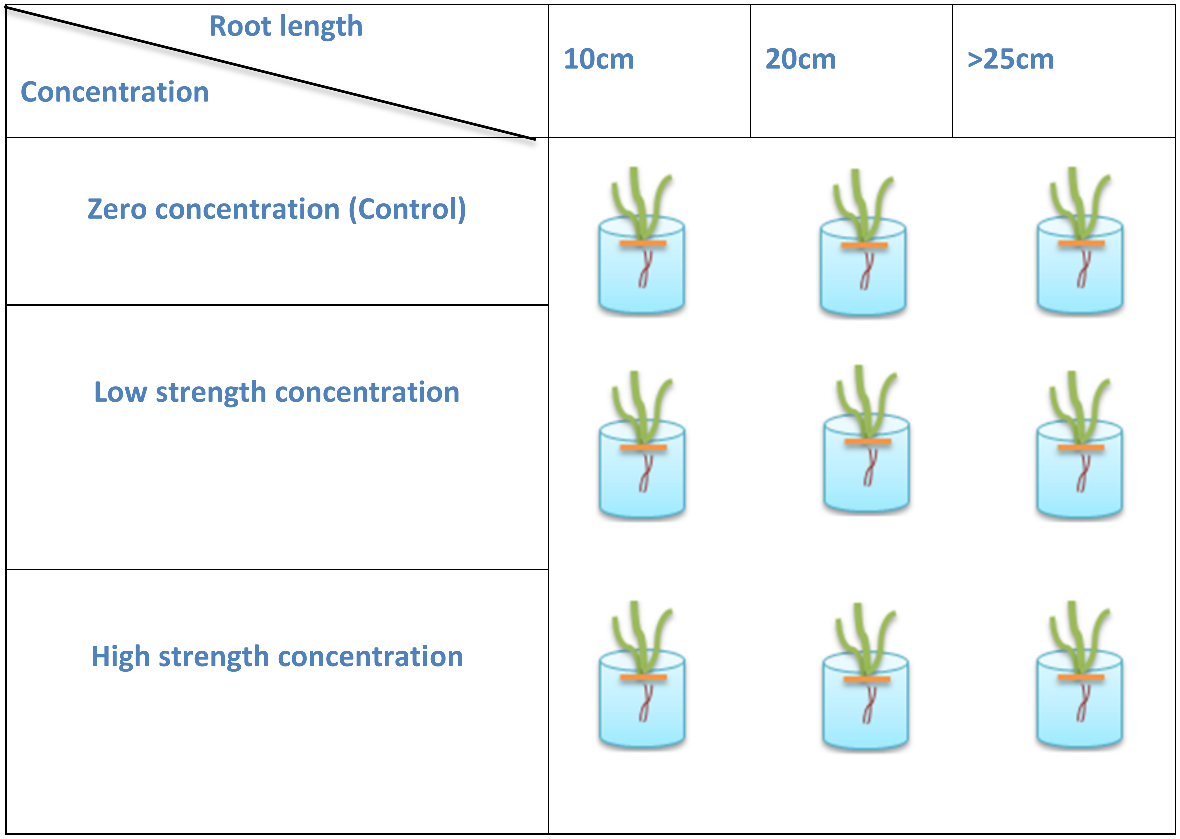

Figure 3.6 Experimental set-up consisting of factors – root length and concentration

3.4.4 Experiment 2

The procedure was exactly the same as Experiment 1, except that the factors were slightly different. The experiment was also a two factors random block design, whereby the factors were the heavy metal concentration and the number of tillers/density. The tillers that were too young was ignored and not counted as a tiller. All VGs were trimmed accordingly - height of 25-cm and root length of 20-cm. Water was sampled at days interval of 0, 1, 3, 5, 7 and 10. Distilled water was added to the original water level in order to prevent the diminution of water because of evapotranspiration process while the experiment is carried out (Ladislas *et al.*, 2013), in which it is assumed that the addition of water would not affect the concentration of the synthetic mixture.

No. of tillers / Concentration	25	50	100
Zero concentration (Control)			
Low strength concentration			
High strength concentration			

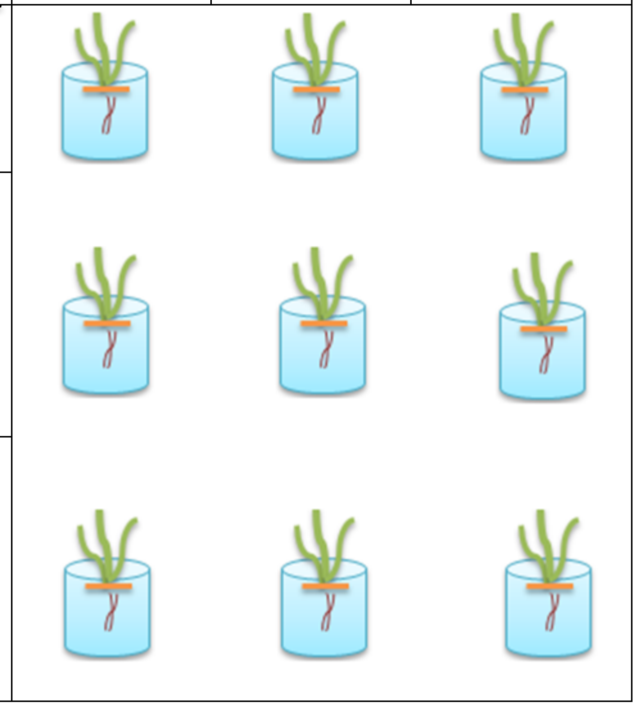

Figure 3.7 Experimental Set-up consisting of factors – tiller density and concentration

3.4.5 Setting up Experiment 1 and 2

The real experiment was being set-up outside the ENFORCE lab. The plants were chosen carefully based on the experimental requirement and transferred to new polystyrene. Meanwhile, they were allowed to acclimatize once again for a few days before the experiment as the transferring might affect some of the roots.

Figure 3.8 Experimental set-up in preparation for Experiment 1 and Experiment 2 respectively (Day 0); and the plant acclimatization of selected plants prior to the experiment

3.5 Laboratory analysis

3.5.1 Water sampling

The water sampling was done at each time frame accordingly for both experiments. There were two parameters involved in this study which are *in-situ* and *ex-situ*. The *in-situ* parameters were such as pH, temperature, electrical conductivity (EC), and salinity. On the other hand, the *ex-situ* parameter was the analysis of selected heavy metals in the synthetic mixture.

50-mL of water sample was obtained in a centrifuge tube and brought immediately to the laboratory. The water samples were filtered by using 0.45µm cellulose acetate filter paper in order to remove large biological materials, which could cause interference by reacting with the analyte during storage as well as the pre-requirement to be run by AAS. The water samples were then acidified to pH<2 by addition of 0.7-ml HNO_3 Suprapur 65% (Appelo and Postma, 1993) in order to preserve most elements by reducing precipitation and adsorption losses to container walls as well as to prohibit microbial activity.

3.5.2 Plant harvesting

Each plant was harvested from each treatment for the respective time frame for Experiment 1. The plants were rinsed with tap water followed by distilled water in order to wash off any dust or dirt deposited on the shoots and residual HM on the root surface. The wet weight of each sample was weighed using analytical balance and recorded in a sampling record sheet. Plants biomass was separated in two samples which consist of shoots and roots. The VG root crowns were not harvested due to the assumption for its inability for metal accumulation. It is believed that they could regrow back, in which it may result in a potential application of on-site applications via pontoons in the river (Ladislas *et al.*, 2013). The shoots and roots were dried at 70 – 85 °C in the oven to a constant weight. The production of total harvestable dry biomass was then determined by the dry weight (DW) of each sample. Then, the plants were separated in terms of shoots and roots, are grinded using mortar and pestle.

0.1g of the grinded samples was then digested with 10 mL of 65% HNO_3 (AR grade; BDH). The acid digestion was done in a fume cupboard using a digestion block at 90°C for about one and half hours/takes about 2 hours. The samples were placed into a beaker and covered with a glass Petri dish when heated on a hot plate. This method was to prevent the sample solution from being lost and prevent contamination from the surroundings during the digestion process. The digestion was completed when the colour of the solution has changed from brown to colourless. The digestate was then cooled at room temperature before being filtered using 0.45µm cellulose acetate filter paper via syringe filter. Then, the filtered sample was adjusted with addition of millipore water to a final volume of 50-mL. The samples of digested plants were ready to be analysed by FAAS (AAS model AA-6800 Shimadzu) to determine the heavy metal concentrations. The concentrations of heavy metals in plants were calculated on a DW basis as shown in the equation below:

$$Conc.\,(\frac{mg}{kg}) = \frac{[HM\ conc. from\ AAS\left(\frac{mg}{L}\right) - Blank\ (\frac{mg}{L}) \times MQ\ water(ml)}{dry\ weight\ of\ sample\ (g)}$$

(Equation 1)

3.6 Standard solution preparation

The standard solution of each element is prepared by using the formula as shown below:

$M_1V_1 = M_2V_2,$ (Equation 2)

Whereby, M_1 = Initial concentration
M_2 = Final Concentration
V_1 = Volume of distilled water required
V_2 = Desired volume of mixture

Table 3.2 Concentration of standard element for calibration curve in FAAS

Element	AAS Standard concentration, mg/L	Correlation Coefficient, r	Wavelength, nm	Gas
Cu	1.0, 2.0, 4.00	1.0000	324.8	Air-acetylene
Fe	1.0, 2.0, 4.0	0.9998	248.3	Air-acetylene
Mn	0.5, 1.0, 2.5	0.9995	279.5	Air-acetylene
Pb	2.5, 5.0, 10.0	1.0000	283.3	Air-acetylene
Zn	0.2, 0.5, 1.0	1.0000	213.9	Air-acetylene

3.7 Quality Assurance (QA) and Quality Control (QC)

Since the beginning of the experiment, QA and QC have been carried out in the preliminary cleaning of glassware. For every physical measurement, three readings were taken to obtain the average value. As for analysis, about 15 mL of each sample solution was transferred into vials that will be analysed by the FAAS machine for the detection of selected heavy metal concentration. The total absorption of the selected heavy metals by VG was analysed using AAS model AA-6800 Shimadzu. Nitric acid samples were digested without the addition of any plant material, which acts as the reagent blank for plant samples, was used to check the contamination possibility from the glassware or the acid. Another set consists of Milli-Q water and concentrated nitric acid which acts as the reagent blank for the water samples. The samples were measured three times by AAS for each injection, and QC is carried out at every 10 – 15 samples intervals.

3.8 Data Analysis

3.8.1 Removal efficiency (Darajeh *et al.*, 2014)

The removal efficiency of each heavy metal is calculated to determine the potential uptake of Vetiver grass from the synthetic mixture.

$$\%\, Removal\ efficiency = \frac{C_{ini} - C_{fin}}{C_{ini}} \times 100, \qquad \text{(Equation 3)}$$

where C_{ini} is initial concentration of synthetic mixture and C_{fin} is final concentration of synthetic mixture.

3.8.2 Metal accumulation amount (Ladislas et al., 2013)

The total accumulated metal uptake by the harvestable biomass is determined by the addition of shoots and roots, in which it would then be indicated by the subtraction of harvestable biomass in Control 2. It is calculated as:

[Metal content in harvestable biomass determined for low or high treatment] – [Metal content in harvestable biomass determined for control 2]

(Equation 4)

3.8.3 Bioconcentration factor (BCF) and Translocation factor (TF) (Zhang *et al.*, 2014)

BCF is a ratio of plant capability to accumulate heavy metal with respect to the synthetic mixture concentration. Based on Galal and Shehata (2014), BCF>1 indicates the accumulation of heavy metals in the shoot. As for TF, it is a ratio of plant ability to extract heavy metal from root to shoot. TF<1 means the most of heavy metal are accumulates in the root, and vice versa.

$$BCF = \frac{Metal\ concentration\ in\ plant\ shoot\ (\frac{mg}{kg})}{Metal\ concentration\ in\ water\ (\frac{mg}{L})}$$

(Equation 5)

$$TF = \frac{Metal\ concentration\ in\ plant\ shoot\ (\frac{mg}{kg})}{Metal\ concentration\ in\ plant\ root\ (\frac{mg}{kg})}$$

(Equation 6)

3.8.4 Statistical Analysis

A statistical analysis to determine root length, number of tillers and treatment effects on the performance of phytoremediation is evaluated using higher-order multivariate analysis of variance (MANOVA), Pearson product-moment correlation coefficient to see the relation between heavy metal removal from water and heavy metal uptake by the root, and Student’s t tests to compare differences between treatments and the uptake by plant parts, using IBM SPSS Statistics 22 software. Multiple comparison was performed using MANOVA with degree of significance of 0.01 based on Bonferroni adjustment.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Vetiver grass cultivation

The plants have to be cultivated by the researcher herself to reach the specified root length (15cm, 30cm and 45cm) as one of the experimental factors was root length (Refer to Appendix 2). The plants have been cultivated in water with the support of polystyrene for almost 5 months, however, the longest root length was only up to 35cm. Besides that, the number with high categorical root length was very low. Hence, the interval for the specified root length was reduced, in which, it might affect the results outcome.

Based on the 5 months experience in growing the Vetiver grass, it has been observed that VG tends to grow healthy and dark green in colour when the hydroponic solution was acidic (ranged from 3.5 to 5) with high electrical conductivity (ranged 3.0 – 5.0 mS/cm). On the other hand, it would die easily when the pH is alkaline (above 8), along with invasion of fungus and other organisms. However, it could survive at pH 4.5 when the salinity goes up to 4.9ppt and electrical conductivity up to 9.91mS/cm.

Not only that, during a month of cultivation, the plants started to turn yellowish with red spots on the leaves. One of the VG expert, Dr. Paul Truong has suggested that it could be the fertilizer problem. Another suggestion by Mr. Azmi from 'Pakar Go Green' Company has advised that the young or weak plants will die if in contact with strong UV rays. Hence, a mini experiment was carried out by isolating some plants to another container to examine the real cause of invasion by fungus and other organisms as well as the red spotting on the leaves. It turned out that the sunlight was the problem as well as the pH value, which is in contrast with the statement by Truong (2009) asserting that VG has high tolerance to extreme climatic variance. In conclusion, cultivation of VG has to be done at a sheltered area with suitable amount of sunlight, low pH to prevent growth of other organisms, hydroponic fertilizer that would produce low pH values as well as aerating the hydroponic solution could enhance the Vetiver growth.

4.2 Trial experiment

This experiment is done to evaluate the heavy metal uptake by Vetiver grass in two planting densities and different heavy metal concentrations in order to prepare and decide the experimental design for the big scale experiment (Refer to Appendix 4). Only water samples are analysed because the potential rate of heavy metal is wished to be known from this experiment in order to harvest the plants at the same time as well. This is because Vetiver grass is known for its high uptake of contamination from the environment (Danh et al., 2009; Truong, 2000; Truong, n.d.).

Both series of graph in the figures 1 and 2 depict the line graph of heavy metal removal by 4 tillers and 10 tillers of Vetiver grass respectively. It has shown that all the graphs have shown decreasing trends of heavy metal removal within just 4 days. Not only that, most of the heavy metal uptake is obtained during the early stage of the experiment. Hence, the sampling for water and plants focused on the early stage of the experiment, whereby sampling is done at interval of 0, 24, 72, 120, 168 and 240 hours.

Not only that, it has also shown that most of heavy metal is being removed at a very fast rate for concentration less than 2ppm, it is also proven in Table 2. By the end of 4 days, most of the heavy metal is already taken up by the plants. Since there is only two types of concentration used in real experiment, setting low and high concentration has to be done carefully (Appendix 3).

From this experiment, it can be indicated that the removal rate of heavy metal can be ranked in this order: Fe>Pb>Zn>Cu>Mn. There were a lot of mistakes in the removal rate based on Table 2. This is because spiking is done inaccurately in this experiment as it requires a lot of skills and experiment. Conclusively, it is better to avoid matrix spiking for the upcoming experiment.

Figure 4.1(a) Plants of 4 tillers and (b) plants of 10 tillers were still green on the 5th day of the experiment

In the figures above, it has illustrated that Vetiver grass could withstand concentration of heavy metal up to 10ppm, without any major signs of toxicity, even up to 5 days except those young shoots with a few brown leaves and wilting.

4.3 Visual changes as sign of toxicity (Experiment 1)

Table 4.1 Visual changes observed in plants grown in synthetic mixture of Cu, Fe, Pb, Zn and Mn at two ranges of concentrations and different time intervals

Media	Type of treatment	Time (hours)					
		0	24	72	120	168	240
Distilled water (control)	10cm	G	G	G	PG	PG	YB
	20cm	G	G	G	PG	PG	YB
	>25cm	G	G	G	G	PG	GB
Heavy metal solution (mixtures of Cu, Fe, Mn, Pb and Zn) :							
Low	10cm	G	G	G	Y	Y	YB
	20cm	G	G	G	YB	B	BW
	>25cm	G	G	G	Y	YW	BW
High	10cm	G	G	G	GY	GY	BW
	20cm	G	G	G	YB	YBW	BW
	>25cm	G	G	G	YB	YBW	BW

Note: **G**: the plants are green; **PG**: the plants are pale-green in color; **Y**: the plants have yellow leaves; **B**: the plant have brown leaves; **W**: the plant partially wilting; **MD**: most of the leaves were dead; **D**: the plant completely died

In the control experiment, all the plants seemed all green and healthy up to 72 hours. It has suggested that Vetiver grass (VG) has high adaptability to new environment despite of the absence of macronutrients such as N, P and K. However, all the plants started to show symptoms after 72 hours to indicate either toxicity (excess nutrients) or nutrient deficiency effect. For the control experiment, some of the plants in 10cm and 20 cm treatments have turned pale green in which indicates the deficiency of N. Furthermore, all the plants in control experiment had brown leaves at 240 hours. This suggested that the plants are suffering from chlorosis due to macronutrient deficiency, especially N and K. These two nutrients are essential for proteins, nucleic acid and chlorophyll production, and enzyme activation for photosynthesis and sugar transport as well as protein formation respectively.

In low concentration mixture, the plants appeared healthy until 72 hours, but then, turned yellow only as a sign of chlorosis at 120 hours for treatments of 10cm and >25cm root length, whereas 20cm root length treatment appeared to have brown leaves as well. This has predicted that 20cm had high heavy metal uptake than the others. At 168 hours, the plants in 10-cm treatment remained yellow, 20cm treatment showed prolonged deterioration by turning into brown leaves, and the plants in >25cm treatment has already started to wilt. After 240 hours exposure, all the plants showed evidence of further exposure by exhibiting signs of brown leaves, but the effects were greater at 20cm and >25cm treatments as the plants started to wilt.

In high concentration mixture, the plants all appeared to be healthy up to 72 hours. However, at 120 hours till 240 hours onwards, the plants in 20cm and >25cm treatments exhibited drastic changes as they showed chlorosis and already started to turn brown in the expense of just 48 hours. They continued to exhibit signs of death by wilting. This has suggested to be varying symptoms of heavy metal toxicity, especially Fe, Cu and Zn. Although it can be seen that there is a difference in reducing tolerance as compared to the low concentration, the plants were yet to show signs of necrosis (death) at the end of the experiment. As for the 10cm treatment, it only started to show signs of prolonged exposure of high concentration heavy metal at the end of the experiment.

In general, VG has shown its ability to adapt to high toxicity level of heavy metal in this experiment that is asserted by the fact that it can tolerate high level of heavy metal concentration (Truong, 2000; Truong, n.d.), whereby it did not even exhibit any signs of death. The plants have only showed morphological symptoms such as chlorosis, brown leaves due to prolonged heavy metal exposure and wilting. Other researchers have indicated that different plant species would respond differently to metal toxicity. For example, water lettuce appeared to be healthy up to Day 6with highest concentration of 5ppm (Kamaruzaman, 2011) and *Phylidrum lanuginosum* has showed toxicity symptoms the next day when exposed to heavy metal treatment (Hanidza et al., 2011).

4.4 Heavy metal content in synthetic water

4.4.1 Removal vs root length

The data results for this section are obtained from Experiment 1, in which the two-factor factorial experiment consists of root lengths and concentration. The results are discussed in terms of the different concentration level. (Refer to Appendix 5)

4.4.1.1 Low concentration

Table 4.2: Heavy metal reduction in water (mg/L) at different root lengths in Low Concentration

Heavy Metals/ Root length	Operation time (day)						Removal efficiency (%)				
	0	1	3	5	7	10	0 – 1	0 – 3	0 – 5	0 – 7	0 – 10
Cu: 10 cm	**1.93**	**1.62**	**1.40**	**1.28**	**1.26**	**1.10**	16.06	27.46	33.68	34.72	43.01
20 cm	**1.96**	**1.58**	**1.37**	**1.25**	**1.20**	**1.02**	19.39	30.10	36.22	38.78	47.96
>25 cm	**1.92**	**1.54**	**1.28**	**1.14**	**1.01**	**0.84**	19.79	33.33	40.63	47.40	56.30
Fe: 10 cm	**0.86**	**0.65**	**0.47**	**0.36**	**0.29**	**0.04**	24.80	45.75	58.09	66.82	94.99
20 cm	**0.81**	**0.51**	**0.30**	**0.22**	**0.17**	**0.09**	36.80	63.32	73.11	78.69	89.47
> 25 cm	**0.85**	**0.55**	**0.28**	**0.22**	**0.18**	**0.10**	35.29	67.29	73.86	79.13	88.51
Mn: 10 cm	**2.81**	**2.30**	**2.16**	**2.09**	**2.15**	**1.97**	18.24	23.22	25.76	23.51	30.16
20 cm	**2.79**	**2.34**	**2.18**	**2.14**	**2.17**	**2.00**	16.11	21.84	23.35	22.39	28.33
> 25 cm	**2.72**	**2.30**	**2.17**	**2.12**	**2.05**	**1.91**	15.36	20.30	22.07	24.43	29.68
Pb: 10 cm	**0.72**	**0.54**	**0.44**	**0.37**	**0.38**	**0.31**	25.68	39.19	48.65	47.31	56.75
20 cm	**0.68**	**0.46**	**0.37**	**0.32**	**0.33**	**0.29**	32.85	45.71	52.86	51.42	57.15
> 25 cm	**0.60**	**0.39**	**0.31**	**0.32**	**0.22**	**0.21**	35.48	48.38	46.78	62.90	64.52
Zn: 10 cm	**1.03**	**0.95**	**0.90**	**0.89**	**0.89**	**0.79**	7.43	12.03	13.57	13.42	22.89
20 cm	**1.01**	**0.95**	**0.91**	**0.89**	**0.85**	**0.76**	5.50	10.00	12.09	15.74	24.84
> 25 cm	**1.01**	**0.95**	**0.89**	**0.85**	**0.80**	**0.73**	6.37	12.35	16.03	20.65	28.15

From the bar chart above, it can be seen that Fe had the highest removal efficiency up to 94.99%, followed up by Pb with 64.52% removal efficiency and Cu with 56.30% removal efficiency, regardless of their root length. Zn had the lowest removal efficiency of 28.15% as well as Mn with 30.16%. In short, the removal efficiency can be ranked in this order: Fe>Pb>Cu>Mn>Zn. Fe has the highest removal efficiency in this experiment which is the same as reported in Roongtanakiat et al. (2007).

Theoretically, it is assumed that the removal rate of heavy metal would increase as the root length increases due to higher surface area for metal absorption by the root into the plants. All of the plants in this experiment illustrated a trend whereby the removal efficiency had increased when the root length increased, except for Fe and Mn. The Fe removal efficiency has decreased as the root length increased; whereas Mn removal efficiency was almost constant although the root length has increased.

Table 4.3 Compliance of data results in this experiment with MOH standard

Element	**Concentration, ppm**			**Percent reduction, %**	**Compliance with the standard**
	MOH Standard	Initial	Final (after 10 days)		
Cu	1	1.94	0.99	48.97	yes
Fe	1	0.84	0.08	90.48	yes
Mn	0.2	2.77	1.96	29.24	no
Pb	0.1	0.67	0.31	53.73	no
Zn	5	1.02	0.76	25.49	yes

Based on the table above, it has shown that only Cu, Fe and Zn have met the requirement for the MOH quality standard. In fact, it supposed that only Cu has successfully been taken up by Vetiver as Fe and Zn have initial concentration of lower than the MOH standard. As for Mn and Pb, both elements showed percent reduction, however, it is suggested that they required a longer time to be taken up by the plant as Mn is only micronutrient by plants, whereas Pb is not required for plant growth but a toxicity at high concentration (McCauley, 2011; Bennett, 1993, as cited in Khalil, 2011).

