PHYTOACCUMULATION OF HEAVY METALS FROM CONTAMINATED SOILS BY VETIVER GRASS (*Vetiveria zizanioides*) IN MALAYSIA

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FACULTY OF SCIENCE
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2017

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2017

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **Ng Chuck Chuan**

Registration/Matric No: **SHC120081**

Name of Degree: **Doctor of Philosophy**

Title of Project Paper/Research Report/Dissertation/Thesis (“this Work”):

PHYTOACCUMULATION OF HEAVY METALS FROM CONTAMINATED SOILS BY VETIVER GRASS (*Vetiveria zizanioides*) IN MALAYSIA

Field of Study: **Soil Pollution and Plant Physiology (Biology and Biochemistry)**

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ABSTRACT

A phytoremediation study was carried out to assess the capability of selected plant species to bio-accumulate contaminated heavy metals in soils. The relative soil-plant metal transfer coefficients such as biological concentration factor (BCF), bioaccumulation coefficient (BAC), translocation factor (TF) and percentage of metal uptake efficacy were employed to determine the mobility and potential phytoavailability of heavy metals in soil. Preliminary evaluation of potential tropical plant species with water spinach (*I. aquatica*), okra (*A. esculentus*), acacia (*A. mangium*), mucuna (*M. bracteata*), imperata (*I. cylindrical*), pennisetum (*P. purpureum*) and Vetiver (*V. zizanioides*) were conducted to assess the Cd, Cu, Pb and Zn accumulations with different levels of metal contamination in soils. Among these species, Vetiver grass was identified to be most promising due to its positive biological characteristics of fast growth, good tolerance to environmental stress, ability to withstand and bio-accumulate high levels of contaminated heavy metals in soils. Consequently, continuous phytoassessment of Vetiver grass with the application of higher levels of Cd and Pb concentrations were performed to evaluate its effectiveness for soil-plant metal accumulations. The threshold capability of heavy metals accumulation in Vetiver grass was found to be < 150 mg/kg for Cd and > 800 mg/kg for Pb, respectively. Under both single and mixed spiked heavy metals contamination in soils, Vetiver grass grown in mixed Cd+Pb, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments accumulated higher heavy metal concentrations, specifically in the roots section than the single spiked treatments. With different types and composition levels of low cost soil amendments namely; ethylene-diamine-tetra-acetic acid (EDTA) disodium salt, elemental sulfur (S) and nitrogen (N) fertilizer; 25 mmol/kg EDTA and 300 mmol/kg N-fertilizer were found to be able to enhance the accumulation of both Cd and Pb in Vetiver grass. Phytoevaluation of Vetiver grass with EDTA soil amendment under both single and

mixed enhanced heavy metals contaminated soils showed single Zn+EDTA enhanced treatment exhibited the highest Zn uptake whilst mixed Cd+Pb+EDTA, Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments were tolerably effective to accumulate higher overall total concentration for Cd, Pb and Cu, respectively. It can be concluded that Vetiver grass is potentially the most viable plant species to be developed and used for phytoremediation, as both phytostabilizer and phytoextractor, in single and/or mixed heavy metals contaminated soils.

ABSTRAK

Kajian fitoremediasi telah dijalankan untuk menilai keupayaan tumbuh-tumbuhan terpilih dalam proses bio-akumulasi logam berat dalam keadaan tanah tercemar. Indikator pekali pemindahan logam dalam tanah-tumbuhan seperti faktor biologi kepekatan (BCF), pekali bio-akumulasi (BAC), faktor translokasi (TF) dan kadar keberkesanan akumulasi logam telah digunakan untuk menentusahkan pergerakan dan potensi fitoketersediaan logam berat di dalam tanah. Penilaian awal potensi spesies tumbuh-tumbuhan tropika seperti kangkung (*I. aquatica*), bendi (*A. esculentus*), acacia (*A. mangium*), mucuna (*M. bracteata*), imperata (*I. cylindrical*), pennisetum (*P. purpureum*) dan Vetiver (*V. zizanioides*) telah dijalankan untuk menilai keupayaan akumulasi Cd, Cu, Pb dan Zn dengan tahap pencemaran logam berat yang berbeza di dalam tanah. Di antara semua spesies tumbuhan yang dikaji, Vetiver telah dikenal pasti sebagai tumbuhan yang paling sesuai disebabkan oleh ciri-ciri biologinya yang positif seperti kadar tumbesaran yang pantas, toleransi baik terhadap tekanan persekitaran, keupayaan untuk menahan dan bio-akumuasi logam berat yang jauh lebih tinggi dalam keadaan tanah tercemar. Oleh itu, kajian fitopenilaian berterusan dalam Vetiver dengan penggunaan tahap pencemaran Cd dan Pb yang lebih tinggi telah dijalankan untuk menilai keberkesanan kadar akumulasi logam beratnya. Had kadar keupayaan akumulasi logam berat dalam Vetiver didapati <150 mg/kg untuk logam Cd dan > 800 mg/kg untuk logam Pb. Di antara kedua-dua keadaan pencemaran tanah oleh logam berat secara tunggal dan campuran, Vetiver di bawah keadaan pencemaran tanah oleh logam berat secara campuran Cd+Pb, Cu+Zn dan Cd+Pb+Cu +Zn berupaya untuk mengumpul kadar kepekatan logam berat yang lebih tinggi, khususnya di dalam bahagian akar berbanding dengan keadaan pencemaran tanah secara tunggal. Dengan penggunaan pelbagai jenis dan tahap komposisi pindaan tanah berkos rendah seperti garam dinatrium asid etilena-diamine-tetra-asetik (EDTA), unsur sulfur (S) dan baja

bernitrogen (N); 25 mmol/kg EDTA dan 300 mmol/kg baja bernitrogen mampu meningkatkan kadar kecekapan akumulasi kedua-dua logam Cd dan Pb dalam Vetiver. Fitopenilaian Vetiver dengan pindaan tanah EDTA dalam kedua-dua keadaan pencemaran tanah oleh logam berat secara tunggal dan campuran menunjukkan pengambilan logam Zn tertinggi diperoleh dalam keadaan Zn+EDTA tunggal manakala keadaan Cd+Pb+EDTA, Cu+Zn+EDTA dan Cd+Pb+Cu+Zn+EDTA campuran melaporkan keberkesanan untuk mengumpul jumlah kepekatan keseluruhan yang lebih tinggi bagi akumulasi Cd, Pb dan Cu. Ia boleh disimpulkan bahawa Vetiver merupakan spesies tumbuhan paling berdaya maju yang boleh dikembangkan dan diguna untuk fitoremediasi sebagai fitopenstabil dan fitoekstrator dalam pemulihan tanah yang disabitkan dengan keadaan pencemaran logam berat secara tunggal dan/atau campuran.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my utmost appreciation to all of my supervisors; Assoc. Prof. Dr. Noor Zalina Mahmood, Prof. Datuk Dr. Amru Nasrulhaq Boyce, Prof. Dr. Mohd. Radzi Abas and Assoc. Prof. Dr. Md. Motior Rahman for all their invaluable patience, ideas sharing, cooperative facilitation, continuous support and guidance throughout my entire journey of completing the doctorate degree.

Nevertheless, I am indeed thankful and indebted to my late father, beloved mother and all of my family members who have always supported and believed in me. Besides, a special thanks to my ever-supportive soul-mate, Ms. Tan Bee Luan who has steadily accompanied and encouraged me through thick and thin over the years. My heartfelt gratitude for all of the words of wisdom, devoted encouragement, unwavering prayers and unconditional love that they have given to me.

Moreover, a big thanks to Rimba Ilmu, Institute of Biological Sciences, Chemistry Department, Faculty of Science, University of Malaya, Kuala Lumpur and Malaysia Toray Science Foundation for providing me the research grants (PG006-2013A, RK001-2016 and STRG15/G251) as well as a conducive work-place (planthouses and laboratories) of all necessary use of facilities and instruments to carry out my research activities.

Last but never least, I would like to thank everyone especially my fellow friends who have given me in one-way or another for their cooperation and moral support to make this thesis a reality.

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LIST OF SYMBOLS AND ABBREVIATIONS

BAM Germany	:	Bundesanstalt für Materialforschung und –prüfung Germany
BOPRC	:	Bay of Plenty Regional Council
CCME	:	Canadian Council of Ministers of the Environment
DOE	:	Department of Environment, Malaysia
FAO	:	Food and Agriculture Organization of the United Nations
IUPAC	:	International Union of Pure and Applied Chemistry
JECFA	:	Codex Alimentarius Commission - Joint FAO/WHO Expert Committee on Food Additives
JPBD	:	Federal Department of Town and Country Planning Peninsular Malaysia
MARDI	:	Malaysian Agricultural Research and Development Institute
UNEP	:	United Nations Environment Programme
US EPA	:	United States Environmental Protection Agency
US FRTR	:	United States Federal Remediation Technologies Roundtable
WHO	:	World Health Organization
ANOVA	:	Analysis of variance
CRD	:	Completely randomized design
LSD	:	Least significant difference
BAC	:	Biological accumulation coefficient
BCF	:	Biological concentration factor
TF	:	Translocation factor
TI	:	Tolerance index
MAQ	:	Metal accumulation quotient
RGR	:	Relative growth rate
R/S	:	Root-shoot quotient
R/T	:	Root-tiller quotient

E longitude	:	East Longitude
N Latitude	:	North Latitude
EDDS	:	Ethylene-diamine-N,N'-disuccinic acid
EDTA	:	Ethylene-diamine-tetraacetic-acid
F-AAS	:	Flame atomic absorption spectrometer
CRM	:	Certified Reference Material
NA	:	Not Applicable / Not Available
N-fertilizer	:	Nitrogen-fertilizer
PVC	:	Polyvinyl chloride
R&M	:	Reichle & De-Massari
$Cd(NO_3)_2.4H_2O$	:	Cadmium nitrate tetrahydrate
$CuSO_4$	:	Copper (II) sulfate
HCl	:	Hydrochloric acid
HNO_3	:	Nitric acid
H_2O_2	:	Hydrogen peroxide
H_2SO_4	:	Sulfuric acid
$Pb(NO_3)_2$	:	Lead (II) nitrate
$ZnSO_4$	:	Zinc sulphate
$ZnSO_4.7H_2O$	:	Zinc sulphate heptahydrate
Al	:	Aluminium
As	:	Arsenic
Ba	:	Barium
Be	:	Beryllium
Bi	:	Bismuth
Ca	:	Calcium
Cd	:	Cadmium

Co	:	Cobalt
Cr	:	Chromium
Cu	:	Copper
Fe	:	Iron
Hg	:	Mercury
K	:	Potassium
Mg	:	Magnesium
Mn	:	Manganese
N	:	Nitrogen
Na	:	Sodium
Ni	:	Nickel
Pb	:	Lead
S	:	Sulfur
Sb	:	Antimony
Se	:	Selenium
Sn	:	Tin
V	:	Vanadium
Zn	:	Zinc
mm	:	Millimeter
cm	:	Centimetre
m	:	Meter
g	:	Gram
mg	:	Milligram
kg	:	Kilogram
mL	:	Milliliter
mmol	:	Millimole

g/m^2	:	Gram per meter square
mg/kg	:	Milligram per kilogram
mg/L	:	Milligram per liter
g/day	:	Gram per day
H^+	:	Hydrogen ion
pH	:	Potential hydrogen
C	:	Celsius (Centigrade)
p	:	Probability (statistics)
r	:	Regression (statistics)
R^2	:	Coefficients of determination
et al.	:	Et alii (and others)
%	:	Percentage
&	:	And
x	:	Multiply
/	:	Or
°	:	Degree
–	:	To
<	:	Less than
>	:	Greater than
>>>	:	Extremely greater than
>>	:	Much greater than
=	:	Equal to
±	:	Plus-minus
'	:	Arcminute
,	:	Comma

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background of study

Soil is an essential component for all living organisms, consisting layers of minerals and organic matters. Nevertheless, soils are often considered as limited natural resources due to the fact that 97% of the Earth's surface is covered by ocean while only 3% constitute land which is made up of different mixture of soils. As history has shown, fertile soil has predominantly acted the starting point for human civilization with massive agricultural productivity.

However, the rapid development of human urbanization has brought about the serious threat of soil pollution especially in the context of heavy metal contamination due to various kinds of uncontrolled anthropogenic activities and natural changes (Duruibe *et al*., 2007; Christoforidis & Stamatis, 2009; Nagajyoti *et al*., 2010; Mmolawa *et al*., 2011). In comparison to air and water, the widespread of land mass is easily the target for contaminants released into the land and is less diluted and tends to accumulate in high amounts due to the small coverage area of land on Earth.

Since the late 1970s, soil contamination was addressed as a global issue, as most of the industrialized countries in North America and the European Union were beset with soil contamination by heavy metal and chemical substances (Nriagu, 1990; GEM-WRI, 1993; Brännvall *et al*., 1999). At the international level, the United Nations Environment Programme (UNEP) as well as the Food and Agriculture Organization of the United Nations (FAO) acknowledged the problem of soil contamination and subsequently established the World Soil Charter in 1981 to institutionalize sustainable soil management at all levels. As a result, a myriad of remediation programmes and legislative actions took place worldwide.

Soil contaminated with heavy metals in Malaysia gained serious public concern recently and the Malaysian Department of Environment (DOE, 2009) classified heavy metal contaminated land to be any site in which substances present exceeded the natural occurring metal concentrations and are likely to pose an immediate or long term health risk to either human and/or the environment.

In Malaysia, activities from both the agriculture and industrial sectors brought a drastic increase in contaminated soils over the last three decades, especially during the implementation of the Malaysian Industrial Master Plan back in the 1980s (Sani, 1993; Aini *et al*., 2001; Othman *et al*., 2011). The scenario in Malaysia further worsened due to lack of enforcement by the authorities, coupled with the rather finite adoptions of relevant technologies for contaminated soil remediation, compared to developed countries (Yin *et al*., 2006; Othman *et al*., 2011). Moreover, the utilization of most of the national technologies were on a trial basis in the past. However, these technologies turned out to be useful in light of the formulation of holistic regulations and policies on sustainable soil remediation management earmarked under the Ninth Malaysia Plan (Yin *et al*., 2006, Yin & Abdul-Talib, 2006). The Ninth Malaysia Plan (2006-2010) pushed for the creation of the contaminated land management framework by the Malaysian Department of Environment, in order to assess and restore contaminated soils throughout the country. The first ever documented paper entitled *"Contaminated land management and control guidelines number 1: Malaysian recommended site screening levels for contaminated land"* outlined the site screening levels of criteria and standards for contaminated soil conditions (DOE, 2009).

Several factors, such as the types of pollutants, hydro-geological and local soil conditions, are required to be taken into consideration in order to select an appropriate remediation strategy for heavy metal contaminated soil. The most commonly available remediation methods include excavation (remove contaminants using an excavator), forced leaching (artificially increase water infiltration through the contaminated site to leach out contaminants), microbial remediation (using microbes to breaking down contaminants which are present in soil and groundwater) as well as the phytoremediation. Although there are a variety of remediation technologies being introduced, phytoremediation is the alternative environmentally friendly and inexpensive solution for heavy metal soil contamination (Raskin, & Ensley, 2000; Jadia & Fulekar, 2009; Purakayastha & Chhonkar, 2010).

Ultimately, the fast-growing tropical plant species Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash was selected, after a series of preliminary trials, for heavy metal phytoassessment. The use of Vetiver grass is central to a wide range of applications which have recorded many significant benefits such as for the prevention and treatment of contaminated water and land, soil erosion control, agriculture improvement, and landscaping purposes (Danh *et al*., 2009; Chomchalow, 2011; Donjadee & Chinnarasri, 2012).

1.2 Problem statement

The rapid development of industrialization and growth of the human population has allowed the production of considerable amounts of contaminated heavy metals released into the environment (Sarkar, 2002; Sarma, 2011). Heavy metal contamination in the soil has caused a major concern for the ecosystem and human health as it is extremely toxic even in low concentrations and is difficult to be degraded (Järup, 2003; Rascio & Navari-Izzo, 2011). These metals are harmful as it can be easily bio-accumulated and subsequently contaminate the food chain.

Globally, there have been numerous types of soil heavy metal remediation technologies, including phytoremediation. However, the development of phytoremediation for heavy metal contamination is still in its infancy stage, especially in Malaysia (Yin *et al*., 2006). There is huge interest in developing biological treatment through phytoremediation strategy to clean up heavy metal contaminated soils, given the high costs and number of contaminated sites in Malaysia (Ismail *et al*., 1993; Zarcinas *et al*., 2004; Shazili *et al*., 2006; Sany *et al*., 2013).

Acknowledging the phytoremediation approach has been less favourable in Malaysia, a comprehensive heavy metal phytoremediation study needs to be carried out to gauge its application feasibility. Although phytoremediation has received substantial research attention, the development of an eco-friendly and cost-effective heavy metal remediation technique still requires further consideration. Thus, there is a need to focus on the phytoassessment of a wide range of heavy metal soil contamination using available tropical plant species in Malaysia.

Despite the abundant variety of plants that have been used for phytoremediation (Sarma, 2011; Van der Ent *et al.*, 2013; Mahar *et al.*, 2016), the selection of suitable plant species and specific types for low cost soil amendments to enhance heavy metal uptakes is a concern that needs to be addressed. Consequently, identifying a prospective plant species that is resilient and has highly adaptable characteristics to withstand contaminated heavy metal soil conditions is required.

Nonetheless, the relatively slow and low efficiency process to remediate heavy metal contaminated soils using phytoremediation alone may not be practical (Glick, 2003; Ward & Singh, 2004; Karami & Shamsuddin, 2010). For this reason, recent studies by Evangelou *et al.* (2007), Kotrba *et al.* (2009), Rajkumar *et al.* (2012), Bhargava *et al.* (2012) and Sharma *et al.* (2016) have reported the successful use of various efficiency aided techniques to facilitate the uptakes of heavy metal in plants. However, many of these techniques are habitually complicated, difficult to handle and are not cost-effective. Hence, it is necessary to examine the possible application of low cost soil amendments for the enhancement of heavy metal accumulation from soil towards different plant parts.

Unlike other forms of water and air pollution, heavy metal contaminations in soil are often persistent and non-biodegradable over a long period of time (Duruibe *et al*., 2007; Singh *et al*., 2011). Nevertheless, the persistent environmental contaminants, such as cadmium (Cd) and lead (Pb) commonly exist in the environment and are of great concern as both metals are exceedingly hazardous to human well-being compared to the other kinds of heavy metal. Both the hazardous Cd and Pb can be easily taken in through direct ingestion of soil and dust, inhalation and/or consumption of contaminated plants, which can in turn substantially affect human health (Hough et al., 2004; Wuana & Okieimen, 2011).

Previous studies (Prasad *et al*., 2014; Singh *et al*., 2015; Banerjee *et al*., 2016; Vargas *et al*., 2016) have solely emphasized on the limited types of single metals and inadequately provide empirical evidence to explain the heavy metal phytoassessment of Vetiver grass in the plant parts (lower and upper sections of roots and tillers). As a result, there is a lack of findings addressing the phytoevaluation of Vetiver grass subjected to both single and mixed heavy metal contaminated soil conditions. Consequently, a comparative study on both single and mixed heavy metal contamination in the lower and upper plant parts of roots and tillers in Vetiver grass would provide new knowledge beneficial for the phytoremediation of heavy metal contaminated soils.

1.3 Significance of research study

Due to the wide selection of remediation technologies available now, the development as well as the *in-situ* application of biological remediation of soil heavy metal contamination using plants has been less popular in many developing countries including Malaysia (Singh *et al*., 2009) compared to other physical and chemical assisted remediation methods. As a result, it is hoped that this study will be able to highlight the importance of identifying an ideal phytoaccumulator plant species (Vetiver grass) with enhanced capability and capacity to bio-remediate heavy metal contaminated soils in Malaysia.

Over the years, Vetiver grass has been widely applied as a biological ground enhancer to control soil erosion and increase stability for affected slope conditions in Malaysia. This research study is significant as it will provide information and findings about the proficiency use of Vetiver grass for heavy metals phytoremediation in Malaysia. In addition, there have been a numerous studies conducted about heavy metal phytoremediation yet limited investigation made available on mixed heavy metal contamination. Despite the growing concern between single and mixed heavy metal spiked contamination, the phytoassessment studies using Vetiver grass require an urgent clarification. Thus, the main highlight from this study is to investigate the response and effects of both single and mixed heavy metal phytoaccumulation using Vetiver grass in different plant parts.

Besides, this study is of significant practical and scientific relevance, and it is hoped that the results will allow us to understand the trends of accumulation and translocation characteristics of heavy metals movement in Vetiver grass and other different types of plant species. Furthermore, this study will also provide a more comprehensive phytoassessment of the different plant parts; lower roots, upper roots, lower tillers and upper tillers in Vetiver grass growing under both single and mixed heavy metal contamination soil conditions.

Nonetheless, the present study is expected to provide incremental information on the mobility and bio-availability of enhanced heavy metal phytoremediation across different plant parts of Vetiver grass with the optimum application of different types of low cost soil amendments. Moreover, the results from these studies will contribute to developing specific guidelines and baseline data for phytoremediation of heavy metal contaminated soil using Vetiver grass in Malaysia. Ultimately, the findings of this study may possibly offer as alternative biological phytosolution to clean up (bio-remediate) as well as allow the regeneration of other human activities such as agriculture planting and other form of development at selected heavy metal contaminated soil sites in Malaysia.

1.4 Research questions and objectives

With reference to the problem statement outlined above, this research study will address the following research questions and objectives.

The research questions in this study are:

a) Among assorted types of tropical plants, is Vetiver grass suitable to be used as a phytoremediator for heavy metal contaminated soil conditions? (Chapter 3)

b) Is Vetiver grass able to withstand high concentration levels of heavy metal in contaminated soil? (Chapter 4)

c) What are the responses of Vetiver grass to bioaccumulate from soil-to-root and root-to-shoot (tiller) under both single and mixed heavy metal contaminated soil conditions? (Chapters 5 – 7)

d) Will the application of low cost soil amendments enhance the phytoremediation of heavy metals in Vetiver grass? (Chapter 6)

e) What are the optimum types and compositions level of soil amendments to maximize the overall total heavy metals phytoaccumulation in Vetiver grass? (Chapters 6 and 7)

The main research objectives of this study are:

1) To ascertain the suitability and viability of Vetiver grass to be used for phytoremediation (Chapter 3) under heavy metal contaminated soil conditions.
2) To evaluate the phytotolerance ability (Chapter 4) and trend of plant responses to bio-accumulate through phytoremediation (all Chapters) under both single and mixed heavy metal contaminated soil conditions using various soil-plant transfer co-efficients.
3) To investigate the capability and efficiency of bioaccumulation in both the lower and upper roots and tillers of Vetiver grass (Chapters 5 – 7) between different types of single and mixed heavy metal contaminated soil conditions.
4) To examine the effects and influence of different types of soil amendments on the enhancement of phytoavailability in Vetiver grass (Chapters 6 and 7) growing under both single and mixed heavy metal contaminated soil conditions.

1.5 Organization of dissertation

The flow of this dissertation is presented in a total of ten chapters addressing different aspects of specific topics in relation to the phytoremediation of heavy metal contaminated soils by Vetiver grass.

Chapter 1: General Introduction consisting of the background of study, problem statement, research questions and objectives as well as the significance of the research study.

Chapter 2: Literature Review covering the overall synthesis of the scientific research and development relevant to heavy metals soil pollution, biological remediation and phytoremediation systems.

Chapters 3 to 7 describe the different research experiments carried out and consist of a short introduction specific to the study, adopted materials and methods, followed by the presentation of results, discussion and conclusions.

Chapter 3 emphasises the preliminary evaluation and comparative studies between Vetiver grass and various types of selected tropical plants species used for heavy metal phytoremediation.

Chapter 4 addresses the phytotolerance and threshold level of Vetiver grass growing under both Cd and Pb contamination in soils.

Chapter 5 reports the comparative phytoassessment of Vetiver grass in both single and mixed heavy metal contaminated soil conditions.

Chapter 6 examines the effects of enhanced heavy metal phytoassessment of Vetiver grass in different types and levels of soil amendments.

Chapter 7 describes the influence of EDTA as an effective soil amendment for phytoevaluation on both single and mixed heavy metal contaminated soil conditions.

Chapter 8 focuses on the general discussion of phytoaccumulation using Vetiver grass in both single and mixed heavy metal contaminated soil conditions.

Lastly, Chapter 9 sums up the general conclusions and recommendations based on the results of the studies and general discussion from Chapter 3 to Chapter 8. It also outlines the achievement of all of the research objectives set at the beginning of the study and discusses the scientific contribution and offers positive recommendations for further study.

Notwithstanding, the study can be briefly summarized into three different phases of research activities which include the following:

Phase I: Preliminary phytoevaluation between Vetiver grass and other selected tropical plants growing under heavy metals contaminated soil conditions which aimed to fulfil the first and second research objectives.

a) Phytoremediation trials with selected tropical vegetables: Water spinach (*Ipomoea aquatica*) and okra (*Abelmoschus esculentus*) (Chapter 3).

b) Phytoremediation trials with selected tropical plants: Kenaf (*Hibiscus cannabinus*), antidesma (*Antidesma salicinum*), ficus (*Ficus trichnopoda*) and fern (*Cyatheaceae sp.*) (Chapter 3).

c) Phytoevaluation of selected tropical plants: Vetiver (*Vetiveria zizanioides*), acacia (*Acacia mangium*) and mucuna (*Mucuna bracteata*) growing under hydrotoxic soil-leachate conditions (Chapter 3).

d) Phytoassessment of three tropical grasses: Vetiver (*Vetiveria zizanioides*), imperata (*Imperata cylindrical)* and pennisetum (*Pennisetum purpureum)* growing under heavy metals contaminated soil conditions (Chapter 3).

Phase II: The advancement of higher concentration levels in Vetiver grass growing under both single and mixed heavy metals contaminated soil conditions which targeted to achieve the second and third research objectives.

a) Phytotolerance and threshold level of Vetiver grass (*Vetiveria zizanioides*) growing under single Cd and Pb contaminated soil conditions (Chapter 4).

b) Comparative phytoassessment of Vetiver grass (*Vetiveria zizanioides*) growing under both single and mixed heavy metal contaminated soil conditions (Chapter 5).

Phase III: The enhancement of heavy metals accumulation in Vetiver grass using low cost soil amendments under both single and mixed heavy metals contaminated soil conditions which intended to meet the third and fourth research objectives.

a) Enhanced phytoassessment of Vetiver grass (*Vetiveria zizanioides*) using three different types of low cost soil amendments growing under the mixed Cd-Pb contaminated soil conditions (Chapter 6).

b) Enhanced phytoevaluation of Vetiver grass (*Vetiveria zizanioides*) using EDTA growing under both single and mixed heavy metal contaminated soil conditions (Chapter 7).

CHAPTER 2: LITERATURE REVIEW

2.1 Soil contamination in Malaysia

In the past five decades, Malaysia has progressed rapidly with massive economic growth in various sectors ranging from agriculture to manufacturing industrial. These growths have led to the presence of many contaminated soils have become a subject of concern (Eng *et al*., 1989; Sani, 1993; Zarcinas *et al*., 2004; Sundaram, 2007; Jomo, 2013). At the international perspective, contaminated soil is closely attributed to be known as Brownfield where the terminology is coined to be any vacant and derelict land which was previously developed or is currently not fully used or partially occupied, that might be complicated by the presence of potential hazardous contaminants derived from past and present day transport systems, industries and domestic use (Alker *et al*., 2000; US EPA, 2002; Burnham-Howard, 2004).

However, the understanding of Brownfield in Malaysia is slightly varied with both government and private premise land areas that have been developed or abandoned as well as development areas that are not fully completed which might or might not to be contaminated by various sources of contaminants (JPBD, 2012). In point of fact, the Federal Department of Town and Country Planning Peninsular Malaysia (JPBD) has classified six different categories of Brownfield in Malaysia which include ex-mining and ex-quarry sites (category A), ex-landfills and dumpsites (category B), housing and industrial premises that has been abandoned for more than 10 years (category C), incomplete development projects that exceeded 10 years (category D), buildings that have been abandoned and unoccupied for more than 10 years (category E) as well as ex-depot or station of any infrastructure and utilities land areas (category F).

Malaysia is estimated to have over 10,000 Brownfield sites. However, there is no full listing and registration of these sites in Malaysia till the present day where most of these Brownfield sites have a high possibility to be polluted by many unknown contaminants including heavy metals (Yin & Abdul-Talib, 2006; Lee *et al*., 2009; JPBD, 2012). In this regard, many studies have shown that former industrial, ex-agricultural land and abandoned buildings sites and land that was previously used for residential activities are commonly contaminated with potentially hazardous heavy metals (Yusuf *et al*., 2003; Romic & Romic, 2013; Maas *et al*., 2010; Nagajyoti *et al*., 2010; Chabukdhara & Nema, 2013). Moreover, previous studies (Fauziah *et al*., 2001; Yin *et al*., 2006; Yin & Abdul-Talib, 2006; Ishak *et al*., 2011; Darus *et al*., 2011) have reported that many heavy metal contaminated soils are located in the Peninsular Malaysia including some enormous land areas that were used for agricultural production, such as Cameron Highlands, Pahang and ex-mining sites in Perak.

The existence of contaminated Brownfield sites in Malaysia have created many detrimental consequences such potential health hazards to the public, loss of ecological value, increase of unemployment and emigration of human population in all of the affected areas (Rowan & Fridgen, 2003; Yin & Abdul-Talib, 2006; Ellerbusch, 2006; Howland, 2007; Bambra *et al*., 2014). Nonetheless, the development of Brownfield remediation is still at a very infant stage as not many studies and information exposure have been conducted in Malaysia, unlike in other developed countries like the United States of America and United Kingdom.

2.2 Types of soil contaminants

Generally, all sources of soil contaminants can be divided into two mainstreams of organic and inorganic groups. Both of the organic and inorganic soil contaminants can be further separated into nine and seven different types of sub-categories, respectively.

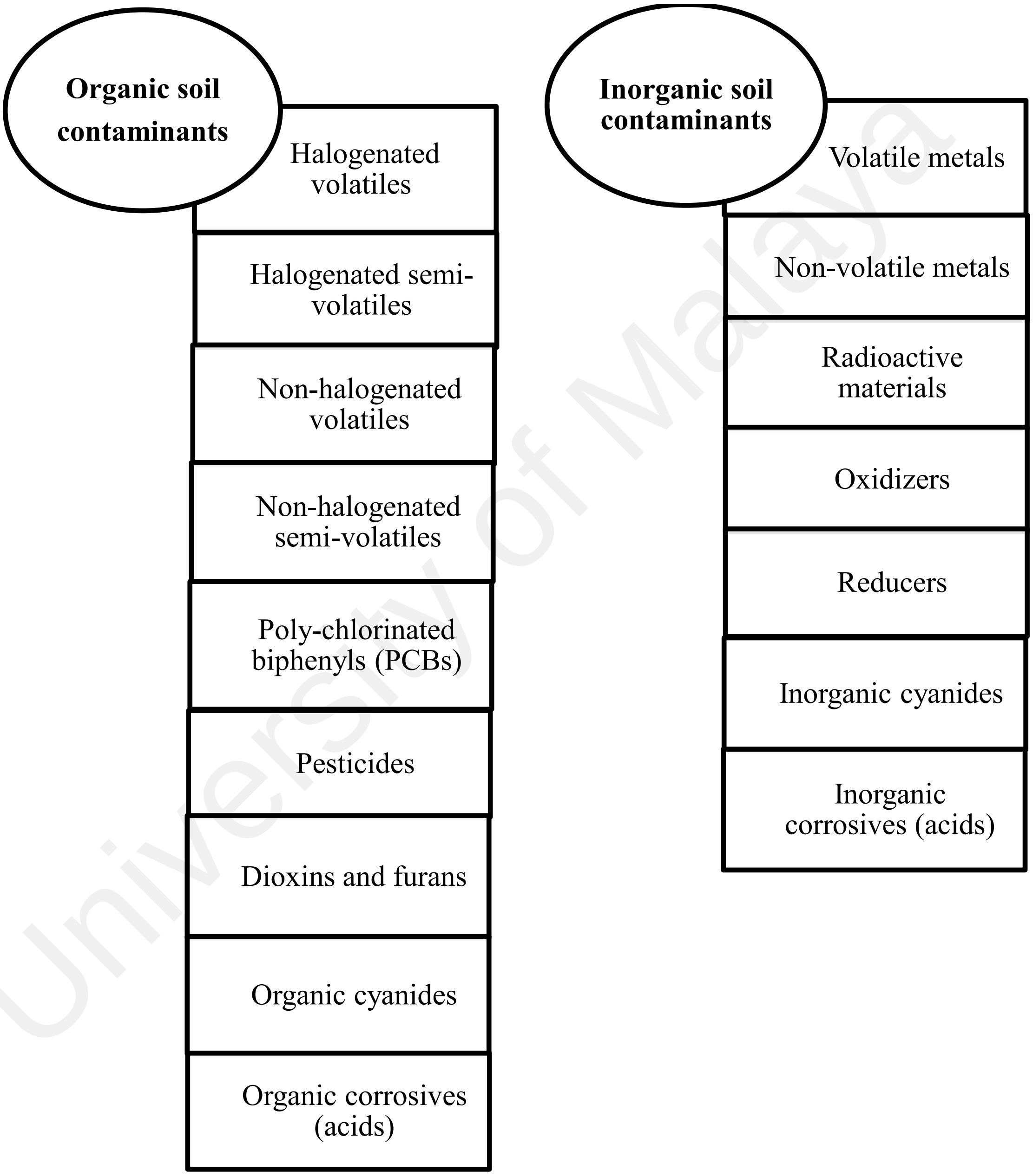

Figure 2.1: Different types of organic and inorganic sources of soil contaminants. Adopted and modified from Haines & Harris (1987), Harris *et al*. (1995), Mirsal *et al*. (2008), Shayler *et al*. (2009), Morrison & Murphy (2010) and Nathanail *et al*. (2011).

However, hazardous pollutants such as heavy metals, poly-aromatic hydrocarbons (PAH), cyanides and radioactive materials are a few of the most common and popular known sources of soil contaminants. As a matter of fact, heavy metals are inorganic soil contaminants and are widely sub-categorized under both volatile metals and non-volatile metals (Haines & Harris, 1987; Harris *et al*., 1995; Morrison & Murphy, 2010; Nathanail *et al*., 2011). Arsenic (As), bismuth (Bi), lead (Pb), mercury (Hg), tin (Sn) and selenium (Se) are examples of inorganic volatile metals. On the other hand, inorganic non-volatile metals consist of aluminium (Al), antimony (Sb), barium (Ba), beryllium (Be), cadmium (Cd), calcium (Ca), chromium (Cr), copper (Cu), cobalt (Co), Iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K), sodium (Na), vanadium (V) and zinc (Zn).

2.3 Heavy metals in soil

Over the years, the terminology for heavy metal is widely recognized to describe a large elemental group of metals and metalloids in the periodic table with relative atomic mass over 20 and specific density greater than 5 g/cm^3 (Hakers, 1997; Kemp, 1998; Hasanuzzaman & Fujita, 2012; Järup, 2003; Alloway, 2013). However, it is very difficult to define heavy metal precisely as there is a lot of confusion in the scientific literature where heavy metal is often being synonymously linked with toxic metals, toxic elements, trace metals and trace elements (Duffus, 2002; Appenroth, 2010; Kabata-Pendias, 2010; Hasanuzzaman & Fujita, 2012).

From the biological point of view, heavy metals are regarded as environmental pollutants of a series of toxic metals and metalloids which are not naturally perishable and are harmful to both plants and animals even at very low concentration levels (Abbasi *et al*., 1998; Rascio & Navari-Izzo, 2011; Hasanuzzaman & Fujita, 2012). This understanding is somehow contrary to the characterization provided by the International Union of Pure and Applied Chemistry (IUPAC) as discussed by Duffus (2002) and Alloway (2013). On the other hand, trace elements simply cover broader perspectives which include the abundance of various micronutrients, heavy metals and other non-metal elements such as halogens (Kabata-Pendias, 2010; Hasanuzzaman & Fujita, 2012; Alloway, 2013).

Typically, heavy metal can be classified into two main groups of essential and non-essential heavy metals. Copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), Nickel (Ni) and zinc (Zn) are the examples of essential metals which are commonly required for growth and metabolism of living organisms at low concentrations. In contrast, non-essential metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and mercury (Hg) have no vital biological functions and are not needed by living organisms for growth development (Leavitt *et al*., 1979; Sharma & Agrawal, 2005; Kabata-Pendias, 2010; Manara, 2012). Although the essential metals play an important role for the promotion of growth, these heavy metals can be health hazardous if it exceeded certain concentration levels.

2.4 Sources of heavy metals in soil

The sources of heavy metals in the environment can be due to both natural and anthropogenic causes. The natural sources of heavy metals are derived through possible channels of water flows and dusts from weathering of crustal materials, volcanic eruption, decaying vegetation, soil and rock cycle formation as well as forest fire.

On the other hand, there are a multitude of heavy metals being released through urban human development activities such as agriculture, construction, metal mining and smelting, road traffics, fossil fuel combustion and other various chemical based industrial processes. Human activities have acted as an essential natural driver for the geochemical cycling of elements as it triggers the mobilization and redistribution of heavy metals into the biosphere through water, air and soil pollution. Besides, the anthropogenic sources of heavy metals are often released in the form of aqueous, gaseous, particulate or solid from both diffuse and point sources (Han *et al*., 2002; Callender, 2003; Järup, 2003; Bradl, 2005; Wei & Yang, 2010; Hasanuzzaman & Fujita, 2012; Alloway, 2013).

2.4.1 Cadmium

Cadmium (Cd) is a metallic element in group 12 of the Periodic Table with an atomic number of 48, density of 8.65 g/cm^3 and relative atomic mass of 112.40. Cadmium is a soft and ductile with silvery-white lustrous metal that has a relatively high melting (321°C) and boiling (767°C) points and has the stable +2 oxidation state in aqueous solution. Cadmium ranks as 64th in order of abundance in the Earth's crust with an average concentration between 0.15 and 0.2 mg/kg. Cadmium is commonly found in natural deposition of aggregate ores containing other elements. The primary use of cadmium include of nickel-cadmium and silver-cadmium batteries, anti-corrosive metal coating (electroplating), plastic stabilizers, alloys, coal combustion, machinery and baking enamels and neutron absorbers in reactors (Siegel, 2002; Bradl, 2005; Naja & Volesky, 2009; Hasanuzzaman & Fujita, 2012; Mudhoo, 2012).

The main anthropogenic pathways for cadmium transportation are through waste dumping, suspended sediments, land application, atmospheric diffusion and wastewater from the different sources to the reservoirs. Cadmium is one of the most toxic heavy metals and is regarded as non-essential for living organisms. Nevertheless, after a long period of time, cadmium has been identified as one of the most toxic among the big three categories of heavy metals together with lead and mercury due to its lethal toxicity. Global attention on cadmium toxicity and contamination was only noticed after the environmental pollution outbreak of the 1912 *itai-itai* incidence in Japan due to severe cadmium poisoning. Chronic cadmium poisoning is believed to cause bone fraction, osteomalacia (softening of the bone), skeletal deformation, muscular rheumatism, kidney damage and renal disorder (Siegel, 2002; Bradl, 2005; Naja & Volesky, 2009; Hasanuzzaman & Fujita, 2012; Mudhoo, 2012).

2.4.2 Copper

Copper (Cu) is a reddish-brown and malleable metal with general melting point of 1084°C and a boiling point of 2562°C. Copper belongs to group 11 in the Periodic Table with the atomic number of 29, density of 8.94 g/cm^3 and 63.5 relative atomic mass. In aqueous solution, copper often exist in divalent (+2) oxidation state although some of the univalent and multivalent (+1, +3 and +4) copper complexes and compounds can occur in nature. Besides, copper is a good conductor as it possesses both high electrical and thermal conductivity. Copper is a moderately abundant source in the Earth's crust as it is an essential trace dietary mineral required by all living organisms (Abbasi *et al*., 1998; Dameron & Howe, 1998 Bradl, 2005; Hasanuzzaman & Fujita, 2012).

Copper is widely used to manufacture kitchenware, alloys, water pipes, roofing, electronic wiring as well as chemical and pharmaceutical equipment. The primary sources of copper release to land are from tailings and overloaded copper mines, electronic waste recycling industries and sewage sludge. The exposure of copper has been associated with toxic properties at high doses through topical deposition of dust, inhalation and ingestion in its various states. Copper toxicity often occur due to consumption of food that been cooked in an non-coated copper cookware and from exposure of contaminated copper in drinking water as well as other environmental sources. Acute copper poisoning may result in nausea, vomiting, diarrhea, hypotension, coma, hematemesis (vomiting of blood) and melena (black tarry feces) while long term exposure will cause extensive liver damage and kidney failure (Flemming & Trevors; 1989; Abbasi *et al*., 1998; Dameron & Howe, 1998; Gaetke & Chow, 2003; Bradl, 2005; Hasanuzzaman & Fujita, 2012).

2.4.3 Lead

Lead (Pb) is a bluish-white metal with atomic number of 82, density of 11.35 g/cm^3 and a relative atomic mass of 207.2 that found in group 14 of the Periodic Table. It is estimated that the concentration of lead in the Earth's crust is at 12.5 mg/kg and thus ranks as the 36^{th} element in abundance. Lead is a soft, very malleable, ductile but poor conductor of electricity which melts at 327°C and boils at 1749°C. Lead is widely used as car batteries, lead acid batteries, glassware, ceramics, alloys, cable sheathings, pesticides, plumbing, crystal glass and plastics productions. In the past, lead was wrongly used as pigments in paints and anti-knocking agents in gasoline until it was banned in the early 1990s as it caused severely hazardous health concerns. The historical application of lead in both paints and gasoline has led to an increased concentration of contaminated lead in the environment (Pirkle *et al*., 1998; Abbasi *et al*., 1998; Needleman, 2004; Bradl, 2005; Naja & Volesky, 2009; Morrison & Murphy, 2010; Hasanuzzaman & Fujita, 2012).

It is estimated that the emissions of lead per annum from both natural and man-made sources is about 24500 and 500000 metric tonnes, respectively. Lead is a non-essential metal that has no vital function for human biological systems and is recognized as a cumulative poisonous element. Many of the lead compounds are rather insoluble and easily bio-accumulate in cell tissues resulting to acute lead poisoning that will cause anaemia, headaches, irritability, loss of memory and coordination, abdominal discomfort, and tiredness. Nonetheless, the groups that are most sensitive to the exposure of lead are infants (including the unborn foetus), children and pregnant women. Children have a higher ability to absorb ingested lead and also have a higher susceptibility to the metal because of their rapid growth rate that eventually affect behavioural changes such as mental retardation and neurological deformation (Moncrieff *et al*., 1964; Abbasi *et al*., 1998; Naja & Volesky, 2009; Morrison & Murphy, 2010; Bradl, 2005; Hasanuzzaman & Fujita, 2012; Villarreal & Castro, 2016).

2.4.4 Zinc

Zinc (Zn) is a bluish-white metal that has an atomic number of 30, density of 7.133 g/cm^3 and relative atomic mass of 65.39. Zinc is an element that belongs to group 12 in the periodic table and has a melting point of 420°C and a boiling point of 907°C. Zinc presents solely as a divalent (+2) in all of it compounds and consists of give stable isotopes. Zinc is the 24th most abundance element with the average concentration in the Earth's crust estimated to be 70 mg/kg. Besides, zinc is a transitional element and is able to form complexes with a variety of organic ligands (Simon-Hettich *et al*., 2001; Abbasi *et al*., 1998; Hasanuzzaman & Fujita, 2012; Alloway, 2013).