4.4.1.2 High concentration

Table 4.4 Heavy metal reduction in water (mg/L) at different root lengths in High Concentration

Heavy Metals/ Root length	Operation time (day)						Removal efficiency (%)				
	0	1	3	5	7	10	0 – 1	0 – 3	0 – 5	0 – 7	0 – 10
Cu: 10 cm	**9.70**	**8.32**	**8.02**	**7.92**	**7.87**	**7.40**	14.23	17.32	18.35	18.87	23.71
20 cm	**9.75**	**8.84**	**8.77**	**8.73**	**8.78**	**8.21**	9.33	10.05	10.46	9.95	15.79
>25 cm	**8.95**	**8.53**	**8.28**	**8.19**	**8.37**	**7.95**	4.69	7.49	8.49	6.48	11.17
Fe: 10 cm	**29.44**	**28.98**	**25.98**	**11.74**	**2.51**	**0.79**	1.56	11.74	60.12	91.47	97.33
20 cm	**29.85**	**30.41**	**29.98**	**26.63**	**4.47**	**1.75**	-1.89	-0.45	10.77	85.04	94.15
> 25 cm	**31.59**	**28.73**	**26.42**	**22.08**	**2.91**	**2.43**	9.07	16.37	30.12	90.80	92.31
Mn: 10 cm	**6.74**	**6.02**	**5.94**	**5.97**	**6.00**	**5.80**	10.65	11.88	11.43	11.02	13.95
20 cm	**6.81**	**6.20**	**6.20**	**6.27**	**6.24**	**5.98**	8.98	8.91	7.94	8.31	12.21
> 25 cm	**6.64**	**6.09**	**6.05**	**6.03**	**6.11**	5.88	8.30	8.82	9.14	8.03	11.38
Pb: 10 cm	**2.09**	**1.60**	**1.38**	**1.39**	**1.41**	**1.43**	23.36	34.11	33.18	32.24	31.31
20 cm	**2.19**	**1.79**	**1.66**	**1.69**	**1.77**	**1.71**	18.31	24.11	22.77	19.20	21.88
> 25 cm	**2.18**	**1.74**	**1.55**	**1.54**	**1.64**	**1.68**	20.18	28.70	29.15	24.66	22.87
Zn: 10 cm	**6.64**	**6.37**	**6.41**	**6.27**	**6.11**	**5.84**	3.99	3.38	5.54	7.91	11.98
20 cm	**6.56**	**6.47**	**6.53**	**6.49**	**6.53**	**5.93**	1.42	0.54	1.17	0.58	9.71
> 25 cm	**6.64**	**6.44**	**6.40**	**6.07**	**6.44**	**6.03**	3.06	3.59	8.56	3.06	9.17

In high concentration of synthetic mixture, it has also depicted that Fe also has the highest efficiency among the five elements with removal efficiency of 97.33%, followed by Pb with 31.31% removal efficiency and Cu with 23.71% removal efficiency, regardless of their root length. Zn, like the low concentration, has the lowest removal effiency of 11.98%, as well as Mn with 13.95% removal efficiency. Just like the ones in low concentration as discussed previously, the removal efficiency can be ranked in this order : Fe>Pb>Cu>Mn>Zn. Fe has the highest removal efficiency in this experiment (Refer to Appendix 5), which is the same as reported in Roongtanakiat et al. (2007).

For high concentration, it seemed that the root length did not affect the removal efficiency as the removal efficiency decreases when the root length increases, which is in contrast with those in low concentration. Not only that, Pb had the best removal efficiency for all the root lengths at 72 hours (Day 3), on the other hand, the others heavy metal element had the highest removal efficiency at 240 hours which is Day 10 – the final day of the experiment.

Based on that Roongtanakiat (2007), the order was Fe>Mn>Zn>Cu>Pb in which Zn and Mn have the higher plant uptake as compared to this experiment. This could be due to the fact that excessive iron has reduced the zinc uptake and excessive zinc has reduced the manganese uptake (Malvi, 2011).

Table 4.5 Compliance of data results in this experiment with MOH standard

Element	**Concentration, ppm**			**Percent reduction, %**	**Compliance with the standard**
	MOH Standard	Initial	Final (after 10 days)		
Cu	1	9.47	7.85	17.11	no
Fe	1	30.29	1.66	94.52	no
Mn	0.2	6.73	5.89	12.48	no
Pb	0.1	2.15	1.61	25.12	no
Zn	5	6.61	5.93	10.29	no

Based on the table above, it has shown that all the elements did not meet the requirement for the MOH quality standard. This is because the initial concentration of these elements are very high, hence it is suggested that they required a longer time to be taken up by the plant although there are high percent reduction, especially in Fe.

4.4.1.3 Statistical analysis for Experiment 1

Table 4.6 Comparison between trial experiment and real experiment

Heavy metal element	Removal efficiency (%)			
	Trial experiment (4 days)		Real experiment (10 days)	
	4 tillers	10 tillers	Low	High
Cu	51.03	72.2	49.09	16.89
Fe	89.1	99.2	90.99	94.60
Mn	28.8	20.15	29.39	12.51
Pb	74	78.1	59.47	25.35
Zn	76.6	75.69	25.29	10.29

From the table above, it can be seen that the heavy metal removal is not as efficient as the trial experiment, especially for Zn and Pb. The set-up in the open area could be the reason for the uncertainties as the trial experiment was carried out in the laboratory, whereas the real experiment was carried out outside the laboratory. There were too many uncontrollable factors in terms of physical, meteorological and ecological, for example contribution by the rain or wind, sunlight which affected the heavy metal content, and biological organisms from microbes to large animals. It is supported by Agunbiade et al. (2009) that the climatic conditions have controlled the efficiency of phytoremediation, hence it depends on factors which include metal dissolution in aqueous state for metal uptake into roots and interaction between the environment, metal and the plant. Furthermore, Zhang et al. (2010) also asserted that plant age and seasonal variation could affect the plant uptake ability, in which younger plants could translocate heavy metal faster than that in older plants. Since the plants are of the same batch, this could be the reason why the removal efficiency has decreased compared to the ones in trial experiment.

A one-way between-groups multivariate analysis of variance (MANOVA) was performed to investigate root length differences in heavy metal removal for low concentration. Five dependent variables were used: Cu, Fe, Mn, Pb and Zn; whereas the dependent variables were root lengths. Preliminary assumption testing was conducted to check for normality, linearity, univariate and multivariate outliers and homogeneity of variance-covariance matrices, with no serious violations noted. There was a statistically significant difference between different root length on the combined dependent variables which are the heavy metal elements, $F(10, 22) = 5.532$, $p < 0.005$; Wilks' Lambda = 0.081; partial eta squared = 0.715. A separate ANOVA was conducted for each dependent variable, with each ANOVA evaluated at an alpha level of 0.01, using Bonferroni adjustment. Based on the Tests of Between-Subjects Effects table in Appendix 6, there was no significant difference between different root length (10cm, 20cm and >25cm) in the removal of each heavy metal. This may be due to low interval between the root length as well as harvesting period, hence, it could be the contribution for results insignificance although it could be seen that there could be relationship between root length and the removal efficiency. This is suggested because most of the studies only harvest the plants after a certain period of time in weeks or months (Ladislas, 2013; Roongtanakiat, 2007; Roongtanakiat, 2001). Pearson product-moment correlation coefficient was done to investigate the relationship, however, there was no correlation as $p>0.05$ (Appendix 7).

Another MANOVA was also performed for high concentration. There was no statistically significant difference between different root length on the combined dependent variables which are the heavy metal elements, $F(10, 22) = 1.316$, $p = 0.282$; Wilks' Lambda = 0.392; partial eta squared = 0.374. A separate ANOVA was conducted for each dependent variable, with each ANOVA evaluated at an alpha level of 0.01, using Bonferroni adjustment. Based on the Tests of Between-Subjects Effects table in Appendix 8, there was no significant difference between different root length in the removal of each heavy metal for High concentration.

Table 4.7 Mean and significant values for the MANOVA results

Concentration	Heavy metal	Density, tillers	Mean	Significance between group
Low	Cu	10	1.4317	0.756
		20	1.3967	
		>25	1.2882	
	Fe	10	0.4432	0.821
		20	0.3478	
		>25	0.3638	
	Mn	10	2.2474	0.933
		20	2.2730	
		>25	2.2120	
	Pb	10	0.4601	0.411
		20	0.4097	
		>25	0.3447	
	Zn	10	0.9094	0.769
		20	0.8932	
		>25	0.8719	
High	Cu	10	8.2050	0.169
		20	8.8467	
		>25	8.3783	
	Fe	10	16.5708	0.875
		20	20.5125	
		>25	19.0242	
	Mn	10	6.0779	0.476
		20	6.2818	
		>25	6.1341	
	Pb	10	1.5510	0.216
		20	1.7981	
		>25	1.7201	
	Zn	10	6.2752	0.627
		20	6.4173	
		>25	6.3368	

4.4.2 Removal vs density

The data results for this section are obtained from Experiment 2, in which the two-factor factorial experiment consists of density (number of density) and concentration. The results were discussed in terms of the different concentration level in sub-chapters below (Refer to Appendix 10).

4.4.2.1 Low concentration

Table 4.8 Heavy metal reduction in water (mg/l) at different number of vetiver (density) for low concentration

Heavy Metals/ No. of vetiver	Operation time (day)						Removal efficiency (%)				
	0	1	3	5	7	10	0 – 1	0 – 3	0 – 5	0 – 7	0 – 10
Cu: 25	**1.96**	**1.75**	**1.66**	**1.57**	**1.51**	**1.31**	10.71	15.31	19.90	22.96	33.16
50	**1.94**	**1.51**	**1.30**	**1.20**	**1.13**	**0.98**	22.16	32.99	38.14	41.75	49.33
100	**1.98**	**1.24**	**0.65**	**0.82**	**0.80**	**0.65**	37.37	66.97	58.79	59.60	67.07
Fe: 25	**0.83**	**0.69**	**0.52**	**0.36**	**0.14**	**0.13**	16.69	37.33	57.14	83.43	84.27
50	**0.79**	**0.54**	**0.28**	**0.19**	**0.16**	**0.13**	32.11	65.11	76.11	79.90	84.20
100	**0.82**	**0.33**	**0.12**	**0.15**	**0.14**	**0.12**	59.75	84.91	81.96	82.58	85.28
Mn: 25	**2.90**	**2.41**	**2.37**	**2.33**	**2.28**	**2.07**	16.88	18.13	19.50	21.12	28.50
50	**2.82**	**2.31**	**2.21**	**2.14**	**2.15**	**1.99**	18.28	21.63	24.08	23.75	29.30
100	**2.90**	**2.48**	**2.06**	**2.09**	**2.03**	**1.72**	14.25	28.93	28.01	29.90	40.52
Pb: 25	**0.68**	**0.59**	**0.46**	**0.48**	**0.33**	**0.34**	14.28	32.85	29.99	51.42	50.00
50	**0.63**	**0.45**	**0.33**	**0.24**	**0.32**	**0.21**	29.24	47.69	61.54	49.24	66.16
100	**0.70**	**0.32**	**0.20**	**0.30**	**0.21**	**0.17**	54.16	72.22	56.94	69.44	76.39
Zn: 25	**1.05**	**1.01**	**0.99**	**0.97**	**0.96**	**0.84**	3.50	5.50	7.90	9.03	20.35
50	**1.02**	**0.95**	**0.92**	**0.90**	**0.88**	**0.78**	6.52	9.37	12.14	13.17	23.24
100	**1.04**	**0.94**	**0.81**	**0.82**	**0.79**	**0.65**	10.22	22.61	21.23	24.08	38.06

Based on Figure 4.7 above, it can be seen that Fe had the highest removal efficiency up to 85.28%, followed up by Pb with 76.39% removal efficiency and Cu with 67.07% removal efficiency, regardless of their density. Zn had the lowest removal efficiency of 38.06% as well as Mn with 40.52%. In short, the removal efficiency can be ranked in this order: Fe>Pb>Cu>Mn>Zn. Fe has the highest removal efficiency in this experiment which is the same as reported in Roongtanakiat et al. (2007).

Theoretically, it is assumed that the removal rate of heavy metal would increase as the density (number of Vetiver tillers) increases due to higher surface area for metal absorption by the root into the plants and more plants to absorb the heavy metal from the synthetic mixture. From the figure above, it has been proven that there was an increasing trend in heavy metal removal as the density of VG increased, however, it still had to be verified via statistics to see its significance which will be discussed in the section 4.4.2.3.

Table 4.9 Compliance of data results in this experiment with MOH standard

Element	**Concentration, ppm**			**Percent reduction, %**	**Compliance with the standard**
	MOH Standard	Initial	Final (after 10 days)		
Cu	1	1.9	0.98	48.42	yes
Fe	1	0.81	0.13	83.95	yes
Mn	0.2	2.87	1.93	32.75	no
Pb	0.1	0.67	0.24	64.18	no
Zn	5	1.04	0.76	26.92	yes

From the table shown above, it has also shown the same results like in Experiment 1, whereby only Cu has met the requirement for MOH quality standard for raw untreated water. Although Fe and Zn also said to be complied with the standard, this is due to the initial concentration which is already lower than the standard requirement. It is suggested that the heavy metal has to be monitored for a longer time to get higher reduction.

4.4.2.2 High concentration

Table 4.10 Heavy metal reduction in water (mg/L) at different number of vetiver (density) for high concentration

Heavy Metals/ No. of vetiver	Operation time (day)						Removal efficiency (%)				
	0	1	3	5	7	10	0 – 1	0 – 3	0 – 5	0 – 7	0 – 10
Cu: 25	**10.75**	**9.48**	**9.37**	**9.36**	**9.40**	**8.60**	11.81	12.84	12.93	12.56	20.00
50	**9.40**	**8.47**	**8.33**	**8.30**	**8.24**	**7.40**	9.89	11.38	11.70	12.34	21.28
100	**10.25**	**8.51**	**8.58**	**8.84**	**8.87**	**7.86**	16.98	16.29	13.76	13.46	23.32
Fe: 25	**34.49**	**32.91**	**30.08**	**19.69**	**3.86**	**2.39**	4.58	12.79	42.91	88.81	93.08
50	**33.27**	**29.41**	**25.09**	**10.20**	**2.30**	**2.42**	11.60	24.60	69.36	93.10	92.73
100	**32.69**	**30.00**	**26.66**	**6.16**	**2.55**	**0.64**	8.24	18.45	81.17	92.20	98.06
Mn: 25	**7.03**	**6.48**	**6.45**	**6.49**	**6.53**	**6.18**	7.80	8.22	7.61	7.04	12.10
50	**6.81**	**5.99**	**6.08**	**6.17**	**6.24**	**5.91**	12.05	10.74	9.42	8.49	13.35
100	**6.89**	**6.23**	**6.40**	**6.54**	**6.69**	**6.17**	9.59	7.03	4.98	2.92	10.37
Pb: 25	**2.72**	**2.49**	**2.36**	**2.35**	**2.44**	**2.14**	8.60	13.26	13.62	10.39	21.51
50	**2.54**	**2.18**	**2.06**	**2.04**	**2.03**	**1.66**	14.23	18.85	19.62	20.00	34.61
100	**2.66**	**1.85**	**1.66**	**1.78**	**1.89**	**1.52**	30.40	37.73	33.33	28.94	42.86
Zn: 25	**6.98**	**6.95**	**6.72**	**6.74**	**6.82**	**5.71**	0.37	3.73	3.35	2.19	18.20
50	**6.48**	**6.30**	**6.33**	**6.33**	**6.51**	**8.01**	2.80	2.35	2.25	-0.48	-23.60
100	**6.82**	**6.69**	**6.80**	**6.60**	**6.99**	**6.38**	2.03	0.32	3.25	-2.45	6.58

In high concentration of synthetic mixture for Experiment 2, it has also exhibited that Fe also has the highest efficiency, which is similar to Roongtanakiat et al. (2007). The removal efficiency was as high as 98.06%, followed by Pb with 42.86% removal efficiency and Cu with 23.52% removal efficiency, regardless of their density. In this experiment, it has shown that Mn had the lowest removal efficiency of 13.35%, instead of Zn with 18.20% removal efficiency. In bried, the removal efficiency can be ranked in this order : Fe>Pb>Cu>Zn>Mn.

For high concentration, it seemed that the density did not affect the removal efficiency. This may be due to the extremely high heavy metal concentration that has obliged the plants to adapt to the heavy metal of different concentration. Based on Wuana and Okieimen (2011), the acclimatized plants should be adapted to the pollutant first then substituted for real experiment. However, it was directly being carried out in the experiment. All the treatments had the highest removal efficiency at 240 hours (Day 10).

Based on that Roongtanakiat (2007), the order was Fe>Mn>Zn>Cu>Pb in which Zn and Mn have the higher plant uptake as compared to this experiment. This could be due to the fact that excessive iron has reduced the zinc uptake and excessive zinc has reduced the manganese uptake (Malvi, 2011).

Table 4.11. Compliance of data results in this experiment with MOH standard

Element	**Concentration, ppm**			**Percent reduction, %**	**Compliance with the standard**
	MOH Standard	Initial	Final (after 10 days)		
Cu	1	10.13	7.95	21.52	no
Fe	1	33.48	1.82	94.56	no
Mn	0.2	6.91	6.09	11.87	no
Pb	0.1	2.64	1.77	32.95	no
Zn	5	6.76	6.7	0.89	no

Based on the table above, all the heavy metal element did not meet the standard requirement for raw untreated water quality. Although there are high percent reduction, especially for Fe, they failed to meet the standard as the initial concentration was very high to simulate the real condition in the river. It is predicted that they could meet the requirement if the experiment is monitored after a long time since most of the studies carried out in long terms have significance for their results such as in Ladislas et al. (2013) and Roongtanakiat (2007).

4.4.2.3 Statistical analysis for Experiment 2

Table 4.12 Comparison between trial experiment and real experiment

Heavy metal element	Removal efficiency (%)			
	Trial experiment (4 days)		Real experiment (10 days)	
	4 tillers	10 tillers	Low	High
Cu	51.03	72.2	49.9	21.5
Fe	89.1	99.2	84.6	94.6
Mn	28.8	20.15	32.8	11.9
Pb	74	78.1	64.2	33.0
Zn	76.6	75.69	27.2	8.3

From the table above, it can be seen that the heavy metal removal is not as efficient as the trial experiment, especially for Zn. The set-up in the open area could be the reason for the uncertainties as the trial experiment was carried out in the laboratory, whereas the real experiment was carried out outside the laboratory. According to Agunbiade et al. (2009) that the climatic conditions have controlled the efficiency of phytoremediation, hence it depends on factors which include metal dissolution in aqueous state for metal uptake into roots and interaction between the environment, metal and the plant. In this experiment, here were too many uncontrollable factors in terms of physical, meteorological and ecological, for example contribution by the rain or wind, sunlight which affected the heavy metal content, and biological organisms from microbes to large animals. Furthermore, Zhang et al. (2010) also asserted that plant age and seasonal variation could affect the plant uptake ability, in which younger plants could translocate heavy metal faster than that in older plants. Since the plants are of the same batch, this could be the reason why the removal efficiency has decreased compared to the ones in trial experiment.

A one-way between-groups multivariate analysis of variance (MANOVA) was performed to investigate density differences in heavy metal removal for Low concentration. Five dependent variables were used: Cu, Fe, Mn, Pb and Zn; whereas the dependent variables were density. Preliminary assumption testing was conducted to check for normality, linearity, univariate and multivariate outliers, and homogeneity of variance-covariance matrices, with no serious violations noted. There was a statistically significant difference between different densities on the combined dependent variables which are the heavy metal elements, $F (10, 22) = 2.904$, $p = 0.018$; Wilks' Lambda = 0.186; partial eta squared = 0.569. A separate ANOVA was conducted for each dependent variable, with each ANOVA evaluated at an alpha level of 0.01, using Bonferroni adjustment. Based on the Tests of Between-Subjects Effects table in Appendix 9, there was no significant difference between different densities (25 tillers, 50 tillers and 100 tillers) in the removal of each heavy metal although it can be observed that there is an increasing trend of removal efficiency when the density increased.

MANOVA was also performed for High concentration. There was a statistically significant difference between combined dependent variables which are the heavy metal elements, $F (10, 22) = 5.460$, $p < 0.005$; Wilks' Lambda = 0.082; partial eta squared = 0.713. A separate ANOVA was conducted for each dependent variable, with each ANOVA evaluated at an alpha level of 0.01, using Bonferroni adjustment. Based on the Tests of Between-Subjects Effects table in Appendix 10, there was no significant difference between different densities in the removal of each heavy metal.

Table 4.13 Mean and significant values for the MANOVA results for Experiment 2

Type of treatment	Heavy metal	Density, tillers	Mean	Significance between group
Low conc.	Cu	25	1.63	0.046
		50	1.34	
		100	1.02	
	Fe	25	0.45	0.592
		50	0.35	
		100	0.28	
	Mn	25	2.39	0.639
		50	2.27	
		100	2.21	
	Pb	25	0.48	0.243
		50	0.36	
		100	0.32	
	Zn	25	0.97	0.114
		50	0.91	
		100	0.84	
High conc.	Cu	25	9.49	0.044
		50	8.36	
		100	8.82	
	Fe	25	20.57	0.869
		50	17.12	
		100	16.45	
	Mn	25	6.53	0.142
		50	6.20	
		100	6.49	
	Pb	25	2.42	0.028
		50	2.09	
		100	1.89	
	Zn	25	6.65	0.973
		50	6.66	
		100	6.71	

4.5 Removal efficiency of heavy metal from synthetic mixture

4.5.1 Removal efficiency vs root length

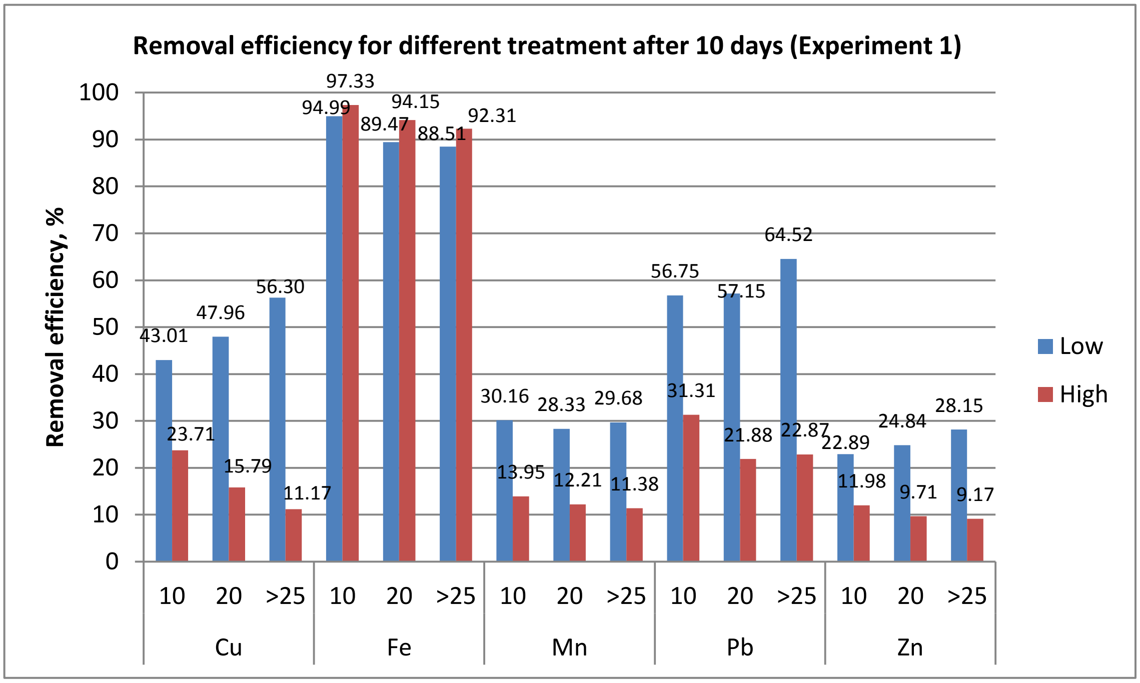

Figure 4.2 Removal efficiency for different treatment by root length after 10 days

From the bar chart above, the highest removal efficiency is reflected by Fe with a percentage of almost up to 95% and 98% for low and high concentration respectively. This suggests that Fe can be removed from water the most because Fe is one of the major element required for plant growth, along with the macronutrients which are N, P and K. Studies have shown that Fe and N has high correlation in regards to plant growth as Fe contributes in photosynthesis reaction, whereby N is also required for photosynthesis. However, the removal efficiency of Fe only increased drastically at the end of the experiment as Vetiver would use up other micronutrients such as Cu, Mn and Zn for they are enzyme activators. The removal efficiency for Experiment 1 can be ranked in this order: Fe>Pb>Cu>Mn>Zn for both low and high concentration.

An independent-samples t-test was also conducted to compare each the heavy metal removal efficiency in water for low and high concentration (Refer to Appendix 11). For Cu element, there was a statistically significant difference in removal efficiency for low concentration (M = 34.99, SD = 11.36) and high concentration (M = 12.43, SD = 5.37);

t(20) = 6.96, p < 0.005, two-tailed. The magnitude of the differences in the means (mean difference = 22.56, 95% CI: 15.92 to 29.21) was very large (eta squared = 0.63).

As for Fe element, there was no significant difference in removal efficiency for low concentration (M = 65.06, SD = 21.22) and high concentration (M = 46.06, SD = 41.41); t(21) = 1.582, p = 0.129, two-tailed. The magnitude of the differences in the means (mean difference = 19.00, 95% CI: -5.99 to 44.00) was moderate (eta suqared = 0.08).

For Mn element, there was a statistically significant difference in removal efficiency for low concentration (M = 22.98, SD = 4.41) and high concentration (M =10.06, SD = 1.84); t(19) = 10.46, p < 0.005, two-tailed. The magnitude of the differences in the means (mean difference = 1.24, 95% CI: 10.33 to 15.51) was very large (eta squared = 0.79).

For Pb element, there was a statistically significant difference in removal efficiency for low concentration (M = 47.71, SD = 10.88) and high concentration (M = 25.74, SD = 5.28); t(28) = 7.04, p < 0.005, two-tailed. The magnitude of the differences in the means (mean difference = 21.97, 95% CI: 15.57 to 28.37) was very large (eta squared = 0.64).

For Zn element, there was a statistically significant difference in removal efficiency for low concentration (M = 14.74, SD = 6.76) and high concentration (M = 4.91, SD = 3.68); t(28) = 4.94, p < 0.005, two-tailed. The magnitude of the differences in the means (mean difference = 9.83, 95% CI: 5.75 to13.90) was very large (eta squared = 0.47).

Table 4.15 T-test results for removal efficiency from water by different root lengths

Element	Significance	eta squared	Magnitude of difference
Cu	0.000	0.63	very large
Fe	0.129	0.08	moderate
Mn	0.000	0.79	very large
Pb	0.000	0.64	very large
Zn	0.000	0.47	very large

4.5.2 Removal efficiency vs density (no. of tillers)

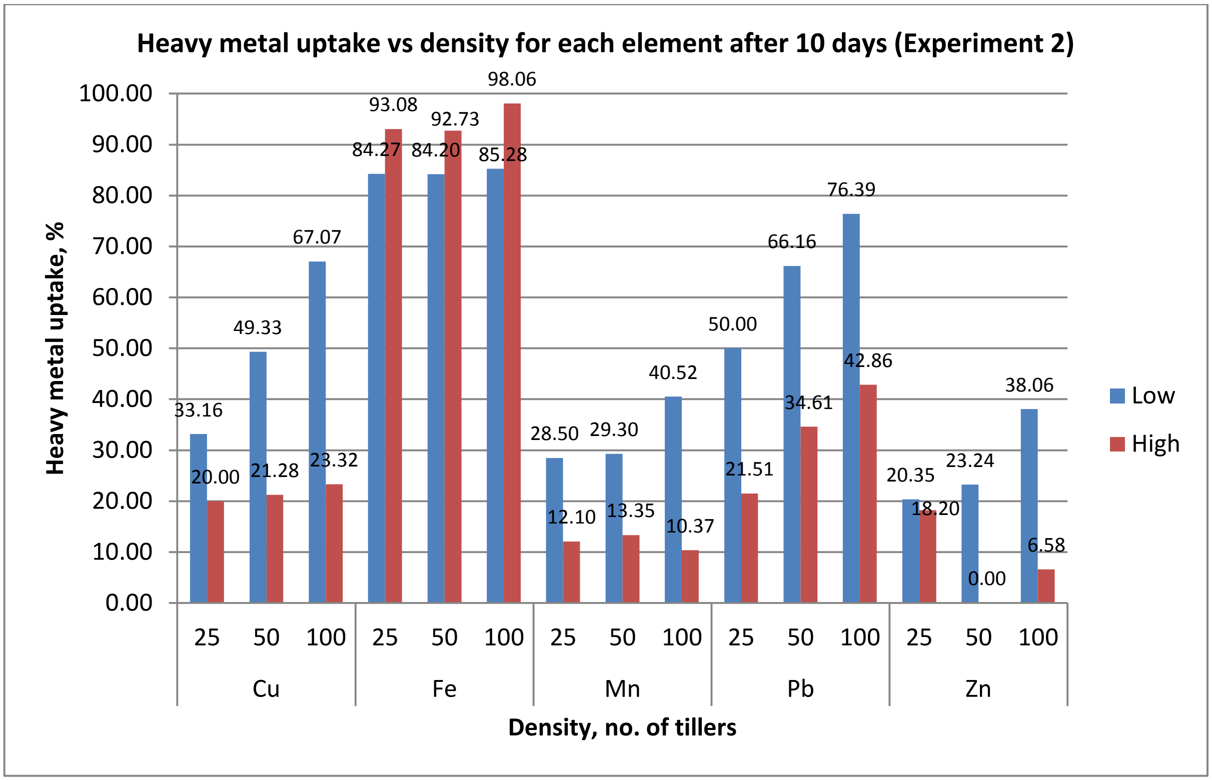

Figure 4.3 Removal efficiency for different treatment by density after 10 days

From the figure above, the highest removal efficiency for Experiment 2 is also reflected by Fe with a percentage of almost up to 93% and 98% for low and high concentration respectively. In short, the removal efficiency for Experiment 2 can be ranked in this order: Fe>Pb>Cu>Mn>Zn and Fe>Pb>Cu>Zn>Mn for low and high concentration respectively.

An independent-samples t-test was also conducted to compare each the heavy metal removal efficiency in water by different densities for low and high concentration (Appendix 12). For Cu element, there was a statistically significant difference in removal efficiency for low concentration ($M = 38.41$, $SD = 18.59$) and high concentration ($M = 14.70$, $SD = 4.00$); $t(15) = 4.831$, $p < 0.005$, two-tailed. The magnitude of the differences in the means (mean difference = 23.71, 95% CI: 13.27 to 34.16) was very large (eta squared = 0.71).

As for Fe element, there was no significant difference in removal efficiency for low concentration (M = 67.38, SD = 22.38) and high concentration (M = 55.45, SD = 38.17); t(23) = 1.045, p = 0.307, two-tailed. The magnitude of the differences in the means (mean difference = 11.42, 95% CI: -11.72 to 35.60) was small (eta suqared = 0.04).

For Mn element, there was a statistically significant difference in removal efficiency for low concentration (M = 24.18, SD = 6.79) and high concentration (M =8.78, SD = 2.77); t(19) = 8.146, p < 0.005, two-tailed. The magnitude of the differences in the means (mean difference = 15.40, 95% CI: 11.44 to 19.37) was very large (eta squared = 0.70).

For Pb element, there was a statistically significant difference in removal efficiency for low concentration (M = 50.77, SD = 17.79) and high concentration (M = 23.20, SD = 10.72); t(28) = 5.143, p < 0.005, two-tailed. The magnitude of the differences in the means (mean difference = 27.57, 95% CI: 16.59 to 38.56) was very large (eta squared = 0.49).

For Zn element, there was a statistically significant difference in removal efficiency for low concentration (M = 15.13, SD = 9.46) and high concentration (M = 3.16, SD = 4.54); t(20) = 4.42, p < 0.005, two-tailed. The magnitude of the differences in the means (mean difference = 11.97, 95% CI: 6.32 to17.62) was very large (eta squared = 0.41).

Table 4.17 T-test results for removal efficiency from water by different density

Element	Significance	eta squared	Magnitude of difference
Cu	0.000	0.71	very large
Fe	0.307	0.04	small
Mn	0.000	0.70	very large
Pb	0.000	0.49	very large
Zn	0.000	0.41	very large

4.6 Heavy metal uptake by plant parts (only for Experiment 1)

4.6.1 Heavy metal content in plant root

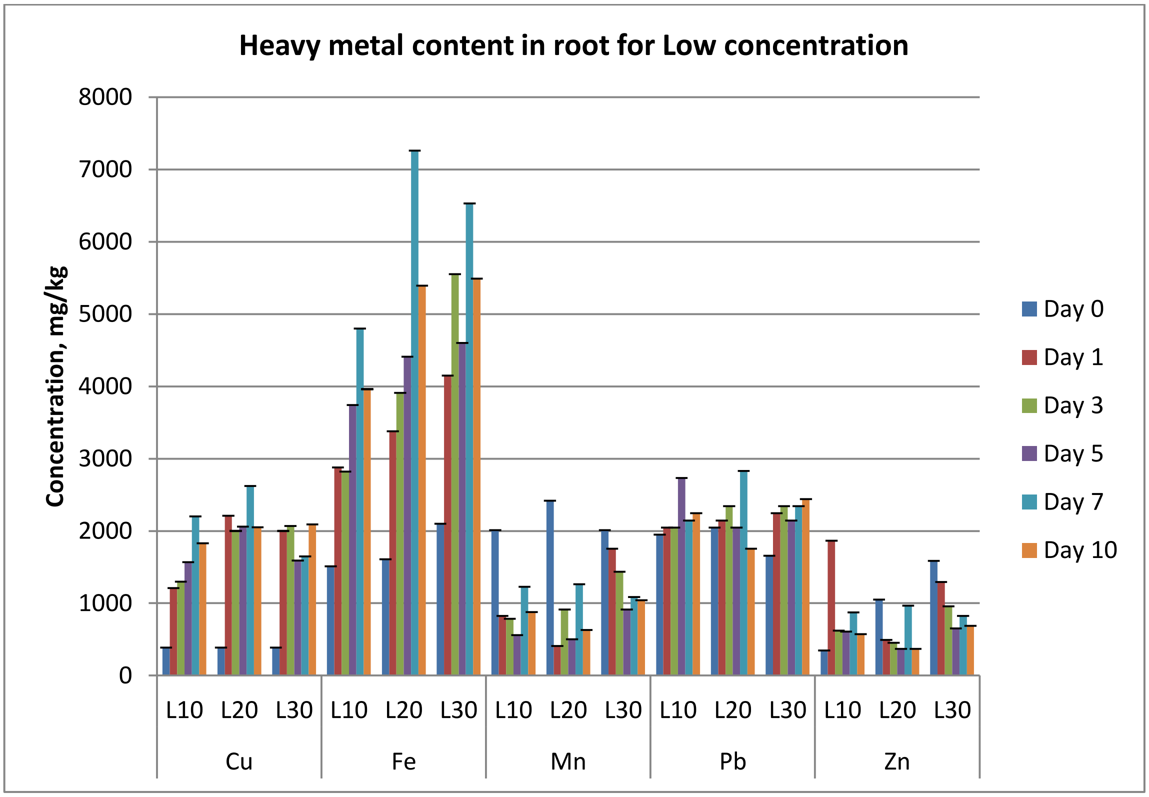

Figure 4.4 Heavy metal content in root for Low concentration in terms of dry weight basis. Error bars represent SDs; n=6.

In low concentration, Fe had the highest concentration of 7260 mg/kg in the root for all treatments at Day 7. Pb content in the root was the second highest with 2829 mg/kg, followed closely by Cu at 2620 mg/kg. Mn and Zn had the lowest content in the root among the 5 elements with 2420 mg/kg and 1586 mg/kg respectively. Overall, all elements had the highest content in the root at 168 hours (Day 7), except for Mn and Zn were highest at the early stage of the experiment. This suggested that plant absorbed most of the nutrients such as Mn and Zn first, as both Mn and Zn are responsible for enzyme activation for photosynthesis and growth respectively (McCauley, 2011; Bennett (1993), as cited in Khalil, 2011). In short, the heavy metal content in the root for low concentration can be ranked in this order: Fe>Pb>Cu>Mn>Zn, which has corresponded with the removal efficiency from the synthetic mixture.

The relationship between heavy metal removal from synthetic mixture and heavy metal content in the root was investigated using Pearson product-moment correlation coefficient (Refer to Appendix 13). All the elements had strong correlation between the variables, except Pb and Zn, in which the heavy metal removal from synthetic mixture corresponded with the heavy metal content in the root. However, there was no significant in correlation for Zn in water and Zn in plant root.

Table 4.18 Correlation coefficient of HM element in synthetic water and in root

	Cu root	Fe root	Mn root	Pb root	Zn root
Cu water	-0.745**				
Fe water		-0.828**			
Mn water			0.755**		
Pb water				-0.480*	
Zn water					0.371

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

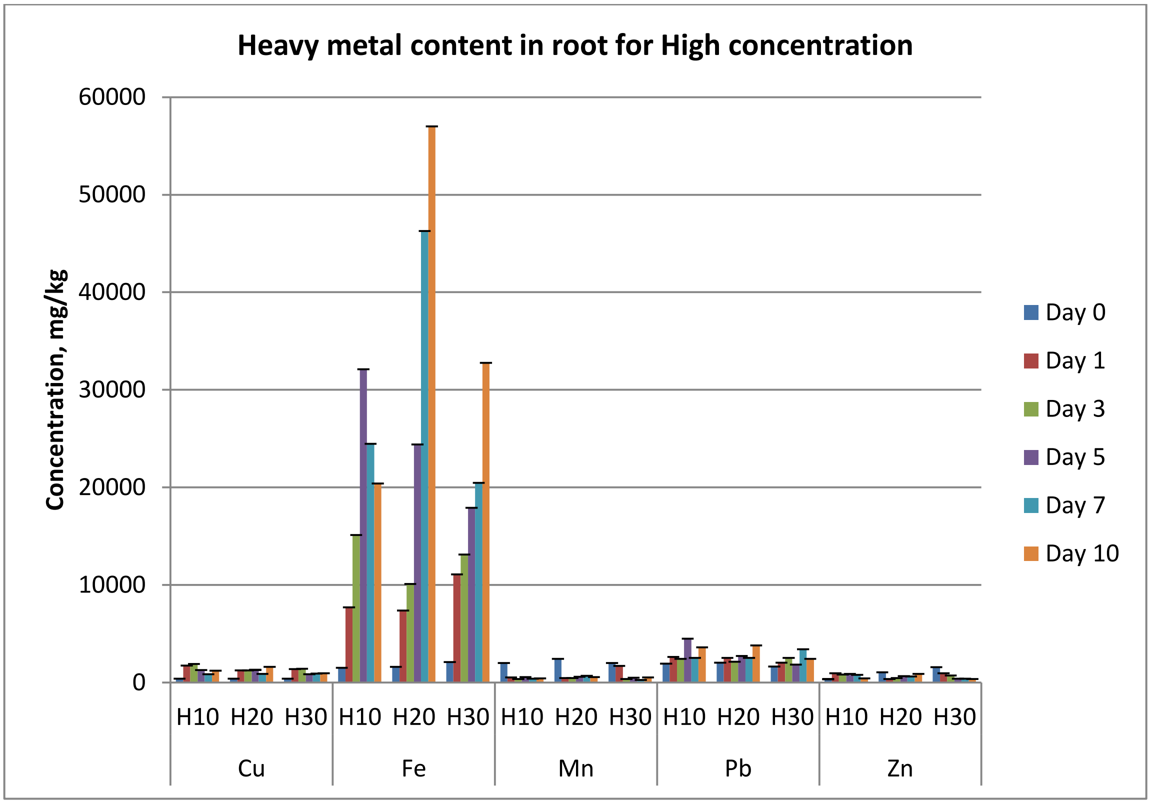

Figure 4.5 Heavy metal content in root for High concentration treatment in terms of dry weight basis. Error bars represent SDs, n = 6.

In high concentration, Fe had the highest concentration of 57000 mg/kg in the root for at 240 hours (Day 10). Pb content in the root was the second highest with 4487 mg/kg, followed by Mn at 2420 mg/kg and Cu at 1900 mg/kg. Zn had the lowest content in the root among the 5 elements with 1586 mg/kg. All elements had different concentration in the root for high concentration treatment. However, Fe still exhibited constant behavior, in which its content is normally high at the end of the experiment as the plant would use up other micronutrients first than Fe. This is because Fe is used for photosynthesis reactions but the plant required essential micronutrients for enzyme activation before photosynthesis can be taken place. In brief, the heavy metal content in the root for high concentration can be ranked in this order: Fe>Pb>Mn >Cu >Zn, which is different than that of removal efficiency from the synthetic mixture.

4.6.2 Heavy metal content in shoot

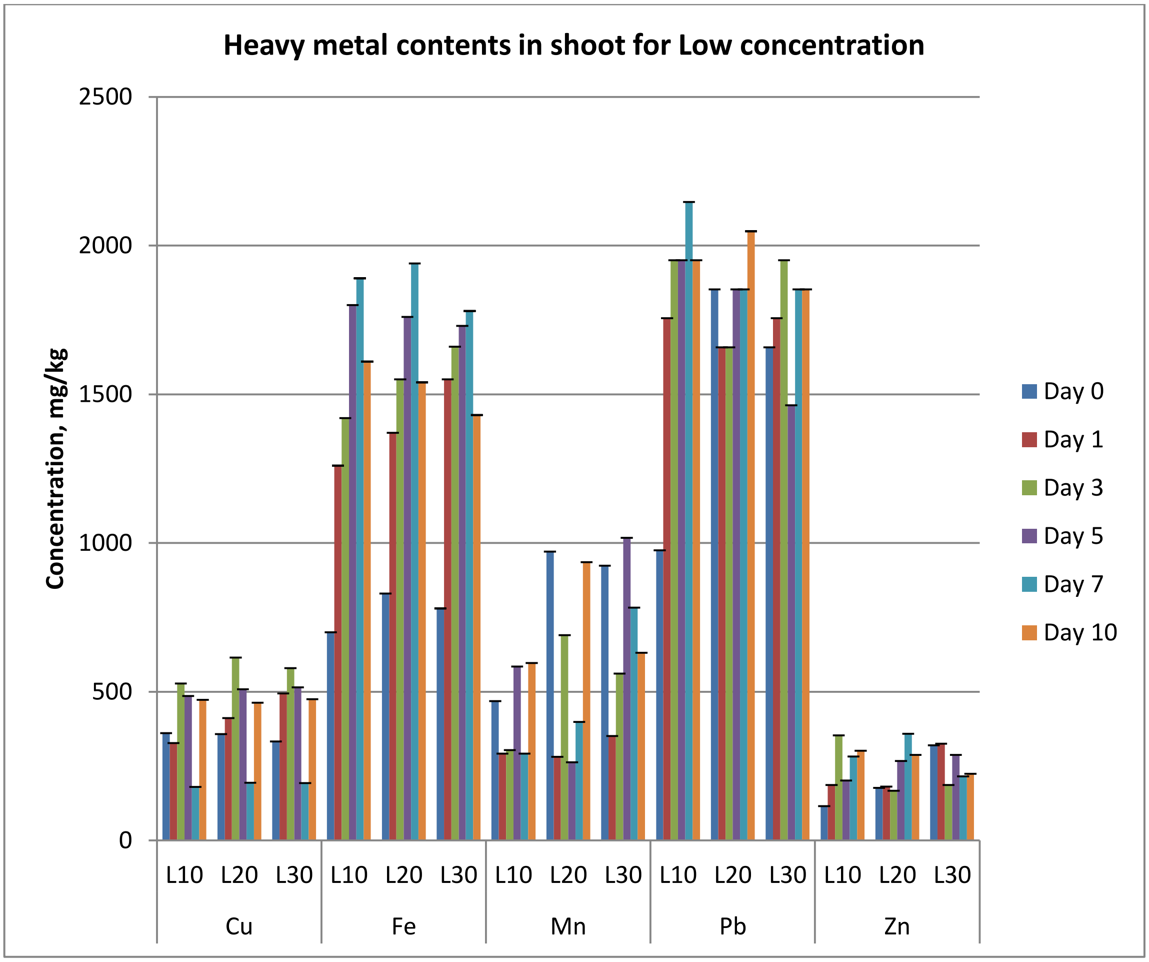

Figure 4.6 Heavy metal content in shoot for Low concentration treatment in terms of dry weight basis. Error bars represent SDs, n = 6.

In contrast, Pb has the highest concentration of 2146 mg/kg in the shoot at 168 hours (Day 7), instead of Fe with concentration of 1940 mg/kg. Not only that, Mn content was the third highest with 1017mg/kg, followed by Cu with 615 mg/kg and lastly Zn with 358mg/kg. This suggested that plant tends to translocate the heavy metal into the shoot, especially for Pb and Mn. The translocation factor will be further discussed in Section 4.9. In short, the heavy metal content in the shoot for low concentration can be ranked in this order: Pb>Fe>Mn>Cu> Zn.

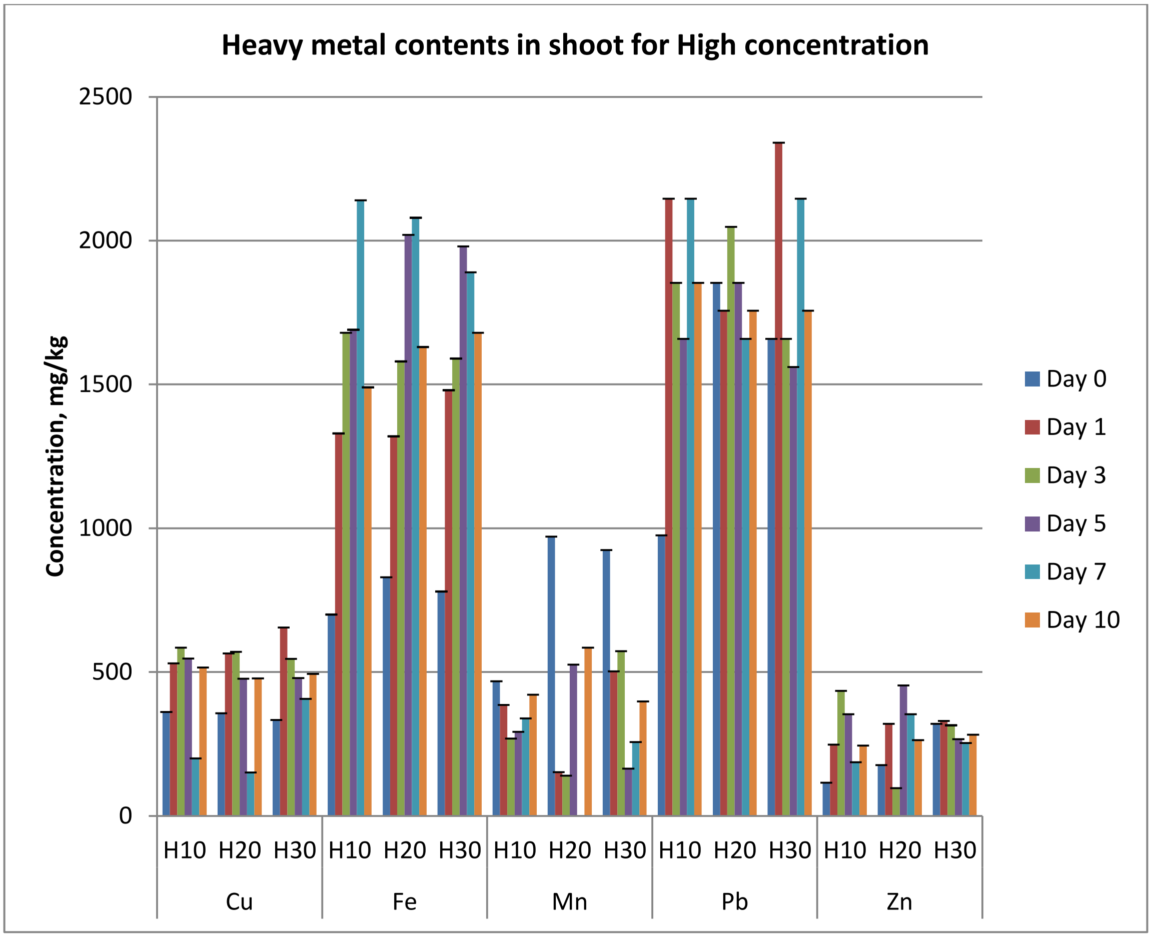

Figure 4.7 Heavy metal content in root for High concentration treatment in terms of dry weight basis. Error bars represent SDs, n = 6

As for high concentration, it has also displayed the same trend of heavy metal content in the shoot: Pb>Fe>Mn>Cu> Zn. Pb has the highest concentration of 2341 mg/kg in the shoot at 24 hours (Day 1), instead of Fe with concentration of 2140 mg/kg at 168 hours (Day 7). Not only that, Mn content was the third highest with 971mg/kg at 0 hour (Day 0), followed by Cu with 655 mg/kg and lastly Zn with 454 mg/kg at the early stage of the experiment. As Cu and Mn are enzyme component for photosynthesis (McCauley, 2011), this has further explained why these elements had higher contents in the shoot at the early stage of the experiment than that of Fe concentration.