Zinc is commonly used to make alloys (bronze and brass), anti-corrosion coating, batteries, cans, paints, welding fluxes and polyvinyl chloride (PVC) stabilizers. The major anthropogenic sources of zinc are often released to the environment through mining, zinc production facilities, iron and steel production, corrosion of galvanized structures, coal and fuel combustion, waste disposal and incineration and the use of zinc-containing fertilizers and pesticides. Zinc is an essential metal and at a very low concentration, acts as an important element for biological development. Nevertheless, exposure to high concentration levels of zinc will affect human well-being such as nausea, vomiting, stomach pains, dehydration, lethargy, dizziness and muscle incoordination (Fosmire, 1990; Abbasi *et al*., 1998; Simon-Hettich *et al*., 2001; Bradl, 2005; Duruibe *et al*., 2007; Plum *et al*., 2010; Hasanuzzaman & Fujita, 2012).

2.5 Toxicity of heavy metals in biological systems

Heavy metals are geological compartments of the Earth's materials and the majority of these metals are detrimental due its non-biodegradability in nature, thus having the ability to stay persistently in all parts of to the environment (Lombi *et al*., 2001; Duruibe *et al*., 2007; Massaquoi *et al*., 2015). Most of the heavy metals cannot be destroyed and degraded naturally. However, it can be transform from one oxidation state to another. Nevertheless, heavy metals are often present freely in the soil, water and atmosphere where it can cause harmful effects, even at very low concentrations, due to bioaccumulation and bio-magnification in food chain (Peralta-Videa *et al*., 2009; Chen *et al*., 2010; De Vries *et al*., 2013).

Human health is easily affected by the presence of heavy metals through soil, water and air pollution in natural bio-geochemical cycles. The exposure of heavy metals are mainly affected through direct atmospheric inhalation of dust particles as well as accidental food ingestion which may contain high levels of heavy metals particularly in contaminated seafood and vegetables (Duffus, 2002; Järup, 2003; Duruibe *et al*., 2007; Martin & Griswold, 2009; Singh *et al*., 2011).

Moreover, the intake of a small amount of heavy metals can seriously cause fatal damage and disturb the overall biological reactions. The negative implications of heavy metals accumulation can lead to the suppression of vital human organs through both soft and hard body tissues as it can cause various situations of sickness, depending on the overall exposure of heavy metal concentration levels. Similarly, high concentration of heavy metals will directly affect plant physiological and biochemical processes resulting in inhibition of growth, reduction in respiration and photosynthetic rate as well as degeneration of the main cell organelles (Foy *et al*., 1978; Clijsters & Assche, 1985;

Vangronsveld & Clijsters, 1994; Nagajyoti *et al*., 2010). In addition, excessive exposure to heavy metal will also increase the probability of potentially lethal diseases such as cardiovascular, nervous, kidney and skeletal system illnesses (Hu, 2002; Järup, 2003; Duruibe *et al*., 2007; Tchounwou *et al*., 2012).

2.6 International heavy metal soil permissible limits

The comparison of various international standards of allowable concentration levels of heavy metal in soil are shown in Table 2.1. Generally, there are huge variations in terms of the overall permissible levels for soil heavy metal standards stipulated in all the different international countries.

The specific and in-detail categorization of different soil types and the consideration of numerous tested parameters would contribute to the vast variations among all of the international standards (CCME, 1999; Lacatusu, 2000; Heinegg *et al*., 2000; US EPA, 2005; Provoost *et al*., 2006; Kabata-Pendias, 2010). However, the permissible limits set by the Department of Environment, Malaysia is the most stringent with the lowest maximum permissible levels for Cu (19.8 mg/kg), Pb (36.0 mg/kg) and Zn (54.3 mg/kg) as compared to all other international standards.

Table 2.1: International heavy metal soil permissible standards

	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
DOE[1]				
Min	0.09	4.00	0.18	6.90
Max	11.9	19.8	36.0	54.3
European[2]				
Normal	0.1 - 1.0	1.0 - 20.0	0.1 - 20.0	3.0 - 50.0
Max	3	100	100	300
US EPA[3]				
Soil	140	80	1700	120
Plant	32	70	120	160
CCME[4]				
Agricultural	3	150	375	600
Residential	5	100	500	500
Commercial	20	500	1000	1500
Industrial	20	500	1000	1500
International[5]				
Residential (Min)	0	63	60	100
Residential (Max)	37	3100	1000	23000
Industrial (Min)	1	91	300	360
Industrial (Max)	1400	41000	2500	100000
Kabata-Pendias[6]				
MAC	1 - 5	60 - 150	20 - 300	100 - 300

[1] Malaysian Department of Environment typical range of natural occurring metals concentrations (DOE, 2009)
[2] European Soil Bureau normal content intervals and maximum allowable limits (Lacatusu, 2000)
[3] Interim ecological soil screening levels for inorganic contaminants (US EPA, 2005)
[4] Canadian environmental quality guidelines interim soil quality criterion (CCME, 1999)
[5] International soil clean-up standards (Provoost *et al*., 2006)
[6] Ranges of maximum allowable concentrations (MAC) in agricultural soil (Kabata-Pendias, 2010)

2.7 Heavy metal contamination in plants

Since the 1970s, various studies of heavy metal contamination in all types of plants including grasses, shrubs and trees have been reported (Antonovics *et al*., 1971; Steffens, 1990; Killham & Firestone, 1983; Zen, 1996; Tsao, 2003; Ashraf *et al*., 2010; Golubev, 2011; Furini, 2012). The contamination of heavy metals in edible crop species such as vegetables have also been carried out in many places including Malaysia and other Asia-Pacific countries (Nadal *et al.*, 2005; Nordin & Selamat, 2013). It is well documented that heavy metals can inhibit many enzymes and thus able to disrupt the metabolic processes, including photosynthesis in most of the plants.

Mohamed *et al.* (2003) and Sharma *et al.* (2007) reported that heavy metals have different effects on various types of plants. Distinct plant species have the ability to accumulate metals from the environment and can be categorized as unsafe for consumption and other general uses if the plants are cultivated on or near to contaminated land. All plants require many sorts of essential macro and micro (trace) mineral nutrients for normal growth and development and these include nitrogen, phosphorus, potassium, magnesium, calcium, iron, zinc and sulphur. However, edible plant species like vegetables can easily absorb and take in heavy metals naturally into their vacuoles (Ismail *et al.*, 2005). It has been reported that arsenic, cadmium and lead are the most commonly available toxic heavy metals in soils while all other types of heavy metals are significantly toxic in high concentration amounts (Radwan & Salama, 2006). The ingestion of contaminated edible plants species (herbs, vegetables and fruits) grown in such contaminated soils will pose a danger to both animal and human health.

Generally, most people assume that all edible plants are healthy for consumption and are uncontaminated, not aware that some parts of the plant may be contaminated with heavy metals and other sources of contaminants. Heavy metals are non-biodegradable and can be very persistent in the environment and have the possibility to accumulate in different body organs (Radwan & Salama, 2006; Chailapakul *et al.*, 2008; Qishlaqi *et al.*, 2008). The long-term exposure (absorption, inhalation and ingestion) of contaminated plants, may lead to excessive heavy metals bioaccumulation of which eventually cause severe health problems in humans (Calderón *et al.*, 2003; Zhuang *et al.*, 2009; Woimant & Trocello, 2014). Bioaccumulation refers to the increase in concentration of a particular chemical or element in biological organisms over time and can be a threat to the well-being of plants, animals and human beings (Sharma *et al.*, 2006; Shi & Cai, 2009).

2.8 Phytoremediation strategies

In the early 1980s, the emergence of green plant based treatment or commonly known as phytoremediation started to receive considerable attention as plants were widely used for environmental clean-up. Phytoremediation is derived from the Greek word of *phyto* which refers to plant and the Latin suffix of *remedium* which simply means remediation or restoration. Phytoremediation involves the use of different plant species including trees, grasses and vegetables to remove, destroy and transform hazardous contaminants from various media of air, soil and water (Prasad, 2003; Mench *et al*., 2009; Karami & Shamsuddin, 2010; Ali *et al*., 2013).

2.8.1 Categories of phytoremediation

Generally, there are five main categories of phytoremediation (Figure 2.2) based on the nature of its remediation process which includes of rhizofiltration, phytostabilization, phytodegradation, phytoextraction and phytovolatilization.

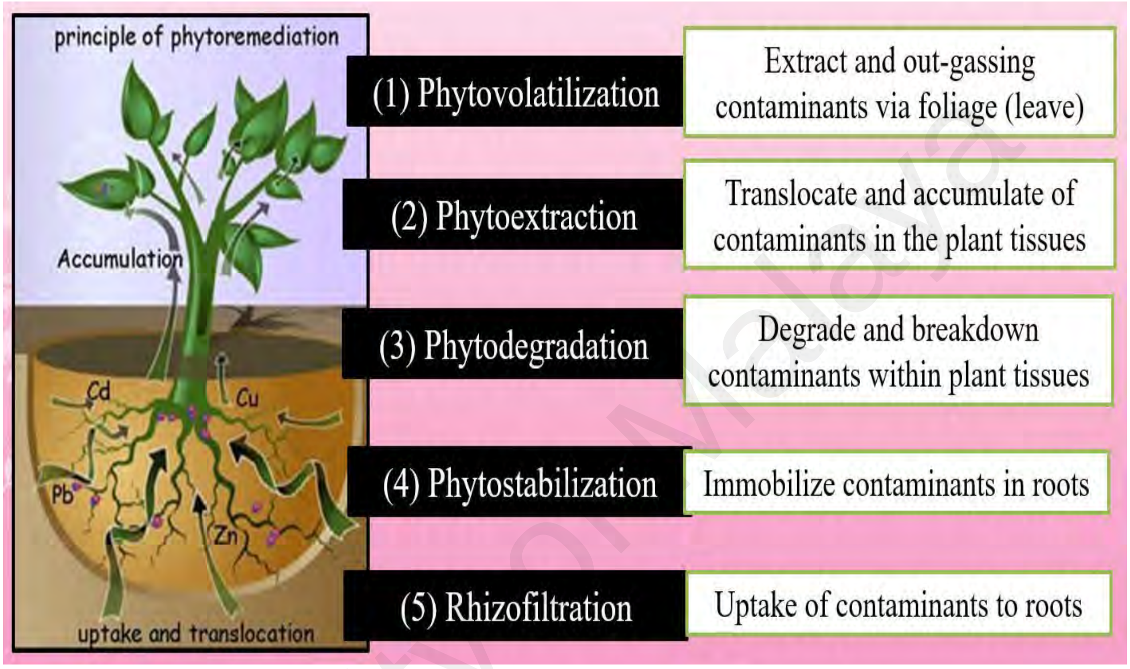

Figure 2.2: Major categories of phytoremediation. Adopted and modified from Änderung (2009), Gomes (2012), Anjum *et al.* (2012) and Hasanuzzaman & Fujita (2012).

Rhizofiltration or commonly also known as phytofiltration refers to the use of plants roots to absorb, uptake and precipitate toxic contaminants via the rhizosphere. Nevertheless, rhizofiltration involves the removal of contaminants in both terrestrial plants grown in soils as well as in aquatic plants under aqueous conditions within the root zones (Padmavathiamma & Li, 2007; Hasanuzzaman & Fujita, 2012; Aisien *et al.*, 2012). However, rhizofiltration is limited to selected plants species with extensive root systems and root biomass in order to have high ability to absorb large amount of contaminants through the root zone (Bradl, 2005; Hasanuzzaman & Fujita, 2012).

Phytostabilization is the use of plants to immobilize harmful contaminants in the soil through absorption and accumulation by roots, within the root zone. It is also referred to as phytoimmobilization as it helps to prevent the mobility of contaminants in the soil to be bio-accumulated through the food chain and groundwater contamination (Padmavathiamma & Li, 2007; Hasanuzzaman & Fujita, 2012; Mahar *et al*., 2016). Plants used for phytostabilization are generally poor translocators from roots to shoots as it primarily works to stabilize contaminants in the roots (Bradl, 2005; Alkorta *et al*., 2010; Hasanuzzaman & Fujita, 2012).

Phytodegradation or phytotransformation involves the breakdown of contaminants taken up by plants through metabolic processes that help to catalyse degradation (Newman & Reynolds, 2004; Aisien *et al*., 2012; Hasanuzzaman & Fujita, 2012). The contaminants are degraded and broken down into simpler and less toxic molecules which are then incorporated into the vascular plant systems. The process of phytodegradation is often aided by enzymes synthesized by plants to catalyse the degradation of contaminants in within plant tissues (Newman & Reynolds, 2004; Saxena & Misra, 2010; Hasanuzzaman & Fujita, 2012).

Phytoextraction or also known as phytoaccumulation is the uptake of contaminants by plant roots to the above ground plant tissues via a translocation pathway (Bradl, 2005; Padmavathiamma & Li, 2007; Hasanuzzaman & Fujita, 2012; Mahar *et al*., 2016). Plants with unusual capability to accumulate high concentration levels of contaminants are called hyperaccumulator (Baker and Brooks, 1989; Bradl, 2005). Phytoextraction appears to be the most common phytoremediation technique for the removal of contaminants in soils with the incorporation of various potential hyperaccumulator plant species.

Lastly, phytovolatilization is the uptake and evapotranspiration of contaminants by plants in volatile form which are then released into the atmosphere (Bradl, 2005; Padmavathiamma & Li, 2007; Hasanuzzaman & Fujita, 2012). It is also known as phytoevaporation and the process of phytovolatilization occur as contaminants translocate to the shoots of the plant and are released through the foliage to the environment. Phytovolatilization is commonly used to remove inorganic volatile contaminants such as arsenic, mercury and selenium which are present in the contaminated soil (Bradl, 2005; Padmavathiamma & Li, 2007; Hasanuzzaman & Fujita, 2012; Mahar *et al*., 2016).

2.9 Advantages and disadvantages of phytoremediation

There are both advantages and disadvantages in the application of phytoremediation technology. Although phytoremediation has not been extensively used, it provides potential advantages in various standpoints. In general, phytoremediation technology itself is naturally a feasible and easily adopted biological treatment method in most of the contaminated areas (Erakhrumen, 2007; Farid *et al*., 2013; Dong *et al*., 2014). It acts as an alternative remediation solution for both the physical and chemical treated strategies as phytoremediation is aesthetically pleasing and requires very minimum maintenance and monitoring (Pulford & Watson, 2003; Prasad & Freitas, 2003; Padmavathiamma & Li, 2007; Purakayastha & Chhonkar, 2010; Jan *et al*., 2014).

In terms of economic, phytoremediation is a relatively inexpensive and cost-effective technology compared to the other treatment methods (Garbisu & Alkorta, 2001; Pulford & Watson, 2003; Prasad & Freitas, 2003; Ali *et al*., 2013; Wan *et al*., 2016). As phytoremediation is a biological treated strategy driven by solar power, it significantly reduces operational expenses for both man-power and purchase of necessary equipment. As a result, it requires a low capital to kick-start the phytoremediation process and it is estimated to be about 10-20% of the total operation cost of a mechanical (physical) treatment of a similar remediation site (Susarla *et al*., 2002; Collins, 2007; Patil & Rao, 2014; Aisien *et al*., 2015). Furthermore, it gives added value as there can be potential recovery of valuable resources from harvested plants for bio-energy (bio-fuels), essential oils and fibres for animal feeds and handicrafts through phytoremediation treatment (Van Ginneken *et al*., 2007; Truong, 2008; Rockwood *et al*., 2013; Truong & Danh, 2015; Prasad, 2015).

From the environmental perspective, phytoremediation is certainly an environmentally friendly (soil cleaning) technology as it is potentially the least harmful method that provides minimum site destruction and uses naturally occurring plant species to remediate the affected areas (Barceló & Poschenrieder, 2003; Suresh & Ravishankar, 2004; Purakayastha & Chhonkar, 2010; Ali *et al*., 2013; Pirzadah *et al*., 2015; Haque & Khan, 2016). Phytoremediation technology is a safe treatment as it preserves the environment in a more natural state by reducing any potential creation of new source of pollution such as air, dust, odour and waste emissions into the environment.

Besides, phytoremediation also helps to build existing and create new natural habitats for different varieties of living organisms (Cunningham & Ow, 1996; Lin & Mendelssohn, 1998; Barceló & Poschenrieder, 2003; Weir & Doty, 2016). The application of plants using phytoremediation has indirectly given rise to the sequestration of carbon dioxide and other greenhouse gases as well as vegetation which are able to prevent runoff and soil erosion (Gomes, 2012; Witters *et al*., 2012; Marañón *et al*., 2016; Pandey *et al*., 2016).

On the other hand, there are a list of major limitations of phytoremediaiton as it is often a slower process in relation to the performance of plant growth compared to other physical and chemical remediation (Glass, 2000; Ward & Singh, 2004; Gomes, 2010; Surriya *et al*., 2014). Moreover, the process of phytoremediation is strictly restricted to the surface area and depth occupied by the roots (root zone) of the plant (Susarla *et al*., 2002; Pilon-Smits, 2005). As a result, phytoremediation is unable to completely clean up a contaminated area and is unable to prevent the possibility of contaminants leaching into the aquifers. Furthermore, not all plants are suitable for phytoremediation due to the wide range of natural physiological and adaptability variances. As a result, the phytoremediation strategy has gained a low level of public acceptance as many developing countries have limited resources and knowledge about the application of phytotechnology (Tucker & Shaw, 2000; Marmiroli & McCutcheon, 2003; Prasad, 2007; Weir & Doty, 2016). In terms of health concern, post-treatment of phytoremediation requires sanitary and safe disposal of the harvested plant materials as bio-magnification of contaminants in plants can be easily transmitted into the food chain if it is not properly handle (Mulligan *et al*., 2001; Ghosh & Singh, 2005; Paz-Ferreiro *et al*., 2014; Mahar *et al*., 2016).

2.10 Vetiver grass for heavy metal phytoremediation

Vetiver grass or scientifically known as *Vetiveria zizanioides* (L.) Nash is a perennial bunch grass which belongs to the grass (Poaceae) family. Although Vetiver grass is widely accepted as a common name, it has other local alternate names such as kus-kus, cuscus and khas-khas which refer to the same plant species (Maffei, 2002; Joy, 2009; Danh *et al*., 2009, Danh *et al*., 2012). The detailed scientific taxonomic classification of Vetiver grass is described as follow:

Kingdom: Plantae (Plants)

Phylum: Tracheobionta (Vascular plants)

Super-division: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants)

Class: Liliopsida (Mono-cotyledons)

Sub-class: Commelinidae

Order: Cyperales

Family: Poaceae (Grass family)

Subfamily: Panicoideae

Genus: *Vetiveria* Bory

Species: *Vetiveria zizanioides* (L.) Nash

Figure 2.3: The overall plant length of Vetiver grass (roots and tillers) of newly harvested plants at the end of 60-day of a control sample from study in Chapter 3.

Vetiver grass is believed to have originated from south India, where it was also domesticated, but is now found available throughout many tropical and sub-tropical countries including Malaysia (Maffei, 2002; Joy, 2009). Vetiver grass is often regarded as a magical plant as it possesses many unique morphological and physiological characteristics which enable it to be used for a wide range of purposes (Truong, 2008; Truong & Danh, 2015).

2.10.1 Morphological and physiological characteristics of Vetiver grass

Vetiver grass is a tall and tufted scented grass with straight stiff stems (tillers) which are able to grow up to three meters. It has a deep, massive and fast growing root system which is capable of reaching up three to four meters in the first year of planting (Grimshaw, 1995; Truong, 2002; Maffei, 2002; Truong, 2008). Most of the root systems in Vetiver grass are abundantly complex and extensive which allow great support and tolerance to extreme drought conditions by penetrating compacted soil layers to enhance deep drainage and allow infiltration of soil moisture (Truong, 2008; Danh *et al*., 2009; Truong & Danh, 2015). Nonetheless, Vetiver grass has another interesting characteristic as it has the ability to form thick hedges when planted close together which can act as an effective biological barrier to spread water runoff as well as a bio-filter to trap fine and coarse agricultural sediments (Greenfield, 1990; Dalton *et al*., 1996; Truong, 2002; Carlin *et al*., 2003; Truong, 2008; Danh *et al*., 2009).

In terms of its physiology, Vetiver grass is highly resilient to survive under many extreme environmental conditions such as droughts, floods, frosts and heat waves. It thrives under a wide range of soil pH (3.5 – 11.5), temperature ranging between -15°C and 55°C and tolerant to a high level of salinity (Truong, 2002; Truong, 2008; Danh *et al*., 2009; Edelstein *et al*., 2009). Moreover, Vetiver grass has good adaptability to elevate concentrations of various heavy metals and other sources of contaminants in both soil and water conditions (Maffei, 2002; Truong, 2008; Danh *et al*., 2009, Danh *et al*., 2012; Truong & Danh, 2015).

2.10.2 Multiple uses of Vetiver grass

Over the years, Vetiver grass has been widely used in both developed and developing countries where it has offered an extensive range of applications besides being able to remediate contaminated heavy metals in both soil and water (Maffei, 2002; Truong, 2008; Joy, 2009; Danh *et al*., 2012; Truong & Danh, 2015). Vetiver grass is holistically a beneficial plant species which provides significant economic, environmental and social values to mankind. The different applications of Vetiver grass are presented below:

a) **Prevention and treatment of contaminated land and water**

Vetiver grass has good adaptability to high concentration levels of heavy metals, agricultural chemicals and other sources of both organic and inorganic contaminants as it can be used to remediate and rehabilitate affected land and wastewaters (Maffei, 2002; Chen *et al*., 2004; Truong, 2008; Joy, 2009; Danh *et al*., 2012; Truong & Danh, 2015).

b) **Soil erosion control**

The dense hedges and extensive root systems of Vetiver grass enables it to form natural terraces across slope areas where it can act as vegetative barriers to minimize rainfall runoff as well as trapping erosion residues (Greenfield, 1990; Kemper *et al*., 1992; NRC, 1993; Xia *et al*., 1997; Truong, 2008; Joy, 2009; Chen *et al*., 2015b).

c) **Agriculture improvement**

Vetiver grass provides a variety of agricultural functions such as cleaning up contaminated agro-wastes, improving soil moisture content, recycling of soil nutrients and acting as a pest control in the farmlands. In addition, Vetiver grasses can be harvested for mulching, animal feeds (forage) and composts purposes (Greenfield, 2002; Truong, 2008; Lavania & Lavania, 2010; Danh *et al*., 2012; Truong & Danh, 2015; Donjadee & Tingsanchali; 2016).

d) **Landscaping**

Vetiver grass has also been utilized for various urban landscaping purposes such as to provide garden beautification, traffic separators, delineation of walk-ways, slope stabilization and many others (Greenfield, 2002; Chong & Chu; 2007; Truong, 2008; Danh *et al*., 2012; Chomchalow, 2012; Truong & Danh, 2015).

e) **Handicrafts**

Unlike many other plants, Vetiver grass provides a good source of exceptional materials (tillers and roots) to improve the quality of life for many communities living in developing countries where it has been used in handicrafts such as ornaments, coarse mats, bracelets, necklaces, shoes, hats, belts, baskets, roofs, perfumes and many others. Besides, Vetiver grass is also applied as a traditional medicine, ingredient in curry cooking and biomass fuels for generating electricity in certain particularly rural areas (Greenfield, 2002; Joy, 2009; Truong, 2008; Danh *et al*., 2012; Truong & Danh, 2015).

Ultimately, all of the application of Vetiver grass provides positive impacts towards the sustainable management of natural resources as well as improving human welfare and quality of life. Although inevitably that there are many other remediation techniques which can be used to tackle heavy metal contaminated soil problems, the heavy metal phytoremediation strategy is universally accepted as it offers a sophisticated biological solution which is cost-effective and able to remediate affected site without dramatically disturbing the landscape. As a result, heavy metal phytoremediation using Vetiver grass is an ideally reliable and feasible method that is able to improve contaminated soil conditions.

CHAPTER 3: [1] PRELIMINARY PLANT SCREENING: HEAVY METAL PHYTOEVALUATION OF VETIVER GRASS AND OTHER TROPICAL PLANTS

3.1 Introduction

Soil is commonly regarded as one of the significant natural resources that provide numerous essential elements and acts as a store for biodiversity, as a natural habitat for living organisms, food and biomass production as well as a relatively stable reservoir for the whole ecosystem. Soil is a limited resource that can easily deteriorate by both anthropogenic and natural changes. Soil contamination is the form in which pollutant materials are present at concentrations above naturally occurring levels and are likely to cause a direct and/or long-term danger to humans and the environment (DOE, 2009). Urban soil contamination has greatly affected many countries, including the United States, Germany, United Kingdom, China and India (Belluck *et al*., 2006; Meuser, 2010), and while heavy metal soil contamination itself has gained a serious attention globally.

Heavy metals can be very toxic even in low concentration and not easily degraded or destroyed. Heavy metal is generally harmful to humans and other living organisms as heavy metals can easily bio-accumulate and cause food chain contamination. Nevertheless, heavy metals often exist in small amounts in soils and plants as some of the trace metals play an essential role in promoting biological growth.

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[1] Two versions of this chapter have been published as: (1) Ng, C. C., Rahman, M. M., Boyce, A. N., & Abas, M. R. (2016). Heavy metals phyto-assessment in commonly grown vegetables: Water spinach (*I. aquatica*) and okra (*A. esculentus*). *SpringerPlus*, *5*(1), 469. and (2) Ng, C. C., Law, S.H., Boyce, A. N., Rahman, M.M, & Abas, M. R. (2016). Phyto-assessment of soil heavy metal accumulation in tropical grasses. *Journal of Animal and Plant Sciences*, *26*(3), 686-696.

In general, heavy metals can be categorized into essential and non-essential. Essential heavy metals such as nickel (Ni), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) are required by living organisms in trace amounts to support their metabolic functions while non-essential heavy metals such as chromium (Cr), arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd) are not needed for the growth of living organisms (Kabata-Pendias, 2011; Cuypers *et al*., 2013). Heavy metals and metalloids such as arsenic (As), chromium (Cr), mercury (Hg), cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) are hazardous and the metal toxicity can be severely hazardous if the concentration of a heavy metal exceeds its threshold level (DOE, 2009; Ng *et al.*, 2016a). Among all heavy metals; cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) are the most commonly found metals in contaminated sites (Wang *et al.*, 2009).

Many soil remediation technologies have been used over the last few decades, and phytoremediation has emerged to be one of the most cost-effective and eco-friendly solution for soil metal contamination (Glass, 2000; Purakayastha & Chhonkar, 2010). In phytoremediation, plants are utilized to remove various hazardous substances present in the environment including organic compounds, inorganic ions, heavy metals and radioactive materials. As a consequence, the phytoremediation approach has gained much attention and numerous plants species have been tested for phytoremediation characteristics, including vegetable crops, ornamental flowers, trees, weeds and grasses.

Correspondingly, the aims of this chapter were to evaluate the preliminary feasibility and viability of Vetiver grass (*V. zizanioides*) to be selected for heavy metal phytoassessment, compared with other different types of tropical plant species growing under contaminated soil conditions. To augment these findings, three different sets of experimental studies were specifically designed as follow:

a) Phytoassessment of heavy metals in tropical vegetables: Water spinach (*I. aquatica*) and okra (*A. esculentus*) growing under heavy metal contaminated soil conditions.
b) Phytoassessment of heavy metals in tropical plants: Vetiver (*V. zizanioides*), acacia (*A. mangium*) and mucuna (*M. bracteata*) growing under hydrotoxic soil-leachate conditions.
c) Phytoassessment of heavy metals in tropical grasses: Vetiver (*V. zizanioides*), imperata (*I. cylindrical*) and pennisetum (*P. purpureum*) growing under heavy metal contaminated soil conditions.

3.2 Materials and Methods

3.2.1 Site description and experimental design

All of three experimental studies were conducted at the planthouse located in Rimba Ilmu, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur with the average temperature ranging between 23.5°C and 34.5°C and relative humidity around 76.0%, as recorded by a RR Group Data Logger. Top soil (0 – 20 cm) for planting, was taken from a field situated at 3° 7' N latitude and 101° 39' E longitude. On the other hand, the treated leachate was collected from the closed Taman Beringin landfill, Jinjang, Kuala Lumpur, Malaysia. Water spinach and okra seeds were provided by the Malaysian Agricultural Research and Development Institute (MARDI) sown in control soil for about 21 days to acclimatize the seedlings. The plantlets of acacia as well as mucuna and Vetiver were obtained from the Lentang Seed and Planting Material Center, Forestry Department of Peninsular Malaysia and Humibox Malaysia, respectively.

The experimental study of heavy metals phytoassessment in tropical vegetables using water spinach and okra were placed under four different treatments (Table 3.1) of spiked heavy metals: Control, Pb (50 mg Pb/kg soil), Zn (50 mg Zn/kg soil), Cu (50 mg Cu/kg soil) while the phytoassessment of heavy metals in tropical plants using Vetiver, acacia and mucuna were placed under six different levels of hydrotoxic soil-leachate treatments (Table 3.2). Subsequently, the experimental study of heavy metals phytoassessment in tropical grasses using Vetiver, imperata and pennisetum were placed under five different treatments (Table 3.3) of spiked heavy metals: Control, Cd (15 mg Cd/kg soil), Pb (140 mg Pb/kg soil), Zn (250 mg Zn/kg soil) and Cu (20 mg Cu/kg soil). All the treatments were conducted under a completely randomized design (CRD) with three (n = 3) and four (n = 4) replications.

Table 3.1: Experimental treatment variables for heavy metals phytoassessment in tropical vegetables using water spinach and okra.

Treatment	Spiked treatment (mg/kg)
Control	No heavy metal added
Pb	50 Pb
Zn	50 Zn
Cu	50 Cu

Table 3.2: Experimental treatment variables for heavy metals phytoassessment in tropical plants using Vetiver, acacia and mucuna.

Treatment	Soil and treated leachate (%)
Control	100% soil
80S + 20L	80% soil + 20% treated leachate
60S + 40L	60% soil + 40% treated leachate
50S + 50L	50% soil + 50% treated leachate
40S + 60L	40% soil + 60% treated leachate
20S + 80L	20% soil + 80% treated leachate
100L	100% treated leachate

Table 3.3: Experimental treatment variables for heavy metals phytoassessment in tropical grasses using Vetiver, imperata and pennisetum

Treatment	Spiked treatment (mg/kg)
Control	No heavy metal added
Cd	15 Cd
Pb	140 Pb
Zn	250 Zn
Cu	20 Cu

3.2.2 Soil pre-treatment and plant preparation

Preliminary soil assessment (physical, biological and chemical) was carried out on the collected soil before it was air-dried in a large container for a week. This was followed by <4mm sieving, using a stainless steel test sieve to remove gravel and large non-soil particles. The concentrations of artificially spiked metal treatments were prepared based on the range of heavy metal as proposed by the Malaysian guidelines for soil contamination (DOE, 2009), the European Union heavy metals threshold limits (Lado *et al.*, 2008) and the Canadian Council of Ministers of the Environment soil quality guidelines (CCME, 1999) which exceed the median permissible natural occurring levels using the cadmium nitrate tetrahydrate [$Cd(NO_3)_2.4H_2O$], lead (II) nitrate [$Pb(NO_3)_2$], zinc sulphate heptahydrate [$ZnSO_4.7H_2O$] and copper (II) sulfate [$CuSO_4$] salt compounds. All chemicals used were of analytical reagent standard or of the best grade available.

The amended soil treatments were then continuously stirred and incubated for a week to ensure the homogeneity of the desired spiked heavy metal treatments are obtained. An initial uniform height of all plantlets was then planted in a plastic pot (0.1m x 0.12m) that filled with 2 kilograms of soil, for all the treatments. All treatments were watered evenly with 50mL of tap water once a day and their growth performance observed throughout the 49-day, 60-day and 75-day of experimental periods.

3.2.3 Preparation of samples and chemical analysis

Freshly harvested plants were washed in running filter water and rinsed thoroughly with deionized water to remove any adhering soil particles before separating them into roots and shoots (tillers). Fresh weights of plant samples were determined before the samples were oven-dried for 72 hours at 70°C until it achieved a constant weight. Then the dry weight of the plant samples was determined before it was homogenized in a mortar and pestle and digested with hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) and nitric acid (HNO_3). Approximately, 0.5g of the homogenized dried root and shoot samples underwent acid digestion according to Method 3050B (US EPA, 1996) followed by the Method 7000B (US EPA, 2007a) for the elemental analysis using the Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (F-AAS). Soil samples were also air-dried for 72 hours until it reached a constant weight before it was analysed following similar analytical procedures. The precise chemical analysis method was controlled using the Bundesanstalt für Materialforschung und –prüfung (BAM Germany): German Federal Institute for Materials Research and Testing (BRM#12-mixed sandy soil) certified reference material with the Cd (95.79 ± 1.17 %), Pb (97.03 ± 3.63 %), Zn (119.34 ± 12.82 %) and Cu (93.47 ± 0.81 %) rate of metal recovery.

3.2.4 Statistical analysis and data interpretation

The experimental data were analysed by one-way analysis of variance (ANOVA) to evaluate the growth performance and metal accumulation in all of the three different sets of experimental studies. Further statistical validity test for significant differences among treatment means, was carried out using Fisher's least significant difference (LSD) tests at the 95% and 99% level of significance.

Growth performance was evaluated using the root-shoot (R/S) quotient, tolerance index (TI) and relative growth rate (RGR) formula (Watson, 1952; Hunt, 1990; Poorter & Garier, 2007) whilst the ability for metal accumulation and translocation upwards in the plant species were assessed by determining the biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF), metal accumulation quotient (MAQ) and metal uptake efficacy (Kabata-Pendias, 2010; Alloway, 2013; Ali *et al*., 2013) as follows:

R/S quotient = Dry matter content in root / Dry matter content in shoot;

TI = Dry matter content in heavy metal treatment / Dry matter content in control;

RGR = [ln (Final biomass of treatment) – ln (Initial biomass of treatment)] / Days of growth;

BCF = Concentration of heavy metal in root / Concentration of heavy metal in soil;

BAC = Concentration of heavy metal in shoot / Concentration of heavy metal in soil;

TF = Concentration of heavy metal in shoot / Concentration of heavy metal in root;

MAQ = [Metal concentration accumulated in shoot x Dry matter content in shoot] / [Metal concentration accumulated in root x Dry matter content in root]; and

Metal uptake efficacy (%) = [Metal concentration accumulated in shoot / Total metal concentration removed from the growth media] x 100.

3.3 Results

3.3.1 Heavy metal phytoassessment in tropical vegetables (water spinach and okra)

3.3.1.1 Preliminary soil assessment

Preliminary soil assessment and initial concentrations of Pb, Zn and Cu metals (Table 3.4) from the collected control and spiked soils were examined using the flame atomic absorption spectrometer (F-AAS). The soil texture of the growth media composed of 71.6% clay, 3.9% silt and 24.5% sand. The texture of the soil is an essential aspect for plant growth as it influences soil fertility, soil porosity, soil stability, ease of tillage and nutrient retention. Soil pH and growth parameters such as number of leaves and plant height were measured throughout the experiments. Water spinach and okra were tested as individual experiments.

Table 3.4: Preliminary growth media soil parameters

Characteristic (Unit)	Mean ± SD
Soil texture	
Sand (%)	24.5
Silt (%)	3.9
Clay (%)	71.6
Soil pH	5.06 ± 0.43
Soil moisture content (%)	19.41 ± 3.62
Soil metal contents (mg/kg)	
Pb	1.23 ± 0.19
Zn	0.41 ± 0.05
Cu	0.55 ± 0.01

SD = standard deviation

3.3.1.2 Soil characterization

Before planting, the pH of the soil varied from 4.88 to 6.08, where the control soil in water spinach recorded the highest pH of 6.08 while the lowest pH of 4.88 was observed in the okra spiked Cu soil. Upon harvesting, all of the water spinach and okra treatment soils showed a decline in pH ranging from 4.63 to 5.01 where the highest pH reduction (-1.27 pH units) was recorded in spiked Pb of water spinach treatment. However, as can be seen in Figure 3.1 and Figure 3.2, there were no significant differences (*p>0.01*) observed between soil pH for water spinach and okra despite the fact that both plants were grown in non-optimum soil pH levels.

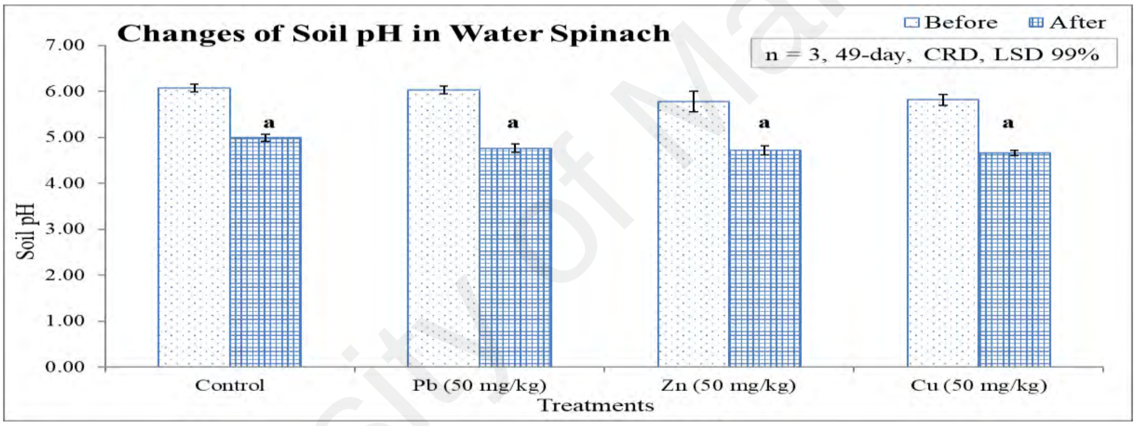

Figure 3.1: Changes in soil pH in water spinach as influenced by different treatments. Vertical bars represent ± standard deviation in treatment means and same letters are not significantly different at 0.01 levels of probability.

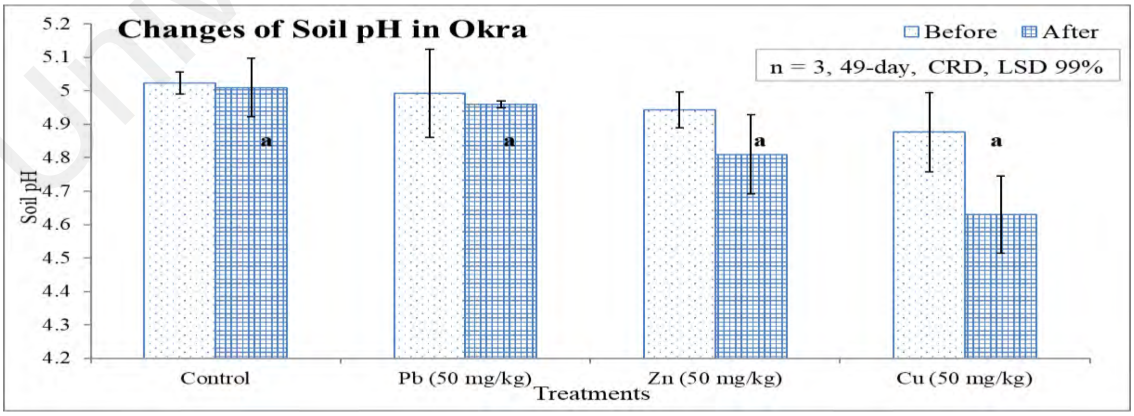

Figure 3.2: Changes in soil pH in okra as influenced by different treatments. Vertical bars represent ± standard deviation in treatment means and same letters are not significantly different at 0.01 levels of probability.

3.3.1.3 Plant growth performance

Plant growth performance was monitored by three parameters, namely plant height, number of leaves and dry matter content for each treatment. There were no significant differences (*p>0.01*) found between plant height for both water spinach and okra even though all the plants were grown in different spiked metal soils. Both water spinach and okra recorded average plant height ranging from 11.4 cm to 36.0 cm in all the treatments throughout the growth period (Figure 3.3 and Figure 3.4). However, opposite trend was observed in okra, whereby all the okra plants recorded a slower growth (plant height and number of leaves) compared to water spinach. Only the spiked Cu treatment in water spinach showed a significant decreased (*p<0.01*) in terms of the total number of leaves (Figure 3.5 and Figure 3.6).

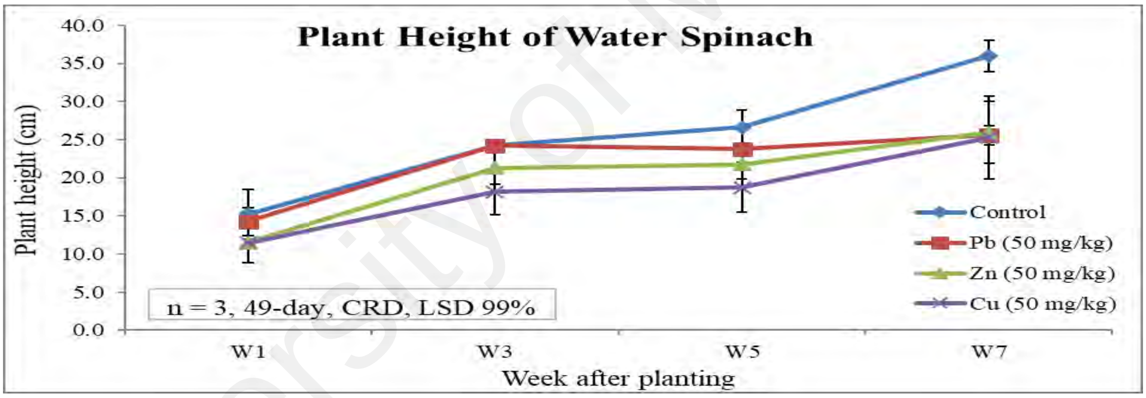

Figure 3.3: Plant height (cm) of water spinach as influenced by different types of treatments. Vertical bars represent ± standard deviation in treatment means and same letters are not significantly different at 0.01 levels of probability.

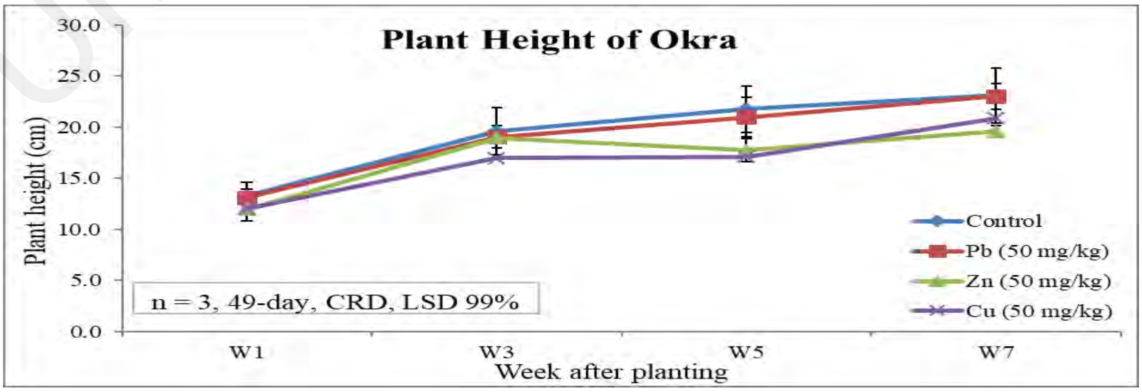

Figure 3.4: Plant height (cm) of okra as influenced by different types of treatments. Vertical bars represent ± standard deviation in treatment means and same letters are not significantly different at 0.01 levels of probability.

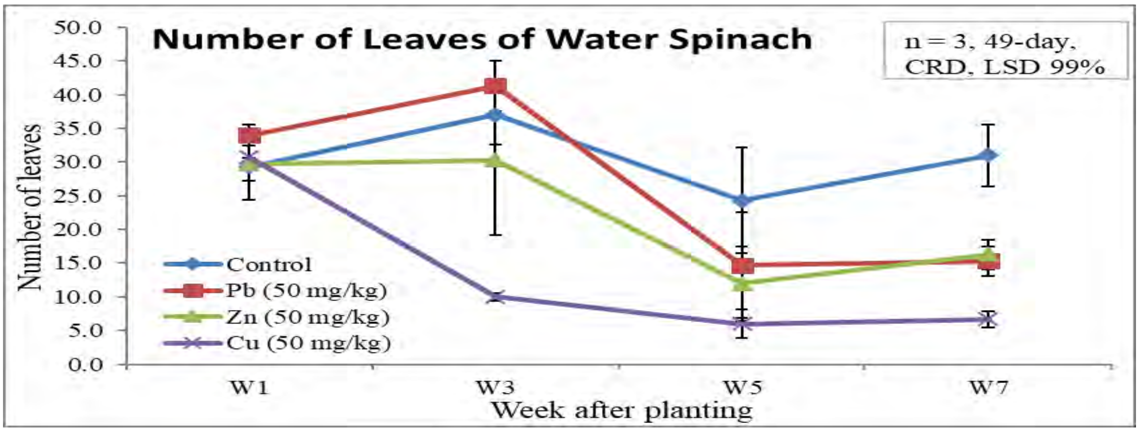

Figure 3.5: Number of leaves of water spinach as influenced by different types of treatments. Vertical bars represent ± standard deviation in treatment means and same letters are not significantly different at 0.01 levels of probability.

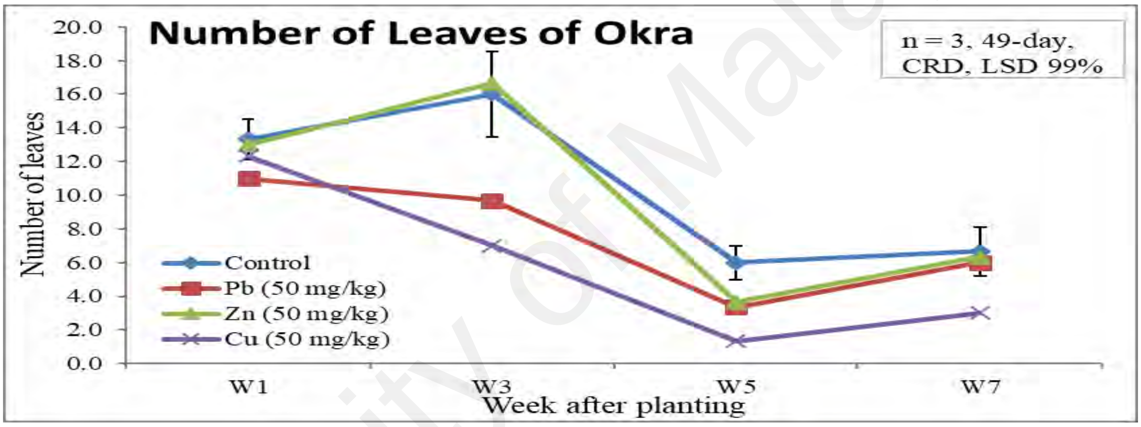

Figure 3.6: Number of leaves of okra as influenced by different types of treatments. Vertical bars represent ± standard deviation in treatment means and same letters are not significantly different at 0.01 levels of probability.

Okra in spiked Cu treatment recorded the lowest number of leaves with a total average of 1.3 leaves on week 5 while the highest number of 41.3 leaves was observed in water spinach control treatment on week 3. The average number of leaves in water spinach ranged from 6.0 to 41.3, while in okra it ranged between 1.3 and 16.7, throughout the entire growth period. It can be clearly seen that both water spinach and okra grown in the spiked Cu treatment, recorded the lowest number of leaves as compared to other spiked metal treatments.

Similarly, with regard to dry matter content, there were no significant differences (*p>0.01*) found between the dry matter contents among all water spinach and okra treatments despite the fact that both plants were grown in different spiked heavy metal contents (Figure 3.7). The water spinach control treatment recorded the maximum dry matter content per pot area (122.08 ± 17.1 g/m^2) followed by the spiked Pb treatment (93.25 ± 6.7 g/m^2), while okra grown in the spiked Cu treatment recorded the lowest dry matter content per pot area (31.08 ± 4.5 g/m^2).

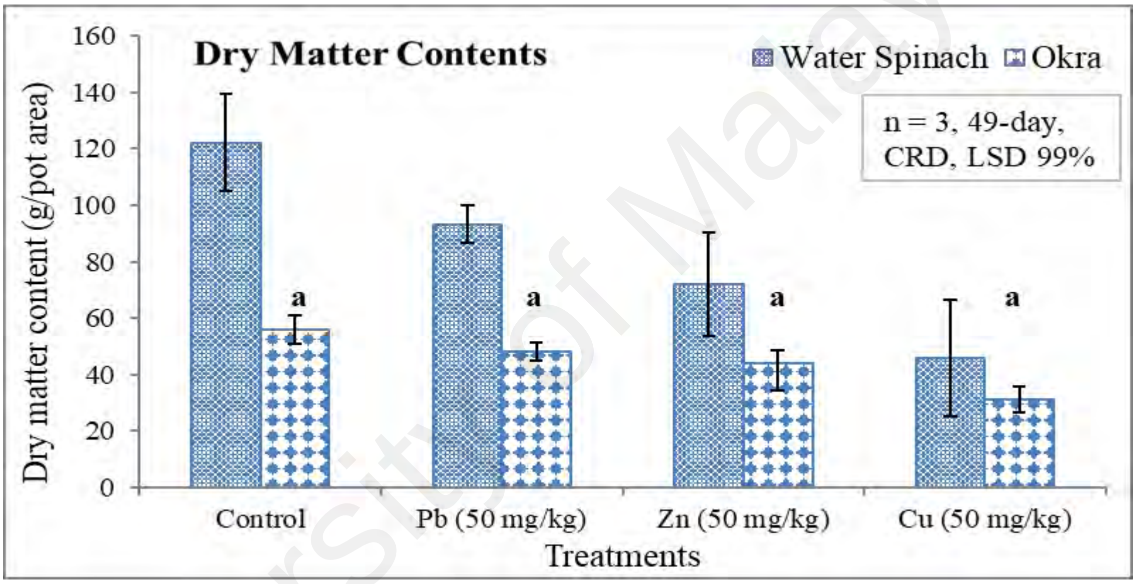

Figure 3.7: Dry matter content (g/m^2) of water spinach and okra as influenced by different types of treatments. Vertical bars represent ± standard deviation in treatment means and same letters are not significantly different at 0.01 levels of probability.

3.3.1.4 Heavy metal accumulation in plants

Table 3.5 – Table 3.10 show the concentration of metal ions accumulated in the roots and shoots of both water spinach and okra in the different types of spiked metal treatments. Edible sections of shoots in both vegetables include the stems and leaves for water spinach whereas okra contain steams, leaves and fruits. All three Pb, Zn, Cu metals accumulated in different amounts in both water spinach and okra plants. Heavy metal accumulation of Pb, Zn and Cu was significantly higher ($p<0.01$) in all spiked metal treatments, compared to the control for both water spinach and okra.

Between shoot and root, the Pb, Zn and Cu accumulation was relatively higher in the roots of both water spinach and okra. Among the treatments, Pb treated okra (80.20 ± 4.7 mg/kg) and water spinach (27.69 ± 3.5 mg/kg) recorded a significant increase ($p<0.01$) of Pb accumulation in the roots compared to the control. Similarly, Pb treated water spinach (30.31 ± 4.1 mg/kg) and okra (18.51 ± 5.2 mg/kg) recorded significantly higher ($p<0.01$) accumulation of Pb in the shoots, compared to the non-metal spiked control treatments.

On the other hand, the highest Zn metal accumulation in water spinach and okra were recorded in both roots and shoots of Zn treated plants. All spiked metal treated okra showed significantly higher ($p<0.01$) Zn accumulation in the roots when compared to the control treatment. However, only Zn treated water spinach (35.10 ± 2.7 mg/kg) and okra (5.18 ± 1.2 mg/kg) recorded a significant increase ($p<0.01$) compared to the control treatment for Zn accumulation in the shoots.

A significant increase (*p<0.01*) of Cu accumulation was observed in the roots of Cu treated water spinach (34.80 ± 3.4 mg/kg) and okra (10.08 ± 2.4 mg/kg) among other treatments. The shoots of Cu treated water spinach (18.87 ± 2.6 mg/kg) and okra (2.62 ± 2.4 mg/kg) also showed significantly higher (*p<0.01*) Cu accumulation compared to the control. Between shoot and root, a greater accumulation of Cu was observed in the roots of all treatments for both water spinach and okra. However, the opposite was found in some of the treatments with Pb and Zn metal accumulation. Amongst all three different types of heavy metal, water spinach recorded the highest metal accumulation for Pb (30.31 ± 4.1 mg/kg), Zn (35.10 ± 2.7 mg/kg) and Cu (18.87 ± 2.6 mg/kg) in the shoots.

Table 3.5: Concentration of Pb (mg/kg) in water spinach as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Pb (mg/kg)		
	Root	Shoot	Total
Control	5.67 ± 4.6 b	4.31 ± 4.1 b	9.98 b
Pb	27.69 ± 3.5 a	30.31 ± 4.1 a	58.00 a
Zn	3.77 ± 1.7 c	3.29 ± 2.3 c	7.06 d
Cu	3.92 ± 0.7 c	3.94 ± 1.7 bc	7.86 c

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.6: Concentration of Pb (mg/kg) in okra as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Pb (mg/kg)		
	Root	Shoot	Total
Control	5.00 ± 1.2 d	4.18 ± 2.9 c	9.18 c
Pb	80.20 ± 4.7 a	18.51 ± 5.2 a	98.71 a
Zn	6.12 ± 3.5 b	13.97 ± 2.1 b	20.09 b
Cu	5.25 ± 2.3 bc	4.01 ± 2.9 d	9.26 c

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.7: Concentration of Zn (mg/kg) in water spinach as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Zn (mg/kg)		
	Root	**Shoot**	**Total**
Control	1.71 ± 1.6 b	1.42 ± 2.6 b	3.13 b
Pb	1.43 ± 0.7 bc	1.26 ± 0.6 bc	2.69 bc
Zn	35.70 ± 3.7 a	35.10 ± 2.7 a	70.80 a
Cu	0.94 ± 0.7 bc	1.34 ± 1.7 bc	2.28 c

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.8: Concentration of Zn (mg/kg) in okra as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Zn (mg/kg)		
	Root	**Shoot**	**Total**
Control	1.10 ± 0.6 d	1.89 ± 1.2 bc	2.99 d
Pb	1.31 ± 0.7 b	1.98 ± 2.9 b	3.29 b
Zn	9.32 ± 2.9 a	5.18 ± 1.2 a	14.50 a
Cu	1.23 ± 0.7 c	1.95 ± 1.7 bc	3.18 bc

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.9: Concentration of Cu (mg/kg) in water spinach as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Cu (mg/kg)		
	Root	Shoot	Total
Control	0.70 ± 1.2 d	0.24 ± 0.4 d	0.94 d
Pb	1.70 ± 0.3 c	1.10 ± 0.5 c	2.80 c
Zn	4.20 ± 2.0 b	1.52 ± 0.3 b	5.72 b
Cu	34.80 ± 3.4 a	18.87 ± 2.6 a	53.60 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.10: Concentration of Cu (mg/kg) in okra as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Cu (mg/kg)		
	Root	Shoot	Total
Control	1.03 ± 0.6 c	0.25± 0.1 c	1.28 d
Pb	1.25 ± 0.7 b	0.43 ± 0.2 b	1.68 b
Zn	1.16 ± 0.6 bc	0.23 ± 0.1 c	1.39 c
Cu	10.08 ± 2.4 a	2.62 ± 0.7 a	12.70 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

3.3.1.5 Heavy metal translocation

The different heavy metal accumulation in the roots and shoots of both water spinach and okra are presented together with the associated translocation factor (TF) and biological concentration factor (BCF) as shown in Table 3.9 – Table 3.16. Despite the poor bioavailability of metals in the soil, plants have a high ability to accumulate metals into different plant parts and this may subsequently pose risks to human health, especially when the plants are cultivated on or near heavy metal contaminated areas.

The amount of metal translocated into the different parts of the plant, especially into the edible portion, are crucial and hence, the soil-plant transfer coefficients of the translocation factor (TF) and biological concentration factor (BCF) have been used to determine the overall metal concentrations in the different plant parts, namely the roots and shoots. The accumulations of Pb and Zn in both water spinach and okra have recorded high TF values. The Zn treated okra recorded a TF value of 2.28 for Pb accumulation while the control and Cu treated okra recorded 1.72 and 1.59 TF values, respectively for Zn accumulation. The lowest BCF value was observed in Zn treated okra (0.19) for Zn accumulation and the highest BCF value was found in Zn treated water spinach (7.65) for Cu accumulation.

The TF values of >1 in both water spinach and okra were high, suggesting that the metal translocation of Pb and Zn from root to shoot was substantial. Furthermore, both water spinach and okra cultivated on all the non-metal spiked treatments showed high BCF values whilst spiked metal treatments exhibited lower BCF values (<1) in the Pb, Zn and Cu treatments. Both water spinach and okra in Pb spiked treatments showed highest tolerance index (TI) in the control (Table 3.17).

Table 3.11: Translocation factor (TF) and biological concentration factor (BCF) of Pb in water spinach as influenced by different treatments of control, Pb (50 mg Pb /kg soil), Zn (50 mg Zn/kg soil) and Cu (50 mg Cu/kg soil)

Treatment	Pb accumulation	
	TF	BCF
Control	0.76 d	4.61 a
Pb	1.09 a	0.55 d
Zn	0.87 c	3.06 c
Cu	1.01 b	3.18 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.12: Translocation factor (TF) and biological concentration factor (BCF) of Pb in okra as influenced by different treatments of control, Pb (50 mg Pb /kg soil), Zn (50 mg Zn/kg soil) and Cu (50 mg Cu/kg soil)

Treatment	Pb accumulation	
	TF	BCF
Control	0.84 b	4.06 b
Pb	0.23 d	1.61 d
Zn	2.28 a	4.97 a
Cu	0.76 c	4.26 c

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.13: Translocation factor (TF) and biological concentration factor (BCF) of Zn in water spinach as influenced by different treatments of control, Pb (50 mg Pb /kg soil), Zn (50 mg Zn/kg soil) and Cu (50 mg Cu/kg soil)

Treatment	Zn accumulation	
	TF	BCF
Control	0.83 bc	4.17 a
Pb	0.88 bc	3.49 b
Zn	0.98 b	0.71 d
Cu	1.43 a	2.29 c

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.14: Translocation factor (TF) and biological concentration factor (BCF) of Zn in okra as influenced by different treatments of control, Pb (50 mg Pb /kg soil), Zn (50 mg Zn/kg soil) and Cu (50 mg Cu/kg soil)

Treatment	Zn accumulation	
	TF	BCF
Control	1.72 a	2.68 c
Pb	1.51 bc	3.20 a
Zn	0.56 d	0.19 d
Cu	1.59 b	3.00 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.15: Translocation factor (TF) and biological concentration factor (BCF) of Cu in water spinach as influenced by different treatments of control, Pb (50 mg Pb /kg soil), Zn (50 mg Zn/kg soil) and Cu (50 mg Cu/kg soil)

Treatment	Cu accumulation	
	TF	BCF
Control	0.34 c	1.28 c
Pb	0.65 a	3.10 b
Zn	0.36 c	7.65 a
Cu	0.54 b	0.70 d

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.16: Translocation factor (TF) and biological concentration factor (BCF) of Cu in okra as influenced by different treatments of control, Pb (50 mg Pb /kg soil), Zn (50 mg Zn/kg soil) and Cu (50 mg Cu/kg soil)

Treatment	Cu accumulation	
	TF	BCF
Control	0.24 bc	1.88 c
Pb	0.35 a	2.28 a
Zn	0.20 d	2.11 ab
Cu	0.26 b	0.20 d

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

Table 3.17: Tolerance index (TI) of Pb, Zn and Cu in water spinach and okra as influenced by different treatments of control, Pb (50 mg Pb /kg soil), Zn (50 mg Zn/kg soil) and Cu (50 mg Cu/kg soil)

Plant species	Treatment	TI
Water spinach	Pb	0.764 a
	Zn	0.591 ab
	Cu	0.376 ab
Okra	Pb	0.860 a
	Zn	0.786 ab
	Cu	0.555 ab

Mean followed by the same letters is not significantly different for each treatment means at 0.01 levels of probability

3.3.1.6 Food and metal contamination

Table 3.18 and Table 3.19 show the permissible levels of soil and food concentration limits for Pb, Zn and Cu for both water spinach and okra. The initial soil concentrations of Pb (1.23 mg/kg), Zn (0.41 mg/kg) and Cu (0.55 mg/kg) were below the limit set by the Department of Environment, Malaysia (2009), before all the metal treatments were spiked with 50 mg/kg of metals, to be used as a contaminated soil. Regardless of the high level of metal spiking in the soil, only Pb accumulation in the shoots of water spinach (30.31 mg/kg) and okra (18.51 mg/kg) exceeded the permissible levels for both the National Malaysia Food Act 1983 & Food Regulations 1985 and the International Codex Alimentarius Commission - Joint FAO/WHO Expert Committee on Food Additives (JECFA) standards. The international Codex Alimentarius Commission has set the permissible levels of Pb (5 mg/kg), Zn (60 mg/kg) and Cu (40 mg/kg) concentrations in food which are slightly less stringent compared to the Malaysia Food Act 1983 & Food Regulations 1985, which has the maximum allowable limits of 2, 40 and 30 (mg/kg); respectively for Pb, Zn and Cu metals.

Table 3.18: Permissible levels of soil and food standards for Pb, Zn and Cu in water spinach

Heavy metals	Final soil (mg/kg)	Spiked heavy metal accumulation (mg/kg)	Soil limit[a] (mg/kg)	Food limit (mg/kg)	
				Msia[b]	FAO/WHO[c]
Pb	0.622 ± 0.115	30.31 ± 1.23	10.4	2	5
Zn	0.187 ± 0.031	35.10 ± 3.58	21.9	40	60
Cu	0.408 ± 0.007	18.87 ± 0.93	13.8	30	40

[a] Department of Environment (DOE), Malaysia 2009
[b] Malaysian Food Act 1983 & Food Regulations 1985 (2006)
[c] Codex Alimentarius Commission 1984

Table 3.19: Permissible levels of soil and food standards for Pb, Zn and Cu in okra

Heavy metals	Final soil (mg/kg)	Spiked heavy metal accumulation (mg/kg)	Soil limit[a] (mg/kg)	Food limit (mg/kg)	
				Msia[b]	FAO/WHO[c]
Pb	1.163 ± 0.542	18.51 ± 5.37	10.4	2	5
Zn	0.151 ± 0.073	5.18 ± 1.92	21.9	40	60
Cu	0.094 ± 0.003	2.62 ± 0.41	13.8	30	40

[a] Department of Environment (DOE), Malaysia 2009
[b] Malaysian Food Act 1983 & Food Regulations 1985 (2006)
[c] Codex Alimentarius Commission 1984

3.3.2 Heavy metal phytoassessment in tropical plants (Vetiver, acacia and mucuna)

3.3.2.1 Preliminary assessment of hydrotoxic leachate

Table 3.20 showed the preliminary hydro-toxicity composition of leachate characteristics with the concentration levels of As, Cd, Fe and Pb, higher than the national and international maximum permissible effluent discharge standards.

Table 3.20: Characteristics of treated leachate compared with national and international maximum permissible effluent discharge standards

Parameter	Treated leachate	MY		TH[3]	SG[4]	JP[5]	US[6,7]
		A[1]	B[2]				
pH at 25°C	7.8	6.0 – 9.0	5.5 – 9.0	5.5 – 9.0	6.0 – 9.0	5.0 – 9.0	6.0 – 9.0
Temperature (°C)	28.6	40.0	40.0	40.0	45.0	NA	NA
Al (mg/L)	0.008	10.0	15.0	NA	NA	NA	NA
As (mg/L)	0.202	0.05	0.1	0.25	0.01 – 0.1	0.1	1.1 – 5.0
Cd (mg/L)	**0.609**	0.01	0.02	0.03	0.003 – 0.1	0.03	1.0
Cr (mg/L)	0.097	0.2	1.0	0.2 – 0.75	0.05 – 1.0	0.5	1.1
Cu (mg/L)	0.055	0.2	1.0	2.0	0.1	3.0	NA
Fe (mg/L)	6.545	1.0	5.0	NA	1.0 – 10.0	10.0	NA
Mn (mg/L)	0.844	0.2	1.0	5.0	0.5 – 5.0	10.0	NA
Ni (mg/L)	0.294	0.2	1.0	1.0	0.1 – 1.0	NA	NA
Pb (mg/L)	**0.897**	0.1	0.5	0.2	0.1 – 1.0	0.1	5.0
Zn (mg/L)	0.004	2.0	2.0	5.0	0.5 – 1.0	2.0	0.5
Se (mg/L)	0.359	NA	NA	0.02	0.5 – 0.01	NA	1.0
Mg (mg/L)	58.771	NA	NA	NA	150 – 200	NA	NA
Ca (mg/L)	348.009	NA	NA	NA	150 – 200	NA	NA
K (mg/L)	628.967	NA	NA	NA	NA	NA	NA
Na (mg/L)	727.371	NA	NA	NA	NA	NA	NA

[1] Malaysian Environmental Quality (Industrial Effluent) Regulations 2009 Standard A (upstream discharge from a water intake point)
[2] Malaysian Environmental Quality (Industrial Effluent) Regulations 2009 Standard B (downstream discharge from a water intake point)
[3] Thailand National Environmental Quality Act 1992, Water Quality (Industrial Effluent) Standards
[4] Singaporean Environmental Protection and Management (Trade Effluent) Regulations 2008
[5] Japanese Water Pollution Control Law, Uniform National Effluent Standards 2015
[6] United States Environmental Protection Agency, Effluent Limitations Guidelines and Standards for Landfill Category 2000
[7] United States Environmental Protection Agency, Maximum Concentration of Contaminants for Toxicity Characteristic Leaching Procedure 2004
NA = Not Available

3.3.2.2 Preliminary trials

As a preliminary phytoevaluation trial, antidesma (*Antidesma salicinum*), ficus (*Ficus trichnopoda*), kenaf (*Hibiscus cannabinus*) and fern (*Cyatheaceae sp.*) were tested as possible candidates, together with acacia, mucuna and Vetiver. All the tropical plant species were selected due to their positive plant growth characteristics (e.g.: fast-growing, low maintenance, non-invasiveness, *et cetera*). Unfortunately, antidesma, ficus, kenaf and the fern species were not able to successfully withstand heavy metal contaminated soil conditions and eventually died before the designed experimental period.

3.3.2.3 Effects on plant growth

During the 75-day experimental period, all the three tropical plants recorded different growth trends (Table 3.21 – Table 3.23). A significantly lower ($p<0.05$) leaf number was recorded in all hydrotoxic treatments of mucuna compared to the control. All hydrotoxic treatments with the exception of the 80S + 20L for mucuna showed significantly reduced ($p<0.05$) percentage plant survivorship and plant height compared to the control.

However, no significant differences ($p>0.05$) were observed in all the hydrotoxic treatments with regard to plant height and leaf number between acacia and the control. Similarly, Vetiver showed no significant difference ($p>0.05$) in terms of plant height and percentage survivorship in the treatments grown in hydrotoxic conditions compared with the control.

Between the three types of plant species, Vetiver exhibited appreciably higher plant height, leaf number and percentage survivorship than both acacia and mucuna. Nevertheless, only selected hydrotoxic treatments (60S + 40L, 50S + 50L, 40S + 60L and 100L) in Vetiver displayed significantly lower ($p<0.05$) leaf number compared to the control.

The overall relative growth rate (RGR) for acacia and mucuna was significantly decreased ($p<0.05$) in all the treatments compared to the control. The decrease in RGR may possibly be due to the accumulation of the metals and its hydro-toxicity. Moreover, due to the high number of withered plants, both the hydrotoxic 60S + 40L (-0.00107 g/day) and 20S + 80L (-0.00006 g/day) treatments in acacia recorded a negative RGR compared to the other treatments.

However, only hydrotoxic 60S + 40L (0.01161 g/day) and 100L (0.01238 g/day) treatments in Vetiver demonstrated significantly lower ($p<0.05$) RGR compared with the control. Nonetheless, among all the three plants, Vetiver (0.01161 – 0.01600 g/day) exhibited a reasonably higher RGR than both acacia (0.01238 – 0.01075 g/day) and mucuna (0.00353 – 0.02337 g/day). The effects of the plant growth parameters such as plant height, leaf number and percentage survivorship contributed to the overall RGR of the plant.

Table 3.21: Plant height (cm), leaf number, plant survivorship (%) and relative growth rate (g/day) of acacia as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Plant height (cm)	Leaf number	Plant survivorship (%)	RGR (g/day)
Control	45.97 ± 1.88 abc	9.29 ± 3.42 ab	100.00 ± 0.00 a	0.01075 a
80S + 20L	47.63 ± 11.12 ab	9.19 ± 3.4 ab	100.00 ± 0.00 a	0.00606 b
60S + 40L	43.05 ± 8.40 bc	11.24 ± 5.28 ab	24.29 ± 10.95 b	- 0.00107 c
50S + 50L	43.10 ± 12.75 bc	10.33 ± 4.22 ab	22.86 ± 16.57 b	0.00225 bc
40S + 60L	40.72 ± 10.02 c	7.52 ± 3.48 b	21.43 ± 15.47 b	0.00290 bc
20S + 80L	46.62 ± 9.58 abc	7.86 ± 3.74 ab	23.57 ± 17.22 b	- 0.00006 c
100L	49.71 ± 6.72 a	12.71 ± 3.96 a	22.14 ± 15.98 b	0.00323 bc

Mean followed by same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.22: Plant height (cm), leaf number, plant survivorship (%) and relative growth rate (g/day) of mucuna as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Plant height (cm)	Leaf number	Plant survivorship (%)	RGR (g/day)
Control	91.21 ± 13.62 a	27.00 ± 14.60 a	100.00 ± 0.00 a	0.02337 a
80S + 20L	74.00 ± 24.16 a	20.05 ± 10.05 b	85.71 ± 10.23 a	0.00501 bcd
60S + 40L	28.21 ± 12.46 b	2.76 ± 1.24 c	35.71 ± 22.61 b	0.00353 d
50S + 50L	24.04 ± 16.62 b	2.48 ± 1.51 c	31.86 ± 17.55 b	0.00721 bc
40S + 60L	32.10 ± 10.50 b	1.05 ± 0.85 c	23.86 ± 15.69 b	0.00729 bc
20S + 80L	33.36 ± 15.63 b	1.62 ± 0.56 c	28.57 ± 19.29 b	0.00771 b
100L	35.52 ± 19.25 b	0.95 ± 0.42 c	19.00 ± 14.27 b	0.00459 cd

Mean followed by same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.23: Plant height (cm), leaf number, plant survivorship (%) and relative growth rate (g/day) of Vetiver as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Plant height (cm)	Number of leaves	Plant survivorship (%)	RGR (g/day)
Control	81.43 ± 4.29 ab	25.95 ± 9.34 ab	100.00 ± 0.00 a	0.01600 ab
80S + 20L	93.71 ± 3.92 a	22.48 ± 3.25 abc	100.00 ± 0.00 a	0.01753 a
60S + 40L	90.83 ± 12.05 ab	13.19 ± 2.54 d	98.57 ± 14.29 a	0.01161 c
50S + 50L	75.95 ± 6.38 ab	16.86 ± 10.21 cd	97.14 ± 27.11 a	0.01439 abc
40S + 60L	82.07 ± 7.36 a	13.81 ± 7.28 d	98.29 ± 20.57 a	0.01413 abc
20S + 80L	74.99 ± 13.51 ab	28.29 ± 14.53 a	100.00 ± 0.00 a	0.01276 bc
100L	53.83 ± 10.32 b	16.19 ± 9.85 cd	100.00 ± 0.00 a	0.01238 c

Mean followed by same letters are not significantly different for each treatment means at 0.05 levels of probability

Both acacia (Figure 3.11 & Figure 3.14) and mucuna (Figure 3.12 & Figure 3.15) under hydrotoxic treatments, exhibited a decline in leaf number and percentage plant survivorship throughout the entire period of the experiment. Both acacia and mucuna showed a significant decrease in the two parameters starting from day 7 onwards and eventually the plants withered between day 21 and day 48. On the other hand, Vetiver showed a comparatively positive growth trend (plant height, leaf number and percentage survivorship) in all the different levels of hydrotoxic conditions (Figure 3.10, Figure 3.13 and Figure 3.16). Almost 100% plant survivorship was demonstrated by Vetiver in all the different levels of hydrotoxic treatments whilst for acacia and mucuna, it was only recorded in the 80S + 20L and control treatments, respectively. All the three plant species showed progressive growth performance with regard to plant height, leaf number and percentage survivorship, particularly in the hydrotoxic 80S + 20L treatment.

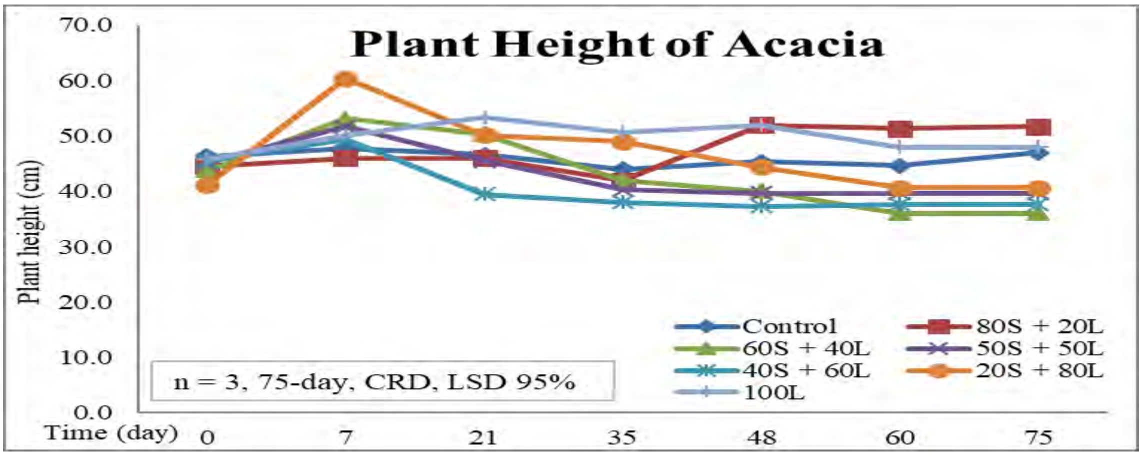

Figure 3.8: Plant growth performance over time for plant height (cm) of acacia as influenced by different levels of hydrotoxic soil-leachate treatments.

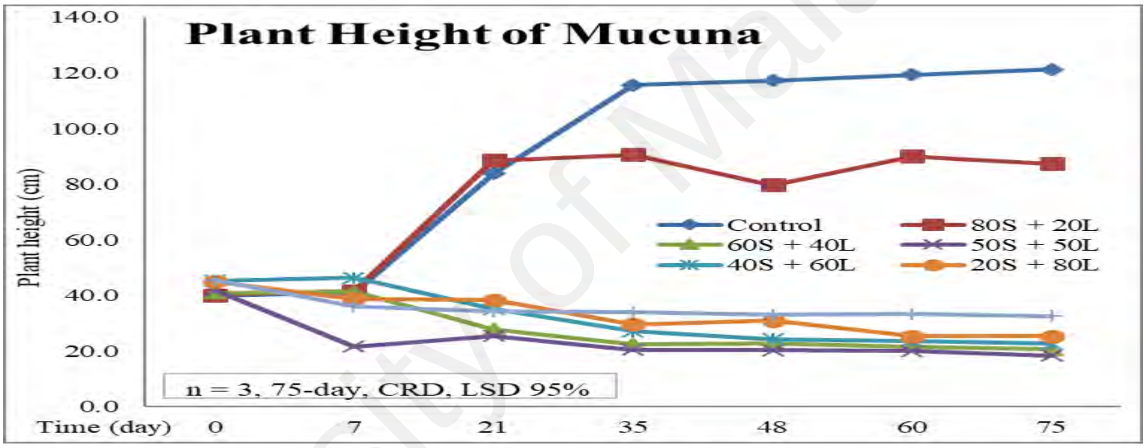

Figure 3.9: Plant growth performance over time for plant height (cm) of mucuna as influenced by different levels of hydrotoxic soil-leachate treatments.

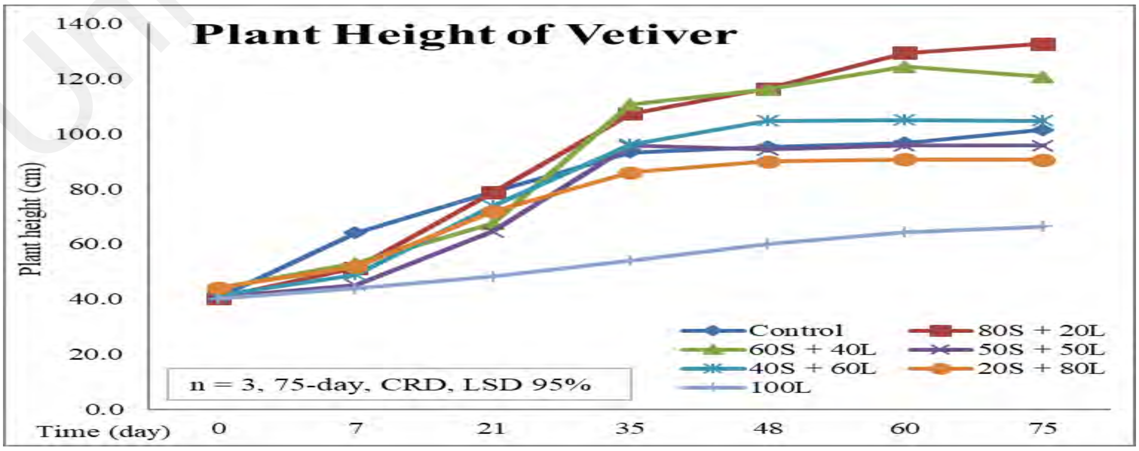

Figure 3.10: Plant growth performance over time for plant height (cm) of Vetiver as influenced by different levels of hydrotoxic soil-leachate treatments.

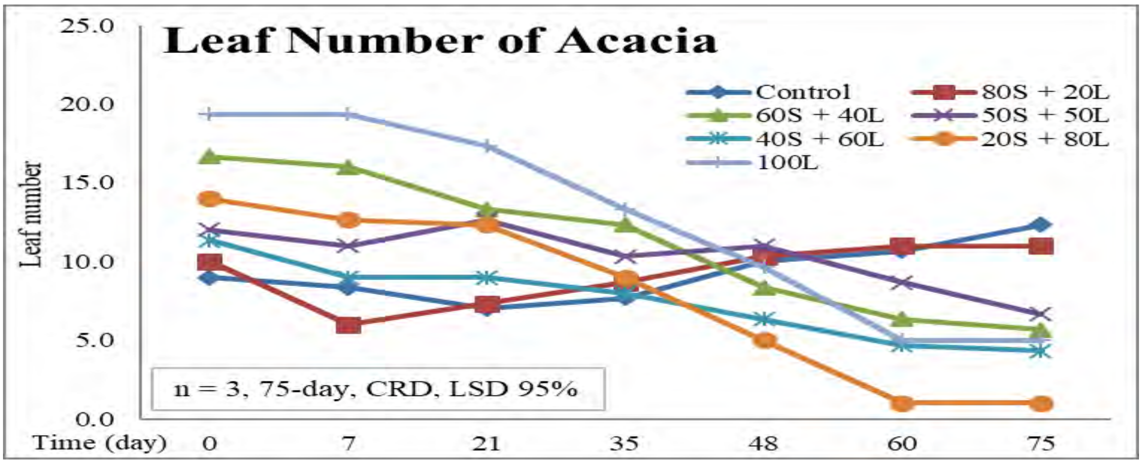

Figure 3.11: Plant growth performance over time for leaf number of acacia as influenced by different levels of hydrotoxic soil-leachate treatments.

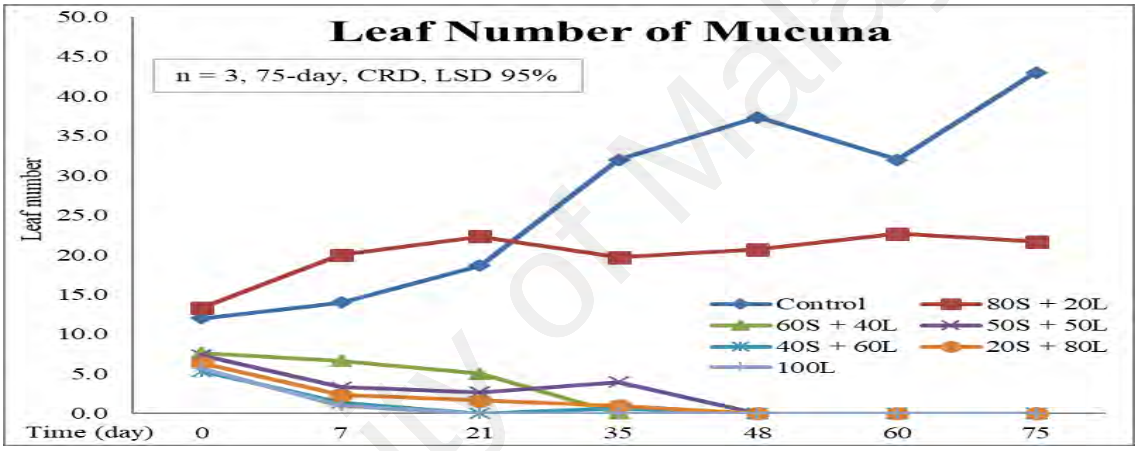

Figure 3.12: Plant growth performance over time for leaf number of mucuna as influenced by different levels of hydrotoxic soil-leachate treatments.

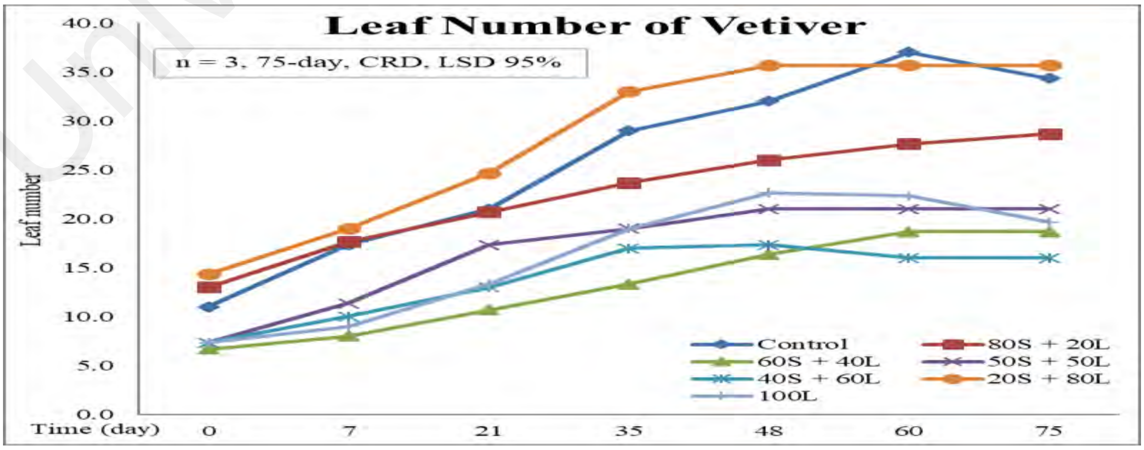

Figure 3.13: Plant growth performance over time for leaf number of Vetiver as influenced by different levels of hydrotoxic soil-leachate treatments.

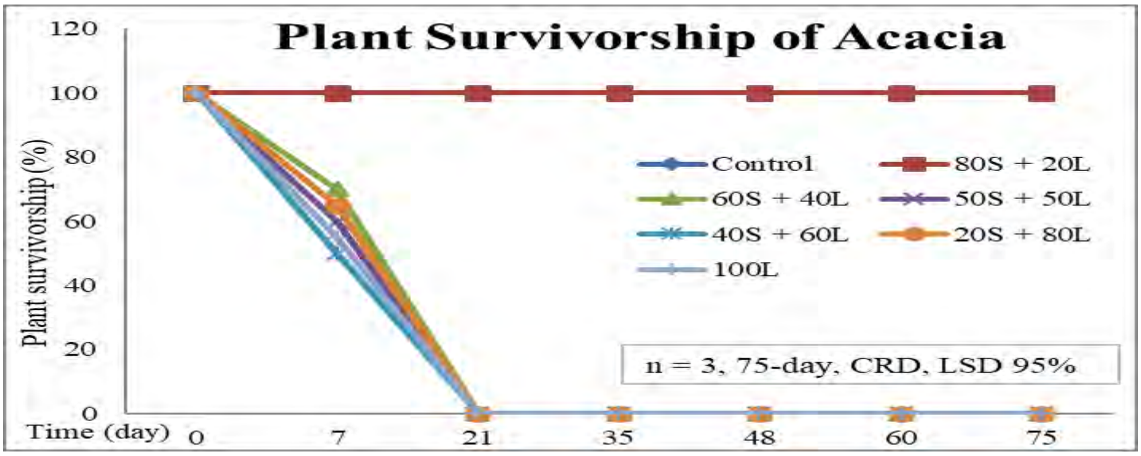

Figure 3.14: Plant growth performance over time for plant survivorship (%) of acacia as influenced by different levels of hydrotoxic soil-leachate treatments.

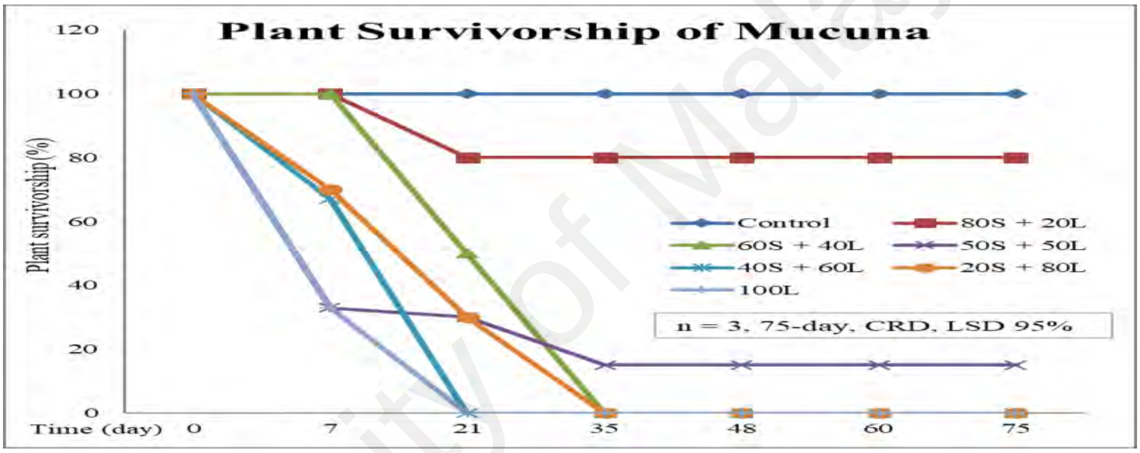

Figure 3.15: Plant growth performance over time for plant survivorship (%) of mucuna as influenced by different levels of hydrotoxic soil-leachate treatments.