Table 4.19 Highest concentration recorded in past literature and my experiment for hydroponic condition and requirement to be classified as hyperaccumulator

Heavy metal element	Concentration, mg/kg DW (Highest)				
	Hydroponic condition*		Real experiment		Hyperaccumulator**
	root	shoot	root	shoot	shoot
Cu	900	700	2620	655	≥ 1000
Fe	-	-	57000	2140	-
Mn	-	-	2420	1017	10000
Pb	≥ 10000	≥ 3350	4487	2341	≥1000
Zn	>10000	>10000	1586	454	10000

* Source from Anjum et al., 2012
** Unique characteristic to be classified as hyperaccumulator

According to Agunbiade (2009), the special characteristic of hyperaccumulators is that the shoot would accumulate at least 1000 mg/kg dry mass for As, Pb, Cu, Ni and Co. The Pb content in this experiment for both low and high concentration is more than 1000 mg/kg DW (Ladislas et al., 2013; Agunbiade et al, 2009), thus it can be concluded that Vetiver can be known as Pb hyperaccumulator, which corresponds to the statement in Danh et al. (2009).

4.6.3 Heavy metal accumulation in plant

Table 4.20 Heavy metal accumulation in plant (mg/kg after 10) days

Metals/ Treatment Condition	Metal accumulation in Root			Metal accumulation in Leaves			Whole plant accumulation		
	10cm	20cm	>25cm	10cm	20cm	>25cm	10cm	20cm	>25cm
Cu: Low conc.	1445	1663	1703	112	106	142	1557	1769	1845
High conc.	835	1233	576	155	121	161	990	1354	737
Fe: Low conc.	2450	3780	3390	910	710	650	3360	4490	4040
High conc.	18890	55390	30680	790	800	900	19680	56190	31580
Mn: Low conc.	*NA*	*NA*	*NA*	128	*NA*	*NA*	*NA*	*NA*	*NA*
High conc.	*NA*	*NA*	*NA*	NA	*NA*	*NA*	*NA*	*NA*	*NA*
Pb: Low conc.	293	*NA*	781	976	195	195	1269	*NA*	976
High conc.	1658	1756	781	878	NA	98	2536	1659	879
Zn: Low conc.	224	*NA*	*NA*	186	110	*NA*	410	*NA*	*NA*
High conc.	76	*NA*	*NA*	129	86	*NA*	205	*NA*	*NA*

Table 4.21 Heavy metal accumulation in plant after 10 days for low concentration (mg/kg)

Type of treatment	Cu			Fe			Mn			Pb			Zn		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Root	1445	1663	1703	2450	3780	3390	0	0	0	293	0	781	224	0	0
Shoot	112	106	142	910	710	650	128	0	0	976	195	195	186	110	0
Translocation (%)	7.19	5.99	7.69	28.8	15.8	16.09	-	-	-	76.9	100	19.98	45.4	-	-

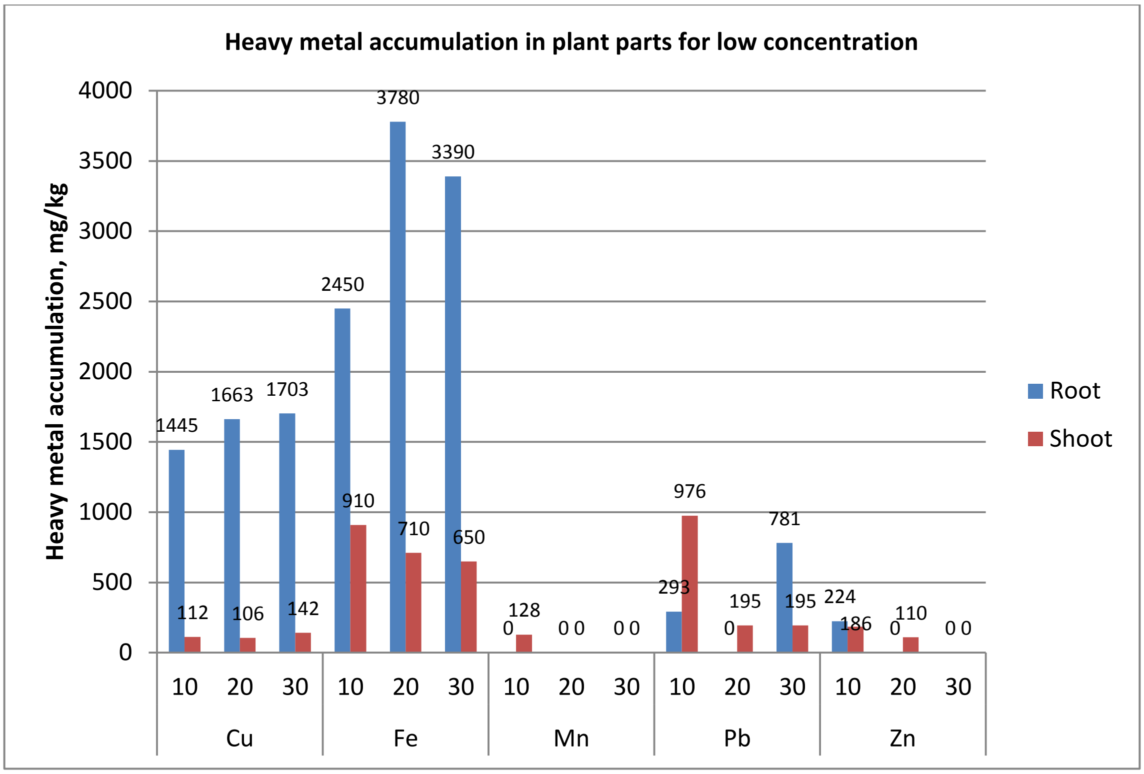

Figure 4.8 Heavy metal accumulation in plant parts for low concentration treatment after 10 days

From the figure above, it has clearly portrayed that the metal content in the root is higher than that in the shoot. However, there were a few exceptions for Mn and Pb element. It has suggested that Pb have the tendency to be translocated to the shoots, whereby it has been proven that a moderate proportion of Pb (33%) could be translocated (Truong, 2000). Not only that, 45.4% of Zn was translocated to the shoot, which is also the same as reported in Truong (2000). In short, it is suggested that Vetiver is more to a rhizofiltrator than a phytoextractor. However, it will be further discussed at the next section.

Furthermore, it can be seen that Mn and Zn have less or no accumulation in the shoot or root. This is because there was a significant Mn and Zn content in the control plants, which contained an average value of 2147.3 ± 236 mg/kg dw and 787.67 ± 278 mg/kg dw; and 995.33 ± 620 mg/kg dw and 204 ± 105 mg/kg dw in root and shoot respectively.

An independent-samples t-test was also conducted to compare each the heavy metal accumulation in plants by different part for low concentration (Refer to Appendix 15). All the elements have shown statistically significant difference in heavy metal content at different plant parts.

Table 4.22 T-tests results for heavy metal accumulation in different plant part after 10 days for Low concentration treatment

Element	Significance	eta squared	Magnitude of difference
Cu	$p < 0.0005$	0.64	very large
Fe	$p < 0.0005$	0.58	very large
Mn	0.001	0.30	very large
Pb	$p < 0.0005$	0.36	very large
Zn	$p < 0.0005$	0.47	very large

Table 4.23 Heavy metal accumulation in plant after 10 days for high concentration (mg/kg)

Type of treatment	Cu			Fe			Mn			Pb			Zn		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Root	835	1233	576	18890	55390	30680	0	0	0	1658	1756	781	76	0	0
Shoot	155	121	161	790	800	900	0	0	0	878	0	98	129	86	0

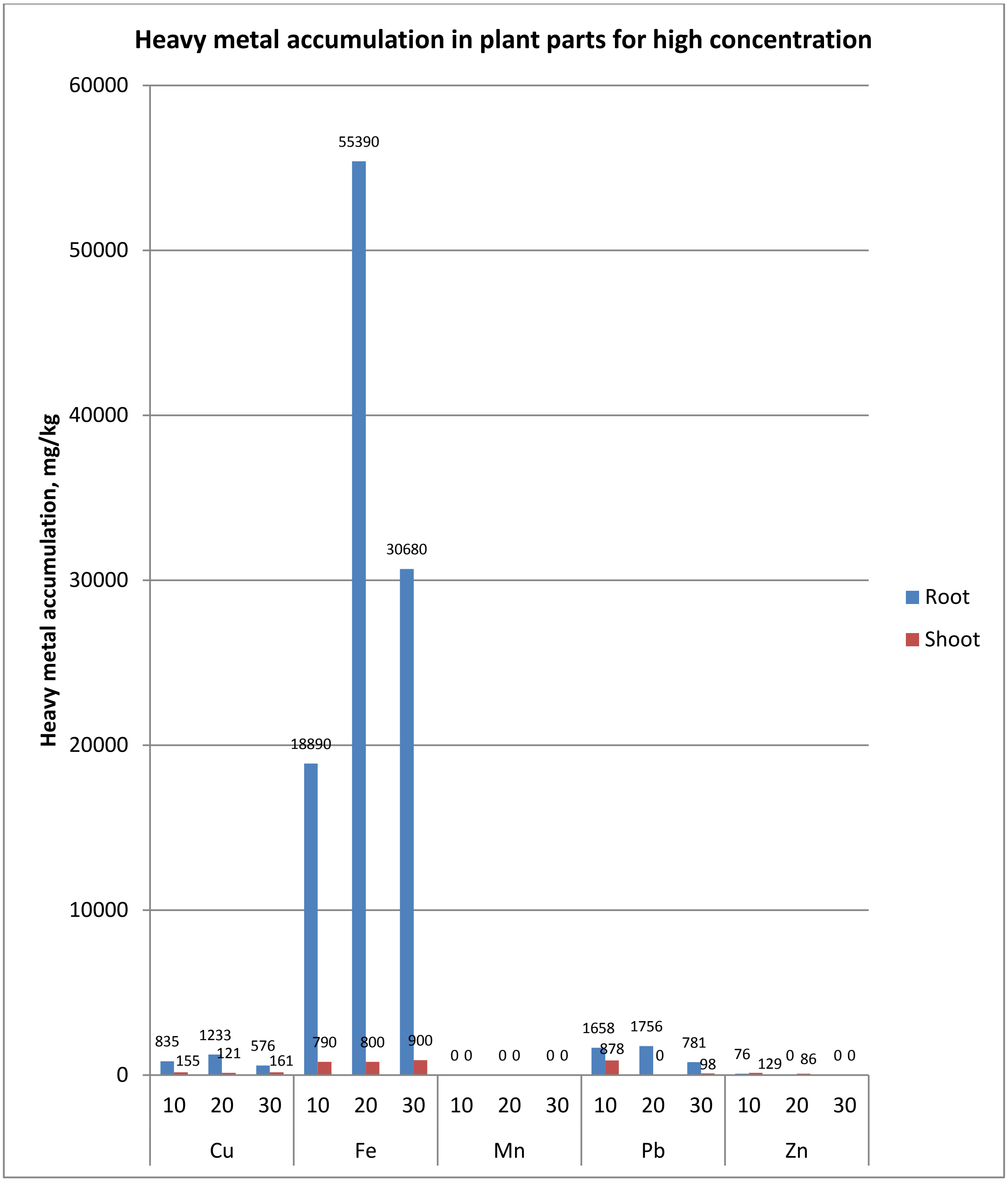

Figure 4.9 Heavy metal accumulation in plant parts in dry weight basis for High concentration

From the figure above, it has clearly portrayed that the metal content dominated in the root than in the shoot. As the synthetic mixture has a very high range of concentration, the plants have the ability to take up high amount of heavy metal contents in the roots. However, not much are being transported to the shoot. This suggested that the plants have higher potential to be rhizofiltrator than phytoextractor, same as the previous studies. However, it will be further discussed at the next section.

Furthermore, it can be seen that Mn and Zn have less or no accumulation in the shoot or root. This is because there was a significant Mn and Zn content in the control plants, which contained an average value of 2147.3 ± 236 mg/kg DW and 787.67 ± 278 mg/kg DW; and 995.33 ± 620 mg/kg DW and 204 ± 105 mg/kg DW in root and shoot respectively.

An independent-samples t-test was also conducted to compare each the heavy metal accumulation in plants by different part for high concentration (Refer to Appendix 15). All the elements have shown statistically significant difference in heavy metal content at different plant parts.

Table 4.24 T-tests for heavy metal accumulation in plant parts after 10 days for High concentration

Element	Significance	eta squared	Magnitude of difference
Cu	p < 0.0005	0.51	very large
Fe	p < 0.0005	0.41	very large
Mn	0.024	0.15	large
Pb	p < 0.0005	0.35	very large
Zn	p < 0.0005	0.46	very large

In both experiments, Mn has showed low concentration in plant uptake and removal from water. As Mn function is associated to redox processes, it plays a vital role in photosynthetic electron transport system. One of the reasons why Mn is low in the plant is the oxidation of Mn to precipitate as MnO_2 in the root (Pendias, 2010). Not only that, it also stated that there are findings about the antagonistic or synergistic effects of Mn on Pb uptake and Zn antagonistic effect on Mn uptake.

4.6.4 Statistical analysis for heavy metal concentration in plants

An independent-samples t-test was also conducted to compare each the heavy metal accumulation in plant roots for different concentration. All the elements have shown statistically significant difference in heavy metal content in plant root, except for Mn and Zn (Refer to Appendix 16).

Table 4.25 T-tests for heavy metal accumulation in plant root for different concentration

Element	Significance	eta squared	Magnitude of difference
Cu	0.008	0.21	large
Fe	0.001	0.34	very large
Mn	0.135	0.06	moderate
Pb	0.030	0.14	large
Zn	0.471	0.02	small

Another independent-samples t-test was also conducted to compare each the heavy metal accumulation in plant shoots for different concentration. The results have exhibited that all the elements had no significant difference in heavy metal content in plant shoot (Refer to Appendix 17).

Table 4.26 T-tests for heavy metal accumulation in plant shoots for different concentration

Element	Significance	eta squared	Magnitude of difference
Cu	0.365	0.02	small
Fe	0.597	0.01	small
Mn	0.063	0.10	moderate
Pb	0.774	0.002	very small
Zn	0.262	0.04	small

From these two analysis, it can be concluded that plant would take up heavy metal from synthetic mixture, which has different heavy metal level, at different amount and rate into the plant roots. However, the ability to translocate them into the shoot is not affected by the heavy metal level in the synthetic mixture.

4.7 Bioconcentration factor (BCF) [only for Experiment 1]

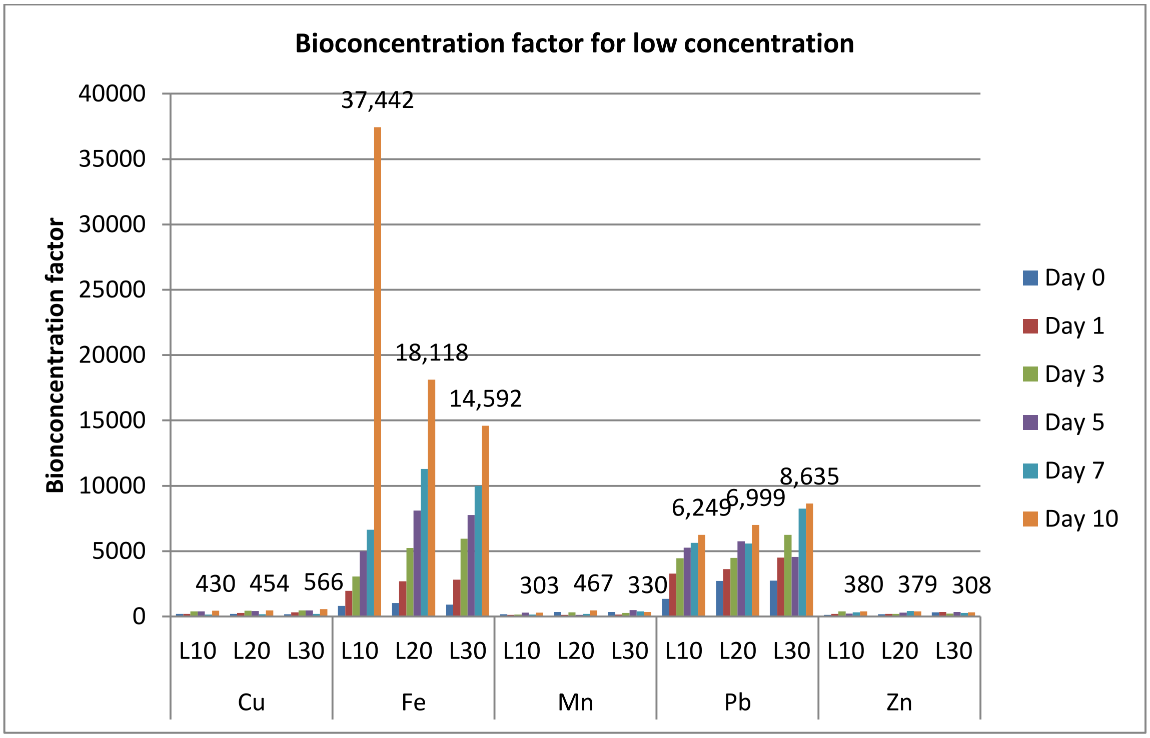

Figure 4.10 Bioconcentration factor (BCF) for Low concentration treatment

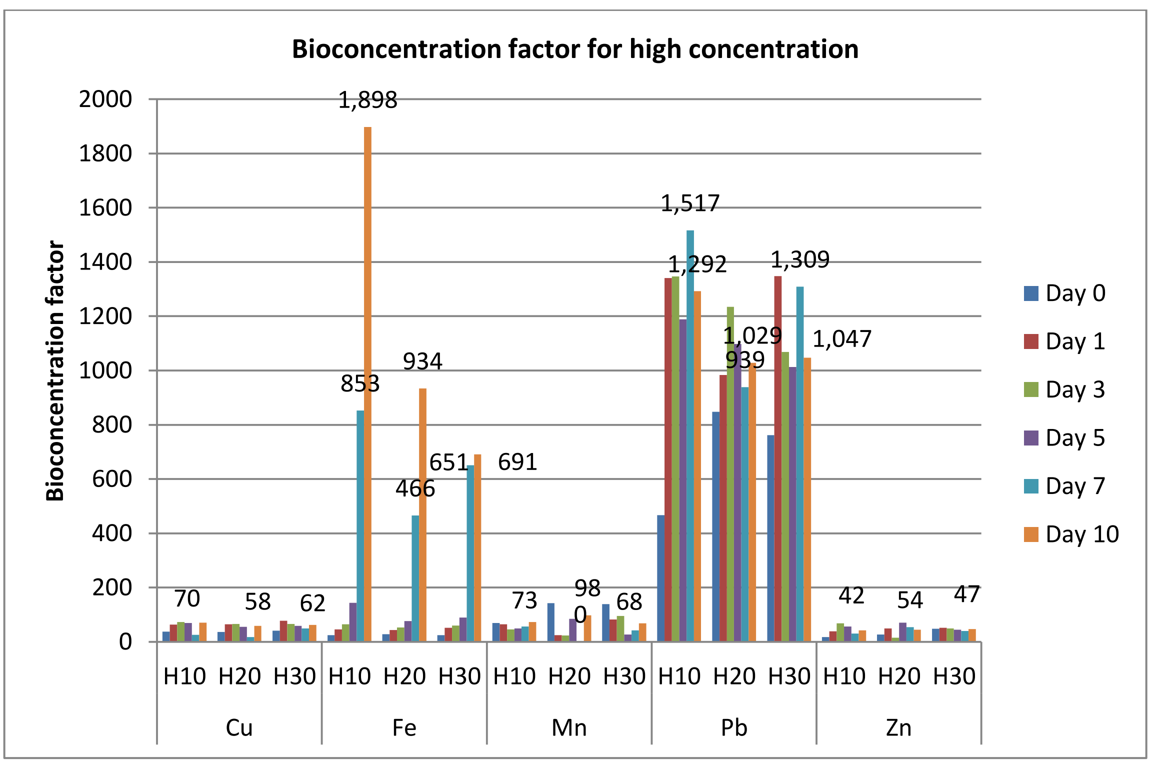

Figure 4.11 Bioconcentration factor (BCF) for High concentration treatment

According to Agunbiade et al. (2009), the enrichment factor > 1, which is represented by concentration in plant/habitat), is one of the feature of an accumulator. Enrichment factor is also known as bioconcentration factor (BCF). Based on Zhang et al. (2014), BCF>1 indicates the accumulation of heavy metals in the shoot. In the two figures above, it has clearly displayed that all the BCFs are more than 1 as the scale could go up to hundreds. Therefore, it can be concluded that Vetiver has the ability to translocate heavy metal in the shoot.