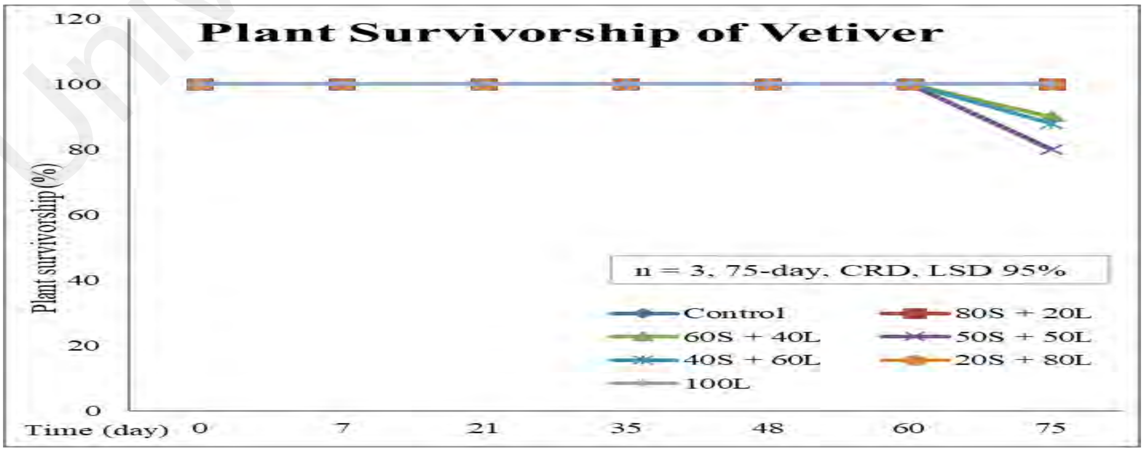

Figure 3.16: Plant growth performance over time for plant survivorship (%) of Vetiver as influenced by different levels of hydrotoxic soil-leachate treatments.

Dry matter content was significantly affected (*p<0.05*) by the hydrotoxic treatment variables (Table 3.24 – Table 3.26). The lowest dry matter content was observed in all of the roots and shoots of hydrotoxic treated mucuna as compared to the control. All the three plants recorded significantly lower (*p<0.05*) total dry matter content in the hydrotoxic treatment compared to the controls. The hydrotoxic 80S + 20L treatments in the shoots of both acacia (7.76 ± 1.37 g/m^2) and Vetiver (14.89 ± 1.83 g/m^2), respectively showed no significant differences (*p>0.05*) in terms of dry matter content compared to the control.

However, the opposite was observed in the other different hydrotoxic level treatments. Among the three plant species, Vetiver recorded an appreciably higher content of dry matter content than both acacia and mucuna. Nevertheless, the root-shoot (R/S) quotients of both mucuna and Vetiver exhibited significant differences (*p<0.05*) under all the hydrotoxic treatments compared with the control. With regard to the tolerance index (TI) that was employed to evaluate the tolerance ability of a plant species to grow under hydrotoxic conditions. Vetiver demonstrated a higher TI than both acacia and mucuna whereby a TI ≥ 1 represents high tolerance proficiency.

Table 3.24: Dry matter content (g/m^2), root-shoot (R/S) quotient and tolerance index (TI) of acacia as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Dry matter content (g/m^2)				
	Acacia			R/S	TI
	Root	Shoot	Total		
Control	4.40 ± 0.52 a	9.00 ± 0.69 a	13.40 ± 0.18 a	0.489 b	
80S + 20L	3.16 ± 0.59 b	7.76 ± 1.37 ab	10.92 ± 1.88 b	0.408 b	0.815 a
60S + 40L	1.57 ± 0.32 d	3.49 ± 0.41 c	5.06 ± 0.68 e	0.451 b	0.378 a
50S + 50L	2.17 ± 0.08 cd	4.69 ± 0.50 c	6.86 ± 0.53 cde	0.464 b	0.512 a
40S + 60L	2.40 ± 0.68 bcd	5.20 ± 1.63 bc	7.60 ± 2.06 cd	0.462 b	0.567 a
20S + 80L	2.45 ± 0.22 bcd	3.46 ± 1.20 c	5.90 ± 1.31 cde	0.709 a	0.441 a
100L	2.68 ± 0.63 bc	5.14 ± 0.49 bc	7.82 ± 0.84 c	0.522 ab	0.584 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.25: Dry matter content (g/m^2), root-shoot (R/S) quotient and tolerance index (TI) of mucuna as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Dry matter content (g/m^2)				
	Mucuna			R/S	TI
	Root	Shoot	Total		
Control	32.49 ± 12.31 a	11.34 ± 0.97 a	43.83 ± 11.35 a	2.865 a	
80S + 20L	1.56 ± 0.29 b	2.55 ± 0.33 b	4.11 ± 0.43 b	0.610 b	0.094 a
60S + 40L	1.40 ± 0.25 b	2.66 ± 0.57 b	4.06 ± 0.32 b	0.525 b	0.093 a
50S + 50L	1.13 ± 0.17 b	2.98 ± 0.31 b	4.11 ± 0.27 b	0.381 b	0.094 a
40S + 60L	1.17 ± 0.39 b	3.30 ± 0.35 b	4.47 ± 0.06 b	0.354 b	0.102 a
20S + 80L	0.88 ± 0.08 b	3.47 ± 0.92 b	4.35 ± 1.00 b	0.254 b	0.099 a
100L	0.73 ± 0.16 b	3.01 ± 0.11 b	3.74 ± 0.17 b	0.242 b	0.085 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.26: Dry matter content (g/m^2), root-shoot (R/S) quotient and tolerance index (TI) of Vetiver as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Dry matter content (g/m^2)				
	Vetiver			R/S	TI
	Root	Shoot	Total		
Control	7.68 ± 1.55 a	18.89 ± 4.56 a	26.57 ± 3.29 a	0.407 d	
80S + 20L	7.10 ± 1.82 a	14.89 ± 1.83 a	21.99 ± 3.39 b	0.476 cd	0.827 a
60S + 40L	8.13 ± 1.98 a	9.32 ± 0.53 b	17.45 ± 2.05 bc	0.872 abcd	0.657 a
50S + 50L	7.82 ± 2.66 a	7.87 ± 1.28 b	15.69 ± 3.95 c	0.994 abc	0.590 a
40S + 60L	8.42 ± 1.72 a	7.82 ± 0.68 b	16.24 ± 1.40 c	1.077 ab	0.611 a
20S + 80L	8.57 ± 2.03 a	10.20 ± 1.61 b	18.77 ± 3.52 bc	0.840 abcd	0.706 a
100L	9.88 ± 2.18 a	7.99 ± 2.11 b	17.87 ± 2.13 bc	1.237 a	0.673 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

3.3.2.4 Distribution of Cd and Pb in roots and shoots

Both Cd and Pb accumulation in the roots and shoots of all the three plant species are shown in Tables 3.27 – Table 3.32. All three plants recorded significantly higher ($p<0.05$) Cd uptake in its roots and shoots and total metal accumulations under the hydrotoxic treatments compared to the controls. Between the roots and shoots, Cd accumulation was considerably greater in the roots than in the shoots. Similarly, Pb uptake was significantly greater ($p<0.05$) in the roots and total metal accumulation under the hydrotoxic treatments of both mucuna and Vetiver than in the control. A significantly higher ($p<0.05$) accumulation of Pb was recorded in the shoots of all the hydrotoxic treatments in mucuna and Vetiver with the exception of the hydrotoxic 100L treatment. However, hydrotoxic 80S + 20L, 60S + 40L and 50S +50L treatments caused a significant increase ($p<0.05$) in Pb uptake in the roots and in total metal accumulation of acacia compared to the control. Nonetheless, only the hydrotoxic 80S + 20L treatment brought about a significantly larger ($p<0.05$) accumulation of Pb in the shoots of acacia.

Comparatively, between roots and shoots, all three plant species accumulated higher amounts of Pb in the roots than in the shoots. The accumulation trend for both Cd and Pb in different plants were in the order of Vetiver > acacia > mucuna for all of the hydrotoxic treatments. The shoots of hydrotoxic 80S + 20L treated Vetiver recorded the highest amount of Cd (27.04 ± 5.84 mg/kg) and Pb (165.24 ± 26.54 mg/kg) accumulation compared to both acacia and mucuna.

Table 3.27: Concentration of Cd (mg/kg) in the roots and shoots of acacia as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Concentration of Cd (mg/kg)		
	Root	Shoot	Total
Control	16.50 ± 3.46 c	3.60 ± 1.44 b	20.10 ± 3.10 b
80S + 20L	66.83 ± 5.01 b	17.55 ± 5.03 a	84.39 ± 7.94 a
60S + 40L	72.93 ± 9.46 ab	16.60 ± 4.44 a	89.53 ± 13.58 a
50S + 50L	83.80 ± 9.78 ab	16.01 ± 1.96 a	99.81 ± 8.56 a
40S + 60L	73.10 ± 11.63 ab	16.25 ± 4.55 a	89.35 ± 9.17 a
20S + 80L	84.93 ± 8.41 ab	15.55 ± 0.82 a	100.48 ± 8.78 a
100L	90.20 ± 15.10 a	13.59 ± 2.18 a	103.79 ± 17.28 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.28: Concentration of Cd (mg/kg) in the roots and shoots of mucuna as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Concentration of Cd (mg/kg)		
	Root	Shoot	Total
Control	4.18 ± 1.94 c	0.91 ± 0.61 b	5.08 ± 2.37 b
80S + 20L	20.95 ± 2.60 b	7.11 ± 0.48 a	28.07 ± 2.94 a
60S + 40L	24.97 ± 7.51 ab	6.58 ± 1.49 a	31.55 ± 7.87 a
50S + 50L	26.73 ± 5.35 ab	5.57 ± 1.11 a	32.31 ± 5.36 a
40S + 60L	23.35 ± 2.17 ab	5.21 ± 1.81 a	28.56 ± 3.48 a
20S + 80L	29.03 ± 4.05 ab	5.75 ± 1.07 a	34.78 ± 4.15 a
100L	30.67 ± 6.22 a	5.62 ± 1.06 a	36.29 ± 7.27 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.29: Concentration of Cd (mg/kg) in the roots and shoots of Vetiver as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Concentration of Cd (mg/kg)		
	Root	Shoot	Total
Control	10.11 ± 2.17 b	5.27 ± 3.07 b	14.77 ± 3.80 b
80S + 20L	89.12 ± 8.65 a	27.04 ± 5.84 a	116.16 ± 11.56 a
60S + 40L	104.03 ± 7.50 a	23.99 ± 6.90 a	128.02 ± 11.39 a
50S + 50L	114.13 ± 24.83 a	24.50 ± 4.20 a	138.64 ± 25.10 a
40S + 60L	122.04 ± 21.56 a	19.43 ± 6.13 a	141.47 ± 26.66 a
20S + 80L	118.63 ± 19.81 a	22.88 ± 4.62 a	141.51 ± 16.45 a
100L	120.93 ± 20.46 a	19.03 ± 3.61 a	139.97 ± 18.61 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.30: Concentration of Pb (mg/kg) in the roots and shoots of acacia as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Concentration of Pb (mg/kg)		
	Root	Shoot	Total
Control	99.33 ± 16.52 d	23.03 ± 11.55 b	122.37 ± 17.74 d
80S + 20L	218.60 ± 38.25 abc	90.33 ± 17.60 a	308.93 ± 21.30 abc
60S + 40L	269.20 ± 86.59 ab	56.10 ± 11.11 ab	325.30 ± 86.08 ab
50S + 50L	281.03 ± 74.57 a	49.47 ± 5.70 b	330.50 ± 71.31 a
40S + 60L	178.87 ± 49.41 bcd	40.80 ± 23.11 b	219.67 ± 72.02 abcd
20S + 80L	146.17 ± 30.55 cd	43.60 ± 26.44 b	189.77 ± 54.35 d
100L	144.13 ± 14.26 cd	46.433 ± 28.38 b	190.57 ± 42.49 d

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.31: Concentration of Pb (mg/kg) in the roots and shoots of mucuna as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Concentration of Pb (mg/kg)		
	Root	Shoot	Total
Control	26.97 ± 7.39 c	13.67 ± 1.05 d	40.63 ± 7.47 c
80S + 20L	50.43 ± 13.20 b	26.87 ± 4.23 a	77.30 ± 10.06 b
60S + 40L	49.30 ± 7.84 b	26.40 ± 4.25 a	75.70 ± 7.82 b
50S + 50L	56.07 ± 12.72 ab	25.73 ± 3.56 a	81.80 ± 16.13 b
40S + 60L	52.83 ± 6.92 ab	22.93 ± 2.99 bc	75.77 ± 6.14 b
20S + 80L	56.33 ± 12.97 ab	24.33 ± 3.36 ab	80.67 ± 14.09 b
100L	72.73 ± 13.59 a	21.07 ± 1.37 c	93.80 ± 12.22 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.32: Concentration of Pb (mg/kg) in the roots and shoots of Vetiver as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Concentration of Pb (mg/kg)		
	Root	Shoot	Total
Control	49.57 ± 17.04 e	32.97 ± 7.74 d	82.53 ± 24.51 e
80S + 20L	200.03 ± 11.07 a	165.24 ± 26.54 a	365.27 ± 35.27 a
60S + 40L	170.33 ± 30.07 abc	138.73 ± 32.13 a	309.07 ± 2.24 b
50S + 50L	180.90 ± 22.68 ab	94.77 ± 8.92 b	275.67 ± 19.02 bc
40S + 60L	137.53 ± 20.68 cd	92.07 ± 7.51 bc	229.60 ± 23.31c
20S + 80L	105.60 ± 14.54 d	69.23 ± 13.80 bc	174.83 ± 15.17 d
100L	95.87 ± 28.85 d	60.50 ± 5.31 cd	156.37 ± 34.10 d

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

3.3.2.5 Metal translocation

The association between Cd and Pb accumulated from the hydrotoxic soil-leachate treatments into the roots and shoots in all the three plants are presented in terms of translocation factor (TF), metal accumulation quotient (MAQ) and percentage of metal uptake efficacy, as shown in Table 3.33 – Table 3.38. Considering the relatively lower accumulation of Cd and Pb in the shoots than the roots in all the three plants, TF was used to assess the capability of the plant to translocate metals from the roots to the shoots. The hydrotoxic 80S + 20L (0.324) and 100L (0.299) treatments in mucuna recorded significant differences ($p<0.05$) of TF values compared with the control for the accumulation of Cd and Pb, respectively. However, no significant differences ($p>0.05$) of TF values were observed in acacia for both Cd and Pb accumulation.

Cd accumulation in all hydrotoxic treatments of Vetiver showed significantly lower ($p<0.05$) TF values compared to the control. The plant response to stressful conditions, caused by both Cd and Pb present in the hydrotoxic conditions, may have affected the translocation of metals from the soil to the above ground parts of the plants (shoots) and hence influence the overall TF values. With the relatively lower TF values, the findings suggest that both Cd and Pb accumulation favoured translocation from the hydrotoxic source into the roots, than to the shoots in all three tropical plants. Although the TF values were < 1, Vetiver (0.531 – 0.852) exhibited appreciably higher TF in the accumulation of Pb than both acacia (0.188 – 0.433) and mucuna (0.299 – 0.566).

The metal accumulation quotient (MAQ) and percentage of metal uptake efficacy were calculated to evaluate the potential and efficiency of the overall metal translocation and bio-accumulation in the plants. The MAQ revealed that the accumulation of both Cd and Pb were significantly greater ($p<0.05$) in all the hydrotoxic treatments of mucuna than the control. Conversely, all hydrotoxic treatments of Cd accumulation together with hydrotoxic 50S +50L, 40S + 60L and 100L treatments of Pb accumulation in Vetiver recorded a significant decrease ($p<0.05$) in MAQ compared with the control. Furthermore, a significantly higher ($p<0.05$) MAQ for Pb accumulation was solely observed in the hydrotoxic 80S + 20L treatment of acacia among other treatments. On the other hand, no significant difference ($p>0.05$) in percentage Cd efficacy was recorded between all hydrotoxic treatments and the control in both acacia and mucuna.

Similarly, acacia and Vetiver exhibited no significant differences ($p>0.05$) in percentage Pb metal efficacy among all hydrotoxic treatments and the control. Despite plant withering in acacia and mucuna, both plants demonstrated reasonably high MAQ and percentage of metal efficacy in the accumulation of Cd and Pb. Between the different plants, Vetiver (13.55 – 32.52% of Cd and 34.51 – 45.05% of Pb) recorded remarkably higher percentages for both Cd and Pb metal efficacy than both mucuna (15.52 – 25.44% of Cd and 22.83 – 35.41% of Pb) and acacia (13.10 – 20.61% of Cd and 15.63 – 29.59% of Pb), respectively.

Table 3.33: Cd accumulation in its translocation factor (TF), metal accumulation quotient (MAQ) and metal uptake efficacy (%) of acacia as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Accumulation of Cd		
	TF	**MAQ**	**Efficacy (%)**
Control	0.230 a	0.471 ab	18.16 a
80S + 20L	0.263 a	0.645 a	20.61 a
60S + 40L	0.226 a	0.501 ab	18.36 a
50S + 50L	0.194 a	0.419 ab	16.18 a
40S + 60L	0.231 a	0.499 ab	18.43 a
20S + 80L	0.184 a	0.260 b	15.53 a
100L	0.151 a	0.289 b	13.10 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.34: Cd accumulation in its translocation factor (TF), metal accumulation quotient (MAQ) and metal uptake efficacy (%) of mucuna as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Accumulation of Cd		
	TF	**MAQ**	**Efficacy (%)**
Control	0.229 bc	0.080 b	17.85 a
80S + 20L	0.342 a	0.559 a	25.44 a
60S + 40L	0.277 b	0.527 a	21.49 a
50S + 50L	0.215 bc	0.568 a	17.55 a
40S + 60L	0.222 bc	0.626 a	18.01 a
20S + 80L	0.201 c	0.793 a	16.64 a
100L	0.184 c	0.758 a	15.52 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.35: Cd accumulation in its translocation factor (TF), metal accumulation quotient (MAQ) and metal uptake efficacy (%) of Vetiver as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Accumulation of Cd		
	TF	MAQ	Efficacy (%)
Control	0.503 a	1.237 a	32.52 a
80S + 20L	0.304 b	0.639 b	23.21 ab
60S + 40L	0.231 b	0.265 bc	18.60 b
50S + 50L	0.221 b	0.222 bc	17.97 b
40S + 60L	0.157 b	0.146 c	13.55 b
20S + 80L	0.200 b	0.238 bc	16.48 b
100L	0.162 b	0.131 c	13.86 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.36: Pb accumulation in its translocation factor (TF), metal accumulation quotient (MAQ) and metal uptake efficacy (%) of acacia as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Accumulation of Pb		
	TF	MAQ	Efficacy (%)
Control	0.239 a	0.489 b	18.63 a
80S + 20L	0.433 a	1.064 a	29.59 a
60S + 40L	0.229 a	0.509 ab	18.22 a
50S + 50L	0.188 a	0.407 b	15.63 a
40S + 60L	0.218 a	0.471 b	17.73 a
20S + 80L	0.284 a	0.400 b	21.32 a
100L	0.312 a	0.479 b	23.07 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.37: Pb accumulation in its translocation factor (TF), metal accumulation quotient (MAQ) and metal uptake efficacy (%) of mucuna as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Accumulation of Pb		
	TF	**MAQ**	**Efficacy (%)**
Control	0.530 ab	0.185 c	34.28 ab
80S + 20L	0.566 a	0.926 b	35.41 a
60S + 40L	0.546 ab	1.037 b	35.01 a
50S + 50L	0.466 ab	1.230 ab	31.74 ab
40S + 60L	0.441 b	1.244 ab	30.40 b
20S + 80L	0.447 b	1.762 a	30.62 b
100L	0.299 c	1.231 b	22.83 c

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.38: Pb accumulation in its translocation factor (TF), metal accumulation quotient (MAQ) and metal uptake efficacy (%) of Vetiver as influenced by different levels of hydrotoxic soil-leachate treatments

Treatment	Accumulation of Pb		
	TF	**MAQ**	**Efficacy (%)**
Control	0.690 a	1.424 a	40.62 a
80S + 20L	0.824 a	1.308 ab	45.05 a
60S + 40L	0.852 a	0.664 abc	44.84 a
50S + 50L	0.531 a	0.447 c	34.51 a
40S + 60L	0.679 a	0.511 bc	40.28 a
20S + 80L	0.671 a	0.634 abc	39.56 a
100L	0.661 a	0.395 c	39.47 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

3.3.3 Heavy metal phytoassessment in tropical grass (Vetiver, imperata and pennisetum)

3.3.3.1 Physico-chemical properties of soil

Preliminary soil analyses (Table 3.39) showed that the colour and texture of the growth media was dull reddish brown with 92.79% sand, 5.56% silt and 1.65% clay. The soil saturation level was relatively dry (12.56%) with high water retention and the percentage of soil porosity was almost the same as the soil field capacity (40.68%) indicating that most of the pore spaces in the soil were filled with water.

Table 3.39: Physical and chemical parameters of the growth media soil

Characteristic(Unit)		Mean
Soil texture		
Sand (%)		92.79
Very coarse sand (%)	0.62	
Coarse sand (%)	46.59	
Medium coarse sand (%)	20.51	
Fine sand (%)	18.38	
Very fine sand (%)	6.69	
Silt (%)		5.56
Clay (%)		1.65
Soil physical		
Bulk density (g/cm^3)		1.54 ± 0.03
Porosity (%)		41.76 ± 0.95
Colour (Munsell colour charts)		2.5YR 5/4 (Dull reddish brown)
Soil biology		
Water content (%)		5.11 ± 0.12
Field capacity (%)		40.68 ± 1.93
Saturation level (%)		12.56
Condition		Dry
Soil chemistry		
pH		5.04 ± 0.07
Metal contents (mg/kg)		
Cd		2.37 ± 1.44
Pb		28.66 ± 10.73
Zn		186.24 ± 56.57
Cu		11.22 ± 4.24

Mean ± Standard deviation

Soil pH was significantly ($p<0.05$) affected by the spiked heavy metal treatments in all the treatments in Vetiver while only Cd and Zn spiked treatments affected pH in imperata and Zn treatment in pennisetum (Figure 3.17 – Figure 3.19). Cd, Pb and Cu spiked treatments did not affect soil pH in pennisetum and neither did Pb and Cu in imperata. The acidic soil pH (5.04 ± 0.07) showed significant fluctuations between the range of 3.69 and 6.67 in all of the spiked metal treatments.

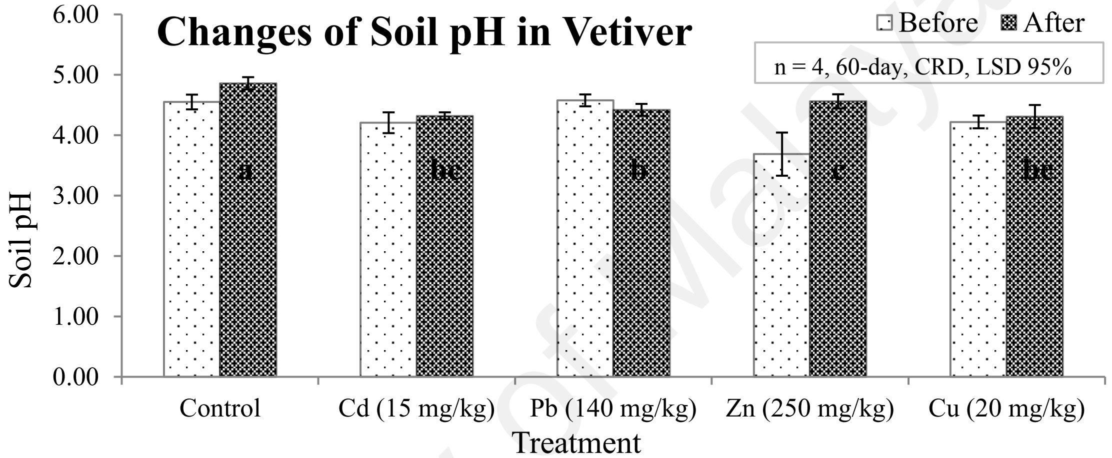

Figure 3.17: Changes in soil pH of Vetiver as influenced by different types of spiked heavy metal treatments. Vertical bars represent standard deviation and same letters are not significantly different for each treatment means at 0.05 levels of probability

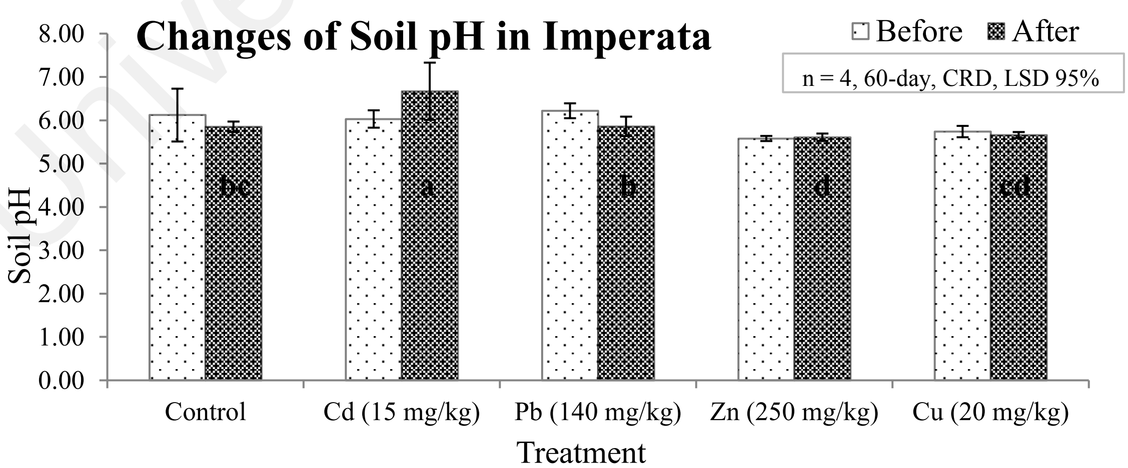

Figure 3.18: Changes in soil pH of imperata as influenced by different types of spiked heavy metal treatments. Vertical bars represent standard deviation and same letters are not significantly different for each treatment means at 0.05 levels of probability

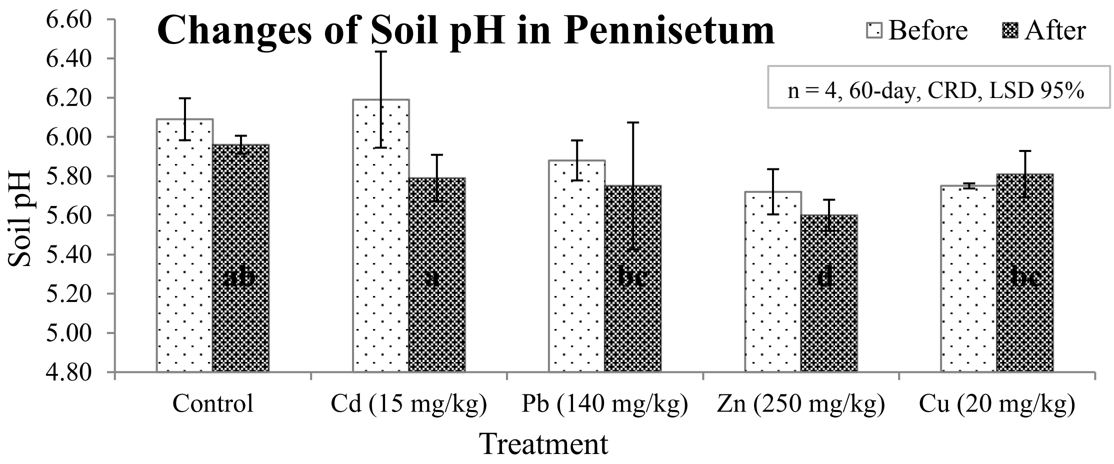

Figure 3.19: Changes in soil pH of pennisetum as influenced by different types of spiked heavy metal treatments. Vertical bars represent standard deviation and same letters are not significantly different for each treatment means at 0.05 levels of probability

3.3.3.2 Plant growth response

The relative growth of all the three grasses, in terms of plant height increased continuously throughout the study, with a significant decrease ($p<0.05$) observed in the case of pennisetum in all the spiked heavy metal treatments when compared with control (Table 3.40). In imperata, only Pb and Cu spiked treatments showed significant decrease in growth ($p<0.05$), while no significant difference ($p>0.05$) in plant height was found in all Vetiver treatments when compared with the control.

Table 3.40: Plant height (cm) of Vetiver, imperata and pennisetum grasses as influenced by different types of spiked heavy metal treatments

Treatments	Plant height (cm)		
	Vetiver	Imperata	Pennisetum
Control	64.86 ± 20.61 a	33.08 ± 6.34 b	72.89 ± 19.48 a
Cd	73.81 ± 24.92 a	29.07 ± 2.11bc	30.48 ± 0.16 cd
Pb	72.33 ± 25.04 a	24.62 ± 1.83bcd	40.36 ± 6.97bc
Zn	61.23 ± 20.19 a	26.11 ± 2.25bcd	32.75 ± 0.66 cd
Cu	60.03 ± 22.63 a	44.89 ± 17.23 a	48.71 ± 10.54 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Yield, in terms of dry matter content (g/m^2) of roots and shoots, in all the treated grasses showed a different picture (Table 3.41 – Table 3.43). Although plant height in Vetiver was not significantly affected, both Vetiver and pennisetum grown under spiked heavy metal treatments exhibited a significant decrease (*$p<0.05$*) in total yield of dry matter content when compared with the controls, even though shoot dry matter did not significantly decrease in Vetiver, but did in the case of pennisetum. The roots of Vetiver and shoots of pennisetum recorded significant reduction (*$p<0.05$*) in dry matter content. Among the three grasses, Vetiver yielded considerably higher root and shoot dry matter content. The application of spiked heavy metals to the soils significantly reduced root growth in Vetiver and shoot growth in pennisetum, but did not significantly affect shoot growth in Vetiver and root growth in both imperata and pennisetum. However, spiked Cu treatment increased shoot growth in imperata.

Dry matter content per pot produced was used to estimate the tolerance index (TI) of the spiked heavy metal treatments in all three grasses. TI acts as an indicator to determine the capability of a plant to grow in heavy metal contaminated soils. imperata was the only grass that displayed a significant difference (*$p<0.05$*) in TI, among the three grasses. All of the spiked heavy metal treatments in imperata exhibited relatively higher TI than both Vetiver and pennisetum, regardless of the plant height and dry matter content recorded. As a result of the high TI, Cu-spiked (2.202), Cd-spiked (1.699) and Zn-spiked (1.303) treatments, imperata showed good tolerance ability of growing under spiked heavy metal conditions, compared to both Vetiver and pennisetum.

Table 3.41: Dry matter content (g/m^2) and tolerance index (TI) of Vetiver as influenced by different types of spiked heavy metal treatments

Treatments	Dry matter content (g/m^2)			
	Vetiver			TI
	Root	Shoot	Total	
Control	27.06 ± 1.39 a	28.13 ± 2.57 a	55.19 ± 2.46 a	
Cd	13.68 ± 1.99bc	23.23 ± 4.00 a	36.91 ± 1.68bc	0.669 a
Pb	15.40 ± 2.76 b	24.01 ± 3.38 a	39.41 ± 3.87 b	0.712 a
Zn	15.22 ± 0.03bc	22.52 ± 1.02 a	37.74 ± 0.83bc	0.686 a
Cu	12.14 ± 1.99bc	21.23 ± 2.11 a	33.37 ± 3.76bc	0.604 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.42: Dry matter content (g/m^2) and tolerance index (TI) of imperata as influenced by different types of spiked heavy metal treatments

Treatments	Dry matter content (g/m^2)			
	Imperata			TI
	Root	Shoot	Total	
Control	1.17 ± 0.77 a	2.86 ± 0.35 bc	4.03 ± 0.10 c	
Cd	2.45 ± 1.07 a	4.32 ± 1.71 b	6.77 ± 0.23 ab	1.699 ab
Pb	2.01 ± 0.98 a	1.84 ± 0.44 c	3.85 ± 0.12 c	0.959 b
Zn	1.07 ± 0.66 a	3.87 ± 0.87 bc	4.94 ± 0.15 bc	1.303 bc
Cu	1.78 ± 0.74 a	6.83 ± 1.14 a	8.61 ± 0.15 a	2.202 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.43: Dry matter content (g/m^2) and tolerance index (TI) of pennisetum as influenced by different types of spiked heavy metal treatments

Treatments	Dry matter content (g/m^2)			
	Pennisetum			TI
	Root	Shoot	Total	
Control	5.33 ± 1.43 a	17.00 ± 3.27 a	22.33 ± 0.37 a	
Cd	3.80 ±1.38 a	3.81 ± 0.31 bc	7.61 ± 0.16 e	0.354 a
Pb	3.40 ± 1.46 a	7.80 ± 4.25 bc	11.20 ± 0.48 c	0.528 a
Zn	7.64 ± 0.92 a	2.72 ± 0.83 bc	10.36 ± 0.88 d	0.511 a
Cu	5.18 ± 0.45 a	8.57 ± 4.45 b	13.75 ± 0.70 b	0.632 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

3.3.3.3 Accumulation of heavy metals

As shown in Tables 3.44 – Table 3.46, the accumulation of heavy metals in the roots and shoots of all three grasses were variable. Cd accumulation in all the three grasses was significantly higher ($p<0.05$) in the Cd-spiked treatments than in the other heavy metal treatments. All grasses recorded higher accumulation of Cd in the Cd-spiked treatments (76.45 – 93.08 mg/kg) compared to the other treatments (0.07 – 8.00 mg/kg). The Cd accumulated in both roots and shoots of Cd-spiked treatments was also significantly higher ($p<0.05$) than other treatments irrespective of the type of grass. Between roots and shoots, Cd accumulations were greater in the roots than in the shoots. The accumulation of Cd in the different type of grasses studied was in the order of Vetiver > pennisetum > imperata for all the treatments.

Table 3.44: Concentration of Cd (mg/kg) in Vetiver as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Cd (mg/kg)		
	Root	**Shoot**	**Total**
Control	0.18 ± 0.04 b	ND	0.18 ± 0.04 b
Cd	89.65 ± 4.31 a	3.43 ± 0.70	93.08 ± 3.81 a
Pb	ND	ND	ND
Zn	0.36 ± 0.11 b	ND	0.36 ± 0.11 b
Cu	0.07 ± 0.01 b	ND	0.07 ± 0.01 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability; ND = Not detected

Table 3.45: Concentration of Cd (mg/kg) in imperata as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Cd (mg/kg)		
	Root	Shoot	Total
Control	4.40 ± 1.11 b	3.60 ± 0.96 b	8.00 ± 2.03 b
Cd	56.30 ± 14.89 a	20.15 ± 5.16 a	76.45 ± 19.73 a
Pb	1.15 ± 0.35 b	0.65 ± 0.21 b	1.80 ± 0.05 b
Zn	0.25 ± 0.07 b	0.70 ± 0.28 b	0.95 ± 0.19 b
Cu	0.35 ± 0.09 b	0.30 ± 0.01 b	0.65 ± 0.07 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.46: Concentration of Cd (mg/kg) in pennisetum as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Cd (mg/kg)		
	Root	Shoot	Total
Control	5.35 ± 1.32 b	2.50 ± 0.91 b	7.85 ± 1.81 b
Cd	44.20 ± 3.68 a	32.45 ± 5.30 a	76.65 ± 7.43 a
Pb	1.80 ± 0.28 b	0.90 ± 0.14 b	2.70 ± 1.56 b
Zn	0.70 ± 0.03 b	0.90 ± 0.05 b	1.60 ± 0.07 b
Cu	0.75 ± 0.09 b	1.05 ± 0.21 b	1.80 ± 0.37 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Similarly, the total accumulation of Pb was significantly higher ($p<0.05$) in all of the Pb-spiked treatments than in the other treatments (Table 3.47 – Table 3.49). All three grasses exhibited higher accumulation of Pb in the Pb-spiked treatments (103.20 – 340.70 mg/kg) compared to the other treatments (2.44 – 20.55 mg/kg). The accumulation of Pb in both roots and shoots of Pb-spiked treatments was significantly higher ($p<0.05$) than other treatments. Between roots and shoots, Pb accumulated more in roots irrespective of the type of grass. The trend for Pb accumulation was in the following order of imperata > pennisetum > Vetiver for all treatments.

Table 3.47: Concentration of Pb (mg/kg) in Vetiver as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Pb (mg/kg)		
	Root	Shoot	Total
Control	2.44 ± 0.17 b	ND	2.44 ± 0.17 b
Cd	4.92 ± 2.32 b	ND	4.92 ± 2.32 b
Pb	77.60 ± 59.96 a	24.60 ± 0.01 a	103.20 ± 59.96 a
Zn	5.62 ± 0.99 b	ND	5.62 ± 0.99 b
Cu	3.51 ± 0.52 b	0.39 ± 0.06 b	3.90 ± 0.43 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability; ND = Not detected

Table 3.48: Concentration of Pb (mg/kg) in imperata as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Pb (mg/kg)		
	Root	Shoot	Total
Control	9.65 ± 3.40 b	3.95 ± 1.76 b	13.60 ± 6.65 b
Cd	11.15 ± 4.27 b	7.75 ± 2.58 b	18.90 ± 2.33 b
Pb	290.45 ± 21.85 a	50.25 ± 3.75 a	340.70 ± 11.87 a
Zn	0.90 ± 0.19 b	1.95 ± 0.34 b	2.85 ± 1.33 b
Cu	4.25 ± 0.21 b	ND	4.25 ± 0.21 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability; ND = Not detected

Table 3.49: Concentration of Pb (mg/kg) in pennisetum as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Pb (mg/kg)		
	Root	Shoot	Total
Control	8.00 ± 3.82 b	3.50 ± 0.85 b	11.50 ± 1.54 b
Cd	15.40 ± 6.93 b	5.15 ± 0.78 b	20.55 ± 4.81 b
Pb	255.40 ± 14.85 a	61.00 ± 4.81 a	316.40 ± 8.48 a
Zn	4.70 ± 0.71 b	7.30 ± 2.18 b	12.00 ± 5.52 b
Cu	2.75 ± 1.34 b	5.00 ± 2.93 b	7.75 ± 3.46 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

All three grasses recorded significantly increased ($p<0.05$) total accumulation of Zn in the Zn-spiked treatments compared to the other treatments (Table 3.50 – Table 3.52). A higher accumulation of Zn was observed in Zn-spiked treatments (393.10 – 1284.00 mg/kg) compared to the other treatments (78.40 – 413.00 mg/kg). Zn accumulated in both roots and shoots of Zn-spiked treatments were significantly higher ($p<0.05$) than in other treatments. Unlike for Cd and Pb, in both imperata and pennisetum, there was a higher accumulation of Zn in the shoots than roots. Accumulation of Zn in all the three grasses was in the following order of Vetiver > pennisetum > imperata.

Table 3.50: Concentration of Zn (mg/kg) in Vetiver as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Zn (mg/kg)		
	Root	**Shoot**	**Total**
Control	185.00 ± 35.79 b	76.10 ± 49.26 b	261.10 ± 32.57 b
Cd	272.00 ± 55.15 b	77.60 ± 16.55 b	349.60 ± 60.43 b
Pb	211.00 ± 123.45 b	144.50 ± 12.02ab	355.50 ± 109.89 b
Zn	883.00 ± 391.74 a	401.00 ± 100.41 a	1284.00 ± 234.83 a
Cu	191.00 ± 86.27 b	222.00 ± 134.76ab	413.00 ± 218.49 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.51: Concentration of Zn (mg/kg) in imperata as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Zn (mg/kg)		
	Root	**Shoot**	**Total**
Control	122.40 ± 21.92ab	56.60 ± 38.33 b	179.00 ± 11.84bc
Cd	81.95 ± 21.43 b	28.60 ± 6.08 b	110.55 ± 17.52 c
Pb	56.80 ± 11.74 b	40.15 ± 4.03 b	96.95 ± 9.04 c
Zn	173.55 ± 38.40 a	219.55 ± 10.11 a	393.10 ± 24.33 a
Cu	160.40 ± 3.82ab	61.45 ± 4.31 b	221.85 ± 1.74 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.52: Concentration of Zn (mg/kg) in pennisetum as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Zn (mg/kg)		
	Root	Shoot	Total
Control	85.25 ± 2.33 c	39.70 ± 10.89 c	121.95 ± 5.47 c
Cd	48.45 ± 8.13 d	29.95 ± 5.73 c	78.40 ± 4.45 c
Pb	69.95 ± 7.57 cd	35.20 ± 0.57 c	105.15 ± 6.23 c
Zn	196.60 ± 7.64 a	236.05 ± 0.49 a	432.65 ± 4.82 a
Cu	128.00 ± 0.14 b	95.50 ± 14.04 b	223.50 ± 16.91 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

With regard to Cu accumulation, Cu-spiked treatments in all three grasses showed significantly higher ($p<0.05$) total Cu accumulation compared to the other treatments (Table 3.53 – Table 3.55). Higher accumulation of Cu was found in Cu-spiked treatments (22.84 – 49.80 mg/kg) compared to the other treatments (1.45 – 12.90 mg/kg). Significantly greater ($p<0.05$) Cu accumulation was observed in the roots whereas no significant differences ($p>0.05$) were observed in shoots of the Cu-spiked treatments. Between roots and shoots, Cu accumulation in the roots was relatively greater than in the shoots of all three grasses. The accumulation trend for Cu was in the following order of pennisetum > imperata > Vetiver.

Table 3.53: Concentration of Cu (mg/kg) in Vetiver as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Cu (mg/kg)		
	Root	Shoot	Total
Control	4.13 ± 1.57 b	3.57 ± 1.64 a	7.70 ± 1.78 b
Cd	9.70 ± 1.27ab	1.89 ± 0.67 a	11.59 ± 0.93ab
Pb	3.69 ± 1.50 b	5.49 ± 3.16 a	9.18 ± 0.66 b
Zn	4.23 ± 1.62 b	3.75 ± 1.53 a	7.98 ± 1.91 b
Cu	17.95 ± 8.98 a	4.89 ± 2.21 a	22.84 ± 5.77 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 3.54: Concentration of Cu (mg/kg) in imperata as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Cu (mg/kg)		
	Root	Shoot	Total
Control	6.60 ± 1.13 b	4.20 ± 1.25 a	10.80 ± 2.12 b
Cd	1.45 ± 0.21 b	ND	1.45 ± 0.21 b
Pb	2.20 ± 0.99 b	ND	2.20 ± 0.99 b
Zn	2.10 ± 1.55 b	2.10 ± 1.69 a	4.20 ± 0.74 b
Cu	45.90 ± 19.66 a	3.85 ± 1.20 a	49.75 ± 13.46 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability; ND = Not detected

Table 3.55: Concentration of Cu (mg/kg) in pennisetum as influenced by different types of spiked heavy metal treatments

Treatment	Concentration of Cu (mg/kg)		
	Root	Shoot	Total
Control	8.75 ± 4.74 b	4.15 ± 0.49 a	12.90 ± 3.62ab
Cd	3.45 ± 2.05 b	4.25 ± 1.20 a	7.70 ± 1.47ab
Pb	1.85 ± 0.07 b	0.70 ± 0.57 a	2.55 ± 1.04 b
Zn	0.40 ± 0.18 b	2.25 ± 1.48 a	2.65 ± 0.58 b
Cu	45.25 ± 21.43 a	4.55 ± 2.31 a	49.80 ± 14.75 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

3.3.3.4 Translocation and efficacy of heavy metals

The association of the different heavy metals accumulated from the soils into the roots and shoots for all the three grasses, in terms of BCF, BAC, TF and efficacy (%) are presented in Tables 3.56 – Table 3.67.

In all the three grasses, relatively more heavy metals were accumulated in the roots than shoots, where it was observed that the root and soil concentration quotient (BCF) was > 1 suggesting that heavy metal translocation from the soil to root was substantially higher and the roots acted as the sink for heavy metal accumulation. All the three grasses recorded remarkably higher BCF (> 1) in the accumulation of Cd (2.947 – 5.977) when grown under Cd-spiked treatments. Zn-spiked treatments in Vetiver (3.532) exhibited appreciably higher accumulation of Zn, followed by Pb-spiked treatments in imperata (2.075) and pennisetum (1.824) that resulted in relatively higher concentration of Pb in the roots compared to the shoots. Comparatively, all the three grasses, showed BAC values < 1, suggesting that the translocation pathway of heavy metals from soils into the shoots may have been inhibited. The accumulation of both Cd and Pb in Vetiver exhibited the highest BAC in the Cd-spiked (0.229) and Pb-spiked (0.176) treatments compared to the other treatments.

Considering the relatively lower accumulation of heavy metals in the shoots than root, in all the three grasses, TF was assessed to gauge the capability of the plant to translocate heavy metals from the roots to the shoots. Zn-spiked treatments of both imperata (1.265) and pennisetum (1.201) recorded relatively higher TF values for the accumulation of Zn. Although the TF value was < 1, Vetiver showed reasonably higher TF in the spiked heavy metal treatments than in other treatments for both the accumulation of Cd (0.038) and Pb (0.317), respectively. A higher TF value was recorded for the accumulation of both Cd (0.358 – 2.800) and Zn (0.349 – 1.265) despite the high TI observed in the spiked heavy metal treatments of imperata.

The efficacy (%) of heavy metal accumulation was calculated in order to evaluate the potential and efficiency of metal translocation and bioaccumulation inside the plant, from roots to shoots. The accumulation efficacy revealed that the spiked heavy metal treatments for Vetiver accumulated reasonably higher Cd (3.69%) and Pb (23.84%) than for other treatments. Between the different grasses, imperata accumulated relatively lower Cd (26.36%), Pb (14.75%), Zn (55.85%) and Cu (7.74%) compared to pennisetum which recorded Cd (42.34%), Pb (19.28%), Zn (54.56%) and Cu (9.14%). The accumulation efficacy of Vetiver was 4.56 – 9.09% higher for Pb and 12.27 – 13.67% higher for Cu when compared to the other grasses, while the efficacy for Cd accumulation in pennisetum was 15.98 – 38.65% higher. A 1.29 – 24.62% higher efficacy for Zn was recorded in imperata compared to the other grasses.

Table 3.56: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cd in Vetiver as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Cd			
	BCF	BAC	TF	Efficacy (%)
Control	0.076 b	0.000	0.000	0.000
Cd	5.977 a	0.229	0.038	3.685
Pb	0.000	0.000	0.000	0.000
Zn	0.152 b	0.000	0.000	0.000
Cu	0.030 b	0.000	0.000	0.000

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.57: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cd in imperata as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Cd			
	BCF	BAC	TF	Efficacy (%)
Control	1.857ab	1.519 a	0.818 b	45.000 b
Cd	3.753 a	1.343ab	0.358 b	26.357 b
Pb	0.485 b	0.274 c	0.565 b	36.111 b
Zn	0.105 b	0.295 c	2.800 a	73.684 a
Cu	0.148 b	0.127 c	0.857 b	46.154ab

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.58: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cd in pennisetum as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Cd			
	BCF	BAC	TF	Efficacy (%)
Control	2.257ab	1.055 b	0.467bc	31.847 a
Cd	2.947 a	2.163 a	0.734ab	42.335 a
Pb	0.759 b	0.380 c	0.500bc	33.333 a
Zn	0.295 b	0.380 c	1.286ab	56.250 a
Cu	0.316 b	0.443 c	1.400 a	58.333 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.59: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Pb in Vetiver as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Pb			
	BCF	BAC	TF	Efficacy (%)
Control	0.337 b	0.138 ab	0.409 ab	29.04 a
Cd	0.389 b	0.270 ab	0.695 a	41.01 a
Pb	2.075 a	0.359 ab	0.173 b	14.75 a
Zn	0.031 b	0.068 b	2.167 b	68.42 a
Cu	0.148 b	0.000	0.000	0.000

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.60 Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Pb in imperata as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Pb			
	BCF	BAC	TF	Efficacy (%)
Control	0.337 b	0.138 ab	0.409 ab	29.04 a
Cd	0.389 b	0.270 ab	0.695 a	41.01 a
Pb	2.075 a	0.359 ab	0.173 b	14.75 a
Zn	0.031 b	0.068 b	2.167 b	68.42 a
Cu	0.148 b	0.000	0.000	0.000

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.61: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Pb in pennisetum as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Pb			
	BCF	BAC	TF	Efficacy (%)
Control	0.279 b	0.122 b	0.438 b	30.43 a
Cd	0.537 b	0.180 ab	0.334 b	25.06 a
Pb	1.824 a	0.436 a	0.239 b	19.28 a
Zn	0.164 b	0.255 ab	1.553 ab	60.83 a
Cu	0.096 b	0.174 b	1.818 a	64.52 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.62: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Zn in Vetiver as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Zn			
	BCF	BAC	TF	Efficacy (%)
Control	0.993 b	0.409 b	0.411 b	29.15 a
Cd	1.460 b	0.417 b	0.285 b	22.20 a
Pb	1.133 b	0.776 ab	0.685 ab	40.65 a
Zn	3.532 a	1.604 a	0.454 b	31.23 a
Cu	1.026 b	1.192 ab	1.162 a	53.75 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.63: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Zn in imperata as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Zn			
	BCF	BAC	TF	Efficacy (%)
Control	0.657 a	0.304 b	0.462 ab	31.62 b
Cd	0.440 a	0.154 b	0.349 b	25.87 b
Pb	0.305 a	0.216 b	0.707 ab	41.41 ab
Zn	0.694 a	0.878 a	1.265 ab	55.85 a
Cu	0.861 a	0.330 b	0.383 b	27.70 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.64: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Zn in pennisetum as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Zn			
	BCF	BAC	TF	Efficacy (%)
Control	0.458 c	0.213 c	0.466 b	32.55 b
Cd	0.260 d	0.161 c	0.618 ab	38.20 b
Pb	0.376 cd	0.189 c	0.503 b	33.48 b
Zn	0.786 a	0.944 a	1.201 a	54.56 a
Cu	0.687 ab	0.513 b	0.746 ab	42.73 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.65: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cu in Vetiver as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Cu			
	BCF	BAC	TF	Efficacy (%)
Control	0.368 c	0.318 a	0.864 ab	46.36 a
Cd	0.865 ab	0.168 a	0.195 b	16.31 a
Pb	0.329 c	0.489 a	1.488 a	59.80 a
Zn	0.377 c	0.334 a	0.887 ab	46.99 a
Cu	0.898 a	0.245 a	0.272 b	21.41 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.66: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cu in imperata as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Cu			
	BCF	BAC	TF	Efficacy (%)
Control	0.588 ab	0.374 a	0.636 a	38.89 ab
Cd	0.129 b	0.000	0.000	0.000
Pb	0.196 b	0.000	0.000	0.000
Zn	0.187 b	0.187 a	1.000 a	50.00 a
Cu	2.295 a	0.193 a	0.084 a	7.74 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 3.67: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cu in pennisetum as influenced by different types of spiked heavy metal treatments

Treatment	Accumulation of Cu			
	BCF	BAC	TF	Efficacy (%)
Control	0.780 ab	0.370 a	0.474 b	32.17 ab
Cd	0.307 ab	0.379 a	1.232 ab	55.19 ab
Pb	0.165 ab	0.062 a	0.378 b	27.45 ab
Zn	0.036 c	0.201 a	5.625 a	84.91 a
Cu	2.263a	0.228 a	0.101 b	9.14 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

3.4 Discussion

3.4.1 Heavy metal phytoassessment in tropical vegetables (water spinach and okra)

Clay soil normally has a high water holding capacity and nutrient retention, but has low aeration and water infiltration properties, due to the small particle size of clay soils (Page, 1952). Soil pH is also significant being part of the important external environmental parameters that can affect plant growth as well as control the solubility and availability of plant nutrients in the soil. All the treatments showed a decrease in soil pH, likely due to the availability of metal (cation) ions exchange in the plants (Motior ***et al***., 2011; Rahman ***et al***., 2013). Generally, most plants are able to grow normally within a certain range of soil pH, however the rate of plant survival will decline when plants are cultivated in extreme acidic and alkaline conditions. As can be seen from the experimental results, the pH range for both water spinach and okra growth, are in the slightly acidic pH range. Although both plants were able to grow, Moyin-Jesu (2007) reported that a higher soil pH would probably be more suitable to increase the growth and contents of vegetables especially okra.

The possible cause for the lower number of leaves in all spiked metal treatments could be the effects of the added spiked metal solutions in the soils, as inhibitory concentrations will retard metabolism in the plant tissues. Past studies by Yang ***et al***. (2002) and Wang ***et al***. (2013) on Cu metal in vegetables showed that the rate of photosynthesis and plant growth are inhibited by excessive amounts of Cu in the plant tissues.

The dry matter content in water spinach was expected to be higher compared to okra due to its higher vegetative growth regardless of the different spiked metal treatments. Okra exhibited the lowest dry matter content in all the treatments, as its growth rate was slow throughout the entire experimental period. Dry matter content is an important plant ecology trait which is closely associated with plant growth and survival (Shipley & Vu, 2002). Grime and Hunt (1975) reported that dry matter content reflects the role of variation in potential relative growth rate and the ecological behavior of the plant. The possible reason for the slower growth rate observed in okra compared to water spinach could be due to the effects of the spiked metals in the contaminated clay soil.

The continuous use of contaminated soil may eventually cause both water spinach and okra to have minimum dry matter content in all the treatments, including the control treatment, due to water stagnation on the clay soil texture. Soil properties and content are probably the contributing factors which inhibited growth as can be seen in the lower plant height and leaf number in both water spinach and okra regardless of the different types of spiked metal treatments. Thus, the presence of additional spiked metals in the Pb, Zn and Cu treatments contributed to lower plant growth, specifically in terms of plant height and number of leaves, hence causing a lower output of plant dry matter content.

Heavy metal accumulation trends for okra were in the order of Pb > Zn > Cu whilst Zn accumulated the highest followed by Pb and Cu in water spinach. Similar trends were reported by Göthberg ***et al***. (2002), Göthberg ***et al***. (2004) and Huang ***et al***. (2014) who observed appreciably higher accumulation of Pb in water spinach for both treated and untreated Pb treatments. However, different water spinach cultivars are likely to have a different range of heavy metal accumulations as was recorded by He ***et al***. (2015) and Alia ***et al***. (2015).The high accumulations of Zn and Cu that were found in the roots of both water spinach and okra was possibly due to the translocation these metal ions from soil into the roots because Zn and Cu are required micronutrients that are routinely taken by plants for life processes (Hopkins, 1999; Mengel ***et al***., 2001).

TF and BCF values are essential indicators that are commonly used to evaluate potential plant species for phytoremediation. Nazir ***et al***. (2011) and Malik ***et al***. (2010) reported that both water spinach and okra can be potential Pb and Zn phytoaccumulators when TF and BCF values >1. Due to the high TF and BCF values obtained in this study, both water spinach and okra shoots (TF = 1.01 to 2.28) can be considered to be the sinks (metal accumulators) for Pb and Zn, whilst the roots of water spinach and okra (BCF = 1.28 to 7.65) act as the sink for the accumulation of Pb, Zn and Cu metals from the source of heavy metals in the soil. Both Pb treated water spinach (0.764) and okra (0.860) showed good tolerance properties for Pb metal accumulation which recorded the highest Tolerance Index (TI), compared to the plants cultivated under Zn and Cu treatments. Although both Pb treated water spinach and okra recorded high TI, the TF and BCF values of water spinach indicated greater translocation of heavy metals from soil to the shoots of the plant. This could be probably due to the high Pb mobility and good phytoaccumulator properties of water spinach.

Even though, both water spinach and okra cultivated in the Zn and Cu treatments did not exceed the allowable levels, the presence of high amounts of Zn and Cu in food is enough to pose health problems, as it has been reported that the major source of these metals are available in almost all urban environmental soils (Thorton, 1991; Li ***et al***., 2001). As a general rule of thumb, any high concentration of heavy metal accumulation in the edible parts of vegetables (shoots of water spinach and fruits of okra) renders it as not recommended for food consumption. Hence, it is not encouraged to grown water spinach and okra in soils which are contaminated with Pb as it may cause high Pb accumulation in the plants. These findings are aligned with the recommendations proposed by Gothberg ***et al***. (2002) and Marcussen ***et al***. (2008).

Although the concentrations of Zn and Cu for both water spinach and okra were lower than the national and international permitted food standards, continuous monitoring and further research in assessing the accumulation of metals in other different types of vegetables is essential in order to avoid excessive bioaccumulation of hazardous metals as well as ensuring the quality of the vegetables. Nevertheless, the study in this study also recognizes that the use of pot assays, instead of field-site experiments would probably give rise to an enhanced accumulation of heavy metals in plants. In addition, the accumulation of heavy metals in plants is extremely complex in nature as various biotic and abiotic factors may likely influence the mechanisms of phytoremediation. It would be worthwhile to extend the further research of this study to other metal elements and a wider range of commonly grown vegetables.

The accumulation of Pb, Zn and Cu varied between both water spinach and okra. For okra, heavy metal accumulation were in the order of Pb > Zn > Cu whilst Zn accumulated the highest followed by Pb and Cu in water spinach. This study has shown that there were significant differences ($p<0.01$) found in both water spinach and okra cultivated under the three spiked metal treatments of Pb, Zn and Cu. It also indicated that both water spinach and okra shoots are the sinks for Pb and Zn accumulation while the roots acted as the sink for the heavy metal accumulation of all three metal ions, the Pb, Zn and Cu, according to the high TF and BCF values obtained.

Among all the three different spiked metal treatments, both water spinach and okra showed great tolerance for Pb accumulation as the accumulation of Pb was high in the roots and shoots of both plants. The concentrations of Pb in the shoots of water spinach and okra exceeded the maximum permissible levels stipulated by the national Malaysian Food Act 1983 and Food Regulations 1985 as well as the international Codex Alimentarius Commission-Joint FAO/WHO Expert Committee on Food Additives (JECFA) standards. The variation of Pb metal accumulation between the different plant parts may be useful for selecting suitable cultivation of vegetable species in order to minimize the intake of potentially harmful elements. Hence, this study of studies strongly suggest that water spinach and okra are not recommended to be cultivated in Pb contaminated soils.

Nevertheless, water spinach and okra were selected in this study for the research study of heavy metal phytoassessment due to its fast growth and being the most commonly grown vegetable crops throughout the year when in Malaysia where the availability of water and soil are sufficiently provided (Tindall, 1983; Cornelis ***et al***., 1985; AVRDC, 1993; Munro & Small, 1997; Van Wyk, 2005; Biggs ***et al***., 2006) Both vegetables also possess favourable phytoaccumulation properties (Göthberg ***et al***., 2002; Cheng, 2003; Chen ***et al***., 2010; Xin ***et al***., 2010; Okieimena ***et al***., 2011; Azeez ***et al***., 2013).

In spite of that, the selection of vegetables to be used as heavy metal phytoremediators for further studies has been discontinued due to the unavoidable controversial perspectives that vegetables are customarily regarded as daily harvested edible crops (Cobb ***et al***., 2000; Yang ***et al.,*** 2011). Nonetheless, phytoremediation using non-edible plant species can ultimately restrict the soil contaminants from being introduced into the food chain.

3.4.2 Heavy metal phytoassessment in tropical plants (Vetiver, acacia and mucuna)

Based on plant growth performance, the results indicated that the application of hydrotoxic soil-leachate had adverse effects on growth in mucuna. Recent studies by Nwaichi and Wegwu (2012) and Azeez ***et al***. (2013) reported similar effects in mucuna (***M. pruriens***) species with regard to its growth rate and phytoaccumulative ability. Truong ***et al***. (2008) and Danh ***et al***. (2009) had earlier reported that Vetiver has high tolerance ability to survive under a wide range of contaminated conditions without affecting its growth.

It has been documented that Vetiver is able to grow under both hydroponic and hydrotoxic conditions as was reported by Chen ***et al***. (2012) and recently by Truong and Danh (2015), whilst both acacia and mucuna were only able to survive under 20% composition of hydrotoxic treated leachate conditions. The results of this study have shown that Vetiver did not exhibit adverse growth effects and is able to withstand hydrotoxic conditions compared to both acacia and mucuna. This demonstrates that Vetiver has great potential for use as phytoremediator in hydrotoxic soil conditions.

Nonetheless, the findings were contrary to the observations made by Majid ***et al***. (2011), Justin ***et al***. (2011) and Maiti ***et al***. (2015) who reported that acacia is a potential heavy metal phytoremediator in both roots and shoots. The possible reasons for the substantial reduction in metal accumulation in the roots and shoots of acacia in this study may be due to the presence of high levels of hydrotoxic soil-leachate conditions. Comparatively, the percentages of plant survivorship in acacia also dropped drastically when the composition of hydrotoxic treated leachate exceeded 20% in this study unlike to the three past studies of Majid ***et al***. (2011), Justin ***et al***. (2011) and Maiti ***et al***. (2015) where acacia were conducted under 100% contaminated soil conditions. The findings for acacia in this study have shown its unsuitability for use for phytoremediation under mixed hydrotoxic soil-leachate conditions.

The appreciable reduction of both Cd and Pb accumulation in acacia and mucuna compared to Vetiver was likely due to the plants withering during the experimental period. The findings also indicated that the concentrations of both Cd and Pb accumulated in the shoots of all the three plants decreased progressively as a result of the increased application of hydrotoxic levels. Similarly, the results have also shown that the application of high levels of hydrotoxic materials can enhance metal accumulation in mucuna. These findings revealed that the uptake of both Cd and Pb may not be strictly inhibited by the plant growth rate over time and its capability to bio-accumulate before the plants gradually withered due the presence of excessive amounts of hydrotoxic materials (Shahandeh & Hossner, 2000; Merkl ***et al***. 2005).

The trend for both Cd and Pb accumulation, under hydrotoxic soil-leachate conditions, in all the three tropical plants studied were in the order of Vetiver > acacia > mucuna. The fast growing Vetiver showed high dry matter content production compared to both acacia and mucuna. All three plants accumulated remarkably higher concentrations of both Cd and Pb in the roots and shoots. However, Vetiver exhibited the greatest potential for phytoremediation under hydrotoxic contaminated conditions due to its good tolerance ability to withstand hydrotoxic soil-leachate and the high percentage of metal efficacy demonstrated for both Cd and Pb. Consequently, a more comprehensive heavy metal phytoassessment of Vetiver will be further explored in the up-coming studies.

3.4.3 Heavy metal phytoassessment in tropical grass (Vetiver, imperata and pennisetum)

The soil used in this study had an ideal bulk density of 1.54 g/cm^3 for plant growth, while its porosity (41.76%) constituted almost half of the soil composition to provide sufficient air and water for good plant growth (BOPRC, 2014). The optimum soil pH for the bioavailability and uptake of essential elements in plants has been reported to be between 5.5 and 7.5 (Moody, 2006). The changes in soil pH observed could be related to the proton ion (H^+) concentration in the soil, whereby a pH reduction would mean more protons are present and vice versa. This is related to the availability of heavy metals in the soil treatments which would subsequently influence the growth performance of these grasses. It is known for example, that the uptake and accumulation of nitrate and sulfate in the roots are accompanied with proton uptake into the roots as well. If these processes are slowed down or inhibited, it would mean that there would be more protons in the soil (Tischner, 2000; Sorgonà ***et al.***, 2011). Soil pH has a strong influence on the availability of plant nutrients and can affect the soil-plant interaction with regard to heavy metal accumulation (Husson, 2013).

The experiments carried out showed that the application of spiked heavy metals in the soil did not affect plant height over dry matter in Vetiver although the opposite was recorded in pennisetum. Ovečka and Takáč (2014) reported recently that the presence of spiked heavy metals in the soil can contribute to reduced plant growth and this was observed in both imperata and pennisetum. However, the growth of Vetiver was not significantly affected, suggesting that the grass was highly adaptable and tolerant to extreme environmental conditions of spiked heavy metals contamination (Danh ***et al.***, 2009). The large increase in plant height observed in the case of imperata (44.89 ± 17.23 cm) could be due to requirement by the plant for Cu as a micronutrient.

It can be seen from the above results that Vetiver accumulated the highest amount of Cd (93.08 ± 3.81 mg/kg) and Zn (1284.00 ± 234.83 mg/kg) in the spiked heavy metal treatments, compared to imperata and pennisetum. All three grasses showed a similar inclination in the order of heavy metal accumulation, with Zn > Pb > Cd > Cu regardless of the total amount of spiked heavy metal put into the soil. The high concentration of heavy metal accumulation found in the roots and shoots of all grasses could be attributed to the method of application of the spiked heavy metals. The use of direct pot assays for spiked heavy metals instead of field-site application is a possible cause for the high concentration of heavy metal accumulation found in the roots and shoots of these plants.

The amount of metal content present in the spiked heavy metal treatments can be considered to be similar to that of a contaminated soil, following recent reports in the literature (CCME, 1999; DOE, 2009). The concentrations of Cd (15.30 mg/kg), Pb (143.30 mg/kg), Zn (258.90 mg/kg) and Cu (22.40 mg/kg) present were above the national and international guidelines for soil contamination permissible levels. Studies by the DOE (2009) observed that, for Malaysian soils, the typical concentration range for naturally occurring heavy metals are as follows: Cd (0.09 – 14.40 mg/kg), Pb (0.18 – 36.00 mg/kg), Zn (6.90 – 54.30 mg/kg) and Cu (4.00 – 19.80 mg/kg). On the other hand, the soil quality guidelines put forward by the Canadian Council of Ministers of the Environment, has set the allowable limits for heavy metal contamination to range from 1.4 – 10.0 mg/kg (Cd), 70.0 – 140.0 mg/kg (Pb), 200.0 – 360.0 mg/kg (Zn) and 63.0 – 91.0 mg/kg (Cu) for both agricultural and urban residential soils (CCME, 1999).

The TF values and heavy metal accumulation efficacy (%) results are vital to estimate the phytoremediation potential of a plant species. Malik ***et al.*** (2010) and Nazir ***et al.*** (2011) suggested that a plant would be suitable for phytoremediation when the BCF, BAC and TF values are > 1. In this study, the TF values and efficacy (%) recorded for Vetiver exhibited the best potential for the accumulation of higher amounts of Pb and Cu than the other two grasses. Nevertheless, imperata exhibited remarkably higher TF values and efficacy (%) for the accumulation of Zn whereas pennisetum showed greater ability for Cd accumulation. However, none of the three grasses tested in this study satisfied the conditions that require all the BCF, BAC and TF values to be > 1.

Despite the low accumulation of heavy metal found in the shoots, all three grasses recorded high BCF values > 1. All the heavy metals greatly accumulated in the roots irrespective of the type of heavy metal. Phytostabilization and phytoextraction are two different categories of phytoremediation which involve the application of different functions and characteristics of plants used to remove heavy metals from contaminated soil (Douchiche ***et al.***, 2012). The primary mechanism involved in phytostabilization is the immobilization of heavy metal ions by storing them at root level without aiming to remove the heavy metals to the upper plant (Berti & Cunningham, 2013; Ali ***et al.***, 2013). On the other hand, phytoextraction mainly refers to the efficiency of heavy metal translocation from the roots to shoots after the accumulation of metals in the roots of the plant. As a result, phytoextraction involves the harvesting of above ground biomass (shoots) for the removal of heavy metals from contaminated soil (Lone ***et al.***, 2008).

A plant is suitable for phytostabilization if its BCF > 1, even if it has a low TF values. However, plants with TF values > 1 and relatively high efficacy (%) are more favorable for phytoextraction. All the three grasses studied can be used for phytostabilization in Cd contaminated soils, whilst Vetiver demonstrated promising phytostabilization traits for the accumulation of Zn. Both imperata and pennisetum showed good phytostabilization properties for Pb and Cu. The findings of this study also showed that both imperata and pennisetum can be utilized for Zn phytoextraction, based on their remarkably high TF and accumulation efficacy (%) values.

The trend for heavy metal accumulation in all the three grasses varied and was in the order of Zn > Pb > Cd > Cu. Vetiver accumulated appreciably higher total concentrations of Cd and Zn than both imperata and pennisetum. All three grasses accumulated relatively higher heavy metal concentrations in the roots than shoots except for Zn accumulation in both imperata and pennisetum. As a result of the BCF values being > 1, the accumulation of Cd, Pb and Cu in all three grasses highlighted that the roots acted as the sink for heavy metals accumulation. The findings of this study indicated that different promising potential for phytostablization was found in Vetiver for Cd and Zn; in imperata for Cd, Pb and Cu; and in pennisetum for Cd, Pb and Cu. Both imperata and pennisetum also exhibited as good Zn phytoextraction properties when grown in contaminated soil.

3.5 Conclusions

Based on all of the findings in this chapter, it can be concluded that Vetiver remained the most promising plant species compared to the other plants studied for use in heavy metals phytoremediation due to its positive characteristics of fast growth, good tolerance towards abiotic stress and its ability to withstand high contaminated heavy metals. Nevertheless, further studies on the advancement of heavy metals phytoremediation under different contaminated soil conditions need to be conducted in the following chapters to conclusively determine the status and extent of Vetiver grass' phytoremediation ability.

CHAPTER 4: [2] PHYTOTOLERANCE AND THRESHOLD OF VETIVER GRASS IN CADMIUM AND LEAD CONTAMINATED SOIL

4.1 Introduction

Over the years, soil contamination has attracted much global attention as it instigates considerable risks to human health and the environment. Anthropogenic sources of soil contaminants such as heavy metals released by human activities via industrial and agricultural practices, urban activities and transportation have caused serious threats to the environment (Sterckeman ***et al***., 2006; Meuser, 2010). Heavy metal is widely used to describe a large group of elements in the periodic table with an atomic density greater than 5 g/cm^3 but can also be defined in relation to its natural chemical properties (Hawkes, 1997; Alloway, 2013). Among various types of heavy metals, both cadmium (Cd) and lead (Pb) are regarded to be highly toxic pollutants even at low concentration levels.

Although naturally occurring, both Cd and Pb are often imperceptible, non-biodegradable and are persistent in soils over a long duration (Bradl, 2005; Tchounwou ***et al.,*** 2012). Unlike other types of heavy metals (as discussed in Chapter 3), both Cd and Pb are extremely hazardous to human health as it is easily bio-accumulated via the food chain including direct inhalation, ingestion of soil and/or consumption of contaminated plants (Kamal ***et al.***, 2016; Ng ***et al.***, 2016a).

[2] A version of this chapter has passed through the scientific peer-review procedures and been accepted for publication in the Chiang Mai Journal of Science (CMJS), http://it.science.cmu.ac.th/ejournal/index.php on July 2016.

The exposure of plants in heavy metal contaminated soil can inhibit plant growth; reduce metabolism and lower biomass due to the toxicity effects of both Cd and Pb (Prasad, 1995; Das ***et al***., 1997; Sharma & Dubey 2005; Benavides ***et al.***, 2005; Nagajyoti ***et al.***, 2010; Pourrut ***et al.***, 2011). As a result, the Priority Pollutant List (US EPA, 2014) has recognised both Cd and Pb to be among the 126 Toxic and Priority Pollutants, due to its lethal characteristic in nature.

There are numerous remediation technologies, including both physical (excavation, containment and fracturing) and chemical (soil washing, solidification-stabilization and chemical redox) assisted methods that have been tested to clean up contaminated heavy metals in soils (Riser-Roberts, 1998; US FRTR, n.d.). Nonetheless, phytoremediation has evolved to be an alternative method that is cost-effective, non-destructive and an environmentally friendly solution for heavy metals soil contamination compared to other techniques (Glass, 2000; Gomes, 2012; Ali ***et al***., 2013).

Among the various types of plants, Vetiver grass, ***Vetiveria zizanioides*** (Linn.) Nash has been widely used for all kinds of contaminated soil phytoremediation including heavy metals. Furthermore, based on the earlier findings in Chapter 3, Vetiver grass has been reported to be one of the most promising species with great potential for heavy metals phytoremediation due to its fast growth, extensive deep root system, high tolerance to a wide range of adverse soil conditions, requires low maintenance and is abundantly available in Malaysia and many other tropical and sub-tropical countries across the Asia-Pacific region (Maffei, 2002; Truong ***et al***., 2008; Truong & Danh, 2015; Ng ***et al***., 2016b,c).

However, at present, there is scarce information available concerning the effect of different levels of spiked heavy metals on Vetiver grass. Recent studies by Banerjee ***et al.*** (2016), Pidatala ***et al.*** (2016) and Chen ***et al.*** (2015a) have solely reported on the phytoextraction and phytostabilization effects of heavy metals uptake in Vetiver grass. Nevertheless, there are currently no robust studies that have been tested to evaluate the comparative and empirical phytoassessment using Vetiver grass growing under different levels of spiked Cd and Pb conditions. Hence, there is a growing need and urgency to conduct this chapter to evaluate the growth performance; assess the metal uptake ability and accumulation trends; and examines the phytotolerance and threshold limits of Vetiver grass growing under different levels of spiked Cd and Pb contaminated soil conditions.

4.2 Materials and Methods

4.2.1 Samples preparation and experimental design

This chapter was conducted using pot experiments in the planthouse of the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur with the average room temperature ranging between 27°C and 36°C throughout the day. Top soil (0 – 20cm) collected from uncontaminated field located in the University of Malaya, Kuala Lumpur (3° 7' N latitude and 101° 39' E longitude) was used as the tested soil in the experiment after undergoing preliminary physico-chemical soil assessment (Table 4.1). The dull reddish brown soil composed of 90.47% sand, 7.89% silt and 1.64% clay. All collected soils were air-dried for a week, followed by <4mm sieving using test sieve to remove gravels and large non-soil particles.

The artificially spiked heavy metal treatments were prepared using cadmium nitrate tetrahydrate [$Cd(NO_3)_2.4H_2O$] and lead (II) nitrate [$Pb(NO_3)_2$] salt compounds that exceed the median permissible in the natural occurring levels of the Malaysian (DOE, 2009) Canadian (CCME, 1999) and European Union (Lado ***et al.***, 2008) soil contamination guidelines. The amended soil was then continuously stirred and incubated for a week to ensure that the homogeneousity of the desired spiked heavy metal concentration is obtained.

Table 4.1: Physico-chemical parameters of growth media soil

Parameter (Unit)		**Mean**
pH		6.62 ± 0.09
Temperature (°C)		31.8 ± 2.4
Soil texture		
Sand (%)		90.47
	Very coarse sand (%)	1.93
	Coarse sand (%)	50.45
	Medium coarse sand (%)	32.19
	Fine sand (%)	10.22
	Very fine sand (%)	5.21
Silt (%)		7.89
Clay (%)		1.64
Metal contents (mg/kg)		
	Cd	1.23 ± 0.46
	Pb	9.25 ± 1.42
Bulk density (g/cm^3)		1.49 ± 0.56
Porosity (%)		43.77 ± 2.14
Water content (%)		3.85 ± 0.71
Field capacity (%)		24.32 ± 2.16
Saturation level (%)	Dry	15.83
Colour (Munsell colour charts)	Dull reddish brown	2.5YR 5/4

Mean ± standard deviation

The control and five different levels of spiked Cd and Pb treatments (Table 4.2) were tested using Vetiver grass in a plastic pot (0.18m diameter x 0.16m depth) filled with 2kg of soil, respectively. Vetiver plantlets were obtained from Humibox Malaysia whereby only fresh and healthy plantlets with an initial average tiller number (10 – 15), plant height (30 – 35cm) and basal diameter (2.5 – 3.5cm) were selected for the experiment.

Table 4.2: Treatment variables

Treatment	**Spiked heavy metal**
Control	No heavy metal added
5Cd	5 mg/kg of Cd
10Cd	10 mg/kg of Cd
50Cd	50 mg/kg of Cd
100Cd	100 mg/kg of Cd
150Cd	150 mg/kg of Cd
50Pb	50 mg/kg of Pb
100Pb	100 mg/kg of Pb
200Pb	200 mg/kg of Pb
400Pb	400 mg/kg of Pb
800Pb	800 mg/kg of Pb

All of the spiked heavy metal treatments were watered evenly with 50mL of tap water once a day and the plant growth parameters such as height, tiller number, basal diameter and percentage plant survivorship were continuously monitored throughout the 60-day period of the experiment. The study of this chapter was conducted under a completely randomized design (CRD) with three replications (n = 3).

4.2.2 Samples and chemical analysis

At the end of the 60-day of the experimental period, all Vetiver treatments were uprooted and brought into the laboratory for chemical analysis. Freshly harvested Vetiver were washed in running filter water followed by deionized water to remove any adhering soil particles before it was sectioned into parts of roots and tillers (shoots). All soil and plant samples were oven-dried for 72 hours at 70°C to obtain a constant weight of dry matter content before it was homogenized in a mortar and pestle.

Approximately, 0.5g of the homogenized samples underwent acid digestion with hydrochloric acid (HCl), nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) according to Method 3050B (US EPA, 1996) followed by Method 7000B (US EPA, 2007a) for total recoverable elemental analysis of both Cd and Pb using the Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (F-AAS). All chemicals used were of analytical reagent standard or of the best grade available. The highly precise technique of chemical analysis was controlled using the Bundesanstalt für Materialforschung und –prüfung (BAM Germany): German Federal Institute for Materials Research and Testing (BRM#12-mixed sandy soil) certified reference material with an average rate of metal recovery for Cd (93.46%) and Pb (108.25%), respectively.

4.2.3 Statistical analysis and data interpretation

Growth performance was evaluated using the root-tiller (R/T) quotient and tolerance index (TI) whilst the ability for metal accumulation and translocation upwards in Vetiver grass were assessed by determining the translocation factor (TF), biological concentration factor (BCF), biological accumulation coefficient (BAC) and percentage of metal uptake efficacy (Kabata-Pendias, 2010; Alloway, 2013; Ali ***et al***., 2013) as follows:

R/T quotient = Dry matter content in root / Dry matter content in tiller

TI = Total dry matter content in spiked heavy metal treatment / Total dry matter content in control

TF = Concentration of heavy metal in tiller / Concentration of heavy metal in root

BCF = Concentration of heavy metal in root / Concentration of heavy metal in soil

BAC = Concentration of heavy metal in tiller / Concentration of heavy metal in soil

Metal uptake efficacy (%) = [Concentration of heavy metal in tiller / Total concentration of heavy metal accumulated in Vetiver] x 100

All experimental data were analysed by performing the one–way analysis of variance (ANOVA) and further statistical validity test for significant differences among treatment means was conducted by employing the Fisher's least significant difference (LSD) tests at the 95% level of confidence.

4.3 Results

4.3.1 Soil pH and plant growth

The initial soil pH varied from 4.12 to 5.57, where control soil recorded the highest pH of 5.57 while the lowest pH of 4.12 was observed in 800Pb treatment (Figure 4.1 and Figure 4.2). Upon harvesting, all spiked Cd and Pb treatments, except for 800Pb treatment, showed a decline in pH ranging from 4.20 to 4.62, where the highest pH reduction (-1.03 pH units) was recorded in 50Pb treatment. No significant difference (***p>0.05***) in soil pH was observed among all Cd spiked treatments. On the other hand; 100Pb, 200Pb, 400Pb and 800Pb treatments significantly (***p<0.05***) affected the soil pH levels compared to the control.

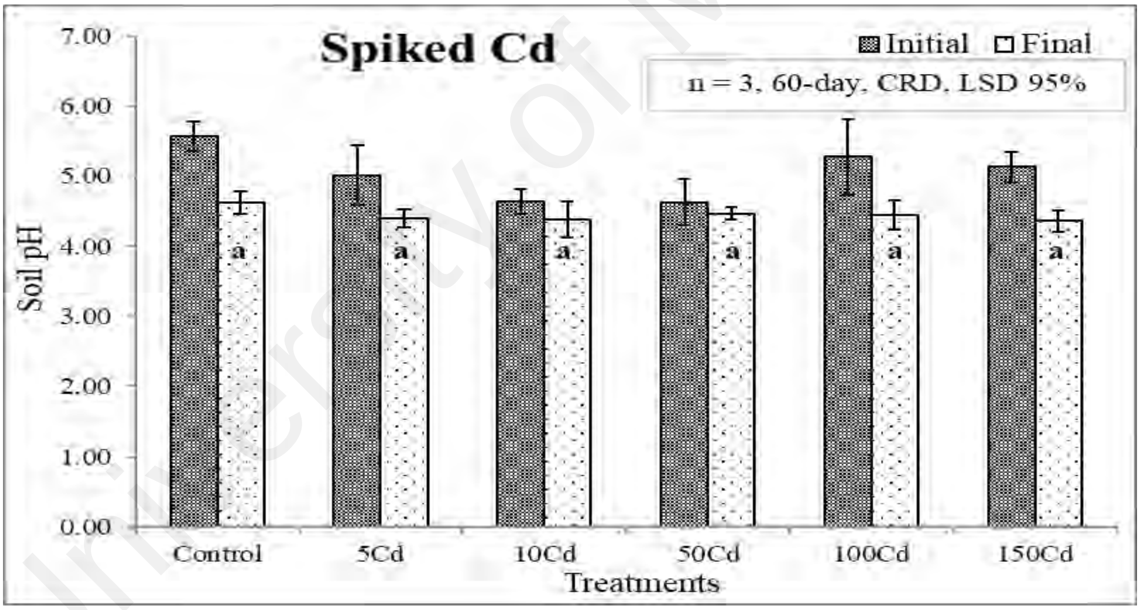

Figure 4.1: Changes in soil pH in Vetiver as influenced by different levels of spiked Cd concentrations. Vertical bars represent standard deviation in treatment means and same letters are not significantly different at 0.05 levels of probability.

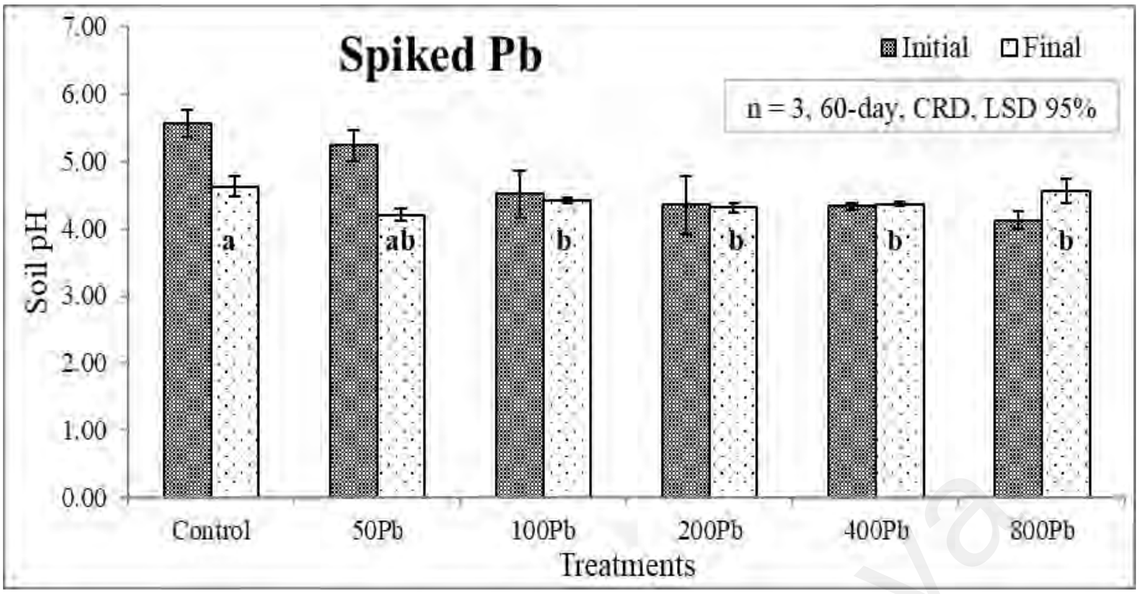

Figure 4.2: Changes in soil pH in Vetiver as influenced by different levels of spiked Pb concentrations. Vertical bars represent standard deviation in treatment means and same letters are not significantly different at 0.05 levels of probability.

Table 4.3 and Table 4.4 show that a significantly lower (***p<0.05***) tiller number and plant height were obtained in 150Cd treatment compared to other spiked Cd treatments and control. Both 100Cd (64.44%) and 150Cd (37.22%) treatments demonstrated significantly lower (***p<0.05***) percentage of survival compared with the control and other spiked Cd treatments. Among spiked Cd treatments, 100Cd and 150Cd recorded significantly lower growth performance, as the plants started to wither during the second week of the experimental period due to the presence of high concentrations of spiked Cd in the soils. In contrast, there was no significant difference (***p>0.05***) in terms of tiller number, plant height, basal diameter and percentage survivorship recorded in all spiked Pb treatments compared with the control.

Table 4.3: Tiller number, plant height (cm), basal diameter (cm) and plant survivorship (%) of Vetiver as influenced by different levels of spiked Cd heavy metal concentrations

Treatment	Tiller number	Plant height (cm)	Basal diameter (cm)	Plant survivorship (%)
Control	27.9 ± 5.8 a	61.49 ± 8.54 ab	7.73 ± 1.26 ab	100.00 ± 0.00 a
5Cd	26.9 ± 7.9 a	73.26 ± 15.25 a	8.71 ± 4.11 ab	98.89 ± 15.81 a
10Cd	32.6 ± 7.1 a	75.58 ± 11.58 a	9.64 ± 5.12 a	98.33 ± 14.25 a
50Cd	21.2 ± 8.5 ab	61.93 ± 12.81 ab	7.95 ± 3.21 ab	97.78 ± 10.86 a
100Cd	20.2 ± 11.7 b	45.02 ± 10.84 b	6.82 ± 3.47ab	64.44 ± 13.55 b
150Cd	8.1 ± 5.8 c	13.64 ± 9.50 c	3.38 ± 1.69 b	37.22 ± 10.82 c

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 4.4: Tiller number, plant height (cm), basal diameter (cm) and plant survivorship (%) of Vetiver as influenced by different levels of spiked Pb heavy metal concentrations

Treatment	Tiller number	Plant height (cm)	Basal diameter (cm)	Plant survivorship (%)
Control	27.9 ± 5.8 a	61.49 ± 8.54 a	7.73 ± 1.26 a	100.00 ± 0.00 a
50Pb	21.0 ± 12.6 a	74.07 ± 12.95 a	8.28 ± 1.95 a	99.44 ± 18.58 a
100Pb	19.0 ± 8.4 a	79.92 ± 11.01 a	8.37 ± 0.87 a	98.89 ± 1.83 a
200Pb	21.4 ± 10.8 a	75.89 ± 9.53 a	8.37 ± 3.58 a	98.89 ± 9.21 a
400Pb	21.1 ± 9.6 a	75.77 ± 15.88 a	8.44 ± 2.54 a	98.33 ± 4.56 a
800Pb	23.0 ± 13.4 a	80.56 ± 20.59 a	8.13 ± 1.05 a	98.33 ± 8.19 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

The roots, tillers and total dry matter contents in all spiked Pb treatments were not significantly affected (***p>0.05***) with the application of different levels of Pb concentrations (Table 4.5 and Table 4.6). Only the 150Cd treatments recorded a significantly lower (***p<0.05***) tiller number and total dry matter content. The 150Cd (9.87 ± 0.12 g/m^2) treatment displayed the lowest total dry matter content with an average of 31.3% reduction as compared to the control. The application of both root-tiller (R/T) r quotients and tolerance index (TI), employed to assess the tolerance ability of Vetiver grass to grow under different levels of spiked heavy metal concentrations, showed that there was no significant difference observed in the R/T quotients (***p>0.05***) among all spiked Cd and Pb treatments. Furthermore, although no significant difference in TI (***p>0.05***) was observed in both spiked Cd and Pb treatments, all spiked Pb treatments recorded remarkably high TI values > 1 (1.021 – 1.099).