4.8 Translocation factor (TF) [only for Experiment 1]

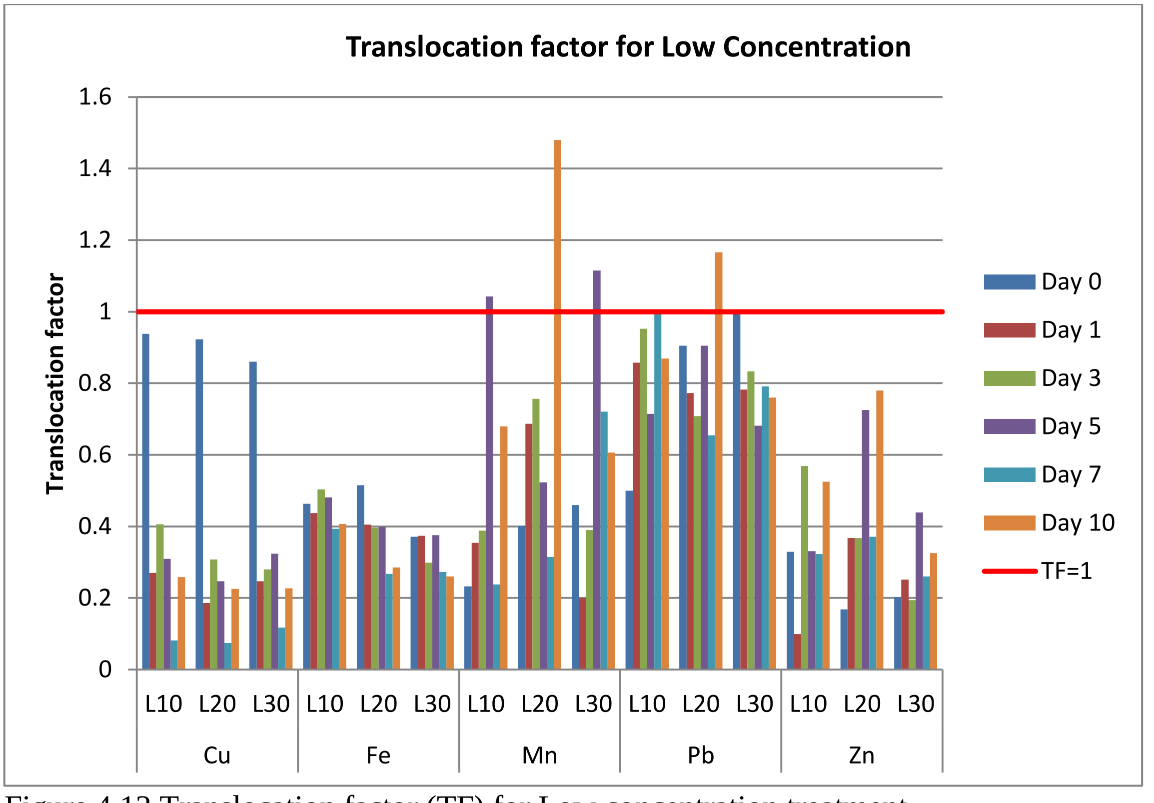

Figure 4.12 Translocation factor (TF) for Low concentration treatment

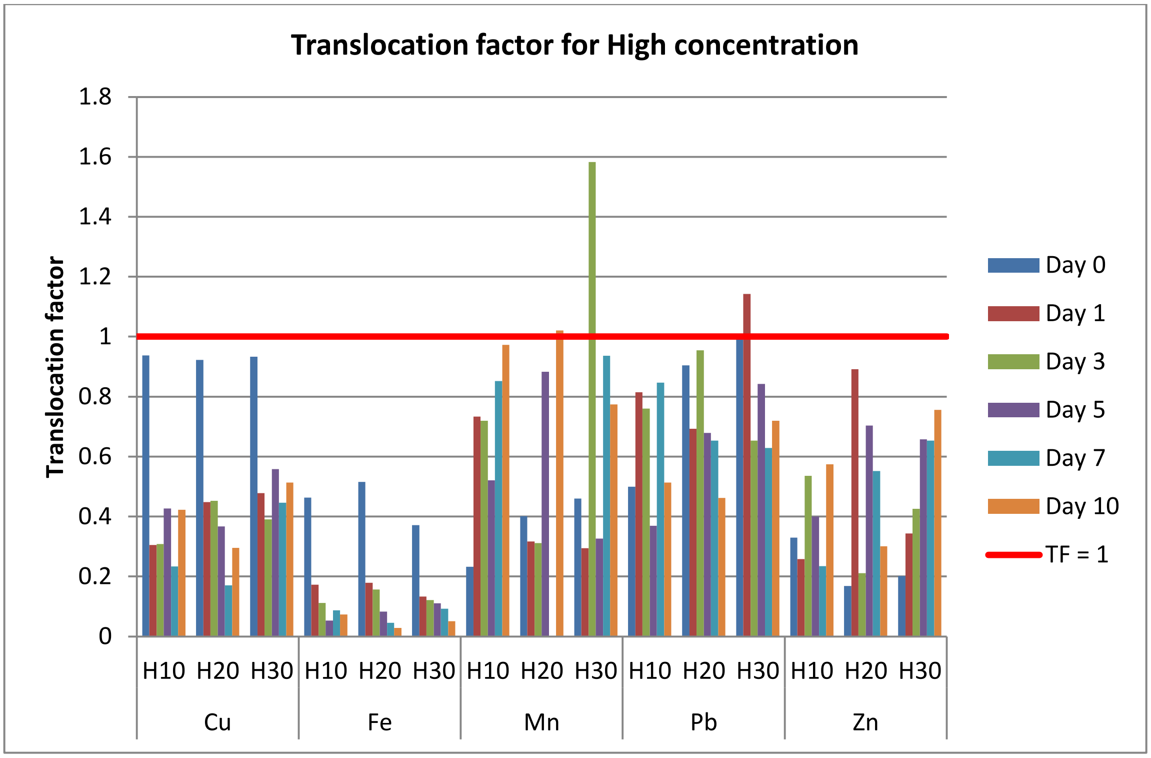

Figure 4.13 Translocation factor (TF) for High concentration treatment

According to Agunbiade et al. (2009) and Zhang et al., 2014), translocation factor (TF) >1 is also one of the features for an accumulator. Based on the two figures above, most of the TF are below 1. This indicates that Vetiver prefers to accumulate heavy metal in the root more than in the shoot, although the bioconcentration factor in the previous section has shown that Vetiver has the ability to translocate the heavy metal into the shoot. In contrast, there a few exceptions, whereby Mn and Pb have TF > 1 in both low and high concentration treatment. This means that Pb can accumulate Pb in the shoot as VG has high affinity towards lead (Danh et al., 2009), however, it is in contrast with Lai & Chen (2004) saying that vetiver cannot take up Pb in soil even at high concentration level of 1000 mg/kg in the soil. This suggests that vetiver would behave differently in terms of heavy metal uptake in different environmental condition.

The important findings of this experiment is that since not much heavy metal are translocated into the shoot, its shoots can be used as food for grazing animals or mulch, as reported in Anjum (2013) and Truong (2000).

CHAPTER 5

SUMMARY OF FINDINGS, CONCLUSIONS, PROJECT LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 Summary of findings

This project is carried out in order to explore and understand more in-depth about the use of phytoremediation technique in water by using Vetiver grass (VG). The objectives are (1) to assess the efficiency of phytoremediation technique by using VG in water, (2) to determine the rate of pollutant uptake by VG in varying pollutant concentrations, root length and density of VG, and (3) to evaluate the removal efficiency of heavy metal uptake by VG. This project is believed to have successfully met the objectives stated in this project.

5.2 Conclusions

For plant cultivation; it is better to grow them at these conditions:(1) avoid strong UV rays/ preferably sheltered with sufficient amount of sunlight, (2) acidic condition below 5 to prevent growth or invasion of fungus and other organisms, (3) provide aeration to enhance the growth, (4) avoid using fertilizer that causes the pH to be alkaline, (5) high electrical conductivity ranging from 2mS/cm to 8 mS/cm, and (6) trimming to enhance new growth.

During the experiment, the toxicity symptoms of the Vetiver grass (VG) towards the treatment for Experiment 1 have been observed. VG is said to have high adaptability to polluted environment as all the plants still looked green and healthy up to 3 days. However, the symptoms started to show up, either due to malnutrition (Controls) or over-nourishment (LCT and HCT), such as chlorosis, browning of leaves due to prolonged exposure and then wilting as a sign of necrosis. In a nutshell, none of the plants died during the experiment even if wilting was present as it only occurred in a very small amount.

The order of heavy metal removal efficiency from water for both high and low concentration in Experiment 1 are: LCT [Fe(90.48) >Pb(53.73) >Cu(48.97) >Mn(29.24) >Zn(25.49)]; HCT [Fe(94.52) >Pb(25.12) >Cu(17.11) >Mn(12.48) >Zn(10.29)]. As for Experiment 2, the order was also the same as in Experiment 1: LCT [Fe(83.95) >Pb(64.18) >Cu(48.42) >Mn(32.75) >Zn(26.92)] and HCT [Fe(98.06) >Pb(42.86) >Cu(23.52) >Mn(13.35) >Zn(18.20)]. This order has corresponded to the heavy metal accumulation in the root for Low Concentration Treatment (LCT), but not for High Concentration Treatment (HCT) of Experiment 1 due to the inconsistent uptake of Cu and Mn by the plants. The order of heavy metal accumulation by roots in mg/kg DW basis are as follows: LCT [Fe(7260) >Pb(2829) >Cu(2620) >Mn(2420) >Zn(1586)]; HCT [Fe(57000) >Pb(4487) >Mn(2420) >Cu(1900) >Zn(1586)]. The order of heavy metal accumulation by shoots in DW basis for both low and high concentration are as follows: LCT [Pb(2146) >Fe(1940) >Mn(1017) >Cu(615) > Zn(358)]; HCT [Pb(2341) >Fe(2140) >Mn(971) >Cu(655) > Zn(454)]. It is suspected to be the antagonistic or synergistic effect of between Fe and Zn, Mn and Pb and Mn and Zn, which has affected the uptake. Not only that, the difference in habitat (soil or water) would affect the bioavailability of the heavy metal.

Throughout these experiments, it has been found that there were no significance in removal efficiency by different root length and Vetiver density. This might be due to the low interval between the root lengths, hence there are obvious significance when the experiment is done for only 10 days. As compared to the trial experiment, the removal efficiency is ranked in this order: Fe>Pb>Zn>Cu>Mn, which is different from the real experiment, the possible reasons could be due to the climatic condition, seasonal variation and the plant age. It is an unexpected findings that the heavy metal in the HCT did not meet the quality standard set by MOH for the potable water. It is the same goes to LCT, except for Cu. Although there were high removal efficiencies in some heavy element, the residual concentration has not met the MOH water quality standard as the experiment is carried out for only 10 days.

From this experiment, Vetiver grass is best to known as rhizofiltrator instead of phyto-extractor due to its ability to accumulate heavy metal in the roots than in the shoot although all the data results have shown BCF>1 but TF<1 as well. In contrast, there is a finding, whereby Vetiver is a Pb hyper-accumulator as most of the heavy metal concentrated in the shoot. Although VG is not a hyper-accumulator except for Pb, VG is still a good accumulator for phytoremediation. Not only that, this experiment also shows that Vetiver grass behave differently in heavy metal uptake, depending on the type of environment (soil or water) and physical condition (pH, salinity, electrical conductivity, etc.).

5.3 Project Limitations

The report would present information that is as accurate and valid as possible. However, there are several potential limitations that would affect the accuracy and precision of some information in the report includes:

- The time allocated to complete the entire research project to be carried out was 8 months (March to October) but the plants only arrived in the middle of June. Hence, the project is delayed and only left 5 months to do the project.

- As the project has been delayed, the interval for harvesting was also shortened. This could be the reason why there were no significance in doing this experiment.

- The plants were supposed to arrive with the root lengths requested (15cm, 45cm and 60cm) earlier on during the plant ordering but they all arrived with root lengths of 12cm so they have to be cultivated once again to get the specified root lengths for the experiment.

- In order to get the root length specified for the experiment, a lot of time has been wasted in growing the plants. Almost 5 months is spent in plant cultivation, however the longest root obtained was only up to 35cm. Hence, the experimental design has to be altered slightly. It was suggested that it could also be the reason why there were no significance in the experimental results as their root length interval was too small.

- The plants are cultivated in water as part of plant acclimatization and due to absence of suitable land for cultivation, thus the plant growth would be slower as compared to planting in the soil.

- The synthetic mixture prepared was done by using dilution of minerals salts. Due to this method, the synthetic mixture obtained did not reach the specified concentration. This may be due to calculation error or the large volume of synthetic mixture which has contributed to more errors. However, the concentration for each element could be obtained for trial experiment.

- Since the budget provided was limited, the experimental set-up at the open area, which is difficult to control as the surroundings are biodiversity-rich environment, could be reason of some data uncertainties as it could be affected by rain water or other contributors such as the air pollutants and other organisms.

- The researcher’s main area of study in the discipline of Environmental Science and Technology is not adequate to analyse certain aspects of the research subject such as the plant cultivation, the study of the plant growth and the handling of the plants as well as experimental preparation and analysis of the samples.

5.4 Recommendations for Future Research

In order to improve the limitations faced in this project, in which the data results did not turn out as expected, there are several recommendations to be suggested in regards of future research. It might be a good opportunity in order to help explore more on the pollutant uptake mechanism in terms of toxicity, tolerance towards heavy metals and accumulation of heavy metals in the plants. The recommendations are listed as shown below:

- The projects should be carried out in a longer time length as the time required for plant cultivation as well as plant acclimatization would take a very long time, especially the plant cultivation which would take up to at least 6 months.
- The plant cultivation should be done in soil as VG grow faster in the soil than water; whereas the plant acclimatization in water can be done for a week time up to a month before the experiment. If there is no land to plant, the soil attached to plants should not be washed out during plant cultivation in water.
- It is suggested to use higher interval of root length (15cm or even up to 30cm) for the experiment, maybe it would produce a more significant results as the interval for this experiment is only up to 10cm, which has not much different.
- It is suggested to increase the harvesting time interval as plants need time to grow and adapt to the synthetic mixture condition. Harvesting at a longer interval of time may produce better results than that in this project.
- For synthetic mixture preparation, it is better to use standard solution to obtain the specified concentration as the concentration using metal salts dilution might be difficult to control, especially the ones found in this experiment although the trial experiment shows a good results. It is suggested that the larger volume has incurred more errors in preparing the solution.
- It is better to use metals salts in the form of nitrates as other salt forms like sulphates, chlorides and others might cause the physical or chemical reaction between the specified heavy metal element in the experiment with the salts.

- Not only that, the method used for acid digestion has to be validated as it is modified from Hanidza et al. (2011) although the results obtained from the experiment has acceptable RSD values as well as constant expected results.

- The time length of the experiment should be longer to see the effective of treatment by the Vetiver grass in order to remove heavy metal from the water to meet the MOH quality standard for raw untreated water.

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APPENDICES
Appendix 1

Review on heavy metal concentration in the river

No.	Type of water body	Country	State/Agency		Heavy metal concentration, ppm						Reference
					Cu	Fe	Mn	Pb	Zn	Cr	
Standard		Malaysian Water quality standard	Interim National Water Quality Standard (2008)	Class I	Natural levels/absent						
				Class II	0.02	1	0.1	0.05	5	0.05	
				Class III	-	1	0.1	0.05	0.4 (at hardness 50mg/L CaCO3)		
				Class IV	0.2	1	0.2	5	2	0.1	
				Class V	-						
			MOH (2009)	Raw untreated water quality	1	1	0.2	0.1	5	0.05	
				Potable water	1	0.3	0.1	0.05	5	0.05	
		USA & world	USEPA (2008) & WHO (2009)	Potable water	1	0.3	0.05	0.05	5	0.05	Ashraf, Maah & Yusoff, 2012
		Malaysia	Sewage & Industrial Effluent	Standard A	0.2	1	0.2	0.1	1	0.2	
				Standard B	1	5	1	0.5	1	1	
1	River	Malaysia	Penang Juru river	sampling	0.005	-	-	0.001	0.052	-	Idriss & Ahmad, 2013
2			FPAS river		NA	0.12	0.05	0.01	0.01	-	
3			TPU river A		NA	0.13	0.02	0.01	0.01	-	
4			TPU river B		NA	0.12	0.08	0.01	0.03	-	
5			Selangor	Langat river	0.02-0.04			0.05-0.07	0.09-0.24	-	Sarmani, 1989
6				Semenyih river	0.00733	0.489	0.0598	0.00249	0.04919	0.00546	Al-Badaii & Othman, 2014
8			Sarawak	Serin river	0.094	**1.208**	**-**	**0.253**	0.33	**0.12**	Ling et al., 2012
9			Sabah (Cu mine discharge)	Mamut river	0.017	0.022	**0.472**	0.005	0.045	0.003	Ali et al., 2004
10				Kipungit river	0.006	0.005	0.001	0.002	0.009	0.0005	
11				Mamut river	**1.02**	-	0.013	0.016	0.207	0.002	Lee & Stuebing, 1990
12				Langanan River	**0.3**	-	0.021	0.019	0.173	0.006	
13				Tabin Wildlife	**0.414**	-	0.01	0.019	0.102	0.007	

				Reserve							
14				Sunsuron Ridge of the Crocker Range National	**0.127**	-	0.004	0.002	0.244	0.011	
15			Perak	Park	-	NA	-	0.019	-	-	Orji et al., 2013
16				Sungai Kinta	-	NA	-	0.037	-	-	
17			Terengganu	Kemaman river	0.004	0.51	-	0.0003	0.036	0.002	Shazili et al., 2006
18			Kedah	Sg Kerian	-	**3.179**	0.004	-	0.048	-	Ibrahim et al., 2015
19		Vietnam	Hanoi To Lich river		0.0045	-	**0.2162**	0.0081	0.0511	0.0029	Thuong et al., 2015
20		China	Songhua river		0.00386	-	-	-	0.02541	0.00122	Li et al., 2012
21			Taihu basin		0.00132	-	-	0.00375	0.09636	0.00314	Bian et al., 2015
22		Iran	East Azerbaijan Province	Stream water	0.0283	-	-	0.0019	0.0212	-	Moore, Esmaelli, Keshavarzi, 2011
23		Czech Republic	Bilina river	surface water	-	-	-	0.00792	0.03526	-	Kohušová et al. 2011
24	Pond	France	Nantes	Stormwater ponds	-	-	-	-	0.06-0.075	-	Ladislas et al., 2015
25		Egypt	Nile river		**0.039**	0.872	**0.436**	**0.057**	0.097	**0.056**	Bouraie et al., 2010
26		China	Beijing	Golf course pond	0.1	-	-	0.018	0.25	0.035	Puyang, Gao & Han, 2015
27	Lake	Malaysia	Miri	Curtin lake	0.00695	**1.608-1.946.83**	0.0136	0.004	0.009	-	Prasanna et al., 2012
28		China	Chaohu lake		0.00155	-	-	-	0.00756	0.00073	Li et al., 2012
29			DongTing lake		0.00456	-	-	-	0.0121	0.00173	
30			XuanWu lake		-	-	-	-	0.007	-	
31		Greece	Doirani lake		0.001-0.013	-	-	-	0.006-0.066	0.001-0.017	
32			Megali Prespa		0.002-0.005	-	-	-	0.002-0.012	0.001-0.019	
33		Turkey	Hazar lake		BDL	-	-	-	0.038-0.071	-	
34			Ataturk dam lake		0.025-0.220	-	-	-	0.064-0.197	NA	
35		USA	Texoma lake		0.011-0.104	-	-	-	0.012-0.246	0.002-0.008	
36		South Africa	Zeekoevlei (surface water)		BDL	-	-	-	0.009-0.012	BDL	

37		Hungary	Balaton lake		0.0002-0.0006	-	-	-	0.00022-0.0019	-	
38		India	Bhopal lake		0.02	-	-	**0.087**	-	**0.087**	
39		Macedonia	Ohrid lake		**0.07**	**1.91**	**0.25**	-	0.45	-	Aliu et al,2011
40	Wetland	China	Hengshuihu wetland		0.00054 - 0.00092	-	-	-	BDL	BDL	Li et al., 2012
41			Zhalong Wetland		0.00024	-	-	-	0.00052	0.00109	Zhang, Zang & Sun, 2014
42	Coastal area / Sea	Malaysia	Johor strait		-	-	-	**0.43-2.25**	0.34-0.75	-	Hadibarata et al., 2012
43			Selangor	Port Klang	0.005	-	-	0.007	0.088	0.007	Sany et al., 2004
44				Kelang estuary	0.004	-	-	0.002	0.004	-	Shazili et al., 2006
45				Port Dickson	0.002	-	-	0.005	0.006	-	
46			Terengganu	Kemaman coast	0.00076	0.035	-	0.0003	0.029	0.002	
47		Egypt	Suez canal		-	-	-	**0.95**	0.13	-	Sharaf & Shehata, 2015
48	Mining area	China	Guangzhou	Lechang Pb/Zn mine	0.35	-	**3.69**	**0.46**	**58.9**	-	Shu, 2003
49		Malaysia	Perak	ex-mining pools	-	0.14	-	**0.075**	-	-	Orji et al., 2013
50			Pahang	Lipis gold mine effluent	-	-	-	-	0.11	-	Bakar et al., 2013
51				AMD gold mine	0.01	**1.26**	**0.59**	0.02	0.01	-	Abu Bakar et al, 2015
52				Lombong Barit	**11.06**	**7.14**	**3.3**	**0.45**	1.58	-	Hatar et al., 2013
53				Sg. Lembing	**9.19**	**36.31**	**7.17**	0.13	**6.56**	-	
54			Terengganu	Lubuk Mandi (active gold mine)	0.17	**2.15**	**1.12**	0.01	**1.12**	-	
55			Sabah (Cu mine discharge)		2.0 - 47.0	0.1 - 7.1	2.7 - 79.8	-	-	-	
56				Mamut river	**14.26**	**2.68**	**23.55**	-	**6.4**	-	Jopony & Tongkul, 2009
57	Leachate	Malaysia		Taman Beringin	0.041	**4.78**	**0.27**	0.07	0.17	0.03	Atta et al., 2015

Note: Concentration above standard water quality ***(numbers in bold)***

Appendix 2

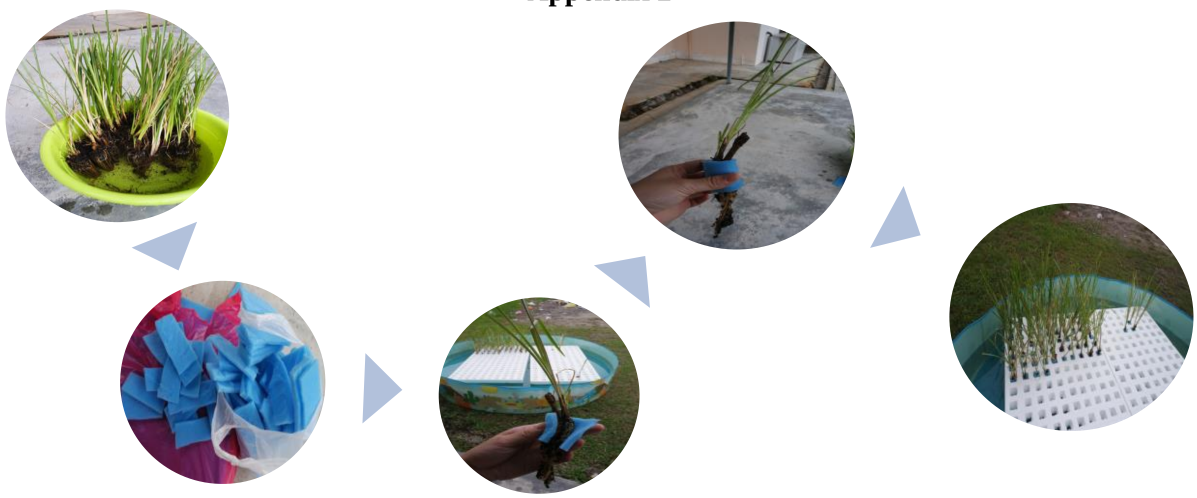

Figure 1: How to set up the plant cultivation in water

Figure 2: Abnormal growth of VG: reddish spots and browning on the leaves tips; fungus-like white growth; parasite-like organism (left to right above); white parasite growing on the stem (left picture)

New set-up for the plan cultivation under shelter

Figure 3: Plants cultivated under shelter in pool S, M, L respectively

Comparison between young and mature plant

Figure 4: : Young plant with only 14 cm root after a month (left), mature plant with 23 cm root (right)

Mini-experiment (Isolation of plants into different container)

Figure 5: Plants grown in S,M, L pools respectively (top left to right); plants isolated from pools with same fertilizer under shelter and different fertilizer under shelter (left pictures)

Appendix 3

Setting high and low concentration

Option 1

Element	Concentration, ppm		River levels	Reference
	Low	High		
Cu	5	15	9.19	[1]Hatar et al., 2013 AIP Conference Proceedings 1571, 641 (2013); doi: 10.1063/1.4858727
Fe	20	60	36.31	
Mn	4	12	7.17	
Pb	0.5	1.5	0.13	
Zn	3.5	10.5	6.56	
	Appox 50% of river level	**Appox 150% of river level**	Sungai Lembing, Pahang	

Using one river as reference (the worst case scenario)

Option 2

Element	Concentration, ppm		River levels	Reference
	Low	High		
Cu	5	15	9.19	[1]Hatar et al., 2013 AIP Conference Proceedings 1571, 641 (2013); doi: 10.1063/1.4858727
Fe	20	60	36.31	
Mn	4	12	7.17	
Pb	1.5	4.5	2.25	[2]Hadibarata et al., 2012
Zn	3.5	10.5	6.56	[1]
	Appox 50% of river level	**Appox 150% of river level**	Sungai Lembing, Pahang	

All use one reference (worst case scenario), except Pb as it has the highest Pb concentration from my read-ups.

Option 3

Element	Low Concentration			High Concentration		
	Reported	Suggested	Reference	Reported	Suggestion	Reference
Cu	1.7519	2.0		9.19	10	[1]
Fe	3.876	**2.0**		36.31	40	
Mn	2.1614	2.5		7.17	8	
Pb	0.04589	0.5		2.25	2.5	[2]
Zn	0.6889	1.0		6.56	7	[1]
	Average of the river concentration from my read-ups			The worst case scenario		

For low concentration, I have averaged all the river concentration all over Malaysia to get the low concentration. Is it relevant to do so?

For the high concentration, I used the one in Option 2.