Table 4.5: Dry matter content (g/m^2), root-tiller quotient (R/T) and tolerance index (TI) of Vetiver as influenced by different levels of spiked Cd heavy metal concentrations

Treatment	Dry matter content (g/m^2)			R/T	TI
	Root	Tiller	Total		
Control	8.20 ± 0.51 a	6.17 ± 0.93 a	14.37 ± 1.43 a	1.343 a	
5Cd	8.10 ± 1.15 a	6.11 ± 0.74 a	14.21 ± 1.14 a	1.341 a	1.001 a
10Cd	8.04 ± 1.68 a	6.06 ± 0.79 a	14.10 ± 2.32 a	1.322 a	0.994 a
50Cd	7.98 ± 1.56 a	5.83 ± 0.33 a	13.81 ± 1.53 a	1.375 a	0.969 a
100Cd	7.56 ± 1.33 a	5.03 ± 0.87 ab	12.60 ± 2.19 ab	1.502 a	0.890 a
150Cd	5.98 ± 0.40 a	3.89 ± 0.48 b	9.87 ± 0.12 b	1.565 a	0.692 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 4.6: Dry matter content (g/m^2), root-tiller quotient (R/T) and tolerance index (TI) of Vetiver as influenced by different levels of spiked Pb heavy metal concentration

Treatment	Dry matter content (g/m^2)			R/T	TI
	Root	Tiller	Total		
Control	8.20 ± 0.51 a	6.17 ± 0.93 a	14.37 ± 1.43 a	1.343 a	
50Pb	9.00 ± 0.81 a	5.66 ± 0.80 a	14.66 ± 1.50 a	1.599 a	1.021 a
100Pb	9.73 ± 1.62 a	5.86 ± 0.41 a	15.60 ± 2.03 a	1.652 a	1.084 a
200Pb	9.78 ± 1.70 a	5.81 ± 0.81 a	15.59 ± 2.47 a	1.680 a	1.087 a
400Pb	9.54 ± 0.50 a	6.01 ± 1.39 a	15.56 ± 1.46 a	1.640 a	1.086 a
800Pb	9.51 ± 0.68 a	6.14 ± 0.87 a	15.65 ± 1.27 a	1.564 a	1.099 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

4.3.2 Distribution of Cd and Pb in plant

The accumulation of both Cd and Pb in the roots and tillers of Vetiver grass was variable (Table 4.7 – Table 4.8). The roots (***p<0.05***) and total accumulation (***p<0.05***) of Cd in 50Cd, 100Cd and 150Cd treatments were found to be significantly higher than the control, 5Cd and 10Cd treatments. There was no Cd accumulation recorded in the tillers for both control and 5Cd treatments as its detection limits were lower than 0.01 mg/kg (dry weight basis), whilst the tillers of 150Cd treatment (66.85 ± 9.73 mg/kg) showed the highest accumulation Cd. Between roots and tillers, Cd accumulation was reasonably greater in the roots (1.28 – 112.67 mg/kg) than the tillers (4.16 – 66.85 mg/kg) in all treatments. The accumulation trend of Cd in Vetiver grass was in the following order: 150Cd > 100Cd > 50Cd > 10Cd > control.

Similarly, with regard to Pb accumulation, the roots (***p< 0.05***) and total accumulation (***p< 0.05***) of Pb in 100Pb, 200Pb, 400Pb and 800Pb treatments were found to be significantly greater than the control and 50Pb treatments. A significantly higher accumulation of Pb was observed in the tillers (***p< 0.05***) of both 400Pb and 800Pb treatments compared to the control and other spiked Pb treatments. Between roots and tillers, Pb accumulated substantially higher in the roots (4.27 – 396.60 mg/kg) compared to the tillers (1.79 – 122.94 mg/kg) in all treatments. The trend for Pb accumulation in Vetiver grass was in the following order of 800Pb > 400Pb > 200Pb > 100Pb > 50Pb > control. In both spiked heavy metal treatments, Vetiver grass accumulated the highest total amount of Cd (179.52 ± 16.74 mg/kg) and Pb (519.54 ± 24.93 mg/kg) in the spiked 150Cd and 800Pb treatments, respectively.

Table 4.7: Concentration of Cd (mg/kg) as influenced by different levels of spiked heavy metal concentrations

Treatment	**Cd concentration (mg/kg)**		
	Root	**Tiller**	**Total**
Control	1.28 ± 0.72 c	ND (< 0.01)	1.28 ± 0.72 d
5Cd	6.71 ± 2.76 c	ND (< 0.01)	6.71 ± 2.76 d
10Cd	17.10 ± 6.05 c	4.16 ± 0.97 c	21.26 ± 6.10 d
50Cd	59.12 ± 10.59 b	13.13 ± 7.79 c	72.24 ± 13.77 c
100Cd	88.57 ± 18.89 a	37.32 ± 10.99 b	125.88 ± 15.50 b
150Cd	112.67 ± 22.34 a	66.85 ± 9.73 a	179.52 ± 16.74 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 4.8: Concentration of Pb (mg/kg) as influenced by different levels of spiked heavy metal concentrations

Treatment	Pb concentration (mg/kg)		
	Root	Tiller	Total
Control	4.27 ± 0.43 e	1.79 ± 1.16 c	6.06 ± 1.13 e
50Pb	36.94 ± 13.44 e	5.08 ± 1.61 c	42.02 ± 14.65 e
100Pb	120.71 ± 16.02 d	12.52 ± 1.43 c	133.23 ± 16.86 d
200Pb	189.58 ± 11.08 c	27.86 ± 9.22 c	217.44 ± 20.20 c
400Pb	235.25 ± 28.49 b	86.57 ± 11.03 b	321.82 ± 37.04 b
800Pb	396.60 ± 30.82 a	122.94 ± 29.11 a	519.54 ± 24.93 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

4.3.3 Association of Cd and Pb uptake in plant

The plant-soil association of both Cd and Pb accumulated from the spiked heavy metal soils into the roots and the tillers of Vetiver grass are shown in terms of biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and percentage of metal uptake efficacy in Table 4.9 and Table 4.10. Considering the tolerable lower accumulation of spiked heavy metals in the tillers than the roots in Vetiver grass, TF and BAC were employed to evaluate the capability of Vetiver to translocate heavy metals from soils and roots into the tillers. All of the recorded TF and BAC values in both Cd and Pb accumulations were < 1. The 100Cd (0.446) and 150Cd (0.615) treatments recorded significantly higher (***p<0.05***) TF values whilst the 50Pb (0.145), 100Pb (0.104) and 200Pb (0.145) treatments showed a significant decrease (***p<0.05***) in TF values compared to the control for both Cd and Pb accumulations, respectively. No significant difference (***p>0.05***) were found in BAC in all Pb spiked treatments. Meanwhile 10Cd, 50Cd, 100Cd and 150Cd treatments exhibited significantly higher (***p<0.05***) BAC values than the control.

Nevertheless, in terms of BCF, no significant difference (***p>0.05***) was observed in all spiked Cd treatments. However, 50Pb (0.739), 100Pb (1.207) and 200Pb (0.948) treatments documented significantly higher (***p<0.05***) values compared to the control. Metal uptake efficacy (%) was used to assess the total efficiency and potential of Cd and Pb uptake in Vetiver grass from the soil to its tillers. A significantly greater (***p<0.05***) percentage of Cd efficacy was recorded in the 10Cd, 50Cd, 100Cd and 150Cd treatments, whilst only 100Pb (9.45%) treatment showed a significantly lower (***p<0.05***) percentage of Pb efficacy compared to the control.

Table 4.9: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver as influenced by different levels of spiked Cd heavy metal concentrations

Treatment	**Cd accumulation**			
	BCF	**BAC**	**TF**	**Efficacy (%)**
Control	1.041 ab	0.008 c	0.010 c	0.98 c
5Cd	1.341 ab	0.002 c	0.002 c	0.17 c
10Cd	1.710 a	0.416 ab	0.262 bc	20.49 b
50Cd	1.182 ab	0.263 b	0.224 bc	17.65 b
100Cd	0.886 ab	0.373 ab	0.446 ab	29.89 ab
150Cd	0.751 b	0.446 a	0.615 a	37.57 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 4.10: Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver as influenced by different levels of spiked Pb heavy metal concentrations

Treatment	**Pb accumulation**			
	BCF	**BAC**	**TF**	**Efficacy (%)**
Control	0.462 d	0.193 a	0.426 a	27.97 a
50Pb	0.739 bc	0.102 a	0.145 bc	12.54 ab
100Pb	1.207 a	0.125 a	0.104 c	9.45 b
200Pb	0.948 ab	0.139 a	0.145 bc	12.63 ab
400Pb	0.588 cd	0.216 a	0.369 ab	26.91 a
800Pb	0.496 cd	0.154 a	0.314 abc	23.64 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Vetiver grass showed a strong and significantly positive relationship with the levels of spiked concentrations for both Cd (***r=0.975***) and Pb (***r=0.952***) accumulations (Table 4.11). The slopes indicated that with each application of 1.0 mg/kg (dry weight basis) level of spiked Cd and Pb concentrations in the soil, an approximately 1.181 mg/kg (Cd) and 0.617mg/kg (Pb) will be accumulated in Vetiver grass, respectively. However, significantly negative correlation relationships were exhibited between dry matter content and Cd accumulations (***r=0.491***) and the levels of spiked Cd concentrations (***r=0.508***). Dry matter content was negatively correlated due to the increased level of spiked Cd concentrations in the soil which affected higher accumulation of Cd in Vetiver grass.

Table 4.11: Regression equation, coefficients of determination (R^2) and F values of different parameters in Vetiver grass

Regression equation	R^2	R	F value
Relationship between level of spiked concentrations and Cd accumulation			
$Y_{Cd} = 6.059 + 1.181X_1$	0.97507	0.98746	586.795**
Relationship between level of spiked concentrations and Pb accumulation			
$Y_{Pb} = 49.690 + 0.617X_1$	0.95158	0.97549	294.792**
Relationship between dry matter contents and Cd accumulation			
$Y_{Cd} = 14.509 - 0.026X_2$	0.49136	0.70097	14.490*
Relationship between dry matter contents and Pb accumulation			
$Y_{Pb} = 380.678 -10.368X_2$	0.00858	0.09262	0.130
Relationship between level of spiked concentrations and dry matter contents			
$Y_{Cd} = 14.509 - 0.026X_1$	0.50833	0.71297	15.508*
$Y_{Pb}= 15.690 - 0.0002X_1$	0.00113	0.09262	0.130

X_1 = Level of spiked concentrations
X_2 = Dry matter contents
* Significant at 0.01 level of probability
** Significant at < 0.001 level of probability

4.4 Discussion

The changes in soil pH observed could be related to the application of high levels of spiked Pb as more positively charge (proton ions) are present in the soils. Similar studies by Husson (2013) and Adamczyk-Szabela ***et al***. (2015) have revealed that the bioavailability of metal uptake in plants is strongly associated with soil pH conditions.

Nonetheless, the findings demonstrated an adverse growth reduction in terms of tiller number (27.6 – 71.0%), plant height (26.8 – 77.8%) and plant survivorship (35.6 – 62.8%) when high amounts of spiked 100Cd and 150Cd treatments were applied to Vetiver grass, respectively. With regard to Pb, similar recent studies by Truong and Danh (2015) and Prasad ***et al***. (2014) reported that Vetiver grass has progressive outgrowth under all the different levels of spiked Pb concentrations.

Moreover, the results also indicated that the different levels of spiked Pb concentrations had no direct influence on the overall growth performance and dry matter content in Vetiver grass. As the TI (1.001 – 1.099) was relatively > 1, Vetiver grass can be regarded to have good adaptability and high tolerance proficiency in Pb concentrated soils. Conversely, it can be concluded that Vetiver grass is not suitable to be used as a phytoremediator for soils contaminated with Cd exceeding the threshold level of 100 mg/kg (dry weight basis). This is in agreement with the findings of recent studies by Truong and Danh (2015), Danh ***et al***. (2009) and Liu ***et al***. (2010).

Besides, the results demonstrated that the accumulation of both Cd and Pb in Vetiver grass increases with the application of higher levels of spiked heavy metals concentrations. Both 100Cd and 150Cd treatments recorded higher accumulation of Cd than the other spiked Cd treatments and the control and this was reflected in the noticeable reduction in plant growth. As a result, these findings indicated that the declining plant growth trends do not affect the overall bioavailability uptake of spiked heavy metals in Vetiver grass.

These findings are supported by the recent similar studies of Danh ***et al***. (2012) and Truong and Danh (2015) which showed that the phytotolerance and growth threshold of Vetiver grass for Cd and Pb accumulations were 20 – 60 mg/kg Cd and > 1500 mg/kg Pb in soil, respectively. The results obtained are similar but it has been further demonstrated to show that the Vetiver grass have limited phytotolerance and threshold levels when the accumulation of heavy metal is higher than 100 mg/kg of Cd in soil whilst no specific threshold level for Pb has been recorded till present. However, the use of direct pot experiments in this chapter for the spiked heavy metals instead of in-situ (field) experiments may have possibly attributed to the high heavy metals accumulation in both the roots and tillers of Vetiver grass.

The overall findings of TF and BAC < 1, whilst BCF > 1, demonstrate that the translocation of both Cd and Pb were more favourably accumulated in the roots than the tillers in Vetiver grass. Furthermore, the noticeably higher Pb accumulation in the roots than the tillers of Vetiver grass could have caused the tolerably lower percentage of Pb efficacy among all spiked Pb treatments. Vetiver grass can be regarded as a suitable phytostabilizer for both Cd and Pb, owing to the BCF >1 value and its considerable ability to immobilize heavy metals in the soil (Gomes, 2012; Berti, 2000; Padmavathiamma & Li, 2007).

4.5 Conclusions

The inclination trend of heavy metals accumulation for both Cd and Pb in Vetiver grass were in the order of 150Cd > 100Cd > 50Cd > 10Cd > control and 800Pb > 400Pb > 200Pb > 100Pb > 50Pb > control, respectively. The accumulation of both Cd and Pb in the roots and tillers of Vetiver grass increased when higher levels of spiked heavy metals concentrations were applied into the soil. Vetiver grass can thus be recommended to be an effective Cd and Pb phytostabilizer, owing to the considerably high heavy metals accumulation in its roots.

However, Vetiver grass was not suitable for phytoremediation if the level of contamination exceeded the threshold amount of 100 mg/kg Cd as it would inhibit overall plant growth. Nonetheless, in terms of Pb accumulation, Vetiver grass could be used as a suitable phytoremediator as its phytotolerance and threshold is expected to be higher than 800 mg/kg Pb. This chapter can be further extended with increased applications of higher spiked concentrations of Pb as well as by covering a wider range of highly hazardous heavy metals such as aluminium (Al), arsenic (As) and mercury (Hg).

CHAPTER 5: PHYTOEVALUATION OF VETIVER GRASS GROWN IN SINGLE AND MIXED HEAVY METAL CONTAMINATED SOIL

5.1 Introduction

Soil contamination has received major global environmental attention as a result of its adverse effects to both human health and the surroundings (Doran, 2002; Azam, 2016; Gómez-Sagasti, 2016). Soils often become contaminated typically due to the past and present emissions from rapidly expanding industrial activities, agricultural chemical runoff and improper disposal of wastes (Waller, 1982; Meuser, 2010; Van der Perk, 2013). The common sources of soil contaminants may include both organic (halogenated volatiles, non-halogenated volatiles, pesticides, dioxin, furan, poly-chlorinated biphenyl and cyanides) and inorganic (volatile metals, non-volatile metals and radioactive materials) components (Harris ***et al***., 1995).

Among the various types of soil contamination, inorganic heavy metal contaminants have turned out to be a huge concern as heavy metals are freely available in soil materials (environment) and are highly hazardous to human health even in trace amounts (Storelli, 2008; Martin & Griswold, 2009; Clemens & Ma, 2016). Generally, the term heavy metal is widely accepted to describe a group of elemental metals in the periodic table which have an atomic weight exceeding that of iron (Fe), often being persistent in environmental bodies over a long duration and are mostly lethal (Gomes, 2010; Kabata-Pendias, 2010).

Heavy metals such as copper (Cu), zinc (Zn), iron (Fe), nickel (Ni) and manganese (Mn) are essential soil micronutrients required by living organisms in trace amounts for biological metabolic processes (Pilbeam & Barker, 2007). Nevertheless, non-essential heavy metals like cadmium (Cd), lead (Pb), chromium (Cr), arsenic (As) and mercury (Hg) are predominantly hazardous and are not needed for the growth of living organisms. Naturally occurring heavy metals are often untraceable, non-biodegradable and can easily bio-accumulate and affect human health through the food chain (Bradl, 2005; Kamal ***et al***., 2016). Among all the different types of heavy metals, Cd, Pb, Cu and Zn are the few commonly available metals found in the soil (Brümmer, 1986; Wuana & Okieimen, 2011; Alloway, 2013). Soil contaminated with heavy metals may severely contribute to the inhibition of growth and reduced metabolic activities in plants over time (Antonovics ***et al.***, 1971; Nagajyoti ***et al***., 2010).

As a consequence, a myriad of soil remediation techniques (physical, chemical and biological assisted methods) for heavy metal contamination have been developed over the years (Garbisu & Alkorta, 2003; Hasegawa ***et al***., 2016). Nonetheless, phytoremediation has successfully developed to be one of the most preferred techniques as a result of its simple, cost-effective and environmentally friendly approach towards heavy metals soil contamination (Ali ***et al***., 2013; Mahar ***et al***., 2016). Correspondingly, Vetiver grass, ***Vetiveria zizanioides*** (Linn.) Nash has been carefully selected among various types of plants based on the earlier research studies in Chapter 3, to be the most favourable species used for phytoremediation owing to its high tolerance towards environmental stress and its ability to withstand a wide range of contaminated heavy metals (Danh ***et al***., 2009; Truong & Danh, 2015).

However, there is a growing interest in the difference of phytoremediation between single and mixed heavy metal spiked contamination with Vetiver grass, which remains poorly studied and requires urgent elucidation. Over the years, little evidence (Khalil ***et al***., 1996; Peralta-Videa ***et al***., 2002; Stolpe & Müller, 2016; Yang ***et al***., 2016) has been made available on studies with mixed heavy metal contamination. Previous studies have solely emphasized on the limited types of heavy metals and inadequately explained phytoassessment in the different plant parts. As a result, this chapter was constructed to evaluate the growth performance, accumulation trend and proficiency of metal uptake between the different types of single and mixed Cd, Pb, Cu and Zn spiked contaminated soil conditions in both the lower and upper roots and tillers of Vetiver grass.

5.2 Materials and Methods

5.2.1 Site description and experimental layout

A pot experimental study in this chapter was conducted in the planthouse situated at the Rimba Ilmu, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur. Vetiver grass (***V. zizanioides***) was selected for this experiment and placed under eight different types of single and mixed heavy metal spiked treatments (Table 5.1). All of the treatments were conducted under a completely randomized design (CRD) with three replications (n = 3).

Table 5.1: Design of treatment variables

Treatment	Spiked heavy metal (mg/kg)
Control	No heavy metal added
Cd	20 Cd
Pb	200 Pb
Cu	100 Cu
Zn	200 Zn
Cd+Pb	20 Cd + 200 Pb
Cu+Zn	100 Cu + 200 Zn
Cd+Pb+Cu+Zn	20 Cd + 200 Pb + 100 Cu + 200 Zn

5.2.2 Soil sampling and sample preparation

Top soil (0 – 20cm) was collected from a field located in the University of Malaya, Kuala Lumpur situated at the 3° 7' N latitude and 101° 39' E longitude for planting purposes. The preliminary physico-chemical soil assessment (Table 5.2) was conducted before the soils were air-dried for a week followed by <4mm sieving to remove gravels and large non-soil particles. The dull reddish brown soil consists of 89.42% sand, 8.27% silt and 2.31% clay.

Table 5.2: Physico-chemical properties of growth media soil

Parameter (Unit)		Mean
Metal contents (mg/kg)		
	Cd	0.87 ± 0.08
	Pb	26.95 ± 1.24
	Cu	7.48 ± 2.35
	Zn	52.51 ± 11.64
Soil texture		
Sand (%)		89.42
	Very coarse sand (%)	4.56
	Coarse sand (%)	39.15
	Medium coarse sand (%)	30.68
	Fine sand (%)	11.55
	Very fine sand (%)	3.48
Silt (%)		8.27
Clay (%)		2.31
Temperature (°C)		32.6 ± 1.2
pH		5.84 ± 0.92
Colour (Munsell colour charts)	Dull reddish brown	2.5YR 5/4
Water content (%)		6.29 ± 1.28
Field capacity (%)		35.16 ± 4.82
Saturation level (%)	Dry	17.89
Bulk density (g/cm^3)		1.96 ± 0.35
Porosity (%)		26.04 ± 3.14

Mean ± standard deviation

Vetiver grass plantlets were purchased from Humibox Malaysia and each fresh plant plantlet with a uniform height (20 – 25cm) was selected for this chapter. Each plant was grown in a plastic pot (0.18m diameter x 0.16m depth) filled with two kilograms of soil, in all the treatments. All plants were watered evenly with 50mL of tap water once a day and their plant growth performance such as height, tiller number and percentage plant survivorship were continuously observed throughout the entire 60-day of experiment.

The single and mixed heavy metal spiked treatments were prepared using cadmium nitrate tetrahydrate [$Cd(NO_3)_2.4H_2O$], lead (II) nitrate [$Pb(NO_3)_2$], copper (II) sulfate [$CuSO_4$] and zinc sulfate heptahydrate [$ZnSO_4.7H_2O$] salt compounds. The amended soil was then continuously stirred and incubated for a week to ensure the homogeneity of the desired single and mixed heavy metal treatments is obtained. The concentrations of both single and mixed heavy metal spiked treatments were determined based on the range of heavy metal concentrations exceeding the median permissible in the natural occurring levels by the Department of Environment, Malaysia (DOE, 2009), Canadian Council of Ministers of Environment (CCME, 1999) and European Union (Lado ***et al***., 2008) soil contamination guidelines.

5.2.3 Soil and plant sample analysis

Freshly harvested plants were brought into the laboratory and washed in running filter water, followed by deionized water to remove any adhering soil particles before separating the plants into four different parts of the lower and upper sections of roots and tillers. All plant samples were oven-dried for 72 hours until it obtained a constant dry weight. The dry matter content (g/m^2) of the plant samples was determined before it was homogenized using a mortar and pestle.

Approximately, 0.5g of the homogenized dried samples underwent acid digestion with hydrochloric acid (HCl), nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) according to Method 3050B (US EPA, 1996) followed by the Method 7000B (US EPA, 2007a) for the total recoverable elemental analysis using the Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (F-AAS). All chemicals used were of analytical reagent standard or of the best grade available.

Soil samples were air-dried for 72 hours until it reached a constant weight before it was analysed following similar analytical procedures. The highly precise technique of chemical analysis was controlled using the Bundesanstalt für Materialforschung und – prüfung (BAM Germany): German Federal Institute for Materials Research and Testing (BRM#12-mixed sandy soil) certified reference material with an average rate of metal recovery for Cd (102.65%), Pb (98.42%), Cu (93.21%) and Zn (105.94%), respectively.

5.2.4 Statistical analysis and data processing

The growth performance of Vetiver grass were evaluated using the root-tiller (R/T) quotient and tolerance index (TI) whilst the ability for metal accumulation and translocation upwards were evaluated by determining the translocation factor (TF), biological concentration factor (BCF), biological accumulation coefficient (BAC) and percentage of metal uptake efficacy (Kabata-Pendias, 2010; Alloway, 2013; Ali ***et al***., 2013) as follows:

R/T quotient = Dry matter content in root / Dry matter content in tiller

TI = Total dry matter content in heavy metal treatment / Total dry matter content in control

TF = Concentration of heavy metal in tiller / Concentration of heavy metal in root

BCF = Concentration of heavy metal in root / Concentration of heavy metal in soil

BAC = Concentration of heavy metal in tiller / Concentration of heavy metal in soil

Metal uptake efficacy (%) = [Concentration of heavy metal in tiller / Total concentration of heavy metal accumulated in Vetiver] x 100

All experimental data were analysed by performing the one–way analysis of variance (ANOVA) and further statistical validity test for significant differences among treatment means was conducted by employing the Fisher's least significant difference (LSD) tests at the 95% level of confidence.

5.3 Results

5.3.1 Responses of plant growth

Soil pH was not significantly affected (***p>0.05***) by the single and mixed spiked heavy metals in all Vetiver treatments (Figure 5.1). During the 60-day of experimental period, all Vetiver treatments recorded fluctuations in the soil pH between initial readings of 4.26 – 4.95 and final readings of 4.17 – 5.74. The control treatment recorded the highest pH of 5.74 while the lowest pH of 4.17 was observed in the Cd+Pb treatment. The results obtained for both single and mixed spiked heavy metals did not considerably influence the overall soil pH changes in all treatments.

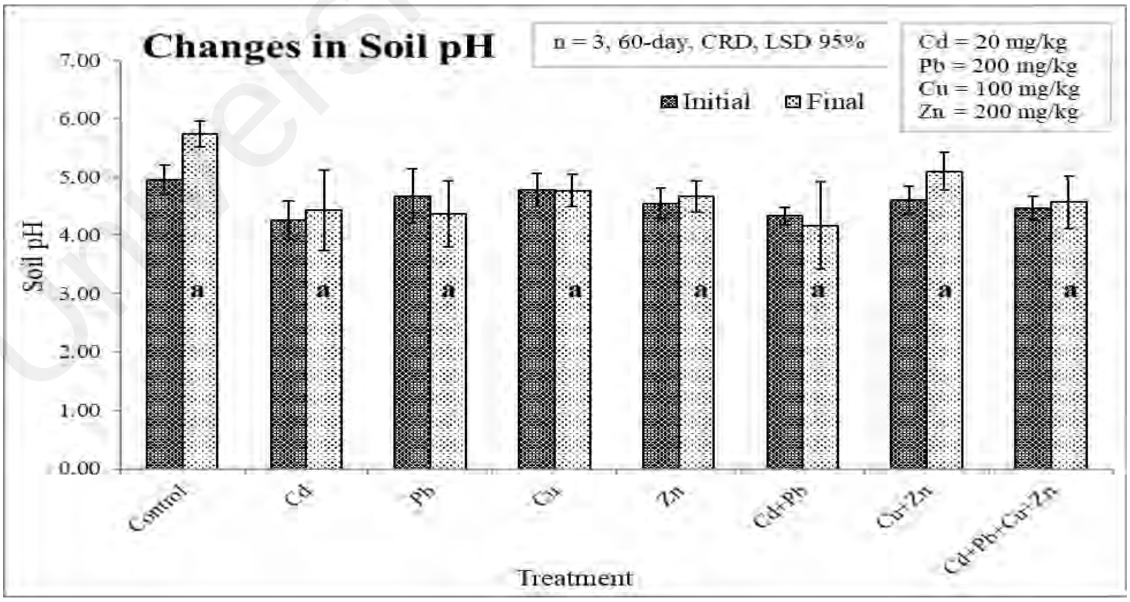

Figure 5.1: Changes in soil pH of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments. Vertical bars represent standard deviation whilst same letters are not significantly different for each treatment means at 0.05 levels of probability.

The relative growth of Vetiver grass in terms of plant height, tiller number and percentage survivorship varied in all the different types of single and mixed heavy metal spiked treatments (Table 5.3). There was no significant difference (***p>0.05***) in the plant height observed among all single and mixed heavy metal spiked treatments compared with the control. Nevertheless, all of the single and mixed heavy metal spiked treatments recorded relatively lower plant height (45.68 cm – 68.48 cm) compared to the control (76.88 cm). In contrast, the Cd, Cu, Zn, Zn+Cu and Cd+Pb+Cu+Zn spiked treatments showed significantly lower (***p<0.05***) tiller number compared to the control. The control recorded the highest tiller number of 26.6 while the lowest tiller number of 12.2 was observed in the Cd+Pb+Cu+Zn treatment. Similarly, with regard to plant survivorship, the Zn, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments demonstrated significantly decreased (***p<0.05***) percentage of survival compared with the control. Among all spiked treatments, both Cu+Zn (77.34%) and Cd+Pb+Cu+Zn (58.67 %) mixed heavy metal treatments recorded the lowest percentage of survivorship.

Table 5.3: Plant height (cm), tiller number and plant survivorship (%) of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Plant height (cm)	Tiller number	Plant survivorship (%)
Control	76.88 ± 12.07 a	26.6 ± 5.5 a	100.00 ± 0.00 a
Cd	60.16 ± 8.40 a	16.8 ± 8.4 b	97.33 ± 14.21 a
Pb	68.48 ± 20.83 a	21.6 ± 11.4 ab	100.00 ± 0.00 a
Cu	52.00 ± 14.95 a	18.0 ± 8.9 b	81.94 ± 5.72 ab
Zn	49.88 ± 11.16 a	17.4 ± 9.3 b	78.67 ± 13.66 b
Cd+Pb	64.48 ± 9.05 a	19.6 ± 7.3 ab	87.33 ± 10.09 ab
Cu+Zn	47.14 ± 22.39 a	17.2 ± 5.4 b	77.34 ± 23.45 b
Cd+Pb+Cu+Zn	45.68 ± 17.73 a	12.2 ± 7.7 b	58.67 ± 19.46 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

The single Cu and Zn spiked treatments as well as the mixed Cu+Zn and Cd+Pb+Cu+Zn spiked treatments exhibited significantly lower (***p<0.05***) dry matter contents in both the roots and tillers compared to the control (Table 5.4). All spiked treatments, with the exception of Pb treatment, showed significantly lower (***p<0.05***) total dry matter content compared with the control. Between spiked treatments, both single Cd (15.50 ± 1.22 g/m^2) and Pb (17.14 ± 0.69 g/m^2) spiked treatments recorded reasonably higher total dry matter content than the other mixed heavy metal treatments. Both root-tiller (R/T) quotient and tolerance index (TI) were employed to evaluate the tolerance ability of Vetiver grass growing under different types of single and mixed heavy metal spiked treatments. In terms of R/T quotient, no significant difference (***p>0.05***) was observed among all treatments. Nonetheless, among all the treatments, single Pb spiked treatment showed the highest TI value of 0.914 while the lowest TI was recorded in the Cd+Pb+Cu+Zn spiked treatment.

Table 5.4: Dry matter content (g/m^2), root-tiller quotient (R/T) and tolerance index (TI) of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Dry matter content (g/m^2)				
	Vetiver			R/T	TI
	Root	Tiller	Total		
Control	8.01 ± 1.37 a	11.17 ± 2.87 a	19.18 ± 3.01 a	0.751 a	
Cd	7.00 ± 0.22 abc	8.51 ± 1.21 ab	15.50 ± 1.22 b	0.833 a	0.817 ab
Pb	7.48 ± 0.90 ab	9.66 ± 1.18 a	17.14 ± 0.69 ab	0.790 a	0.914 a
Cu	5.77 ± 0.60 bc	5.95 ± 1.61 bc	11.72 ± 1.56 c	1.034 a	0.625 bcd
Zn	5.27 ± 1.01 c	4.85 ± 1.54 c	10.12 ± 2.35 c	1.136 a	0.546 bcd
Cd+Pb	6.82 ± 0.76 abc	8.36 ± 0.61 ab	15.19 ± 1.30 b	0.815 a	0.803 abc
Cu+Zn	5.26 ± 0.88 c	4.37 ± 1.13 c	9.63 ± 1.68 c	1.241 a	0.520 cd
Cd+Pb+Cu+Zn	5.24 ± 1.65 c	4.34 ± 0.97 c	9.58 ± 0.70 c	1.321 a	0.506 d

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

5.3.2 Heavy metal uptake in plant

Table 5.5 – Table 5.16 shows the concentration of Cd, Pb, Cu and Zn heavy metal accumulation in the roots, tillers and its total for Vetiver grass in different types of single and mixed heavy metal spiked treatments. The accumulation of all four different types of spiked heavy metals in the lower and upper parts of the roots and tillers was comparatively variable.

In terms of Cd accumulation (Table 5.5 – Table 5.7), all of the Cd, Cd+Pb and Cd+Pb+Cu+Zn spiked treatments showed significantly greater (***$p < 0.05$***) Cd in both the lower and upper roots and tillers of Vetiver grass compared to the control. Similarly, the Cd, Cd+Pb and Cd+Pb+Cu+Zn spiked treatments recorded significantly larger accumulation of Cd (***$p < 0.05$***) in the total root, total tiller and overall total among all other treatments. Unlike the other types of heavy metals (Pb, Cu and Zn), the highest accumulation of Cd were recorded in the lower tillers for Cd+Pb+Cu+Zn (62.53 ± 5.97 mg/kg) and Cd+Pb (58.33 ± 10.06 mg/kg) spiked treatments. Between roots and tillers, Cd accumulation was considerably greater in the roots than in the tillers. The accumulation of Cd was relatively higher in the lower roots and lower tillers for Cd, Cd+Pb and Cd+Pb+Cu+Zn spiked treatments compared with the upper plant parts, respectively. Nevertheless, the accumulation of Cd among the different types of single and mixed Cd spiked treatments was in the order of Cd+Pb+Cu+Zn > Cd+Pb > Cd >> other spiked treatments.

Table 5.5: Concentration of Cd (mg/kg) in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Cd (mg/kg)		
	Root		
	Lower	Upper	Total
Control	0.52 ± 0.20 c	0.37 ± 0.25 c	0.88 ± 0.45 d
Cd	45.87 ± 9.33 b	48.37 ± 7.59 a	94.23 ± 1.75 b
Pb	1.17 ± 0.14 c	0.46 ± 0.13 c	1.63 ± 0.24 d
Cu	0.65 ± 0.21 c	ND (< 0.01)	0.65 ± 0.21 d
Zn	ND (< 0.01)	0.50 ± 0.25 c	0.50 ± 0.25 d
Cd+Pb	52.17 ± 6.07 ab	37.47 ± 7.91 b	89.63 ± 2.15 c
Cu+Zn	0.57 ± 0.27 c	0.95 ± 0.17 c	1.51 ± 0.44 d
Cd+Pb+Cu+Zn	58.10 ± 1.56 a	55.60 ± 2.12 a	113.70 ± 3.67 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability; ND = Not detected

Table 5.6: Concentration of Cd (mg/kg) in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Cd (mg/kg)		
	Tiller		
	Lower	Upper	Total
Control	0.49 ± 0.17 c	0.70 ± 0.16 cd	1.20 ± 0.31 c
Cd	50.27 ± 12.96 b	ND (< 0.01)	50.27 ± 12.96 b
Pb	0.31 ± 0.20 c	0.29 ± 0.09 cd	0.60 ± 0.30 c
Cu	0.40 ± 0.09 c	0.23 ± 0.18 cd	0.64 ± 0.09 c
Zn	0.42 ± 0.15 c	0.55 ± 0.15 cd	0.97 ± 0.30 c
Cd+Pb	58.33 ± 10.06 ab	30.43 ± 2.75 a	88.77 ± 8.26 a
Cu+Zn	1.22 ± 0.27 c	1.17 ± 0.40 c	3.90 ± 0.33 c
Cd+Pb+Cu+Zn	62.53 ± 5.97 a	23.60 ± 4.06 b	86.13 ± 2.25 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability; ND = Not detected

Table 5.7: Total Cd concentration (mg/kg) in the root and tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Cd (mg/kg)		
	Total root	**Total tiller**	**Overall total**
Control	0.88 ± 0.45 d	1.20 ± 0.31 c	2.08 ± 0.75 d
Cd	94.23 ± 1.75 b	50.27 ± 12.96 b	144.50 ± 11.23 c
Pb	1.63 ± 0.24 d	0.60 ± 0.30 c	2.22 ± 0.54 d
Cu	0.65 ± 0.21 d	0.64 ± 0.09 c	1.29 ± 0.13 d
Zn	0.50 ± 0.25 d	0.97 ± 0.30 c	1.47 ± 0.55 d
Cd+Pb	89.63 ± 2.15 c	88.77 ± 8.26 a	178.40 ± 7.28 b
Cu+Zn	1.51 ± 0.44 d	4.72 ± 0.71 c	6.24 ± 0.28 d
Cd+Pb+Cu+Zn	113.70 ± 3.67 a	86.13 ± 2.25 a	199.83 ± 1.42 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

With regard to Pb accumulation (Table 5.8 – Table 5.10), the Pb, Cd+Pb and Cd+Pb+Cu+Zn spiked treatments exhibited significantly higher (***p<0.05***) Pb in both the lower and upper roots and tillers of Vetiver grass compared to the control. A significantly greater (***p<0.05***) Pb accumulation was demonstrated in the total root, total tiller and overall total accumulation for Pb, Cd+Pb and Cd+Pb+Cu+Zn spiked treatments among the treatments. The lower roots of Cd+Pb+Cu+Zn (177.67 ± 20.01 mg/kg) and Cd+Pb (141.83 ± 9.99 mg/kg) spiked treatments recorded the highest accumulation of Pb among all the treatments. Between roots and tillers, an appreciably higher accumulation of Pb was found in the roots than in the tillers for all treatments. The accumulation of Pb was noticeably greater in the lower roots and lower tillers for both Cd+Pb and Cd+Pb+Cu+Zn spiked treatments compared with the upper plant parts, respectively whilst the vice versa trend was observed for Pb spiked treatment. However, among the different types of single and mixed Pb spiked treatments, the accumulation trend for Pb was in the following order of Cd+Pb+Cu+Zn > Cd+Pb > Pb >> other spiked treatments.

Table 5.8: Concentration of Pb (mg/kg) in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Pb (mg/kg)		
	Root		
	Lower	Upper	Total
Control	8.23 ± 0.85 d	10.53 ± 1.00 d	18.77 ± 1.82 d
Cd	9.67 ± 1.72 d	10.33 ± 1.06 d	20.00 ± 2.43 d
Pb	81.67 ± 7.67 c	83.27 ± 8.94 c	164.93 ± 16.34 c
Cu	10.89 ± 1.32 d	5.05 ± 0.50 d	15.94 ± 1.62 d
Zn	1.11 ± 0.27 d	9.40 ± 1.10 d	10.51 ± 0.84 d
Cd+Pb	141.83 ± 9.99 b	121.33 ± 18.16 b	263.17 ± 10.32 b
Cu+Zn	3.79 ± 0.37 d	6.50 ± 0.40 d	10.29 ± 0.12 d
Cd+Pb+Cu+Zn	177.67 ± 20.01 a	138.67 ± 12.53 a	316.33 ± 7.69 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 5.9: Concentration of Pb (mg/kg) in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Pb (mg/kg)		
	Tiller		
	Lower	Upper	Total
Control	0.69 ± 0.20 c	ND (< 0.1)	0.69 ± 0.20 c
Cd	0.63 ± 0.18 c	ND (< 0.1)	0.63 ± 0.18 c
Pb	26.83 ± 2.61 b	29.70 ± 6.32 a	56.53 ± 3.73 b
Cu	0.50 ± 0.18 c	ND (< 0.1)	0.50 ± 0.18 c
Zn	1.29 ± 0.54 c	ND (< 0.1)	1.29 ± 0.54 c
Cd+Pb	55.67 ± 7.31 a	20.29 ± 2.56 b	75.96 ± 9.30 a
Cu+Zn	3.14 ± 0.45 c	ND (< 0.1)	3.14 ± 0.45 c
Cd+Pb+Cu+Zn	57.20 ± 13.00 a	20.10 ± 6.58 b	77.30 ± 19.45 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 5.10: Total Pb concentration (mg/kg) in the root and tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Pb (mg/kg)		
	Total root	**Total tiller**	**Overall total**
Control	18.77 ± 1.82 d	0.69 ± 0.20 c	19.46 ± 2.02 d
Cd	20.00 ± 2.43 d	0.63 ± 0.18 c	20.63 ± 2.54 d
Pb	164.93 ± 16.34 c	56.53 ± 3.73 b	221.47 ± 19.83 c
Cu	15.94 ± 1.62 d	0.50 ± 0.18 c	16.44 ± 1.46 d
Zn	10.51 ± 0.84 d	1.29 ± 0.54 c	11.80 ± 0.42 d
Cd+Pb	263.17 ± 10.32 b	75.96 ± 9.30 a	339.12 ± 10.20 b
Cu+Zn	10.29 ± 0.12 d	3.14 ± 0.45 c	13.43 ± 0.50 d
Cd+Pb+Cu+Zn	316.33 ± 7.69 a	77.30 ± 19.45 a	393.63 ± 27.05 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

The Cu, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments recorded significantly higher (***p<0.05***) accumulation of Cu in both the lower and upper roots and tillers of Vetiver grass compared to the control (Table 5.11 – Table 5.13). Similarly, the total root, total tiller and overall total accumulation for Cu, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments exhibited significantly greater (***p<0.05***) Cu than all other treatments. The lower root (365.64 ± 27.00 mg/kg) and upper root (308.03 ± 10.74 mg/kg) for Cd+Pb+Cu+Zn spiked treatment recorded the highest accumulation of Cu among all the treatments. Between roots and tillers, the accumulation of Cu was substantially higher in the roots than in the tillers. The lower roots and lower tillers for Cu, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments accumulated reasonably higher Cu compared with the upper plant parts, respectively. The accumulation trend for Cu among the different types of single and mixed Cu spiked treatments was in the order of Cd+Pb+Cu+Zn > Cu+Zn > Cu >> other spiked treatments.