Option 4

Element	Low Concentration			High Concentration		
	Reported	Suggested	Reference	Reported	Suggestion	Reference
Cu	2.0	2.0	[1]	9.19	10	[1]
Fe	1.946	2.0	Prasanna et al., 2012	36.31	40	
Mn	0.59	1.0	Abu Bakar et al, 2015	7.17	8	
Pb	0.253	0.5	Ling et al., 2012 World Applied Sciences Journal 16 (4): 550-559, 2012	2.25	2.5	[2]
Zn	0.34	0.5	[2]	6.56	7	[1]
	Low but still above the standard, except Pb or Zn			The worst case scenario (mining area)		

For the low concentration, I chose all the concentration that is not low but lower than the Malaysian standard. Is it relevant?

For the high concentration, I used the one in Option 2 again.

Element	Low Concentration		High Concentration	
	Reported	Suggested	Reported	Suggested
Cu	1.7519	2.0	9.19	10
Fe	3.876	**2.0**	36.31	40
Mn	2.1614	2.5	7.17	8
Pb	0.04589	0.5	2.25	2.5
Zn	0.6889	1.0	6.56	7
	Average of the river concentration from my read-ups		The worst case scenario	

Appendix 4

Results for trial experiment

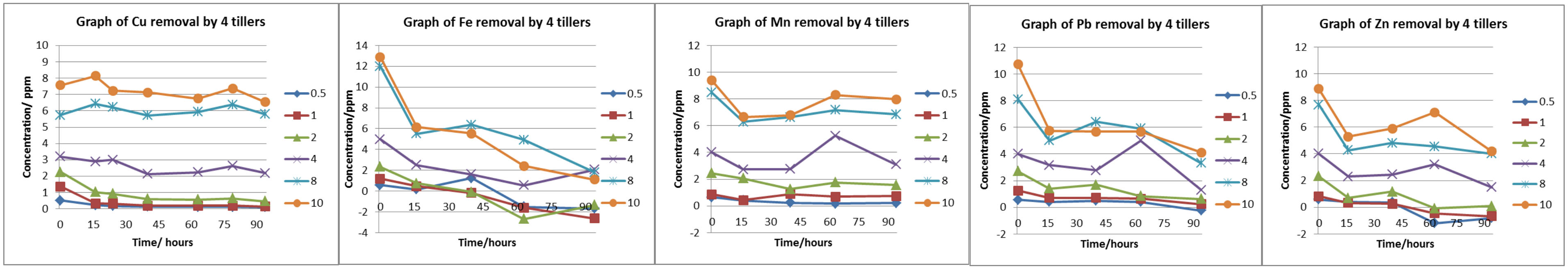

Figure 1: Heavy metal removal (Cu, Fe, Mn, Pb, Zn) by 4 tillers of Vetiver grass in 0.5ppm, 1ppm, 2ppm, 4ppm, 8ppm, 10ppm respectively

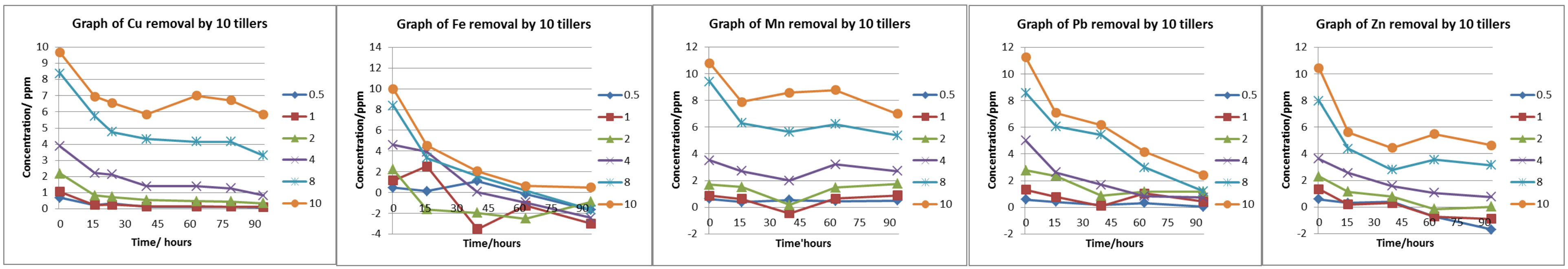

Figure 2: Heavy metal removal (Cu, Fe, Mn, Pb, Zn) by 10 tillers of Vetiver grass in 0.5ppm, 1ppm, 2ppm, 4ppm, 8ppm, 10ppm respective*ly*

Table 2: Removal rate in various concentration with different plant densities in 4 days

Density of tillers	**Metal**	**Removal rate (%) in 4 days in various concentration (ppm)**					
		0.5	**1.0**	**2.0**	**4.0**	**8.0**	**10.0**
4 tillers	Copper (Cu)	79.52	90.07	79.97	31.99	10.92	13.7
	Iron (Fe)	388.58	321.53	157.80	58.43	84.95	91.46
	Manganese (Mn)	65.79	14.05	36.3	22.55	19.06	14.92
	Lead (Pb)	142.42	81.08	77.07	67.64	59.02	61.74
	Zinc (Zn)	235.66	182.61	96.52	62.5	47.69	52.92
10 tillers	Copper (Cu)	84.20	88.24	84.08	78.62	60.62	39.73
	Iron (Fe)	467.14	358.02	140.09	153.20	119.70	95.01
	Manganese (Mn)	20.21	-1.51	-3.51	22.83	42.81	35.11
	Lead (Pb)	91.45	66.67	57.33	85.36	85.84	78.38
	Zinc (Zn)	388.71	165.66	99.21	78.77	60.69	15.41

Note: Assume all the data having >100% removal rate as 100% removal by Vetiver grass

Appendix 5

Experiment 1: The water concentration after treated with different root length at both low and high concentration treatment containing 5 heavy metal elements

Type of heavy metal	Root length, cm	Type of treatment	Operation time (day)					
			0	1	3	5	7	10
Cu		Control B (x plant)						
		Control	0.05 ± 1.2E-4	0.05 ± 3E-3	0.03 ± 5E-3	0.03 ± 1.3E-3	0.05 ± 1.1E-3	0.07 ± 1.8E-3
		Low	1.92 ± 8.5E-3	1.94 ± 0.01	1.92 ± 0.01	1.92 ± 0.01	1.93 ± 0.01	1.93 ± 0.01
		High	9.19 ± 0.03	9.08 ± 0.03	9.08 ± 0.04	9.07 ± 0.01	9.09 ± 1.2E-3	9.01 ± 0.03
	10cm	Control A	0.02 ± 8E-4	0.05 ± 5E-4	0.03 ± 2E-3	0.03 ± 4E-3	0.06 ± 1.2E-3	0.05 ± 1E-3
		Low conc.	1.93 ± 3.7E-3	1.62 ± 4E-3	1.40 ± 3.8E-3	1.28 ± 3.6E-3	1.26 ± 9.1E-3	1.10 ± 0.01
		High conc.	9.70 ± 0.01	8.32 ± 0.01	8.02 ± 0.01	7.92 ± 0.04	7.87 ± 0.02	7.40 ± 0.01
	20cm	Control A	0.01 ± 6E-4	0.04 ± 2.1E-3	0.01 ± 6.7E-4	0.02 ± 1.5E-3	0.05 ± 1.1E-3	0.05 ± 2.8E-3
		Low conc.	1.96 ± 0.01	1.58 ± 0.01	1.37 ± 4E-3	1.25 ± 4E-3	1.2 ± 0.01	1.02 ± 0.01
		High conc.	9.75 ± 3.4E-3	8.84 ± 0.02	8.77 ± 0.01	8.73 ± 0.05	8.78 ± 0.03	8.21± 0.03
	>25cm	Control A	0.02 ± 2E-3	0.05 ± 1.9E-3	0.03 ± 3.2E-3	0.03 ± 2.6E-3	0.06 ± 1.7E-3	0.06 ± 1.8E-3
		Low conc.	1.92 ± 0.01	1.54 ± 3.2E-3	1.28 ± 0.01	1.14 ± 2.1E-3	1.01 ± 3.8E-3	0.84 ± 1.3E-3
		High conc.	8.95 ± 0.02	8.53 ± 0.02	8.28 ± 0.03	8.19 ± 0.04	8.37 ± 0.04	7.95 ± 0.02
Fe		Control B (x plant)						
		Control	0.03 ± 3.8E-3	0.12 ± 0.01	0.12 ± 4E-3	0.16 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
		Low	0.80 ± 0.01	0.86 ± 0.01	0.87 ± 0.01	0.87 ± 0.01	0.86 ± 0.01	0.89 ± 0.01
		High	29.19 ± 0.08	32.41 ± 0.08	31.33 ± 0.08	32.46 ± 0.08	30.25 ± 0.06	30.09 ± 0.03
	10cm	Control A						
			0.08 ± 0.01	0.13 ± 0.01	0.20 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.16 ± 0.02
		Low conc.	0.86 ± 0.02	0.65 ± 0.01	0.47 ± 0.01	0.36 ± 0.01	0.29 ± 0.01	0.04 ± 0.01
		High conc.	29.44 ± 0.07	28.98 ± 0.06	25.98 ± 0.03	11.74 ± 0.03	2.51 ± 0.02	0.79 ± 0.01
	20cm	Control A	0.07 ± 0.01	0.13 ± 0.01	0.12 ± 0.02	0.13 ± 0.02	0.18 ± 0.02	0.12 ± 0.01
		Low conc.	0.81 ± 0.02	0.51 ± 0.01	0.30 ± 0.02	0.22 ± 0.02	0.17 ± 0.01	0.09 ± 0.01
		High conc.	29.85 ± 0.05	30.41 ± 0.09	29.98 ± 0.04	26.63 ± 0.04	4.47 ± 0.02	1.75 ± 0.02
	>25cm	Control A	0.05 ± 0.02	0.09 ± 0.02	0.10 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.04 ± 0.01
		Low conc.	0.85 ± 0.01	0.55 ± 0.01	0.28 ± 0.01	0.22 ± 0.01	0.18 ± 1E-3	0.10 ± 0.01
		High conc.	31.59 ± 0.04	28.73 ± 0.08	26.42 ± 0.08	22.08 ± 0.03	2.91 ± 0.01	2.43 ± 0.02
Mn		Control B (x plant)						
		Control	0.05 ± 0.01	0.15 ± 3E-3	0.14 ± 7E-4	0.14 ± 0.00	0.01 ± 0.00	0.03 ± 2E-4
		Low	2.80 ± 3.2E-3	2.48 ± 2E-4	2.44 ± 2E-3	2.45 ± 2E-3	2.45 ± 7E-4	2.51 ± 2E-3
		High	6.79 ± 0.01	6.21 ± 4E-3	6.12 ± 2E-3	6.18 ± 2E-3	6.63 ± 5E-3	6.19 ± 3E-3
	10cm	Control A	BDL	0.02 ± 8E-4	0.01 ± 8E-4	0.00 ± 0.00	0.03 ± 7E-4	0.02 ± 4E-4
		Low conc.	2.81 ± 4E-3	2.30 ± 2E-3	2.16 ± 2E-3	2.09 ± 2E-3	2.15 ± 2E-3	1.97 ± 2E-3
		High conc.	6.74 ± 8E-3	6.02 ± 5E-3	5.94 ± 5E-3	5.97 ± 4E-3	6.00 ± 3E-3	5.80 ± 2E-3
	20cm	Control A	0.07 ± 1E-3	0.00 ± 0.00	0.04 ± 1E-3	0.05 ± 6E-4	0.10 ± 1E-3	0.05 ± 1E-3

		Low conc.	2.80 ± 3.2E-3	2.35 ± 7E-4	2.18 ± 4E-3	2.14 ± 2E-3	2.17 ± 2E-3	2.00 ± 1E-3
		High conc.	6.81 ± 0.01	6.20 ± 2E-3	6.20 ± 5E-3	6.27 ± 6E-3	6.24 ± 3E-3	5.98 ± 5E-3
	>25cm	Control A	0.03 ± 5E-4	0.04 ± 2E-3	0.05 ± 5E-4	0.08 ± 7E-4	0.07 ± 2E-3	0.10 ± 6E-4
		Low conc.	2.72 ± 3E-3	2.30 ± 2E-3	2.17 ± 2E-3	2.12 ± 2E-3	2.06 ± 6E-4	1.91 ± 2E-3
		High conc.	6.64 ± 7E-3	6.09 ± 7E-3	6.05 ± 4E-4	6.03 ± 6E-3	6.11 ± 5E-3	5.88 ± 0.00
Pb		Control B (x plant)						
		Control	0.22 ± 4E-4	0.14 ± 3E-4	0.18 ± 2E-4	0.19 ± 2E-4	0.26 ± 2E-4	0.16 ± 2E-4
		Low	0.63 ± 3E-4	0.65 ± 2E-4	0.60 ± 1E-4	0.68 ± 6E-4	0.62 ± 1E-4	0.63 ± 2E-4
		High	2.24 ± 4E-4	2.24 ± 1E-4	2.21 ± 3E-4	2.30 ± 5E-4	2.26 ± 1E-4	2.21 ± 1E-4
	10cm	Control A	0.23 ± 2E-4	0.19 ± 2E-4	0.16 ± 2E-4	0.18 ± 4E-4	0.18 ± 2E-4	0.19 ± 2E-4
		Low conc.	0.72 ± 2E-4	0.54 ± 1E-4	0.44 ± 0.00	0.37 ± 2E-4	0.38 ± 1E-4	0.31 ± 2E-4
		High conc.	2.09 ± 4E-4	1.60 ± 4E-4	1.38 ± 3E-4	1.40 ± 1E-3	1.41 ± 4E-4	1.43 ± 2E-4
	20cm	Control A	0.03 ± 5E-4	0.04 ± 2E-3	0.05 ± 5E-4	0.08 ± 7E-4	0.07 ± 2E-3	0.10 ± 6E-4
		Low conc.	2.72 ± 3E-3	2.30 ± 2E-3	2.17 ± 2E-3	2.12 ± 2E-3	2.06 ± 6E-4	1.91 ± 2E-3
		High conc.	6.64 ± 7E-3	6.09 ± 7E-3	6.05 ± 4E-4	6.03 ± 6E-3	6.11 ± 5E-3	5.88 ± 0.00
	>25cm	Control A	0.20 ± 1E-4	0.21 ± 3E-4	0.17 ± 3E-4	0.12 ± 7E-3	0.18 ± 2E-4	0.16 ± 2E-4
		Low conc.	0.61 ± 3E-4	0.39 ± 2E-4	0.31 ± 3E-4	0.32 ± 2E-4	0.22 ± 0.00	0.22 ± 1E-4
		High conc.	2.18 ± 1E-4	1.74 ± 2E-4	1.55 ± 0.00	1.54 ± 3E-4	1.64 ± 2E-4	1.68 ± 4E-4
Zn		Control B (x plant)						
		Control	0.01 ± 9E-4	0.01 ± 1E-3	0.01 ± 4E-4	0.02 ± 7E-4	0.01 ± 7E-4	0.03 ± 9E-4
		Low	1.03 ± 2E-3	1.03 ± 5E-4	1.03 ± 2E-3	1.03 ± 1E-3	1.04 ± 2E-3	1.01 ± 9E-4
		High	6.62 ± 0.01	7.09 ± 0.01	6.43 ± 0.01	6.60 ± 0.02	6.50 ± 0.01	6.67 ± 0.02
	10cm	Control A	2E-3 ± 1E-4	0.03 ± 3E-4	0.04 ± 3E-4	0.03 ± 4E-4	0.05 ± 7E-4	0.06 ± 1E-3
		Low conc.	1.03 ± 1E-3	0.95 ± 8E-4	0.91 ± 6E-4	0.89 ± 6E-4	0.85 ± 2E-3	0.76 ± 5E-3
		High conc.	6.64 ± 0.01	6.37 ± 8E-3	6.41 ± 6E-3	6.27 ± 0.01	6.11 ± 7E-3	5.84 ± 0.02
	20cm	Control A	0.08 ± 6E-4	0.02 ± 2E-3	0.0 ± 5E-4	0.03 ± 5E-4	0.04 ± 2E-4	0.06 ± 4E-4
		Low conc.	1.01 ± 1E-3	0.95 ± 1E-3	0.91 ± 3E-3	0.89 ± 6E-4	0.85 ± 2E-3	0.76 ± 5E-3
		High conc.	6.56 ± 0.01	6.47 ± 0.01	6.53 ± 8E-3	6.49 ± 0.01	6.53 ± 0.02	5.93 ± 0.02
	>25cm	Control A	0.01 ± 2E-4	0.03 ± 4E-3	0.04 ± 1E-4	0.05 ± 6E-4	0.05 ± 8E-4	0.04 ± 1E-3
		Low conc.	1.01 ± 1E-3	0.95 ± 1E-3	0.89 ± 2E-3	0.85 ± 2E-3	0.80 ± 9E-4	0.73 ± 4E-3
		High conc.	6.64 ± 2E-3	6.44 ± 5E-3	6.40 ± 0.01	6.07 ± 8E-3	6.44 ± 9E-3	6.03 ± 0.02

Note:` Control A consists of plant treatment with distilled water; Control B has no plants
BDL *below detection limit*
Mean ± SD

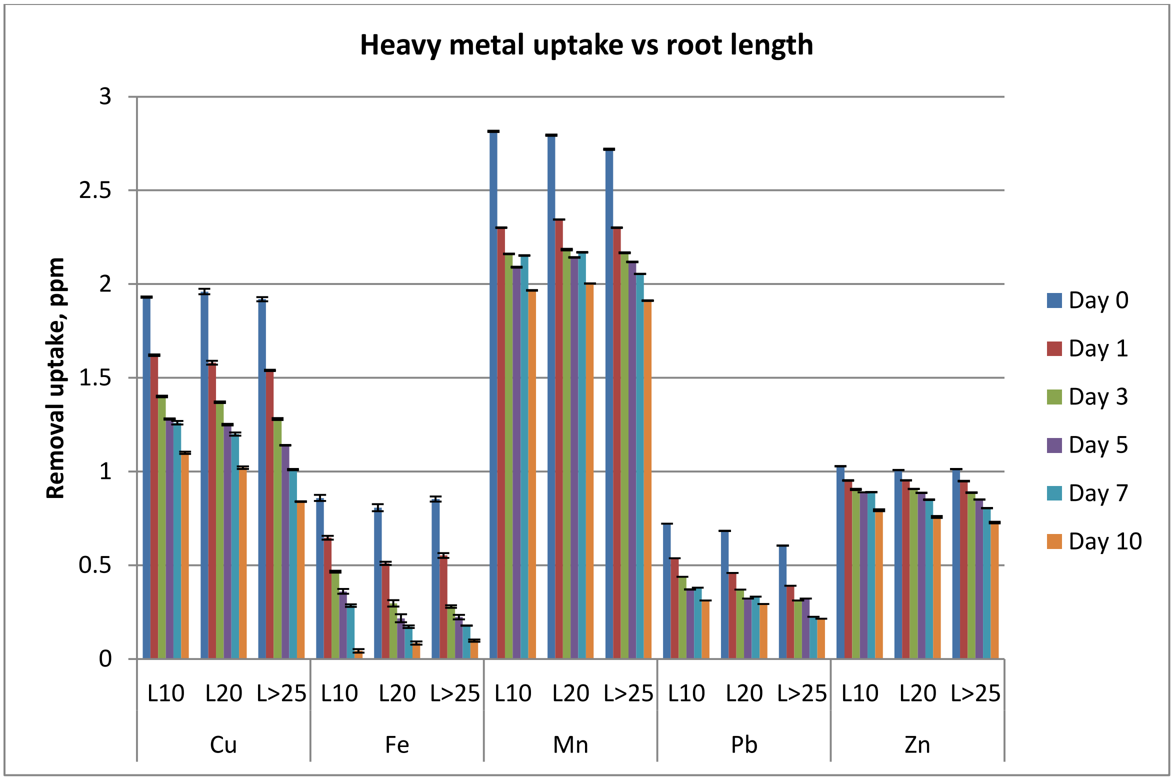

Figure 1: Heavy metal uptake by different root length in Low concentration treatment. Error bars represent SDs; n = 6.

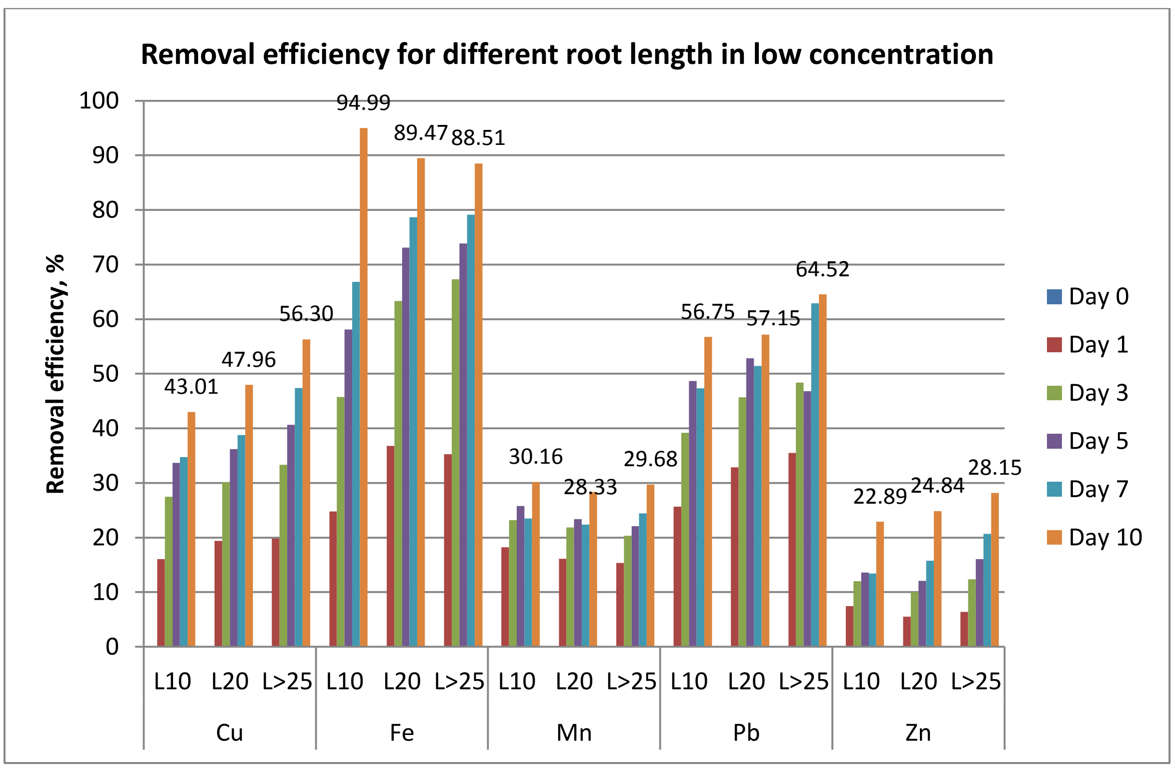

Figure 2: Removal efficiency for different root length in Low concentration treatment

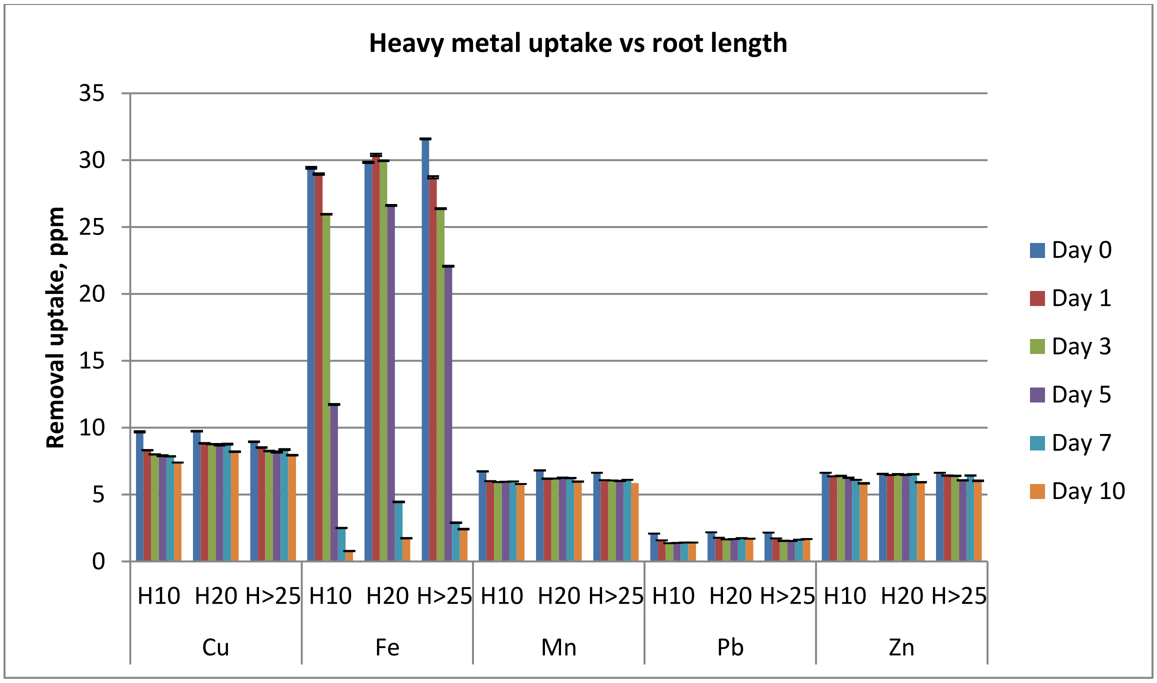

Figure 3: Heavy metal uptake by different root length in High concentration treatment. Error bars represent SDs; n = 6.

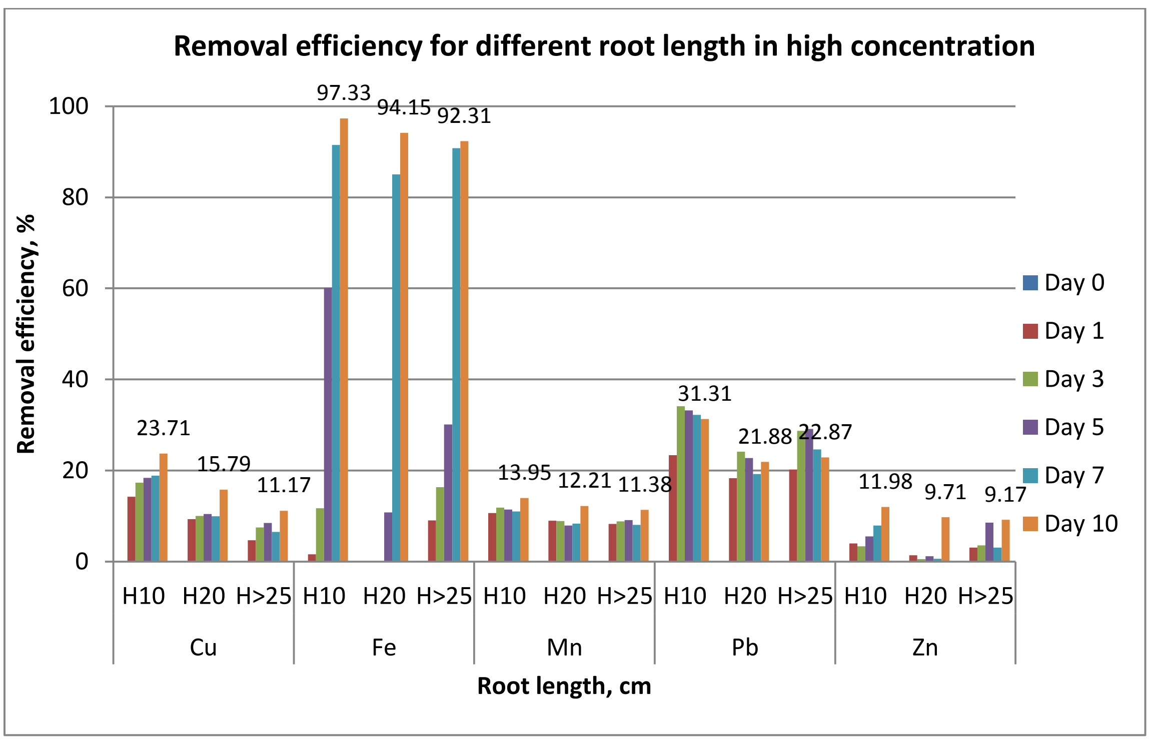

Figure 4: Removal efficiency for different root length in Low concentration treatment

Appendix 6

MANOVA for HM removal in different root length for Low concentration (Experiment 1)

Between-Subjects Factors

		Value Label	N
rootlength	1	10cm	6
	2	20cm	6
	3	>25cm	6

Box's Test of Equality of Covariance Matrices[a]

Box's M	78.061
F	1.259
df1	30
df2	712.958
Sig.	.162

Tests the null hypothesis that the observed covariance matrices of the dependent variables are equal across groups.

a. Design: Intercept + rootlength

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
Cu1	.183	2	15	.835
Fe1	.024	2	15	.976
Mn1	.005	2	15	.995
Pb1	.024	2	15	.977
Zn1	.317	2	15	.733

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + rootlength

Descriptive Statistics

	rootlength	Mean	Std. Deviation	N
Cu1	10cm	1.4317	.29909	6
	20cm	1.3967	.33279	6
	>25cm	1.2882	.39084	6
	Total	1.3722	.32827	18
Fe1	10cm	.4432	.28518	6
	20cm	.3478	.26718	6
	>25cm	.3638	.28528	6
	Total	.3849	.26588	18
Mn1	10cm	2.2474	.29842	6
	20cm	2.2730	.27783	6
	>25cm	2.2120	.27942	6
	Total	2.2441	.26930	18
Pb1	10cm	.4601	.14902	6
	20cm	.4097	.14560	6
	>25cm	.3447	.14332	6
	Total	.4048	.14550	18
Zn1	10cm	.9094	.07788	6
	20cm	.8932	.08629	6
	>25cm	.8719	.10184	6
	Total	.8915	.08529	18

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.999	3557.882[b]	5.000	11.000	.000	.999
	Wilks' Lambda	.001	3557.882[b]	5.000	11.000	.000	.999
	Hotelling's Trace	1617.219	3557.882[b]	5.000	11.000	.000	.999
	Roy's Largest Root	1617.219	3557.882[b]	5.000	11.000	.000	.999
rootlength	Pillai's Trace	1.281	4.280	10.000	24.000	.002	.641
	Wilks' Lambda	.081	5.532[b]	10.000	22.000	.000	.715
	Hotelling's Trace	6.876	6.876	10.000	20.000	.000	.775
	Roy's Largest Root	6.148	14.755[c]	5.000	12.000	.000	.860

a. Design: Intercept + rootlength

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Cu1	.067[a]	2	.034	.285	.756	.037
	Fe1	.031[b]	2	.016	.200	.821	.026
	Mn1	.011[c]	2	.006	.069	.933	.009
	Pb1	.040[d]	2	.020	.943	.411	.112
	Zn1	.004[e]	2	.002	.267	.769	.034
Intercept	Cu1	33.891	1	33.891	288.060	.000	.951
	Fe1	2.667	1	2.667	34.181	.000	.695
	Mn1	90.649	1	90.649	1113.091	.000	.987
	Pb1	2.950	1	2.950	138.400	.000	.902
	Zn1	14.305	1	14.305	1796.857	.000	.992
rootlength	Cu1	.067	2	.034	.285	.756	.037
	Fe1	.031	2	.016	.200	.821	.026
	Mn1	.011	2	.006	.069	.933	.009
	Pb1	.040	2	.020	.943	.411	.112
	Zn1	.004	2	.002	.267	.769	.034
Error	Cu1	1.765	15	.118			
	Fe1	1.171	15	.078			
	Mn1	1.222	15	.081			
	Pb1	.320	15	.021			
	Zn1	.119	15	.008			
Total	Cu1	35.723	18				
	Fe1	3.869	18				
	Mn1	91.881	18				
	Pb1	3.310	18				
	Zn1	14.429	18				
Corrected Total	Cu1	1.832	17				
	Fe1	1.202	17				
	Mn1	1.233	17				
	Pb1	.360	17				
	Zn1	.124	17				

a. R Squared = .037 (Adjusted R Squared = -.092)

b. R Squared = .026 (Adjusted R Squared = -.104)

c. R Squared = .009 (Adjusted R Squared = -.123)

d. R Squared = .112 (Adjusted R Squared = -.007)

e. R Squared = .034 (Adjusted R Squared = -.094)

Appendix 7

Pearson product-moment Correlation Coefficient for Low concentration (Experiment 1)

Descriptive Statistics

	Mean	Std. Deviation	N
rootlength	2.00	.840	18
Cu1	1.3722	.32827	18
Fe1	.3849	.26588	18
Mn1	2.2441	.26930	18
Pb1	.4048	.14550	18
Zn1	.8915	.08529	18

Correlations

		rootlength	Cu1	Fe1	Mn1	Pb1	Zn1
rootlength	Pearson Correlation	1	-.184	-.125	-.055	-.333	-.185
	Sig. (2-tailed)		.466	.620	.827	.177	.463
	N	18	18	18	18	18	18
Cu1	Pearson Correlation	-.184	1	.967**	.956**	.961**	.966**
	Sig. (2-tailed)	.466		.000	.000	.000	.000
	N	18	18	18	18	18	18
Fe1	Pearson Correlation	-.125	.967**	1	.934**	.941**	.933**
	Sig. (2-tailed)	.620	.000		.000	.000	.000
	N	18	18	18	18	18	18
Mn1	Pearson Correlation	-.055	.956**	.934**	1	.945**	.899**
	Sig. (2-tailed)	.827	.000	.000		.000	.000
	N	18	18	18	18	18	18
Pb1	Pearson Correlation	-.333	.961**	.941**	.945**	1	.895**
	Sig. (2-tailed)	.177	.000	.000	.000		.000
	N	18	18	18	18	18	18
Zn1	Pearson Correlation	-.185	.966**	.933**	.899**	.895**	1
	Sig. (2-tailed)	.463	.000	.000	.000	.000	
	N	18	18	18	18	18	18

Appendix 8

MANOVA for HM removal in different root length for High concentration (Experiment 1)

Between-Subjects Factors

		Value Label	N
rootlength	1	10cm	6
	2	20cm	6
	3	>25cm	6

Box's Test of Equality of Covariance Matrices[a]

Box's M	68.602
F	1.106
df1	30
df2	712.958
Sig.	.319

Tests the null hypothesis that the observed covariance matrices of the dependent variables are equal across groups.

a. Design: Intercept + rootlength

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
Cu1	.944	2	15	.411
Fe1	.042	2	15	.959
Mn1	.114	2	15	.893
Pb1	.269	2	15	.768
Zn1	.095	2	15	.910

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + rootlength

Descriptive Statistics

	rootlength	Mean	Std. Deviation	N
Cu1	10cm	8.2050	.79044	6
	20cm	8.8467	.49907	6
	>25cm	8.3783	.34014	6
	Total	8.4767	.60731	18
Fe1	10cm	16.5708	13.25231	6
	20cm	20.5125	13.57833	6
	>25cm	19.0242	13.04765	6
	Total	18.7025	12.59954	18
Mn1	10cm	6.0779	.33354	6
	20cm	6.2818	.27771	6
	>25cm	6.1341	.25982	6
	Total	6.1646	.28825	18
Pb1	10cm	1.5510	.27488	6
	20cm	1.7981	.19546	6
	>25cm	1.7201	.23515	6
	Total	1.6897	.24696	18
Zn1	10cm	6.2752	.27329	6
	20cm	6.4173	.24265	6
	>25cm	6.3368	.23668	6
	Total	6.3431	.24361	18

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	1.000	5888.421[b]	5.000	11.000	.000	1.000
	Wilks' Lambda	.000	5888.421[b]	5.000	11.000	.000	1.000
	Hotelling's Trace	2676.555	5888.421[b]	5.000	11.000	.000	1.000
	Roy's Largest Root	2676.555	5888.421[b]	5.000	11.000	.000	1.000
rootlength	Pillai's Trace	.741	1.413	10.000	24.000	.234	.371
	Wilks' Lambda	.392	1.316[b]	10.000	22.000	.282	.374
	Hotelling's Trace	1.215	1.215	10.000	20.000	.339	.378
	Roy's Largest Root	.781	1.874[c]	5.000	12.000	.173	.438

a. Design: Intercept + rootlength

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Cu1	1.322[a]	2	.661	2.004	.169	.211
	Fe1	47.541[b]	2	23.771	.134	.875	.018
	Mn1	.133[c]	2	.067	.780	.476	.094
	Pb1	.192[d]	2	.096	1.699	.216	.185
	Zn1	.061[e]	2	.030	.482	.627	.060
Intercept	Cu1	1293.370	1	1293.370	3921.072	.000	.996
	Fe1	6296.103	1	6296.103	35.622	.000	.704
	Mn1	684.036	1	684.036	8019.776	.000	.998
	Pb1	51.393	1	51.393	911.997	.000	.984
	Zn1	724.225	1	724.225	11460.043	.000	.999
rootlength	Cu1	1.322	2	.661	2.004	.169	.211
	Fe1	47.541	2	23.771	.134	.875	.018
	Mn1	.133	2	.067	.780	.476	.094
	Pb1	.192	2	.096	1.699	.216	.185
	Zn1	.061	2	.030	.482	.627	.060
Error	Cu1	4.948	15	.330			
	Fe1	2651.180	15	176.745			
	Mn1	1.279	15	.085			
	Pb1	.845	15	.056			
	Zn1	.948	15	.063			
Total	Cu1	1299.640	18				
	Fe1	8994.825	18				
	Mn1	685.449	18				
	Pb1	52.430	18				
	Zn1	725.234	18				
Corrected Total	Cu1	6.270	17				
	Fe1	2698.721	17				
	Mn1	1.412	17				
	Pb1	1.037	17				
	Zn1	1.009	17				

a. R Squared = .211 (Adjusted R Squared = .106)

b. R Squared = .018 (Adjusted R Squared = -.113)

c. R Squared = .094 (Adjusted R Squared = -.027)

d. R Squared = .185 (Adjusted R Squared = .076)

e. R Squared = .060 (Adjusted R Squared = -.065)

Appendix 9

MANOVA for HM removal in different density in Low concentration (Experiment 2)

Between-Subjects Factors

		Value Label	N
density	1	25 tillers	6
	2	50 tillers	6
	3	100 tillers	6

Descriptive Statistics

	density	Mean	Std. Deviation	N
Cu	25 tillers	1.6267	.22115	6
	50 tillers	1.3433	.34168	6
	100 tillers	1.0233	.51617	6
	Total	1.3311	.43749	18
Fe	25 tillers	.4450	.28752	6
	50 tillers	.3483	.26256	6
	100 tillers	.2800	.27633	6
	Total	.3578	.26814	18
Mn	25 tillers	2.3933	.27529	6
	50 tillers	2.2700	.28893	6
	100 tillers	2.2133	.41433	6
	Total	2.2922	.32142	18
Pb	25 tillers	.4800	.13755	6
	50 tillers	.3633	.15513	6
	100 tillers	.3167	.19684	6
	Total	.3867	.17040	18
Zn	25 tillers	.9700	.07127	6
	50 tillers	.9083	.07960	6
	100 tillers	.8417	.13408	6
	Total	.9067	.10748	18

Box's Test of Equality of Covariance Matrices[a]

Box's M	94.704
F	1.527
df1	30
df2	712.958
Sig.	.036

Tests the null hypothesis that the observed covariance matrices of the dependent variables are equal across groups.

a. Design: Intercept + density

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
Cu	1.704	2	15	.215
Fe	.109	2	15	.897
Mn	.845	2	15	.449
Pb	.091	2	15	.913
Zn	1.241	2	15	.317

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + density

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.997	676.385[b]	5.000	11.000	.000	.997
	Wilks' Lambda	.003	676.385[b]	5.000	11.000	.000	.997
	Hotelling's Trace	307.448	676.385[b]	5.000	11.000	.000	.997
	Roy's Largest Root	307.448	676.385[b]	5.000	11.000	.000	.997
density	Pillai's Trace	.954	2.190	10.000	24.000	.056	.477
	Wilks' Lambda	.186	2.904[b]	10.000	22.000	.018	.569
	Hotelling's Trace	3.629	3.629	10.000	20.000	.007	.645
	Roy's Largest Root	3.408	8.179[c]	5.000	12.000	.001	.773

a. Design: Intercept + density

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Cu	1.093[a]	2	.547	3.796	.046	.336
	Fe	.082[b]	2	.041	.543	.592	.067
	Mn	.102[c]	2	.051	.461	.639	.058
	Pb	.085[d]	2	.042	1.559	.243	.172
	Zn	.049[e]	2	.025	2.523	.114	.252
Intercept	Cu	31.893	1	31.893	221.441	.000	.937
	Fe	2.304	1	2.304	30.321	.000	.669
	Mn	94.577	1	94.577	857.367	.000	.983
	Pb	2.691	1	2.691	98.780	.000	.868
	Zn	14.797	1	14.797	1510.220	.000	.990
density	Cu	1.093	2	.547	3.796	.046	.336
	Fe	.082	2	.041	.543	.592	.067
	Mn	.102	2	.051	.461	.639	.058
	Pb	.085	2	.042	1.559	.243	.172
	Zn	.049	2	.025	2.523	.114	.252
Error	Cu	2.160	15	.144			
	Fe	1.140	15	.076			
	Mn	1.655	15	.110			
	Pb	.409	15	.027			
	Zn	.147	15	.010			
Total	Cu	35.147	18				
	Fe	3.526	18				
	Mn	96.333	18				
	Pb	3.185	18				
	Zn	14.993	18				
Corrected Total	Cu	3.254	17				
	Fe	1.222	17				
	Mn	1.756	17				
	Pb	.494	17				
	Zn	.196	17				

a. R Squared = .336 (Adjusted R Squared = .248)

b. R Squared = .067 (Adjusted R Squared = -.057)

c. R Squared = .058 (Adjusted R Squared = -.068)

d. R Squared = .172 (Adjusted R Squared = .062)

e. R Squared = .252 (Adjusted R Squared = .152)

Appendix 10

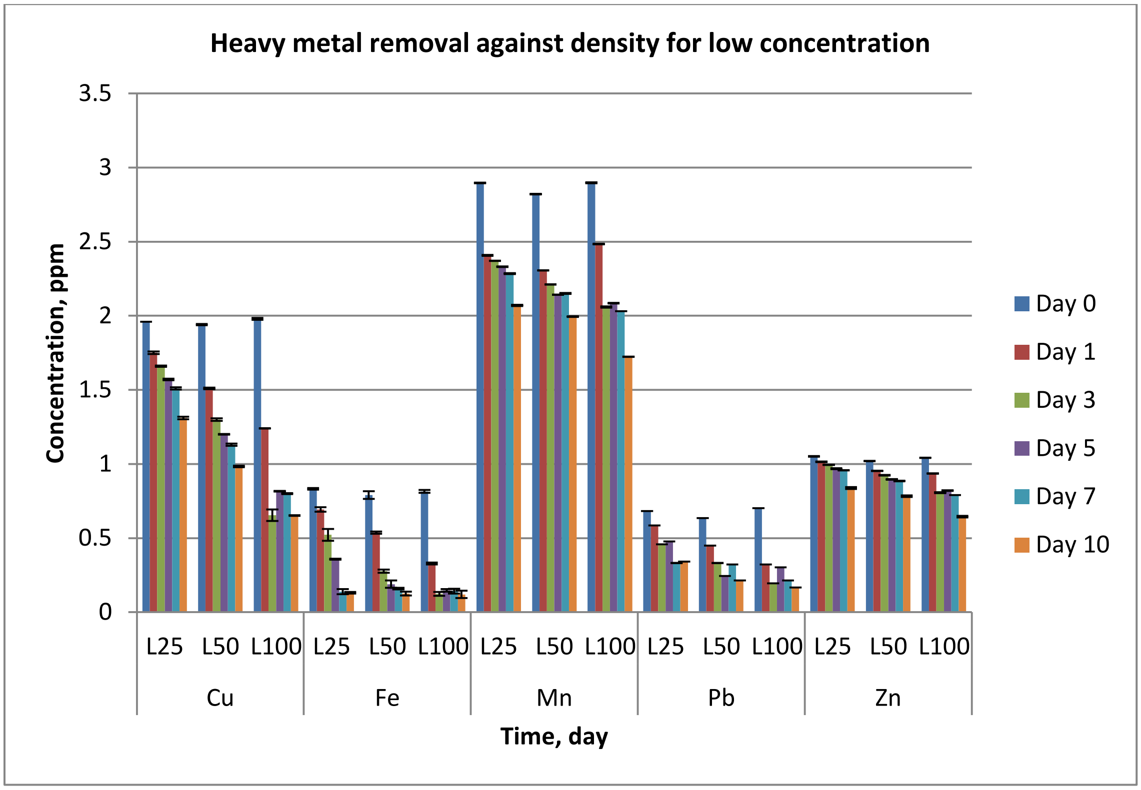

Figure 1: Heavy metal removal in ppm against density for Low concentration. Error bars represent SDs, n=6.

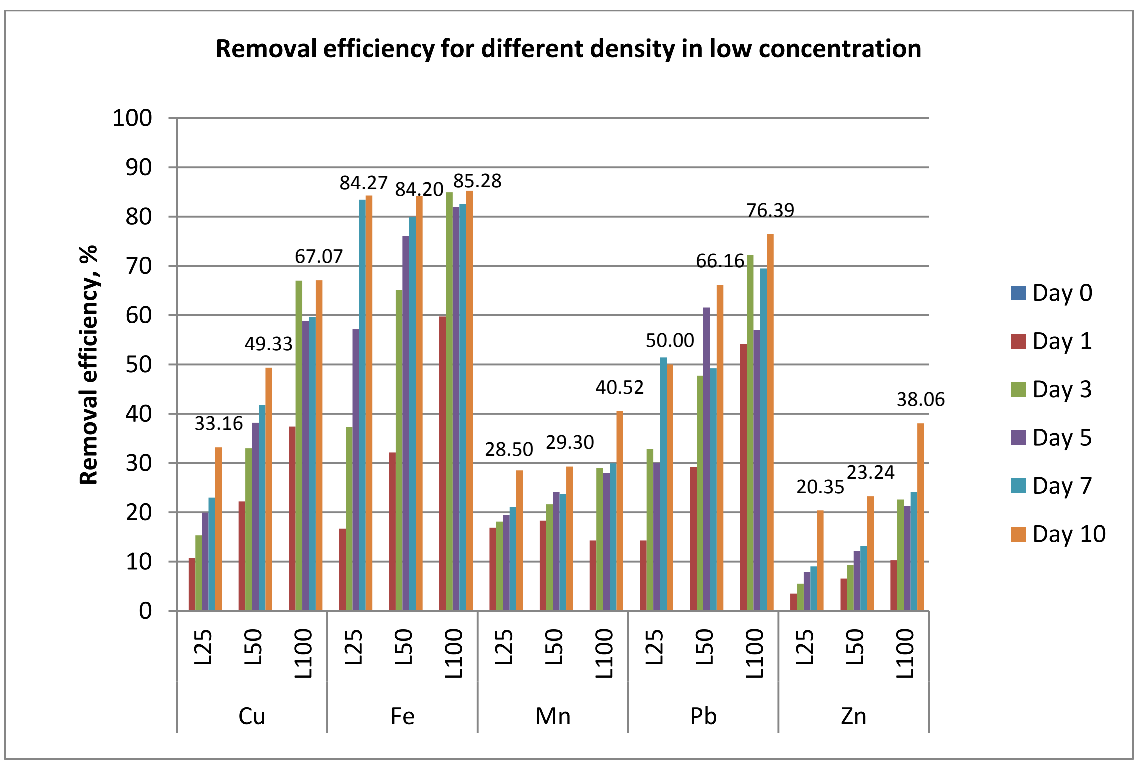

Figure 2: Removal efficiency for different density in Low concentration

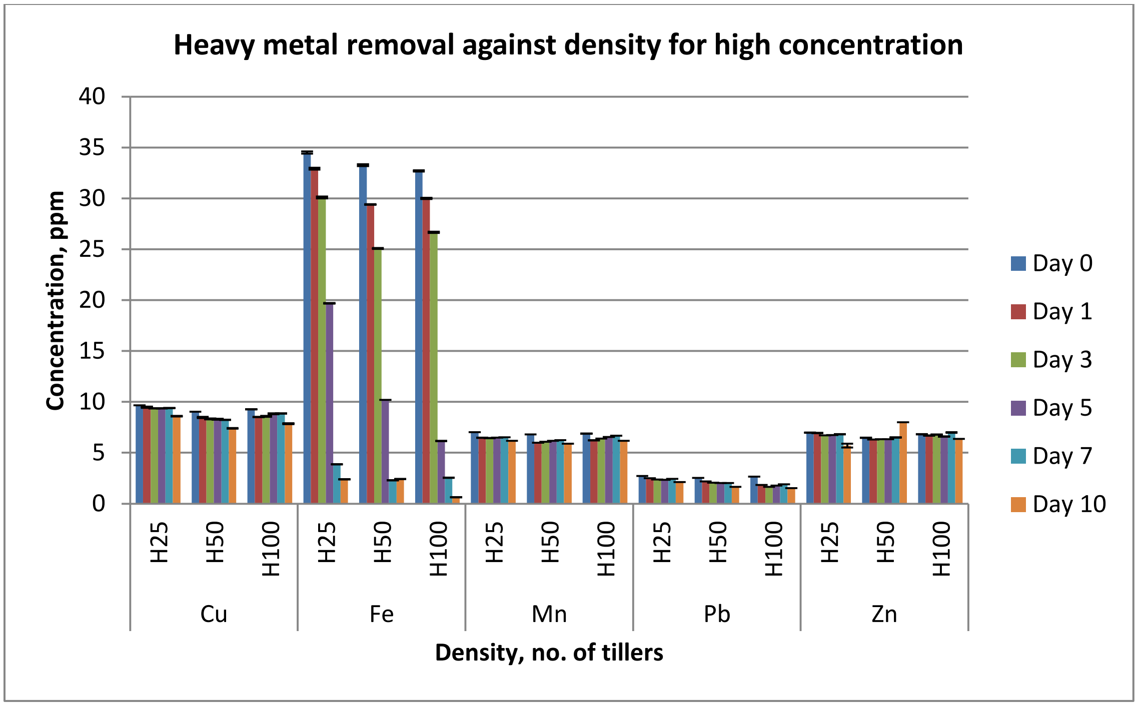

Figure 3: Heavy metal removal in ppm against density for Low concentration. Error bars represent SDs, n=6.