Table 5.11: Concentration of Cu (mg/kg) in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Cu (mg/kg)		
	Root		
	Lower	Upper	Total
Control	11.40 ± 4.69 d	7.56 ± 1.83 d	18.96 ± 2.94 c
Cd	10.97 ± 1.94 d	5.73 ± 2.49 d	16.70 ± 4.14 c
Pb	12.78 ± 4.01 d	8.16 ± 0.77 d	20.94 ± 3.28 c
Cu	178.77 ± 17.42 c	226.00 ± 18.34 c	404.77 ± 5.97 b
Zn	0.80 ± 0.57 d	6.20 ± 0.95 d	7.00 ± 1.51 c
Cd+Pb	5.03 ± 1.36 d	4.42 ± 1.40 d	9.45 ± 0.19 c
Cu+Zn	227.67 ± 31.41 b	276.99 ± 13.56 b	504.66 ± 44.61 a
Cd+Pb+Cu+Zn	365.64 ± 27.00 a	308.03 ± 10.74 a	673.67 ± 19.71 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 5.12: Concentration of Cu (mg/kg) in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Cu (mg/kg)		
	Tiller		
	Lower	Upper	Total
Control	2.76 ± 0.85 d	ND (< 0.1)	2.76 ± 0.85 d
Cd	1.92 ± 1.01 d	ND (< 0.1)	1.92 ± 1.01 d
Pb	5.80 ± 1.43 d	3.72 ± 1.43 c	9.52 ± 2.43 d
Cu	83.07 ± 5.39 c	58.60 ± 14.93 ab	141.67 ± 20.26 c
Zn	2.95 ± 0.60 d	5.15 ± 0.89 c	8.10 ± 0.36 d
Cd+Pb	2.34 ± 0.78 d	2.13 ± 1.86 c	4.47 ± 1.27 d
Cu+Zn	110.80 ± 18.92 b	63.47 ± 11.36 a	174.27 ± 8.72 b
Cd+Pb+Cu+Zn	136.07 ± 9.06 a	49.27 ± 6.51 b	185.33 ± 3.06 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 5.13: Total Cu concentration (mg/kg) in the root and tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Cu (mg/kg)		
	Total root	**Total tiller**	**Overall total**
Control	2.76 ± 0.85 d	18.96 ± 2.94 c	21.72 ± 3.79 d
Cd	1.92 ± 1.01 d	16.70 ± 4.14 c	18.61 ± 5.08 d
Pb	9.52 ± 2.43 d	20.94 ± 3.28 c	29.79 ± 5.30 d
Cu	141.67 ± 20.26 c	404.77 ± 5.97 b	546.43 ± 21.03 c
Zn	8.10 ± 0.36 d	7.00 ± 1.51 c	15.10 ± 1.86 d
Cd+Pb	4.47 ± 1.27 d	9.45 ± 0.19 c	13.92 ± 1.38 d
Cu+Zn	174.27 ± 8.72 b	504.66 ± 44.61 a	678.93 ± 53.18 b
Cd+Pb+Cu+Zn	185.33 ± 3.06 a	673.67 ± 19.71 a	859.01 ± 22.77 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Similarly, in terms of Zn accumulation (Table 5.14 – Table 5.16), a significantly higher (***$p<0.05$***) accumulation was found in both the lower and upper roots and tillers of Vetiver grass for Zn, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments compared to the control. A significantly greater (***$p<0.05$***) concentration of Zn was observed in the total root, total tiller and overall total accumulation for Zn, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments than all other treatments. The lower roots of Cd+Pb+Cu+Zn (2191.33 ± 145.06 mg/kg) and Cu+Zn (2188.00 ± 167.78 mg/kg) recorded the highest accumulation of Zn among all the treatments. Between roots and tillers, the Zn accumulation was noticeably greater in the roots than in the tillers. A considerably higher accumulation of Zn was recorded in the lower roots and lower tillers for Zn, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments compared with their upper plant parts, respectively. Among the different types of single and mixed Cd spiked treatments, the accumulation of Zn was in the order of Cd+Pb+Cu+Zn > Cu+Zn > Zn >> other spiked treatments.

Table 5.14: Concentration of Zn (mg/kg) in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Zn (mg/kg)		
	Root		
	Lower	Upper	Total
Control	315.67 ± 34.26 c	184.67 ± 8.49 d	500.33 ± 41.60 d
Cd	148.47 ± 13.83 c	110.47 ± 10.49 de	258.93 ± 3.36 d
Pb	245.03 ± 41.41 c	181.77 ± 16.73 d	426.81 ± 40.90 d
Cu	209.53 ± 27.79 c	83.90 ± 8.54 e	293.43 ± 36.34 d
Zn	1945.53 ± 144.65 b	1173.27 ± 96.01 c	3118.80 ± 231.57 c
Cd+Pb	200.33 ± 20.48 c	38.87 ± 9.25 e	239.20 ± 13.79 d
Cu+Zn	2188.00 ± 167.78 a	1276.23 ± 66.60 b	3464.23 ± 231.32 b
Cd+Pb+Cu+Zn	2191.33 ± 145.06 a	1849.90 ± 77.04 a	4041.23 ± 216.01 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 5.15: Concentration of Zn (mg/kg) in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Zn (mg/kg)		
	Tiller		
	Lower	Upper	Total
Control	49.23 ± 11.54 d	42.98 ± 2.49 d	92.21 ± 10.41 c
Cd	56.70 ± 4.09 d	23.36 ± 6.62 d	80.06 ± 10.19 c
Pb	46.73 ± 9.96 d	38.60 ± 10.45 d	85.33 ± 18.54 c
Cu	117.07 ± 15.66 d	33.87 ± 9.20 d	150.93 ± 22.66 c
Zn	1342.17 ± 130.21 c	1449.40 ± 153.58 a	2791.57 ± 280.91 ab
Cd+Pb	56.17 ± 5.97 d	18.60 ± 1.47 d	74.77 ± 7.44 c
Cu+Zn	1921.90 ± 130.97 a	1060.77 ± 73.63 b	2982.67 ± 130.20 a
Cd+Pb+Cu+Zn	1703.13 ± 170.40 b	897.27 ± 48.98 c	2600.40 ± 218.90 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 5.16: Total Zn concentration (mg/kg) in the root and tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Concentration of Zn (mg/kg)		
	Total root	**Total tiller**	**Overall total**
Control	500.33 ± 41.60 d	92.21 ± 10.41 c	592.54 ± 52.00 c
Cd	258.93 ± 3.36 d	80.06 ± 10.19 c	338.99 ± 13.55 cd
Pb	426.81 ± 40.90 d	85.33 ± 18.54 c	512.14 ± 59.28 cd
Cu	293.43 ± 36.34 d	150.93 ± 22.66 c	444.37 ± 58.13 cd
Zn	3118.80 ± 231.57 c	2791.57 ± 280.91 ab	5910.37 ± 103.05 b
Cd+Pb	239.20 ± 13.79 d	74.77 ± 7.44 c	313.97 ± 16.92 d
Cu+Zn	3464.23 ± 231.32 b	2982.67 ± 130.20 a	6446.90 ± 353.07 a
Cd+Pb+Cu+Zn	4041.23 ± 216.01 a	2600.40 ± 218.90 b	6644.97 ± 43.27 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Based on the results obtained, the mixed Cd+Pb+Cu+Zn spiked treatment accumulated the highest overall total amount for Zn (6644.97 ± 43.27 mg/kg) followed by Cu (859.01 ± 22.77mg/kg), Pb (393.63 ± 27.05 mg/kg) and Cd (199.83 ± 1.42 mg/kg). The general trends of heavy metal accumulation for all treatments were in the order of Zn >> Cu > Pb > Cd regardless of the total amount of spiked heavy metal put into the soil. On the other hand, between single and mixed spiked treatments, the accumulation for mixed Cd+Pb, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments recorded remarkably higher accumulation of heavy metals compared to all of the single spiked treatments.

5.3.3 Heavy metal translocation

The association between the different types of single and mixed spiked heavy metals accumulated from the soils into the roots and tillers of Vetiver grass, in terms of biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) are presented in Table 5.17 – Table 5.24.

Relatively higher BCF values were found in both lower and upper roots of all single and mixed Cd (1.873 – 2.905), Pb (0.408 – 0.888), Cu (1.788 – 3.656) and Zn (5.866 – 10.957) spiked treatments, respectively, compared with other treatments. Among all the treatments, the lower root for mixed Cd+Pb+Cu+Zn spiked treatment exhibited the highest BCF value in the accumulation for all the four different types of heavy metals. Considering the BCF values > 1 for Cd, Cu and Zn accumulation, all of the single and mixed spiked treatments accumulated appreciably higher metals in the roots than the tillers suggesting that translocation of heavy metals from soil to root was remarkably greater and the roots acted as a sink for heavy metal accumulation.

The BAC, TF and metal efficacy were calculated to evaluate the capability and efficiency of heavy metal translocation from the roots to the tillers. Despite the relatively lower accumulation of all heavy metals in the shoots than the tillers, the BAC values > 1 were recorded in both the lower and upper tillers for single and mixed Cd (1.180 – 3.127) as well as Zn (4.486 – 9.610) spiked treatments. The translocation pathway for heavy metal accumulation from the roots to the tillers may have been inhibited, considering the appreciably high BAC values < 1 in both lower and upper tillers for single and mixed Pb (0.101 – 0.286) and Cu (0.493 – 0.831) spiked treatments.

Similarly, with regard to the TF values < 1, the tolerably lower accumulation in both lower and upper tillers than its roots for all four different types of heavy metals suggest that the movement of metal uptake from the roots to the tillers were hindered regardless of single and/or mixed spiked treatments. Even though the TF values < 1, for the single and mixed spiked treatments demonstrated fairly higher TF in both lower and upper tillers than the other treatments for the accumulation of Pb (0.050 – 0.164) and Zn (0.135 – 0.298), respectively.

The percentages of metal efficacy in both lower and upper tillers for Pb (5.047% – 16.414%) and Zn (13.509% – 29.800%) accumulation for single and mixed spiked treatments were relatively higher compared with the other treatments, respectively. Despite the considerably lower accumulation of Cd found in the tillers compared to the Cu and Zn, the lower tiller for single (34.464%) and mixed (31.306% – 32.601%) Cd spiked treatments recorded the highest percentages of Cd efficacy among all the treatments. Between single and mixed spiked heavy metal treatments, the single spiked treatments for all four different types of heavy metals recorded a relatively higher percentage of metal efficacies compared to the mixed spiked treatments. Nonetheless, the percentages of metal efficacy were remarkably higher in the lower tiller compared to the upper tiller for all four different types of heavy metals accumulation.

Table 5.17: Biological concentration factor (BCF) of Cd accumulation in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy spiked metal treatments

Treatment	Cd accumulation BCF (Root)	
	Lower	Upper
Control	0.594 d	0.421 de
Cd	2.293 b	2.418 a
Pb	1.341 c	0.529 d
Cu	0.747 d	0.011 e
Zn	0.011 e	0.579 d
Cd+Pb	2.608 ab	1.873 b
Cu+Zn	0.651 d	1.088 c
Cd+Pb+Cu+Zn	2.905 a	2.780 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 5.18: Biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cd accumulation in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Cd accumulation BAC (Tiller)		Cd accumulation TF (Tiller)		Cd accumulation Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.567 d	0.808 cd	0.597 ab	0.878 a	23.994 bc	35.014 a
Cd	2.513 ab	0.001 f	0.535 b	0.0001 d	34.464 a	0.007 c
Pb	0.352 d	0.333 ef	0.179 c	0.175 cd	12.880 d	12.892 b
Cu	0.464 d	0.268 ef	0.625 ab	0.437 bc	31.198 ab	18.988 b
Zn	0.479 d	0.636 de	0.848 ab	1.153 a	28.427 ab	38.444 a
Cd+Pb	2.917 ab	1.522 a	0.652 ab	0.339 cd	32.601 a	17.091 b
Cu+Zn	1.406 c	1.341 b	0.885 a	0.809 ab	19.517 cd	18.783 b
Cd+Pb+Cu+Zn	3.127 a	1.180 bc	0.551 ab	0.207 cd	31.306 ab	11.803 bc

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 5.19: Biological concentration factor (BCF) of Pb accumulation in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Pb accumulation	
	BCF (Root)	
	Lower	Upper
Control	0.306 d	0.391 bc
Cd	0.359 cd	0.383 bc
Pb	0.408 c	0.416 b
Cu	0.404 cd	0.188 d
Zn	0.041 f	0.349 bc
Cd+Pb	0.709 b	0.607 a
Cu+Zn	0.141 e	0.241 d
Cd+Pb+Cu+Zn	0.888 a	0.693 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 5.20: Biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Pb accumulation in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Pb accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.026 c	0.004 c	0.035 d	0.005 c	3.499 d	0.518 c
Cd	0.023 c	0.004 c	0.030 d	0.005 c	3.033 d	0.490 c
Pb	0.134 b	0.149 a	0.123 bc	0.133 a	12.252 bc	13.318 a
Cu	0.019 c	0.004 c	0.031 d	0.006 c	3.110 d	0.611 c
Zn	0.048 c	0.004 c	0.110 c	0.008 c	10.968 c	0.848 c
Cd+Pb	0.278 a	0.101 b	0.164 b	0.060 b	16.414 b	5.974 b
Cu+Zn	0.116 b	0.004 c	0.233 a	0.007 c	23.297 a	0.745 c
Cd+Pb+Cu+Zn	0.286 a	0.101 b	0.144 bc	0.050 b	14.430 bc	5.047 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 5.21: Biological concentration factor (BCF) of Cu accumulation in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Cu accumulation	
	BCF (Root)	
	Lower	Upper
Control	1.524 c	1.011 c
Cd	1.467 c	0.766 cd
Pb	1.709 bc	1.091 c
Cu	1.788 bc	2.260 b
Zn	0.107 d	0.829 cd
Cd+Pb	0.673 d	0.591 d
Cu+Zn	2.277 b	2.770 a
Cd+Pb+Cu+Zn	3.656 a	3.080 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 5.22: Biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cu accumulation in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	Cu accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.369 d	0.013 c	0.125 bc	0.005 d	12.524 bc	0.469 d
Cd	0.256 d	0.013 c	0.099 c	0.006 d	9.879 c	0.562 d
Pb	0.775 c	0.497 ab	0.190 ab	0.120 bc	18.979 ab	12.008 c
Cu	0.831 c	0.586 a	0.152 abc	0.107 bc	15.194 abc	10.667 c
Zn	0.394 d	0.689 a	0.200 a	0.340 a	20.012 a	33.989 a
Cd+Pb	0.312 d	0.285 b	0.171 ab	0.147 b	17.074 ab	21.515 b
Cu+Zn	1.108 b	0.635 a	0.162 abc	0.095 bc	16.246 abc	9.461 cd
Cd+Pb+Cu+Zn	1.361 a	0.493 ab	0.158 abc	0.057 cd	15.830 abc	5.749 cd

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 5.23: Biological concentration factor (BCF) of Zn accumulation in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	**Zn accumulation**	
	BCF (Root)	
	Lower	**Upper**
Control	6.012 c	3.517 c
Cd	2.827 e	2.104 d
Pb	4.666 d	3.462 c
Cu	3.990 de	1.598 d
Zn	9.728 b	5.866 b
Cd+Pb	3.815 de	0.740 e
Cu+Zn	10.940 a	6.381 b
Cd+Pb+Cu+Zn	10.957 a	9.250 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 5.24: Biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Zn accumulation in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal spiked treatments

Treatment	**Zn accumulation**					
	BAC (Tiller)		**TF (Tiller)**		**Efficacy (Tiller)**	
	Lower	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**
Control	0.938 e	0.818 d	0.082 e	0.073 d	8.240 e	7.300 d
Cd	1.080 e	0.445 d	0.167 d	0.068 d	16.714 d	6.849 d
Pb	0.890 e	0.735 d	0.091 e	0.075 d	9.060 e	7.463 d
Cu	2.229 d	0.645 d	0.263 b	0.076 d	26.338 b	7.588 d
Zn	6.711 c	7.247 a	0.227 c	0.245 a	22.701 c	24.506 a
Cd+Pb	1.070 e	0.354 d	0.179 d	0.059 d	17.880 d	5.926 d
Cu+Zn	9.610 a	5.304 b	0.298 a	0.165 b	29.800 a	16.487 b
Cd+Pb+Cu+Zn	8.516 b	4.486 c	0.256 b	0.135 c	25.643 b	13.509 c

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

5.4 Discussion

The findings of this chapter have shown that soil pH, plant height and R/T quotient of Vetiver grass are not affected with the different application of single and mixed spiked heavy metal treatments. Nonetheless, the results obtained for tiller number, percentage survivorship and dry matter content in Vetiver grass sharply declined among single and mixed spiked treatments compared to the control.

In the present study of this chapter, a 54.1% and 41.3% reduction was observed in the mixed Cd+Pb+Cu+Zn spiked treatment compared to the control in terms of both tiller number and percentage survivorship, respectively. The significant decrease in tiller number and percentage survivorship in Vetiver grass could be accounted for as a result of the combination application of mixed (multiple) contaminated heavy metals as was suggested by Chiu ***et al***. (2006). In addition, studies by An ***et al***. (2004) with cucumber (***Cucumis sativus***) also recorded similar findings of lower dry matter contents. However, Huang ***et al***. (2009) reported the opposite results for paddy rice plant (***Oryza sativa*** L.) where different single and mixed spiked heavy metals accumulations were applied.

In contrast to other Pb, Cu and Zn accumulations, there was no accumulation of Cd found in the upper tiller for Cd spiked treatment. The highest accumulation of Cd was recorded in the lower tiller compared to the root parts for all mixed spiked treatments, unlike the other types of heavy metals. This trend is supported by earlier studies done by Aibibu ***et al.*** (2010), Zheng ***et al.*** (2014), Christofilopoulos ***et al.*** (2016) and Phusantisampan ***et al.*** (2016) whereby generally, most of the Cd were more likely to be accumulated higher in the roots compared to the tillers. These findings highlight the fact that Vetiver grass could be a potential Cd phytoextractor with regard to its high accumulation capability in both roots and tillers with BCF and BAC values > 1 for single Cd as well as for mixed Cd+Pb and Cd+Pb+Cu+Zn spiked treatments.

Similarly, both lower and upper roots demonstrated positive characteristics of phytostabilization for all different types of heavy metals under both single and mixed spiked treatments due to it as high BCF values of > 1. Generally, there are numerous categories of phytoremediation technology depending on the different types of plants and levels of clean-up required (Padmavathiamma & Li, 2007; Tangahu ***et al.,*** 2011) with the phytoextraction and phytostabilization having its unique mechanism of functions. Phytoextraction refers to the bioaccumulation and translocation uptake of metal contaminants in the soil via the roots of the plant into the above ground components of the plants (Nascimento & Xing, 2006; Sheoran ***et al.,*** 2016). On the other hand, phytostabilization uses the plant to immobilize metal contaminants in soil through bioaccumulation and adsorption by roots within the root zones (Berti & Cunningham, 2000; Mahar ***et al.,*** 2016).

Over the past decades, there have been limited studies that emphasis on the comparison between single and mixed heavy metals accumulation in plants. This chapter has demonstrated the complex interactions in the different applications of single and mixed spiked treatments, affecting the overall heavy metal accumulation trends in Vetiver grass. Similar effects of different application of single and mixed spiked treatments were reported in Peralta-Videa ***et al.*** (2002), Zhou ***et al.*** (2014), Wuana ***et al.*** (2016), He ***et al.*** (2016), Yang ***et al.*** (2016) and Chirakkara ***et al.*** (2016) which have contributed to the variation of metal accumulation in other types of plants such as alfalfa, castor and paddy rice.

On top of that, with reference to Duo ***et al.*** (2010), this chapter was further expanded to cover separate parts of the lower and upper roots and tillers of Vetiver grass in order to provide a more comprehensive phytoassessment for translocation of heavy metals from the lower root upwards to the top of the tiller. Notwithstanding, it is important to note that the mixed heavy metals spiked contamination was expected to be more complex than phytoremediation with only a single type of contamination.

The presence of more than one distinct type of heavy metal contaminants would possibly result in the physico-chemical interactions that will affect phytoability in the plants (Chirakkara ***et al.***, 2016). Many recent studies by Ramamurthy and Memarian (2014), Hechmi ***et al.*** (2014) as well as Chigbo and Batty (2015) reported that the use of two and more different types of soil contaminants could unexpectedly limit its mobility and bioavailability resulting in reduction phytoaccumulation efficiency in the plants.

However, this chapter has demonstrated findings to the contrary with Vetiver grass showing substantially high phytoaccumulation ability under the mixed spiked treatments for all different types of heavy metals. This scenario is possible as the fate and translocation of metal contaminants under the mixed heavy metal conditions are arbitrary, complex and unpredictable (Reddy, 2011).

5.5 Conclusions

The overall findings highlighted that Vetiver grass grown in mixed Cd+Pb, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments is potentially capable of accumulating higher heavy metal concentrations than the single spiked treatments, in the following accumulation order of Zn >> Cu > Pb > Cd. Vetiver grass can be regarded as a promising Cd, Cu and Zn phytostabilizer owing to its high BCF values of > 1 and noticeably higher accumulation of heavy metals found in the roots compared to the tillers for both single and mixed spiked treatments. In terms of different plant parts, the lower roots and lower tillers of Vetiver grass exhibited a strong tendency for greater uptake and accumulation of all the four types of heavy metals, irrespective of single and/or mixed spiked treatments.

CHAPTER 6: [3] ENHANCED HEAVY METAL PHYTOASSESSMENT OF VETIVER GRASS WITH SOIL AMENDMENTS

6.1 Introduction

Soil contamination has become an increasingly important environmental issue in both developed and developing nations. In most cases, soil contamination has been brought about by anthropogenic factors with humans being the culprit, continuously contaminating the soil in the past and present days via industrial and domestic activities. Of these, heavy metal contamination is one of the major types of inorganic soil contamination in the environment. The major contributing factors to anthropogenic heavy metal contamination in soils and the environment include the improper management of agricultural leaching, metalliferous mining and smelting, disposal of metallurgical and electronic commodities, sewage sludge and other chemical manufacturing waste materials (Bradl, 2005; Alloway, 2013). Many remediation technologies, such as landfilling, soil washing, bioleaching and excavation have been attempted to resolve soils with contaminated heavy metals. However, all of these strategies are not cost-effective, extremely complicated and are not economically viable in addition to being intrusive to the environment. As a consequence, phytoremediation has emerged to be the green plant based clean-up solution that is able to remove, metabolize and degrade a wide range of hazardous soil heavy metal contaminants with minimum cost required and is non-destructive to the natural ecosystem (Padmavathiamma & Li, 2007; Ali ***et al***., 2013; Haque & Khan, 2016).

[3] A version of this chapter has been published as Ng, C. C., Boyce, A. N., Rahman, M. M., & Abas, M. R. (2016). Effects of different soil amendments on mixed heavy metals contamination in vetiver grass. ***Bulletin of Environmental Contamination and Toxicology***, ***97***(5), 695-701. doi:10.1007/s00128-016-1921-5

Numerous plants have being studied over the years, with reports suggesting Vetiver grass, ***Vetiveria zizanioides*** (Linn.) Nash to be one of the most promising plants, with a fast growth rate, and the ability to adapt to many environmental conditions and stress, in addition to being able to tolerate a wide range of extreme heavy metal contamination in soils (Truong ***et al***., 2008; Truong & Danh 2015). However, most of these studies and recent ones (Chen ***et al***., 2012; Prasad ***et al***., 2014; Singh ***et al***., 2015; Banerjee ***et al***., 2016; Vargas ***et al***., 2016) have solely focused on the phytoassessment of a single metal accumulation. Little information is available on the growing concern of mixed (Cd-Pb) metal contaminations and the suitability of Vetiver grass as a phytoremediator under these conditions.

Both Pb and Cd metals are extremely toxic even at low concentration levels and humans can be easily exposed to these heavy metals through direct inhalation or ingestion of soil and dust, or consumption of contaminated plants, which can substantially affect human health and well-being. In order to increase the metal accumulation, low cost soil amendments have been used to enhance the phytoavailability of mixed metal uptakes in Vetiver grass (US EPA, 2007b; Karami ***et al***., 2011). With reference to the phytoassessment studies in the earlier chapters, the aims of this chapter are to evaluate the trends and effects of heavy metal accumulation and assess the influence and capability of different types and levels of low cost soil amendments to enhance the accumulation of heavy metals by Vetiver grass grown in mixed Cd-Pb contaminated soil conditions.

6.2 Materials and Methods

6.2.1 Soil preparation and experimental setup

The experiments were conducted in the plant house located at Rimba Ilmu, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur using pot assays with the average room temperature ranging between 25.5ºC and 33.5ºC throughout the day. Top soil (0 – 20 cm depth) for planting was taken from a field situated at 3° 7' N latitude and 101° 39' E longitude and was air-dried for a week before being thoroughly mixed and sieved through <4mm mesh to remove all non-soil particles to obtain a homogenous soil sample. The soil samples underwent a preliminary physico-chemical soil assessment (Table 6.1) prior to the preparation of soils with mixed-contamination of Cd (50 mg/kg) and Pb (100 mg/kg), taking into consideration both national (DOE, 2009) and international (CCME, 1999) permissible soil heavy metals contamination guidelines.

The mixed Cd-Pb contamination were artificially spiked using cadmium nitrate tetrahydrate, $Cd(NO_3)_2.4H_2O$ and lead (II) nitrate, $Pb(NO_3)_2$ salt compounds before being filled up with two kilograms of soil in a plastic pot with height and diameter measurements of 0.18m x 0.16m, respectively for all treatments. Fresh Vetiver plantlets were collected and placed under different individual experiments, conducted with various types of soil amendments such as the disodium ethylene-diamine-tetra-acetate, $C_{10}H_{14}N_2Na_2O_8.2H_2O$ (EDTA), elemental sulfur, S_8 (S) and ammonium nitrate, NH_4NO_3 (N-fertilizer). Four levels of EDTA (1, 5, 10 and 25 mmol EDTA/kg soil), five levels of elemental S (5, 10, 20, 40 and 80 mmol S/kg soil) and six levels of N-fertilizer (10, 25, 50, 100, 200 and 300 mmol N/kg soil) were tested, respectively (Table 6.2). All chemicals used were of analytical reagent standard or of the best grade available.

All of the treatments were watered evenly with 50 mL of tap water once a day and their growth performance continuously monitored throughout the 60-day period of the experiment. The study of this chapter was conducted under a completely randomized design (CRD) with three replications (n = 3).

Table 6.1: Physico-chemical properties of growth media soil

Parameter (Unit)		Mean
Soil texture		
Sand (%)		93.12
Very coarse sand (%)	1.54	
Coarse sand (%)	45.21	
Medium coarse sand (%)	21.87	
Fine sand (%)	17.58	
Very fine sand (%)	6.92	
Silt (%)		4.89
Clay (%)		1.99
Bulk density (g/cm^3)		1.34 ± 0.47
Porosity (%)		49.43 ± 3.45
Colour (Munsell colour charts)		Dull reddish brown 2.5YR 5/4
Water content (%)		5.85 ± 1.09
Field capacity (%)		38.59 ± 8.28
Saturation level (%)		15.16 (Dry)
pH		5.11 ± 0.05
Temperature (ºC)		29.27 ± 0.45
Metal contents (mg/kg)		
	Cd	1.59 ± 0.15
	Pb	52.30 ± 2.77

Mean ± standard deviation

Table 6.2: Soil amendment with treatment variables

Treatment	Cd and Pb (mg/kg soil), EDTA, Elemental S and N (mmol/kg soil)
Control	50 Cd + 100 Pb
1EDTA	50 Cd + 100 Pb + 1 EDTA
5EDTA	50 Cd + 100 Pb + 5 EDTA
10EDTA	50 Cd + 100 Pb + 10 EDTA
25EDTA	50 Cd + 100 Pb + 25 EDTA
5S	50 Cd + 100 Pb + 5 elemental S
10S	50 Cd + 100 Pb + 10 elemental S
20S	50 Cd + 100 Pb + 20 elemental S
40S	50 Cd + 100 Pb + 40 elemental S
80S	50 Cd + 100 Pb + 80 elemental S
10N	50 Cd + 100 Pb + 10 N-fertilizer
25N	50 Cd + 100 Pb + 25 N-fertilizer
50N	50 Cd + 100 Pb + 50 N-fertilizer
100N	50 Cd + 100 Pb + 100 N-fertilizer
200N	50 Cd + 100 Pb + 200 N-fertilizer
300N	50 Cd + 100 Pb + 300 N-fertilizer

6.2.2 Chemical analyses of soil and plant samples

Freshly harvested Vetiver grasses were brought into the laboratory and washed in running filter water followed by deionized water to remove any adhering soil particles before separating them into roots and shoots (tillers). The fresh weights of plant samples were determined before the samples were oven-dried for 72 hours at 70°C until it achieved a constant weight. Then the dry matter content of the Vetiver samples was determined before it was homogenized in a mortar and pestle. Approximately, 0.5g of the homogenized dried root and tiller samples underwent acid digestion with hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) and nitric acid (HNO_3) as according to Method 3050B (US EPA, 1996) followed by Method 7000B (US EPA, 2007a) for the elemental analysis using the Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (F-AAS). The Bundesanstalt für Materialforschung und –prüfung (BAM Germany): German Federal Institute for Materials Research and Testing certified reference material (BRM#12-mixed sandy soil) was used to validate the precision of the chemical analysis technique and the metal recovery rates are recorded in Table 6.3. Soil samples were also air-dried for 72 hours until it reached a constant weight before it was analysed following similar analytical procedures.

Table 6.3: Concentrations of Certified Reference Material (CRM) and metal recovery (%) of Cd and Pb

Metal	Initial soil (mg/kg)	Spiked metal (mg/kg)	CRM* (mg/kg)	Calculated (mg/kg)	Metal recovery (%)
Cd	1.59 ± 0.15	52.14 ± 7.56	4.04 ± 0.22	3.64 ± 1.45	90.09
Pb	52.30 ± 2.77	101.88 ± 13.21	204.0 ± 6.00	217.32 ± 14.32	106.53

* BAM Germany certified reference material BRM#12-mixed sandy soil
Mean ± standard deviation

6.2.3 Data interpretation and statistical analyses

The growth performance of Vetiver was determined using tolerance index (TI) and root-tiller quotient. The ability for heavy metal accumulation and translocation upwards in Vetiver were evaluated by assessing the biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (Kabata-Pendias, 2010; Alloway, 2013; Ali ***et al***., 2013) as follow:

TI = Total dry matter content in heavy metal treatment / Total dry matter content in control;

Root-tiller quotient = Dry matter content in root / Dry matter content in tiller;

BCF = Heavy metal concentration in root / Heavy metal concentration in soil;

BAC = Heavy metal concentration in tiller / Heavy metal concentration in soil;

TF = Heavy metal concentration in tiller / Heavy metal concentration in root; &

Metal uptake efficacy (%) = (Heavy metal concentration in tiller / Total heavy metal concentration removed from the soil) X 100%

Data was analysed by performing one-way analysis of variance (ANOVA) to evaluate the growth performance and metal accumulation in Vetiver growing under different types and levels of treatments. Further statistical validity test for significant differences among treatment means, was carried out using Fisher's least significant difference (LSD) tests at the 95% level of confidence whilst linear regression analysis was undertaken to assess the relationships between the different types of soil amendments and the accumulation of heavy metal concentration in Vetiver.

6.3 Results

6.3.1 Soil pH and plant growth

The initial soil pH varied between 5.10 and 5.78, where 25EDTA treatment recorded the highest pH of 5.78 while the lowest pH of 5.10 was observed in 300N treatment (Figure 6.1 – Figure 6.3). Upon harvesting, fluctuations of soil pH was observed in all the three different types and levels of soil amendments. A significant decrease (***p<0.05***) in the final soil pH was recorded in all the elemental S (4.21 – 4.79) as well as 200N (5.53) and 300N (5.59) treatments compared with the control (5.98).

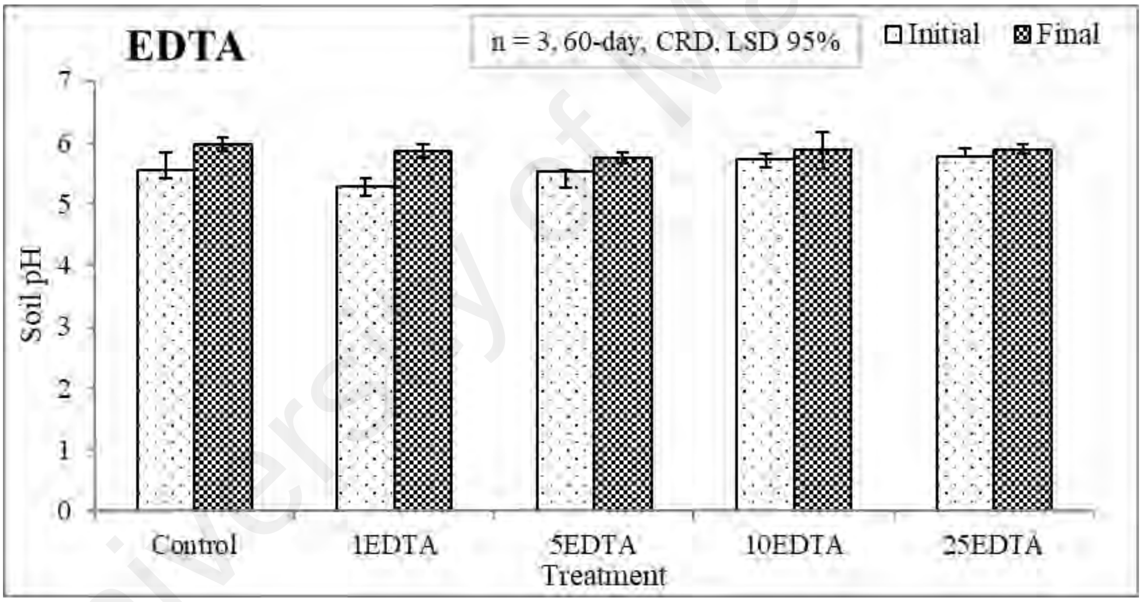

Figure 6.1: Changes in soil pH in Vetiver as influenced by different levels of EDTA soil amendment. Vertical bars represent standard deviation in treatment means and same letters are not significantly different at 0.05 levels of probability.

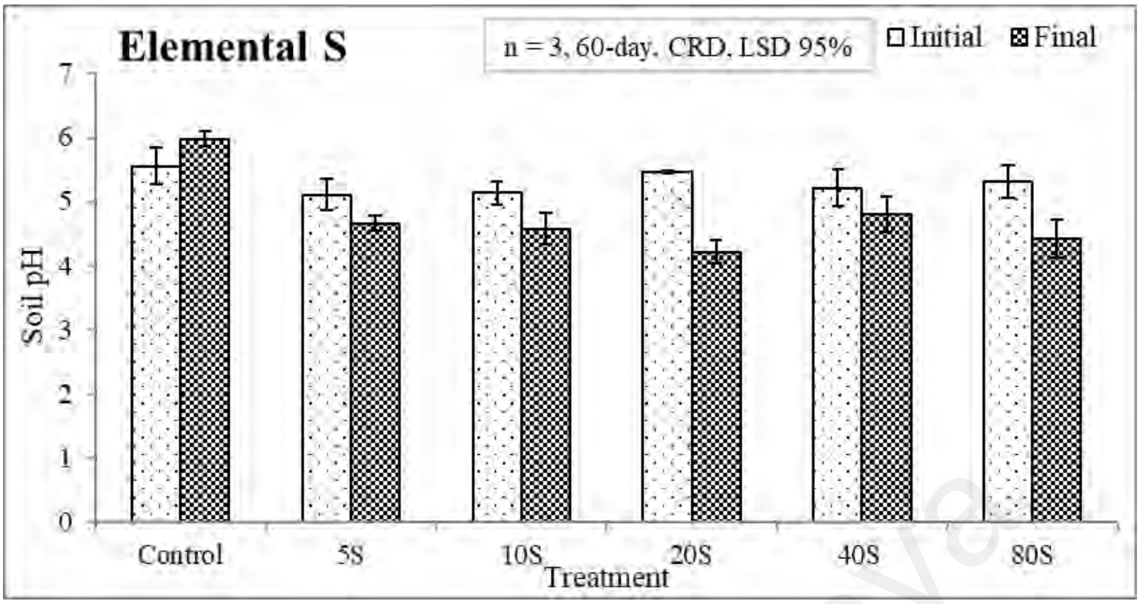

Figure 6.2: Changes in soil pH in Vetiver as influenced by different levels of elemental S soil amendment. Vertical bars represent standard deviation in treatment means and same letters are not significantly different at 0.05 levels of probability.

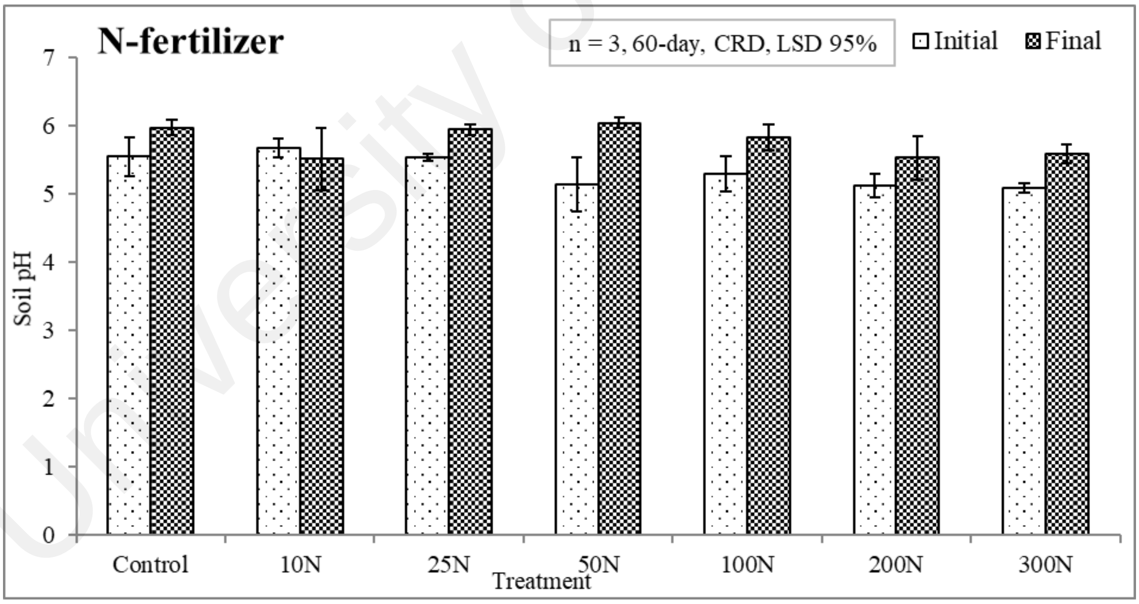

Figure 6.3: Changes in soil pH in Vetiver as influenced by different levels of elemental S soil amendment. Vertical bars represent standard deviation in treatment means and same letters are not significantly different at 0.05 levels of probability.

In terms of plant growth performance, Table 6.4 – Table 6.6 show no significant differences (***p>0.05***) in tiller number and basal diameter among all three different types of soil amendments compared with the control. Similarly, no significant difference (***p>0.05***) was found in plant height among all levels of EDTA treatments with the control. However, a significantly higher (***p<0.05***) plant height was recorded in 40S, 80S, 200N and 300N treatments compared to the other soil amendment levels and the control.

Table 6.4: Tiller number, plant height (cm) and basal diameter (cm) of Vetiver as influenced by different levels of EDTA soil amendment

Treatment	Tiller number	Plant height (cm)	Basal diameter (cm)
Control	14.2 ± 2.8 a	42.20 ± 2.48 a	10.02 ± 0.36 a
1EDTA	13.6 ± 3.4 a	41.74 ± 0.47 a	9.66 ± 3.12 a
5EDTA	13.8 ± 7.4 a	38.03 ± 8.36 a	9.27 ± 2.98 a
10EDTA	13.1 ± 0.7 a	38.37 ± 11.33 a	9.17 ± 2.47 a
25EDTA	15.1 ± 5.4 a	41.71 ± 13.40 a	9.97 ± 1.91 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 6.5: Tiller number, plant height (cm) and basal diameter (cm) of Vetiver as influenced by different levels of elemental S soil amendment

Treatment	Tiller number	Plant height (cm)	Basal diameter (cm)
Control	14.2 ± 0.4 a	42.20 ± 4.70 cd	10.02 ± 0.75 a
5S	15.6 ± 1.3 a	43.35 ± 8.53 cd	9.48 ± 1.46 a
10S	14.9 ± 6.3 a	41.75 ± 6.42 d	10.91 ± 3.22 a
20S	13.5 ± 1.2 a	51.30 ± 0.89 bc	9.35 ± 0.35 a
40S	15.0 ± 4.7 a	60.58 ± 7.11 a	10.51± 2.41 a
80S	13.3 ± 5.2 a	54.50 ± 8.69 ab	9.43 ± 3.08 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 6.6: Tiller number, plant height (cm) and basal diameter (cm) of Vetiver as influenced by different levels of N-fertilizer soil amendment

Treatment	Tiller number	Plant height (cm)	Basal diameter (cm)
Control	14.2 ± 3.5 a	42.20 ± 2.94 bc	10.02 ± 2.54 a
10N	15.7 ± 1.3 a	42.73 ± 3.68 bc	10.43 ± 1.79 a
25N	12.3 ± 0.2 a	41.25 ± 0.52 c	9.12 ± 3.18 a
50N	12.8 ± 4.7 a	43.02 ± 3.44 bc	9.29 ± 2.48 a
100N	16.4 ± 0.4 a	45.93 ± 11.30 abc	9.19 ± 0.36 a
200N	13.9 ± 2.1 a	47.65 ± 9.62 ab	9.15 ± 1.25 a
300N	14.7 ± 1.6 a	51.20 ± 3.77 a	9.37 ± 0.64 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

The dry matter contents were not affected by treatment variables (Table 6.7 – Table 6.9) as there were no significant differences (***p>0.05***) found in all of the three different types and levels of soil amendments. Subsequently, the root-tiller quotient and tolerance index (TI) was employed to assess the capability of the Vetiver grass growing under mixed Cd-Pb contamination conditions. Similarly, no significant differences (***p>0.05***) was observed in the root-tiller quotient and tolerance index (TI) among the treatment variables.

Table 6.7: Dry matter content (g/m^2), root-tiller quotient (R/T) and tolerance index (TI) of Vetiver as influenced by different levels of EDTA soil amendment

Treatment	Dry matter content (g/m^2)			R/T	TI
	Root	Tiller	Total		
Control	3.07 ± 0.16 ab	4.92 ± 0.75 ab	7. 99 ± 0.66 ab	0.624 ab	
1EDTA	3.23 ± 0.36 ab	4.83 ± 0.13 ab	8.07 ± 0.31 ab	0.669 ab	1.010 ab
5EDTA	3.29 ± 0.28 ab	4.19 ± 0.36 ab	7.48 ± 0.30 ab	0.785 a	0.936 ab
10EDTA	3.57 ± 0.21 a	4.75 ± 0.24 ab	8.32 ± 0.16 ab	0.752 ab	1.041 ab
25EDTA	3.45 ± 0.35 ab	5.12 ± 0.52 a	8.57 ± 0.86 a	0.674 ab	1.073 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 6.8: Dry matter content (g/m^2), root-tiller quotient (R/T) and tolerance index (TI) of Vetiver as influenced by different levels of elemental S soil amendment

Treatment	Dry matter content (g/m^2)			R/T	TI
	Root	Tiller	Total		
Control	3.07 ± 0.16 a	4.92 ± 0.75 a	7. 99 ± 0.66 a	0.624 a	
5S	3.20 ± 0.36 a	4.35 ± 0.58 a	7.54 ± 0.93 a	0.736 a	0.944 a
10S	3.79 ± 0.63 a	4.66 ± 0.75 a	8.45 ± 1.31 a	0.813 a	1.058 a
20S	3.68 ± 0.80 a	4.77 ± 1.11 a	8.46 ± 1.81 a	0.771 a	1.059 a
40S	4.58 ± 1.37 a	5.53 ± 2.03 a	10.11 ± 3.39 a	0.828 a	1.265 a
80S	4.15 ± 0.69 a	5.34 ± 0.55 a	9.48 ± 1.09 a	0.777 a	1.186 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 6.9: Dry matter content (g/m^2), root-tiller quotient (R/T) and tolerance index (TI) of Vetiver as influenced by different levels of N-fertilizer soil amendment

Treatment	Dry matter content (g/m^2)			R/T	TI
	Root	Tiller	Total		
Control	3.07 ± 0.16 ab	4.92 ± 0.75 ab	7. 99 ± 0.66 a	0.624 a	
10N	3.73 ± 0.46 ab	4.88 ± 1.40 ab	8.61 ± 0.98 a	0.764 a	1.078 a
25N	2.63 ± 1.16 b	3.75 ± 1.48 b	6.38 ± 2.60 a	0.701 a	0.798 a
50N	3.02 ± 0.12 ab	4.97 ± 2.02 ab	7.99 ± 1.93 a	0.608 a	1.000 a
100N	4.28 ± 0.79 a	5.07 ± 1.06 a	9.35 ± 1.62 a	0.844 a	1.170 a
200N	3.89 ± 0.17 ab	4.45 ± 0.79 ab	8.34 ± 0.91 a	0.874 a	1.044 a
300N	2.86 ± 0.52 b	4.11 ± 1.39 b	6.97 ± 1.82 a	0.696 a	0.872 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

6.3.2 Heavy metal accumulation in plant

Metal accumulation for both Cd and Pb in the roots and tillers of Vetiver grass are shown in Tables 6.10 – Table 6.15. Each level of EDTA, elemental S and N-fertilizer soil amended treatment recorded a distinctive Cd (172.2 – 303.3 mg/kg) and Pb (73.9 – 304.5 mg/kg) concentration pattern in the roots and tillers of Vetiver. The 25 mmol EDTA treatment exhibited the highest accumulation of both Pb (211.3 ± 12.0 mg/kg) and Cd (191.8 ± 1.9 mg/kg) in the tillers. Between roots and tillers, the accumulation of both Cd and Pb were comparatively greater in the tillers than the roots for 10 mmol EDTA and 25 mmol EDTA. A significant increase (***$p<0.05$***) in Cd and Pb accumulation in the tillers were obtained in both 10 mmol EDTA and 25 mmol EDTA treatments compared to the control.