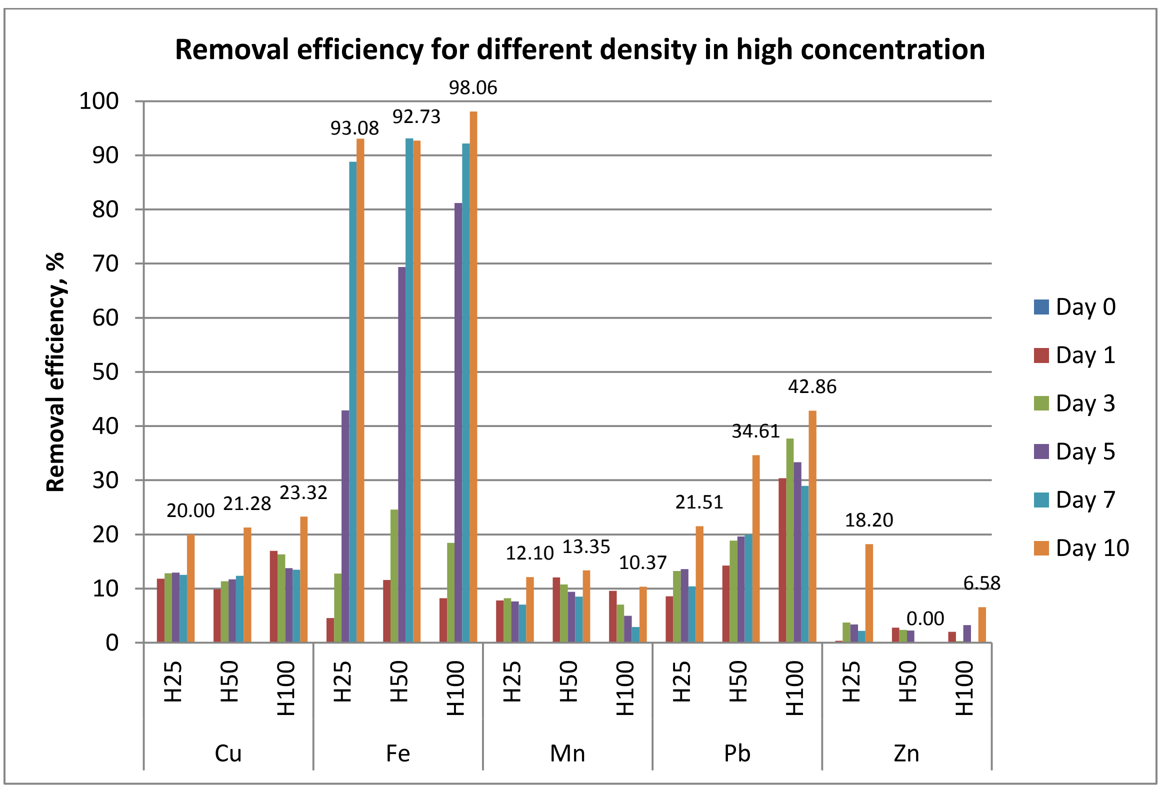

Figure 4: Removal efficiency for different density in High concentration

Appendix 11

MANOVA for HM removal in different density in High concentration (Experiment 2)

Between-Subjects Factors

		Value Label	N
density	1	25 tillers	6
	2	50 tillers	6
	3	100 tillers	6

Box's Test of Equality of Covariance Matrices[a]

Box's M	107.810
F	1.739
df1	30
df2	712.958
Sig.	.009

Tests the null hypothesis that the observed covariance matrices of the dependent variables are equal across groups.

a. Design: Intercept + density

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
Cu2	.078	2	15	.925
Fe2	.171	2	15	.844
Mn2	.137	2	15	.873
Pb2	.499	2	15	.617
Zn2	1.202	2	15	.328

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + density

Descriptive Statistics

	density	Mean	Std. Deviation	N
Cu2	25 tillers	9.4933	.69558	6
	50 tillers	8.3567	.63783	6
	100 tillers	8.8183	.79033	6
	Total	8.8894	.82240	18
Fe2	25 tillers	20.5700	14.47061	6
	50 tillers	17.1150	13.84916	6
	100 tillers	16.4500	14.83667	6
	Total	18.0450	13.64536	18
Mn2	25 tillers	6.5267	.27674	6
	50 tillers	6.2000	.32162	6
	100 tillers	6.4867	.27587	6
	Total	6.4044	.31264	18
Pb2	25 tillers	2.4167	.19086	6
	50 tillers	2.0850	.28367	6
	100 tillers	1.8933	.39908	6
	Total	2.1317	.36154	18
Zn2	25 tillers	6.6533	.47420	6
	50 tillers	6.6600	.66705	6
	100 tillers	6.7133	.20973	6
	Total	6.6756	.45903	18

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.999	2423.883[b]	5.000	11.000	.000	.999
	Wilks' Lambda	.001	2423.883[b]	5.000	11.000	.000	.999
	Hotelling's Trace	1101.765	2423.883[b]	5.000	11.000	.000	.999
	Roy's Largest Root	1101.765	2423.883[b]	5.000	11.000	.000	.999
density	Pillai's Trace	1.411	5.753	10.000	24.000	.000	.706
	Wilks' Lambda	.082	5.460[b]	10.000	22.000	.000	.713
	Hotelling's Trace	5.137	5.137	10.000	20.000	.001	.720
	Roy's Largest Root	3.351	8.041[c]	5.000	12.000	.002	.770

a. Design: Intercept + density

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Cu2	3.922[a]	2	1.961	3.882	.044	.341
	Fe2	58.707[b]	2	29.354	.142	.869	.019
	Mn2	.381[c]	2	.190	2.231	.142	.229
	Pb2	.841[d]	2	.421	4.569	.028	.379
	Zn2	.013[e]	2	.006	.027	.973	.004
Intercept	Cu2	1422.400	1	1422.400	2816.132	.000	.995
	Fe2	5861.196	1	5861.196	28.300	.000	.654
	Mn2	738.304	1	738.304	8647.500	.000	.998
	Pb2	81.792	1	81.792	888.518	.000	.983
	Zn2	802.135	1	802.135	3371.195	.000	.996
density	Cu2	3.922	2	1.961	3.882	.044	.341
	Fe2	58.707	2	29.354	.142	.869	.019
	Mn2	.381	2	.190	2.231	.142	.229
	Pb2	.841	2	.421	4.569	.028	.379
	Zn2	.013	2	.006	.027	.973	.004
Error	Cu2	7.576	15	.505			
	Fe2	3106.624	15	207.108			
	Mn2	1.281	15	.085			
	Pb2	1.381	15	.092			
	Zn2	3.569	15	.238			
Total	Cu2	1433.898	18				
	Fe2	9026.527	18				
	Mn2	739.966	18				
	Pb2	84.014	18				
	Zn2	805.717	18				
Corrected Total	Cu2	11.498	17				
	Fe2	3165.331	17				
	Mn2	1.662	17				
	Pb2	2.222	17				
	Zn2	3.582	17				

a. R Squared = .341 (Adjusted R Squared = .253)

b. R Squared = .019 (Adjusted R Squared = -.112)

c. R Squared = .229 (Adjusted R Squared = .127)

d. R Squared = .379 (Adjusted R Squared = .296)

e. R Squared = .004 (Adjusted R Squared = -.129)

Appendix 12

T-test to compare each heavy metal between low and high concentration (Experiment 1)

Group Statistics

	Conc	N	Mean	Std. Deviation	Std. Error Mean
Cu	Low	15	34.9879	11.35768	2.93254
	High	15	12.4259	5.37372	1.38749
Fe	Low	15	65.0614	21.22364	5.47992
	High	15	46.0568	41.41453	10.69319
Mn	Low	15	22.9827	4.41444	1.13980
	High	15	10.0641	1.84370	.47604
Pb	Low	15	47.7076	10.88492	2.81048
	High	15	25.7352	5.27520	1.36205
Zn	Low	15	14.7375	6.76360	1.74635
	High	15	4.9099	3.68125	.95050

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cu	Equal variances assumed	4.875	.036	6.955	28	.000	22.56208	3.24421	15.91661	29.20755
	Equal variances not assumed			6.955	19.969	.000	22.56208	3.24421	15.79409	29.33007
Fe	Equal variances assumed	25.823	.000	1.582	28	.125	19.00464	12.01556	-5.60813	43.61740
	Equal variances not assumed			1.582	20.879	.129	19.00464	12.01556	-5.99191	44.00118
Mn	Equal variances assumed	4.849	.036	10.459	28	.000	12.91863	1.23522	10.38839	15.44886
	Equal variances not assumed			10.459	18.740	.000	12.91863	1.23522	10.33085	15.50640
Pb	Equal variances assumed	3.571	.069	7.035	28	.000	21.97231	3.12313	15.57486	28.36975
	Equal variances not assumed			7.035	20.233	.000	21.97231	3.12313	15.46237	28.48225
Zn	Equal variances assumed	3.898	.058	4.943	28	.000	9.82760	1.98826	5.75483	13.90037
	Equal variances not assumed			4.943	21.625	.000	9.82760	1.98826	5.70005	13.95515

Appendix 13

T-test to compare each heavy metal between low and high concentration (Experiment 2)

Group Statistics

	Conc	N	Mean	Std. Deviation	Std. Error Mean
cu2	Low	15	38.4147	18.58538	4.79873
	High	15	14.7027	3.99918	1.03258
Fe2	Low	15	67.3847	22.38370	5.77945
	High	15	55.4453	38.16720	9.85473
Mn2	Low	15	24.1841	6.77839	1.75017
	High	15	8.7809	2.77292	.71596
Pb2	Low	15	50.7708	17.78510	4.59209
	High	15	23.1966	10.71766	2.76729
Zn2	Low	15	15.1272	9.46234	2.44316
	High	15	3.1613	4.53558	1.17108

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
cu2	Equal variances assumed	19.628	.000	4.831	28	.000	23.71201	4.90856	13.65727	33.76674
	Equal variances not assumed			4.831	15.294	.000	23.71201	4.90856	13.26712	34.15689
Fe2	Equal variances assumed	16.530	.000	1.045	28	.305	11.93947	11.42443	-11.46241	35.34136
	Equal variances not assumed			1.045	22.612	.307	11.93947	11.42443	-11.71625	35.59519
Mn2	Equal variances assumed	8.413	.007	8.146	28	.000	15.40324	1.89095	11.52979	19.27668
	Equal variances not assumed			8.146	18.558	.000	15.40324	1.89095	11.43904	19.36744
Pb2	Equal variances assumed	2.087	.160	5.143	28	.000	27.57418	5.36145	16.59174	38.55662
	Equal variances not assumed			5.143	22.983	.000	27.57418	5.36145	16.48272	38.66563
Zn2	Equal variances assumed	11.188	.002	4.417	28	.000	11.96582	2.70933	6.41600	17.51564
	Equal variances not assumed			4.417	20.111	.000	11.96582	2.70933	6.31624	17.61540

Appendix 14

Pearson product-moment correlation coefficient for heavy metal removal and accumulation in root

Correlations

			Cu1	CupltR	Mn1	Fe1	Pb1	Zn1	FepltR	MnpltR	PbpltR	ZnpltR
Spearman's rho	Cu1	Correlation Coefficient	1.000	-.523*	.928**	.952**	.951**	.975**	-.834**	.332	-.472*	.226
		Sig. (2-tailed)	.	.026	.000	.000	.000	.000	.000	.179	.048	.367
		N	18	18	18	18	18	18	18	18	18	18
	CupltR	Correlation Coefficient	-.523*	1.000	-.358	-.595**	-.527*	-.507*	.723**	-.290	.528*	-.118
		Sig. (2-tailed)	.026	.	.144	.009	.025	.032	.001	.244	.024	.641
		N	18	18	18	18	18	18	18	18	18	18
	Mn1	Correlation Coefficient	.928**	-.358	1.000	.876**	.889**	.929**	-.642**	.469*	-.397	.261
		Sig. (2-tailed)	.000	.144	.	.000	.000	.000	.004	.049	.103	.295
		N	18	18	18	18	18	18	18	18	18	18
	Fe1	Correlation Coefficient	.952**	-.595**	.876**	1.000	.928**	.971**	-.808**	.363	-.453	.278
		Sig. (2-tailed)	.000	.009	.000	.	.000	.000	.000	.139	.059	.264
		N	18	18	18	18	18	18	18	18	18	18
	Pb1	Correlation Coefficient	.951**	-.527*	.889**	.928**	1.000	.953**	-.850**	.297	-.515*	.225
		Sig. (2-tailed)	.000	.025	.000	.000	.	.000	.000	.231	.029	.370
		N	18	18	18	18	18	18	18	18	18	18
	Zn1	Correlation Coefficient	.975**	-.507*	.929**	.971**	.953**	1.000	-.816**	.339	-.474*	.200
		Sig. (2-tailed)	.000	.032	.000	.000	.000	.	.000	.169	.047	.425
		N	18	18	18	18	18	18	18	18	18	18
	FepltR	Correlation Coefficient	-.834**	.723**	-.642**	-.808**	-.850**	-.816**	1.000	-.069	.594**	.054
		Sig. (2-tailed)	.000	.001	.004	.000	.000	.000	.	.785	.009	.832
		N	18	18	18	18	18	18	18	18	18	18
	MnpltR	Correlation Coefficient	.332	-.290	.469*	.363	.297	.339	-.069	1.000	-.041	.513*
		Sig. (2-tailed)	.179	.244	.049	.139	.231	.169	.785	.	.873	.030
		N	18	18	18	18	18	18	18	18	18	18
	PbpltR	Correlation Coefficient	-.472*	.528*	-.397	-.453	-.515*	-.474*	.594**	-.041	1.000	.093
		Sig. (2-tailed)	.048	.024	.103	.059	.029	.047	.009	.873	.	.713
		N	18	18	18	18	18	18	18	18	18	18
	ZnpltR	Correlation Coefficient	.226	-.118	.261	.278	.225	.200	.054	.513*	.093	1.000
		Sig. (2-tailed)	.367	.641	.295	.264	.370	.425	.832	.030	.713	.
		N	18	18	18	18	18	18	18	18	18	18

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 15

T-tests results for heavy metal accumulation in different plant part after 10 days for Low concentration treatment

Group Statistics

	RoS	N	Mean	Std. Deviation	Std. Error Mean
CuA	1.00	18	1641.6111	671.20157	158.20373
	2.00	18	417.8889	131.03834	30.88603
FeA	1.00	18	4116.1111	1593.40235	375.56854
	2.00	18	1477.7778	372.83580	87.87824
MnA	1.00	18	1148.1389	573.39510	135.15052
	2.00	18	574.5556	262.82318	61.94802
PbA	1.00	18	2194.7222	292.23295	68.87997
	2.00	18	1788.1667	259.24171	61.10386
ZnA	1.00	18	797.2222	423.07009	99.71858
	2.00	18	246.2500	71.46827	16.84523

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
CuA	Equal variances assumed	19.113	.000	7.592	34	.000	1223.72222	161.19047	896.14378	1551.30066
	Equal variances not assumed			7.592	18.294	.000	1223.72222	161.19047	885.46333	1561.98112
FeA	Equal variances assumed	16.835	.000	6.840	34	.000	2638.33333	385.71273	1854.47076	3422.19591
	Equal variances not assumed			6.840	18.856	.000	2638.33333	385.71273	1830.60964	3446.05702
MnA	Equal variances assumed	7.786	.009	3.858	34	.000	573.58333	148.67152	271.44646	875.72021
	Equal variances not assumed			3.858	23.841	.001	573.58333	148.67152	266.63229	880.53438
PbA	Equal variances assumed	.412	.525	4.415	34	.000	406.55556	92.07677	219.43305	593.67806
	Equal variances not assumed			4.415	33.524	.000	406.55556	92.07677	219.33503	593.77608
ZnA	Equal variances assumed	15.909	.000	5.448	34	.000	550.97222	101.13138	345.44853	756.49592
	Equal variances not assumed			5.448	17.969	.000	550.97222	101.13138	338.47718	763.46726

Appendix 16

T-tests for heavy metal accumulation in plant parts after 10 days for High concentration

Group Statistics

	RoS	N	Mean	Std. Deviation	Std. Error Mean
CuH	1.00	18	1110.2222	444.91275	104.86694
	2.00	18	458.3889	133.78830	31.53421
FeH	1.00	18	19197.2222	15265.19563	3598.04112
	2.00	18	1549.4444	430.88790	101.56125
MnH	1.00	18	826.1389	683.81834	161.17753
	2.00	18	409.3333	253.53362	59.75845
PbH	1.00	18	2633.6111	745.15566	175.63487
	2.00	18	1815.2778	301.42579	71.04674
ZnH	1.00	18	705.3889	325.66803	76.76069
	2.00	18	278.3889	95.75529	22.56974

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
CuH	Equal variances assumed	17.809	.000	5.953	34	.000	651.83333	109.50562	429.29113	874.37554
	Equal variances not assumed			5.953	20.050	.000	651.83333	109.50562	423.44475	880.22191
FeH	Equal variances assumed	25.156	.000	4.903	34	.000	17647.77778	3599.47421	10332.76609	24962.78947
	Equal variances not assumed			4.903	17.027	.000	17647.77778	3599.47421	10054.47130	25241.08426
MnH	Equal variances assumed	11.746	.002	2.425	34	.021	416.80556	171.89900	67.46475	766.14636
	Equal variances not assumed			2.425	21.587	.024	416.80556	171.89900	59.91308	773.69803
PbH	Equal variances assumed	6.836	.013	4.319	34	.000	818.33333	189.46041	433.30345	1203.36322
	Equal variances not assumed			4.319	22.418	.000	818.33333	189.46041	425.84121	1210.82546
ZnH	Equal variances assumed	15.270	.000	5.337	34	.000	427.00000	80.00998	264.40016	589.59984
	Equal variances not assumed			5.337	19.918	.000	427.00000	80.00998	260.05782	593.94218

Appendix 17

T-tests for heavy metal accumulation in plant root for different concentration

Group Statistics

	Conc.RS	N	Mean	Std. Deviation	Std. Error Mean
CuR	Low	18	1641.6111	671.20157	158.20373
	high	18	1110.2222	444.91275	104.86694
FeR	Low	18	4116.1111	1593.40235	375.56854
	high	18	19197.2222	15265.19563	3598.04112
MnR	Low	18	1148.1389	573.39510	135.15052
	high	18	826.1389	683.81834	161.17753
PbR	Low	18	2194.7222	292.23295	68.87997
	high	18	2633.6111	745.15566	175.63487
ZnR	Low	18	797.2222	423.07009	99.71858
	high	18	705.3889	325.66803	76.76069

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
CuR	Equal variances assumed	2.205	.147	2.800	34	.008	531.38889	189.80383	145.66109	917.11668
	Equal variances not assumed			2.800	29.522	.009	531.38889	189.80383	143.49419	919.28359
FeR	Equal variances assumed	21.048	.000	-4.169	34	.000	-15081.11111	3617.58920	-22432.93689	-7729.28533
	Equal variances not assumed			-4.169	17.370	.001	-15081.11111	3617.58920	-22701.17810	-7461.04412
MnR	Equal variances assumed	.469	.498	1.531	34	.135	322.00000	210.34224	-105.46687	749.46687
	Equal variances not assumed			1.531	32.997	.135	322.00000	210.34224	-105.94583	749.94583
PbR	Equal variances assumed	6.794	.013	-2.326	34	.026	-438.88889	188.65858	-822.28924	-55.48853
	Equal variances not assumed			-2.326	22.108	.030	-438.88889	188.65858	-830.03158	-47.74620
ZnR	Equal variances assumed	.607	.441	.730	34	.471	91.83333	125.84116	-163.90668	347.57334
	Equal variances not assumed			.730	31.911	.471	91.83333	125.84116	-164.52471	348.19138

Appendix 18

T-tests for heavy metal accumulation in plant shoots for different concentration

Group Statistics

	Conc.RS	N	Mean	Std. Deviation	Std. Error Mean
CuS	Low	18	417.8889	131.03834	30.88603
	high	18	458.3889	133.78830	31.53421
FeS	Low	18	1477.7778	372.83580	87.87824
	high	18	1549.4444	430.88790	101.56125
MnS	Low	18	574.5556	262.82318	61.94802
	high	18	409.3333	253.53362	59.75845
PbS	Low	18	1788.1667	259.24171	61.10386
	high	18	1815.2778	301.42579	71.04674
ZnS	Low	18	246.2500	71.46827	16.84523
	high	18	278.3889	95.75529	22.56974

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
CuS	Equal variances assumed	.013	.910	-.918	34	.365	-40.50000	44.14015	-130.20358	49.20358
	Equal variances not assumed			-.918	33.985	.365	-40.50000	44.14015	-130.20501	49.20501
FeS	Equal variances assumed	.227	.637	-.534	34	.597	-71.66667	134.30292	-344.60303	201.26970
	Equal variances not assumed			-.534	33.312	.597	-71.66667	134.30292	-344.81080	201.47747
MnS	Equal variances assumed	.342	.562	1.920	34	.063	165.22222	86.07339	-9.69996	340.14440
	Equal variances not assumed			1.920	33.956	.063	165.22222	86.07339	-9.70829	340.15274
PbS	Equal variances assumed	.272	.605	-.289	34	.774	-27.11111	93.70870	-217.55010	163.32788
	Equal variances not assumed			-.289	33.255	.774	-27.11111	93.70870	-217.70728	163.48506
ZnS	Equal variances assumed	.426	.518	-1.141	34	.262	-32.13889	28.16301	-89.37300	25.09523
	Equal variances not assumed			-1.141	31.455	.262	-32.13889	28.16301	-89.54410	25.26632

BIODATA OF STUDENT

The author of this thesis, Ashton Lim Suelee was born on 4th October 1992 in Kuching, Sarawak. She lives in Kuching, but currently stays in UPM Serdang in pursuing her Bachelor of Environmental Science and Technology. She is from an average family consisting of her parents, an elder sister and a younger brother, in which their age gap is only 14 months in average. Her parents both work together at her late-grandfather's forwarding agencies company called Natural Service Company – her father is the company manager and her mother is working alongside with him.

She has completed her primary education from year 1999 to 2004 at Sekolah Rendah Bantuan St. Theresa Padungan in Kuching, Sarawak. Then, she continued her secondary education at Sekolah Menengah Kebangsaan St. Teresa Kuching (2005 – 2009) and continued further till Form 6 at Sekolah Menengah Kebangsaan St. Joseph Kuching (2010-2011). Currently, she furthers her study as an undergraduate student in UPM eversince year 2012, whereby she would be graduating soon in year 2016.