For all the levels and types of soil amendments, Pb accumulation in the roots, together with selected Cd roots treatments (25EDTA, 10S, 20S, 40S, 80S, 50N, 100N, 200N and 300N), a significant reduction (***$p<0.05$***) was observed compared to the control. With regard to Pb accumulation, significantly lower (***$p<0.05$***) uptake was observed in all levels of elemental S treatment irrespective of roots, tillers and total metal accumulation compared to the control. However, a significantly larger (***$p<0.05$***) accumulation of Pb in the tillers was observed in all N-fertilizer treatments compared with the control. On the other hand, no significant difference (***$p>0.05$***) was found between the total metal accumulation of Cd in both elemental S and N-fertilizer treatments compared with control.

Table 6.10: Concentration of Cd (mg/kg) in the root and tiller of Vetiver as influenced by different levels of EDTA soil amendment

Treatment	Cd concentration (mg/kg)		
	Root	Tiller	Total
Control	153.6 ± 6.1 ab	73.4 ± 7.6 cd	227.0 ± 1.6 cd
1EDTA	133.3 ± 6.0 bcd	79.2 ± 1.1 cd	212.5 ± 4.9 d
5EDTA	175.5 ± 6.3 a	91.2 ± 8.3 c	266.7 ± 14.6 abc
10EDTA	147.4 ± 16.7 abc	156.4 ± 10.6 b	303.8 ± 27.3 a
25EDTA	111.5 ± 11.8 cd	191.8 ± 1.9 a	303.3 ± 9.9 ab

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 6.11: Concentration of Cd (mg/kg) in the root and tiller of Vetiver as influenced by different levels of elemental S soil amendment

Treatment	Cd concentration (mg/kg)		
	Root	Tiller	Total
Control	153.6 ± 6.1 a	73.4 ± 7.6 c	227.0 ± 1.6 abcd
5S	149.7 ± 8.0 ab	129.2 ± 10.7 abc	278.9 ± 18.7 a
10S	109.8 ± 6.0 c	144.8 ± 32.9 ab	254.6 ± 26.9 ab
20S	93.2 ± 18.9 cd	159.3 ± 16.2 a	252.5 ± 35.1 abc
40S	75.6 ± 1.1 d	97.8 ± 15.0 abc	173.4 ± 16.1 d
80S	82.85 ± 7.0 cd	89.3 ± 8.3 bc	172.2 ± 15.4 d

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 6.12: Concentration of Cd (mg/kg) in the root and tiller of Vetiver as influenced by different levels of N-fertilizer soil amendment

Treatment	Cd concentration (mg/kg)		
	Root	Tiller	Total
Control	153.6 ± 6.1 a	73.4 ± 7.6 d	227.0 ± 1.6 a
10N	140.3 ± 5.2 ab	79.6 ± 10.0 d	219.9 ± 15.2 a
25N	129.9 ± 20.8 abc	93.7 ± 7.4 bcd	223.6 ± 28.2 a
50N	109.4 ± 10.0 bcd	116.9 ± 13.4 abcd	226.3 ± 3.4 a
100N	114.7 ± 6.1 bcd	127.3 ± 16.6 abc	242.0 ± 22.7 a
200N	96.5 ± 5.4 cde	138.1 ± 25.2 ab	234.6 ± 30.6 a
300N	68.9 ± 9.1 e	147.9 ± 5.6 a	216.8 ± 3.5 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 6.13: Concentration of Pb (mg/kg) in the root and tiller of Vetiver as influenced by different levels of EDTA soil amendment

Treatment	Pb concentration (mg/kg)		
	Root	Tiller	Total
Control	165.8 ± 14.0 a	35.5 ± 0.6 d	201.3 ± 14.6 b
1EDTA	80.4 ± 9.0 bc	36.2 ± 0.9 d	116. 6 ± 10.0 c
5EDTA	65.8 ± 4.4 c	74.1 ± 2.1 c	139.9 ± 6.5 c
10EDTA	82.5 ± 2.6 bc	126.7 ± 4.8 b	209.2 ± 7.4 b
25EDTA	93.2 ± 7.1 b	211.3 ± 12.0 a	304.5 ± 19.1 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 6.14: Concentration of Pb (mg/kg) in the root and tiller of Vetiver as influenced by different levels of elemental S soil amendment

Treatment	Pb concentration (mg/kg)		
	Root	Tiller	Total
Control	165.8 ± 14.0 a	35.5 ± 0.6 a	201.3 ± 14.6 a
5S	118.6 ± 10.6 bc	24.3 ± 4.2 bc	142.9 ± 14.8 b
10S	77.5 ± 3.3 d	16.7 ± 1.3 cd	94.2 ± 2.0 c
20S	121.1 ± 10.0 b	25.7 ± 3.5 b	146.8 ± 13.5 b
40S	62.8 ± 3.0 d	11.1 ± 1.5 de	73.9 ± 1.5 c
80S	89.0 ± 6.7 cd	5.2 ± 0.3 e	94.2 ± 6.4 c

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 6.15: Concentration of Pb (mg/kg) in the root and tiller of Vetiver as influenced by different levels of N-fertilizer soil amendment

Treatment	Pb concentration (mg/kg)		
	Root	Tiller	Total
Control	165.8 ± 14.0 a	35.5 ± 0.6 e	201.3 ± 14.6 bc
10N	99.7 ± 5.1 bc	187.3 ± 12.0 a	286.9 ± 17.1 a
25N	114.0 ± 14.4 b	163.8 ± 20.0 ab	277.8 ± 5.6 a
50N	84.5 ± 7.3 bcd	135.6 ± 7.0 bc	220.1 ± 14.3 b
100N	63.9 ± 3.0 de	102.6 ± 8.1 cd	166.5 ± 11.0 c
200N	82.9 ± 8.0 cd	78.4 ± 8.0 d	161.3 ± 16.0 c
300N	38.4 ± 3.7 e	71.1 ± 4.1 d	109.5 ± 0.4 d

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

6.3.3 Translocation and association of metals in plant

Comparatively lower BCF values were obtained in all types of soil amended treatments compared to the control, probably due to the effects of lower accumulation of both Cd and Pb metals in the roots than in the tillers (Table 6.16 – Table 6.21). Among the different types of soil amendments, N-fertilizer (36.20 – 68.22%), EDTA (34.20 – 63.25%) and elemental S (46.32 – 63.09%) recorded higher accumulation efficacy for Cd, compared with other individual levels of treatment, respectively. On the other hand, 25 mmol EDTA (69.40%), 10 mmol N (65.27%) and 300 mmol N (64.96%) exhibited the greatest accumulation efficacy for Pb with more than a two-fold increase compared to the control. However, the application of elemental S showed no significant difference ($p>0.05$) in the enhancement of Pb accumulation regardless of the different concentrations used, compared to the control.

Generally, the inclination trend observed for Cd accumulation, among the different types of soil amendments were in the order of N-fertilizer (300N) > EDTA (25EDTA) > elemental S (20S) for all the treatments. Notwithstanding, the trend for Pb accumulation was in the following order of EDTA (25EDTA) > N-fertilizer >> elemental S among all treatments. Moreover, there were strong and significant positive relationships found between the accumulations of Pb in EDTA (***r=0.998***) and N-fertilizer (***r=0.921***) treatments with the levels of soil amendments used when grown under mixed heavy metal contamination (Table 6.22 and Table 6.23). Nonetheless, the elemental S treatment (***r=0.956***) exhibited strong negative correlation with regard to dry matter content and Cd accumulation due to the appreciably decreased metal uptake in the roots and tillers in selected elemental S treatments.

Table 6.16: Cd accumulation in its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver as influenced by different levels of EDTA soil amendment

Treatment	Cd accumulation			
	BCF	BAC	TF	Efficacy (%)
Control	3.072 ab	1.468 c	0.4778 c	32.33 c
1EDTA	2.665 bc	1.584 c	0.594 c	37.28 c
5EDTA	3.510 a	1.824 c	0.520 c	34.20 c
10EDTA	2.948 abc	3.128 b	1.061 b	51.48 b
25EDTA	2.229 c	3.836 a	1.721 a	63.25 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 6.17: Cd accumulation in its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver as influenced by different levels of elemental S soil amendment

Treatment	Cd accumulation			
	BCF	BAC	TF	Efficacy (%)
Control	3.072 a	1.468 c	0.4778 c	32.33 c
5S	2.994 a	2.583 abc	0.863 bc	46.32 b
10S	2.195 b	2.895 ab	1.319 ab	56.88 ab
20S	1.864 b	3.186 a	1.709 a	63.09 a
40S	1.511 b	1.956 bc	1.295 ab	56.42 ab
80S	1.657 b	1.786 bc	1.078 bc	51.87 ab

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 6.18: Cd accumulation in its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver as influenced by different levels of N-fertilizer soil amendment

Treatment	Cd accumulation			
	BCF	BAC	TF	Efficacy (%)
Control	3.072 a	1.468 d	0.478 c	32.33 e
10N	2.806 ab	1.592 d	0.567 c	36.20 e
25N	2.598 abc	1.874 bcd	0.721 c	41.91 cde
50N	2.187 bcd	2.338 abcd	1.069 bc	51.67 bcd
100N	2.294 bcd	2.546 abc	1.109 bc	52.60 bc
200N	1.929 cde	2.762 ab	1.432 b	58.88 ab
300N	1.378 e	2.958 a	2.147 a	68.22 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 6.19: Pb accumulation in its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver as influenced by different levels of EDTA soil amendment

Treatment	Pb accumulation			
	BCF	BAC	TF	Efficacy (%)
Control	1.658 a	0.355 d	0.214 e	17.64 e
1EDTA	0.804 b	0.362 d	0.451 d	31.06 d
5EDTA	0.658 b	0.741 c	1.126 c	52.97 c
10EDTA	0.825 b	1.267 b	1.536 b	60.56 b
25EDTA	0.932 b	2.113 a	2.268 a	69.40 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 6.20: Pb accumulation in its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver as influenced by different levels of elemental S soil amendment

Treatment	Pb accumulation			
	BCF	**BAC**	**TF**	**Efficacy (%)**
Control	1.658 a	0.355 a	0.214 a	17.64 a
5S	1.186 bc	0.243 bc	0.205 a	17.01 a
10S	0.775 c	0.167 cd	0.215 a	17.68 a
20S	1.211 b	0.257 b	0.212 a	17.51 a
40S	0.628 c	0.111 de	0.177 a	15.03 a
80S	0.890 c	0.051 e	0.058 b	5.46 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 6.21: Pb accumulation in its biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Vetiver as influenced by different levels of N-fertilizer soil amendment

Treatment	Pb accumulation			
	BCF	**BAC**	**TF**	**Efficacy (%)**
Control	1.658 a	0.355 e	0.214 d	17.64 d
10N	0.997 bc	1.873 a	1.880 ab	65.27 a
25N	1.140 b	1.638 ab	1.437 abc	58.96 ab
50N	0.845 bc	1.356 bc	1.605 ab	61.61 ab
100N	0.639 de	1.026 cd	1.607 ab	61.64 ab
200N	0.829 cd	0.784 d	0.946 cd	48.62 c
300N	0.384 e	0.711 e	1.854 a	64.96 ab

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 6.22: Regression equation, coefficients of determination (R^2) and F values of Cd accumulation with different parameters in Vetiver

Regression equation	R^2	R	F value
Relationship between level of soil amendments and Cd accumulation			
Y_{Cd} (EDTA) = 239.579 + 3.119X_1	0.579	0.761	2.746
Y_{Cd} (S) = 271.075 - 1.445X_1	0.779	0.882	10.557*
Y_{Cd} (N) = 227.831 - 0.006X_1	0.005	0.068	0.019
Relationship between dry matter content and Cd accumulation			
Y_{Cd} (EDTA) = - 60.363 + 40.926X_2	0.132	0.363	0.303
Y_{Cd} (S) = 646.832 - 47.748X_2	0.915	0.956	32.185**
Y_{Cd} (N) = 181.232 + 5.787X_2	0.446	0.668	3.224

X_1 = Level of soil amendments; X_2 = Dry matter content
** Significant at 0.01 level of probability
* Significant at 0.05 level of probability

Table 6.23: Regression equation, coefficients of determination (R^2) and F values of Pb accumulation with different parameters in Vetiver

Regression equation	R^2	R	F value
Relationship between level of soil amendments and Pb accumulation			
Y_{Pb} (EDTA) = 111.152 + 7.939X_1	0.977	0.988	83.180**
Y_{Pb} (S) = 127.273 - 0.546X_1	0.261	0.511	1.061
Y_{Pb} (N) = 268.567 - 0.569X_1	0.849	0.921	22.405**
Relationship between dry matter content and Pb accumulation			
Y_{Pb} (EDTA) = 124.487 + 9.154X_2	0.02	0.14	0.04
Y_{Pb} (S) = 344.732 - 26.610X_2	0.668	0.817	6.038
Y_{Pb} (N) = 252.947 - 6.207X_2	0.009	0.096	0.038

X_1 = Level of soil amendments; X_2 = Dry matter content
** Significant at 0.01 level of probability
* Significant at 0.05 level of probability

6.4 Discussion

Among all the three different types of soil amendments, both sulfur and nitrogen can be considered as plant macronutrients that are required for plant growth and metabolism, while EDTA is a colourless, water soluble metal chelating agent that is able to bind and mobilize metal cations (Li & Shuman, 1996; Hong ***et al***., 1999; Saifullah, 2009; Shahid ***et al***., 2014; Grant & Hawkesford, 2015; Pilbeam, 2015).

The drastic dropped in the final soil pH in all levels of elemental S treatments could probably be due to the dilution and oxidation of the sulfur compounds in contact with the soil growth media that eventually turned more acidic, as sulfur is converted to its sulfate and sulfite forms. Although sulfur does not react with water under normal conditions, elemental sulfur can undergo oxidation over time in the soil and this may have contributed to the lowering of the soil pH observed (Motior ***et al***., 2011; Rahman ***et al***., 2011a,b,c).

The findings of this chapter has shown that the application of mixed Cd-Pb contamination in soil growth media did not have much effect on the overall (roots, shoots and total) dry matter yield for Vetiver grass regardless of the different types and levels of treatment combination used. Vetiver grass showed high tolerant and good adaptability properties to the contaminated mixed Cd-Pb soil conditions as was previously reported in Chen ***et al***. (2004c), Rotkittikhun ***et al***. (2007) and Danh ***et al***. (2009).

The use of different types and levels of low cost soil amendments are the major controlling drivers to the overall rate and efficiency for heavy metals phytoaccumulation in plants (Kumpiene ***et al.,*** 2008). The findings showed that application of higher levels of both N-fertilizer and EDTA enhanced the accumulation of Cd in the tillers of Vetiver grass whereas the opposite was found with elemental S. The application of higher levels of EDTA and N-fertilizer could have probably increased Pb accumulation in the tiller of Vetiver grass, as similar trends have been reported previously by Nascimento ***et al***. (2006), Chiu ***et al***. (2006) and Rahman ***et al***. (2013).

Although higher Pb accumulation was recorded with the application of N-fertilizer in the tillers, reasonably all levels of N-fertilizer treatments displayed approximately similar accumulation of Pb. With higher application levels of elemental S (> 20 mmol elemental S), Pb accumulation was more likely to drop in both the roots and tillers of Vetiver grass. Alternatively, all levels of EDTA and N-fertilizer treatments recorded remarkably higher BAC and TF values than the control for both Cd and Pb accumulation, suggesting that the pathway for metal translocation from soil into tillers was more favourable.

The 25 mmol EDTA treatment demonstrated the highest BAC (3.836) and TF (2.268) for Cd and Pb accumulation, respectively. Lai and Chen (2004), Chen ***et al***. (2004c), Chiu ***et al***. (2005) and Chen ***et al***. (2012) also reported similar findings with Vetiver grass showing the accumulation of heavy metals is gradually enhanced with the application EDTA. In addition, the higher accumulation of heavy metals in the tillers than the roots also suggested that the tillers of Vetiver grass act as the sink for both Cd and Pb accumulation.

Nevertheless, the efficiency of Pb metal translocation from soil-to-root and root-to-tiller decreased with increasing amount of elemental S used. These findings are contrary to that reported by Feng ***et al***. (2009), Motior ***et al***. (2011), Rahman ***et al***. (2011a,b,c,), Soaud ***et al***. (2011a,b) as well as Dede and Ozdemir (2016) who used other types of plant species. Despite the lower Pb accumulation in the elemental S treatments, appreciably higher BAC, TF and metal efficacy for Cd accumulation than the control were detected. The highest percentage of metal efficacy was obtained with the optimum use of 25 mmol EDTA and 20 mmol elemental S for both Cd and Pb accumulations whilst 300 mmol N-fertilizer for Cd and 10 mmol N-fertilizer for Pb, respectively.

The regression equations revealed a positive association with the application of EDTA showing a comparably higher influence on dry matter content, as well as Cd and Pb accumulation compared to the other two types of soil amendments. This chapter has demonstrated that 25 mmol EDTA, 300 mmol N-fertilizer and 20 mmol elemental S are the best possible soil amendments for Vetiver grass in mixed Cd-Pb contaminated soil conditions.

6.5 Conclusions

The accumulation of Cd and Pb were appreciably greater in both roots and tillers of Vetiver grass in the presence of soil amendments compared to the control. All three different types of soil amendments acted as a metal translocation inducer (chelator) by enhancing the accumulation of both Cd and Pb in Vetiver grass. The ideal application levels of EDTA (25EDTA), N-fertilizer (300N) and elemental sulfur (20S) has the potential to enhance the accumulation of both Cd and Pb in Vetiver grass.

Nonetheless, cautious measures must be taken into consideration as excessive application of soil amendments may cause growth inhibition and eventually resulting in wilted plant. This chapter concedes that the use of pot experiments for direct spiked mixed Cd-Pb metals for all variable soil amendment treatments (including control) may have relatively given rise to an increase of higher heavy metals accumulation in Vetiver grass. Furthermore, the experimental setup with the application of mixed heavy metals (Cd-Pb) contaminated soil instead of a single metal accumulation may have possibly elevated the soil-to-root and root-to-tiller uptakes of Vetiver grass. Further studies of enhanced mixed heavy metals accumulation using EDTA soil amendment in Vetiver grass will be thoroughly explained in Chapter 7.

CHAPTER 7: ENHANCED PHYTOASSESSMENT OF VETIVER GRASS WITH EDTA GROWN IN SINGLE AND MIXED HEAVY METAL CONTAMINTED SOIL

7.1 Introduction

Heavy metal is the general collective terminology which is widely accepted to describe a series of transitional metals in the periodical table of elements with specific density greater than 5 g/cm^3 and atomic mass over 20 (Pais & Jones, 1997; Rascio & Navari-Izzo, 2011). Typically, heavy metal occurs naturally as an elemental component in the earth's crust and often stays persistent in the environment whereby it cannot be degraded and/or destroyed (Demirbas, 2008; Chopra ***et al***., 2009). Some heavy metals such as copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) are essentially required by all living organisms in trace amount for biological metabolism and growth. In contrast, many other heavy metals such aluminium (Al), cadmium (Cd), lead (Pb), mercury (Hg) and tin (St) have no essential biological function and are highly poisonous and can be freely bio-accumulated through the food chain (Prasad & Hagmeyer, 1999; Kabata-Pendias, 2010).

For years, heavy metal soil contamination has been a global environmental issue as human activities have continuously polluted the surroundings via agrochemical leaching, disposal of toxic wastes and effluents as well as the atmospheric deposition from industrial activities (Bradl, 2005; Meuser, 2010; Hasanuzzaman & Fujita, 2013). Long term exposure via direct respiration (inhalation), drinking water and/or ingestion of food contaminated with heavy metals can be adversely harmful to both the environment (living ecosystem) and human well-being when the tolerance levels are exceeded (Järup, 2003; Duruibe ***et al***., 2007).

Various types of soil remediation such as physical (dig-and-dump, thermal desorption and fracturing), chemical (solidification-stabilization, reduction-oxidation and soil washing) and biological (bio-sorption, bio-leaching and bio-filtration) aided techniques for heavy metal contamination removal have been reported in the past decades (Mulligan ***et al***., 2001; Van Deuren ***et al***., 2002; Sherameti & Varma, 2010; Anjum ***et al***., 2012). Nonetheless, most of these remediation technologies are considerably complicated, cost ineffective and are technically difficult to conduct. As a result, phytoremediation has turned out to be the most viable strategy using plants to clean up heavy metals in contaminated soils. Garbisu and Alkorta (2001), McIntyre (2003) and Ali ***et al***. (2013) have suggested that the application of phytoremediation would be aesthetically non-destructive to the surrounding, environmentally pleasing and often required minimum cost for operation and maintenance.

Among numerous types of plants tested for phytoremediation, Vetiver grass, ***Vetiveria zizanioides*** (Linn.) Nash has proven to be an effective species with quick growth, deep fibrous root system as well as high adaptability and tolerance to many extreme environmental stresses including the elevated high concentration levels of heavy metals (Truong ***et al***., 2008; Danh ***et al***., 2009; Truong & Danh, 2015). To further enhance the accumulation of heavy metals in plants, assorted enrichment materials for instance, disodium ethylene-diamine-tetra-acetate (EDTA) soil amendment has been expansively used as the metal chelating agent for phytoremediation purposes (Luo ***et al***., 2005; Hovsepyan & Greipsson, 2005; Seth ***et al***., 2011; Shahid ***et al***., 2014).

Recent studies by Sinegani ***et al.*** (2015), Özkan ***et al.*** (2016), Vargas ***et al.*** and (2016) have highlighted the results of synthetically designed chemical chelators to enhance metal accumulation and metal translocation in different plant parts for single heavy metal soil contamination. The findings in Chapter 3 till Chapter 6 have reveal the ability of Vetiver grass for heavy metal phytoremediation, however at present, there is still a lack of information about the difference of phytoassessment between single and mixed enhanced accumulation of heavy metals using EDTA in Vetiver grass. To address this uncertainty, this chapter was conducted to analyze the growth performance, accumulation trend and capability of metal uptake between different types of single and mixed Cd, Pb, Cu and Zn enhanced contaminated soil conditions with EDTA in both the lower and upper roots and tillers of Vetiver grass.

7.2 Materials and Methods

7.2.1 Site location and experimental setup

This chapter was conducted using pot experiments in the planthouse located at the Rimba Ilmu, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur. Vetiver grass, ***Vetiveria zizanioides*** (Linn.) Nash was selected for this experiment and placed under nine different types of single and mixed heavy metal enhanced treatments (Table 7.1). All of the treatments were conducted under a completely randomized design (CRD) with three replications (n = 3).

Table 7.1: Design of treatment variables

Treatment	Spiked heavy metal (mg/kg) and EDTA (mmol/kg)
Control	No heavy metal and EDTA added
EDTA	10 EDTA
Cd+EDTA	20 Cd + 10 EDTA
Pb+EDTA	200 Pb + 10 EDTA
Cu+EDTA	100 Cu + 10 EDTA
Zn+EDTA	200 Zn + 10 EDTA
Cd+Pb+EDTA	20 Cd + 200 Pb + 10 EDTA
Cu+Zn+EDTA	100 Cu + 200 Zn + 10 EDTA
Cd+Pb+Cu+Zn+EDTA	20 Cd + 200 Pb + 100 Cu + 200 Zn + 10 EDTA

7.2.2 Soil management and sampling preparation

Top soil (0 – 20cm) was collected from the field located in the University of Malaya, Kuala Lumpur situated at the 3° 7' N latitude and 101° 39' E longitude for planting purposes. The collected soil underwent preliminary physico-chemical soil assessment (Table 7.2) before it was air-dried for a week followed by <4mm sieving to remove gravels and large non-soil particles. The dull reddish brown soil composed of 84.58% sand, 10.48% silt and 4.94% clay.

Table 7.2: Physico-chemical properties of growth media soil

Parameter (Unit)		Mean
Soil texture		
Sand (%)		84.58
	Very coarse sand (%)	9.16
	Coarse sand (%)	31.02
	Medium coarse sand (%)	42.21
	Fine sand (%)	15.54
	Very fine sand (%)	3.07
Silt (%)		10.48
Clay (%)		4.94
Temperature (°C)		30.3 ± 4.5
pH		5.28 ± 1.73
Colour (Munsell colour charts)	Dull reddish brown	2.5YR 5/4
Water content (%)		5.72 ± 1.03
Field capacity (%)		40.93 ± 2.45
Saturation level (%)	Dry	13.97
Bulk density (g/cm^3)		1.62 ± 0.78
Porosity (%)		38.87 ± 4.39
Metal contents (mg/kg)		
	Cd	1.15 ± 0.59
	Pb	32.55 ± 8.01
	Cu	11.94 ± 4.32
	Zn	60.22 ± 18.73

Mean ± standard deviation

Vetiver grass plantlets were purchased from Humibox Malaysia where fresh plantlets with a uniform height (20 – 25cm) were selected for this chapter. Each plant was grown in a plastic pot (0.18m diameter x 0.16m depth) filled with two kilograms of soil, for all the treatments. All plants were watered evenly with 50mL of tap water once a day and plant growth performance such as height, tiller number and percentage plant survivorship were continuously observed throughout the entire 60-day of experiment.

The artificially spiked single and mixed heavy metal enhanced treatments were prepared using cadmium nitrate tetrahydrate [$Cd(NO_3)_2.4H_2O$], lead (II) nitrate [$Pb(NO_3)_2$], copper (II) sulfate [$CuSO_4$] and zinc sulfate heptahydrate [$ZnSO_4.7H_2O$] salt compounds as well as the disodium ethylene-diamine-tetra-acetate, $C_{10}H_{14}N_2Na_2O_8.2H_2O$ (EDTA) soil amendment. The concentration of single and mixed heavy metal enhanced treatments were determined based on the range of heavy metal concentrations exceeding the median permissible in the natural occurring levels by the Department of Environment, Malaysia (DOE, 2009), Canadian Council of Ministers of Environment (CCME, 1999) and European Union (Lado ***et al***., 2008) soil contamination guidelines.

In terms of soil amendment; although the possible outcomes for heavy metals phytoaccumulation may increase with the application of higher amount of EDTA, a standard composition of 10 mmol EDTA/kg was selected in this chapter based on the research findings obtained in Chapter 6 and Grčman ***et al***. (2001). The amended soil was then continuously stirred and incubated for a week to ensure the homogeneity of the desired single and mixed heavy metal enhanced treatments are obtained.

7.2.3 Sample and chemical analysis

All Vetiver treatments were uprooted at the end of the 60-day experimental period and brought into the laboratory and washed in running filter water, followed by deionized water to remove any adhering soil particle before separating the plants into four different parts of lower and upper sections of roots and tillers. All plant samples were oven-dried for 72 hours until it registered a constant dry weight. The dry matter content (g/m^2) of the plant samples was determined before it was homogenized using mortar and pestle.

Approximately, 0.5g of the homogenized dried samples underwent acid digestion with hydrochloric acid (HCl), nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) according to Method 3050B (US EPA, 1996) followed by the Method 7000B (US EPA, 2007a) for the total recoverable elemental analysis using a Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (F-AAS). Soil samples were also air-dried for 72 hours until it reached a constant weight before it was analysed following similar analytical procedures. All chemicals used were of analytical reagent standard or of the best grade available. The highly precise technique of chemical analysis was controlled using the Bundesanstalt für Materialforschung und –prüfung (BAM Germany): German Federal Institute for Materials Research and Testing (BRM#12-mixed sandy soil) certified reference material with an average rate of metal recovery for Cd (96.11%), Pb (106.94%), Cu (102.89%) and Zn (96.82%), respectively.

7.2.4 Data calculation and statistical analysis

The growth performance of Vetiver grass were evaluated using the root-tiller (R/T) quotient and tolerance index (TI) whilst the ability for metal accumulation and translocation upwards were evaluated by determining the translocation factor (TF), biological concentration factor (BCF), biological accumulation coefficient (BAC) and percentage of metal uptake efficacy (Kabata-Pendias, 2013; Alloway, 2013; Ali ***et al***., 2013) as follows:

R/T quotient = Dry matter content in root / Dry matter content in tiller

TI = Total dry matter content in heavy metal treatment / Total dry matter content in control

TF = Concentration of heavy metal in tiller / Concentration of heavy metal in root

BCF = Concentration of heavy metal in root / Concentration of heavy metal in soil

BAC = Concentration of heavy metal in tiller / Concentration of heavy metal in soil

Metal uptake efficacy (%) = [Concentration of heavy metal in tiller / Total concentration of heavy metal accumulated in Vetiver] x 100

All experimental data were analysed by performing the one–way analysis of variance (ANOVA) and further statistical validity test for significant differences among treatment means was conducted by employing the Fisher's least significant difference (LSD) tests at the 95% level of confidence.

7.3 Results

7.3.1 Plant growth performance

The initial soil pH varied from 4.19 to 6.17 where the Cd+Pb+Cu+Zn+EDTA treatment recorded the lowest pH of 4.19 while the highest pH of 6.17 was observed in the control (Figure 7.1). Upon harvesting, Cd+EDTA, Pb+EDTA, Cu+EDTA, Zn+EDTA and Cd+Pb+Cu+Zn+EDTA treatments showed an increased in pH ranging from 4.70 to 5.54, where the highest pH increment (+0.98 pH units) was observed in the Cd+Pb+Cu+Zn+EDTA treatment. The soil pH levels in all the different types of single and mixed heavy metal enhanced treatments were significantly (***$p<0.05$***) affected compared to the control. The application of both enhanced single and mixed heavy metals substantially influenced the overall change in soil pH in all the treatments.

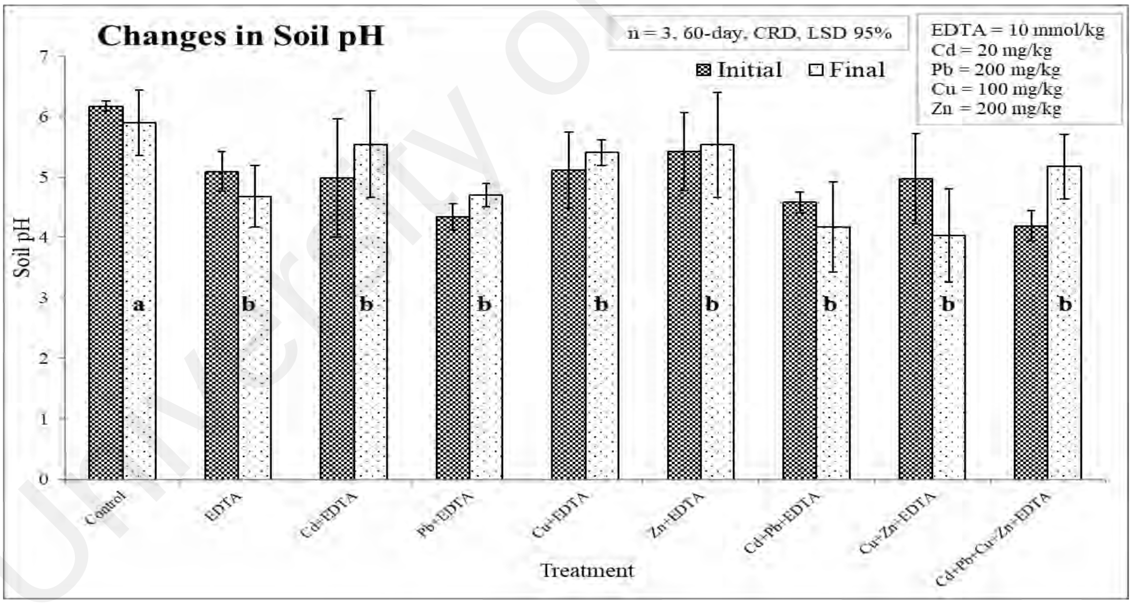

Figure 7.1: Changes in soil pH in Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments. Vertical bars represent standard deviation in treatment means whilst same letters are not significantly different at 0.05 levels of probability

Table 7.3 shows significant differences (***p<0.05***) in tiller number, plant height and percentage of survival of Vetiver grass among the different types of single and mixed heavy metal enhanced treatments. All enhanced treatments with the exception of EDTA (27.0) and Pb+EDTA (27.7) treatments exhibited significantly lower (***p<0.05***) tiller number compared with the control. Both of the mixed Cu+Zn+EDTA (12.8) and Cd+Pb+Cu+Zn+EDTA (13.5) enhanced treatments recorded the lowest tiller number among all the treatments, respectively. Similarly, all enhanced treatments with the exception of Pb+EDTA (56.02cm) treatment displayed significantly lower (***p<0.05***) plant height as compared with the control. Control plant height (69.4cm) was 52.3% higher than the Cd+Pb+Cu+Zn+EDTA enhanced treatment which recorded the lowest plant height of 33.25cm. On the other hand, only EDTA (96.67%) and Pb+EDTA (77.33%) enhanced treatments showed no significant difference (***p>0.05***) of percentage survivorship with the control. Conversely, the percentage of survival among all the other single and mixed heavy metal enhanced treatments (69.33% – 74.67%) were significantly affected (***p<0.05***) compared to the control, with Cu+EDTA treatment recording the lowest (69.33%) percentage survivorship.

Table 7.3: Tiller number, plant height (cm) and plant survivorship (%) of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	**Tiller number**	**Plant height (cm)**	**Plant survivorship (%)**
Control	27.5 ± 1.3 a	69.74 ± 6.45 a	100.00 ± 0.00 a
EDTA	27.0 ± 0.8 a	50.24 ± 3.77 b	96.67 ± 2.27 ab
Cd+EDTA	16.8 ± 0.5 bc	41.13 ± 1.83 b	72.67 ± 4.78 bc
Pb+EDTA	27.7 ± 7.8 a	56.02 ± 13.21 ab	77.33 ± 11.36 abc
Cu+EDTA	14.5 ± 1.5 c	41.71 ± 2.95 b	69.33 ± 9.69 c
Zn+EDTA	16.7 ± 8.3 c	41.86 ± 7.75 b	70.67 ± 2.94 c
Cd+Pb+EDTA	22.5 ± 2.3 ab	49.73 ± 4.46 b	74.67 ± 1.58 bc
Cu+Zn+EDTA	12.8 ± 0.9 c	40.56 ± 2.74 b	67.33 ± 3.74 c
Cd+Pb+Cu+Zn+EDTA	13.5 ± 0.2 c	33.25 ± 6.03 b	71.34 ± 4.60 c

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

The dry matter contents of tiller and total Vetiver grass in all the enhanced treatments were significantly lower (***$p<0.05$***) compared to the control (Table 7.4). The Cu+EDTA enhanced treatment displayed the lowest total dry matter content (9.67 ± 0.11 g/m^2) with an average of 41.2% reduction compared to the control. Between all the enhanced treatments, the single treatments recorded comparatively higher dry matter contents than the mixed heavy metal treatments. In contrast, no significant difference (***$p>0.05$***) was found in the root-tiller (R/T) quotient, tolerance index (TI) and dry matter content in the roots of Vetiver grass in all the treatments.

Table 7.4: Dry matter content (g/m^2), root-tiller quotient (R/T) and tolerance index (TI) of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	**Dry matter content (g/m^2)**				
	Vetiver			**R/T**	**TI**
	Root	**Tiller**	**Total**		
Control	6.75 ± 1.13 a	9.68 ± 1.37 a	16.44 ± 0.35 a	0.718 a	
EDTA	6.06 ± 0.61 a	5.81 ± 0.38 b	11.87 ± 0.27 bc	1.049 a	0.718 a
Cd+EDTA	5.24 ± 0.65 a	4.49 ± 0.92 b	9.72 ± 1.55 c	1.183 a	0.589 a
Pb+EDTA	6.25 ± 0.95 a	5.01 ± 1.06 b	11.26 ± 2.00 b	1.260 a	0.682 a
Cu+EDTA	5.36 ± 1.06 a	4.31 ± 1.11 b	9.67 ± 0.11 c	1.341 a	0.585 a
Zn+EDTA	5.44 ± 0.30 a	4.37 ± 0.47 b	9.82 ± 0.27 c	1.258 a	0.594 a
Cd+Pb+EDTA	5.90 ± 0.42 a	4.52 ± 1.30 b	10.42 ± 1.34 bc	1.380 a	0.631 a
Cu+Zn+EDTA	5.37 ± 0.93 a	4.57 ± 0.87 b	9.94 ± 1.31 bc	1.202 a	0.602 a
Cd+Pb+Cu+Zn+EDTA	5.50 ± 1.08 a	4.45 ± 1.32 b	9.95 ± 0.55 bc	1.351 a	0.602 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

7.3.2 Accumulation of heavy metals

The concentration of Cd, Pb, Cu and Zn heavy metal accumulation in the roots, tillers and total of Vetiver grass in the different types of single and mixed heavy metal enhanced treatments are shown in Table 7.5 – Table 7.16. The accumulation of all four different types of heavy metals in the lower and upper parts of both roots and tillers, for all the enhanced treatments were comparatively variable.

With regard to Cd accumulation, all the Cd+EDTA, Cd+Pb+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments recorded significantly higher ($p < 0.05$) Cd in both the lower and upper roots and tillers of Vetiver grass compared to the control (Table 7.5 – Table 7.7). Similarly, the total root, total tiller and overall total accumulation for Cd+EDTA, Cd+Pb+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments exhibited significantly greater ($p < 0.05$) Cd among all other treatments. The highest accumulation of Cd was found in the upper tiller of Cd+Pb+Cu+Zn+EDTA (128.03 ± 17.95 mg/kg) followed by the lower root of Cd+EDTA (119.60 ± 20.43 mg/kg) enhanced treatments. Between roots and tillers, unlike the other types of heavy metals (Pb, Cu and Zn), the accumulation of Cd was noticeably higher in the tillers than in the roots with the exception of the Cd+EDTA enhanced treatment and the control. A relatively higher Cd accumulation was demonstrated in the upper root and upper tiller of the Cd+Pb+EDTA enhanced treatment compared with its lower plant parts, respectively. In contrast, the accumulation of Cd was appreciably higher in the lower root and lower tiller in the Cd+EDTA enhanced treatment compared to its upper plant parts. Nonetheless, the order of Cd accumulation among the different types of single and mixed Cd enhanced treatments was Cd+Pb+EDTA > Cd+Pb+Cu+Zn+EDTA > Cd+EDTA >> other enhanced treatments.

Table 7.5: Concentration of Cd (mg/kg) in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Cd (mg/kg)		
	Root		
	Lower	Upper	Total
Control	1.83 ± 0.31 c	1.67 ± 0.74 d	3.50 ± 0.90 d
EDTA	0.05 ± 0.02 c	0.55 ± 0.18 d	0.61 ± 0.20 d
Cd+EDTA	119.60 ± 20.43 a	60.10 ± 11.26 b	179.70 ± 31.15 a
Pb+EDTA	0.06 ± 0.03 c	1.43 ± 0.50 d	1.50 ± 0.49 d
Cu+EDTA	0.47 ± 0.14 c	1.19 ± 0.40 d	1.66 ± 0.51 d
Zn+EDTA	0.58 ± 0.18 c	1.41 ± 0.19 d	1.98 ± 0.16 d
Cd+Pb+EDTA	49.43 ± 8.96 b	97.57 ± 5.45 a	147.00 ± 14.08 b
Cu+Zn+EDTA	0.08 ± 0.05 c	1.14 ± 0.37 d	1.22 ± 0.32 d
Cd+Pb+Cu+Zn+EDTA	44.93 ± 8.73 b	36.73 ± 3.43 c	81.67 ± 10.86 c

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 7.6: Concentration of Cd (mg/kg) in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Cd (mg/kg)		
	Tiller		
	Lower	Upper	Total
Control	0.75 ± 0.42 c	0.44 ± 0.21 d	1.19 ± 0.22 d
EDTA	2.30 ± 1.11 c	2.50 ± 1.11 d	4.80 ± 2.23 d
Cd+EDTA	51.13 ± 12.77 ab	30.07 ± 6.95 c	81.20 ± 6.67 c
Pb+EDTA	2.12 ± 0.59 c	2.87 ± 1.53 d	4.99 ± 1.15 d
Cu+EDTA	1.40 ± 0.98 c	2.10 ± 1.41 d	3.50 ± 0.62 d
Zn+EDTA	1.04 ± 0.49 c	1.55 ± 1.00 d	2.60 ± 1.48 d
Cd+Pb+EDTA	49.63 ± 16.70 ab	106.33 ± 21.37 b	155.97 ± 38.05 b
Cu+Zn+EDTA	0.49 ± 0.26 c	5.77 ± 0.91 d	6.26 ± 0.68 d
Cd+Pb+Cu+Zn+EDTA	52.10 ± 14.73 a	128.03 ± 17.95 a	180.13 ± 4.99 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 7.7: Total Cd concentration (mg/kg) in the root and tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Cd (mg/kg)		
	Total root	**Total tiller**	**Overall total**
Control	3.50 ± 0.90 d	1.19 ± 0.22 d	4.69 ± 1.12 c
EDTA	0.61 ± 0.20 d	4.80 ± 2.23 d	5.40 ± 2.41 c
Cd+EDTA	179.70 ± 31.15 a	81.20 ± 6.67 c	260.90 ± 37.34 b
Pb+EDTA	1.50 ± 0.49 d	4.99 ± 1.15 d	6.49 ± 0.76 c
Cu+EDTA	1.66 ± 0.51 d	3.50 ± 0.62 d	5.16 ± 0.56 c
Zn+EDTA	1.98 ± 0.16 d	2.60 ± 1.48 d	4.58 ± 1.43 c
Cd+Pb+EDTA	147.00 ± 14.08 b	155.97 ± 38.05 b	302.97 ± 29.44 a
Cu+Zn+EDTA	1.22 ± 0.32 d	6.26 ± 0.68 d	7.48 ± 0.38 c
Cd+Pb+Cu+Zn+EDTA	81.67 ± 10.86 c	180.13 ± 4.99 a	261.80 ± 7.28 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Similarly, with regard to Pb accumulation, the Pb+EDTA, Cd+Pb+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments recorded significantly larger (***p<0.05***) Pb in both the lower and upper roots and tillers of Vetiver grass compared to the control (Table 7.8 – Table 7.10). A significantly higher (***p<0.05***) amounts of Pb accumulation was observed in the total root, total tiller and overall total accumulation for Pb+EDTA, Cd+Pb+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments among all other treatments. The upper tillers for Cd+Pb+Cu+Zn+EDTA (531.67 ± 36.19 mg/kg) and Cd+Pb+EDTA (368.80 ± 15.09 mg/kg) enhanced treatments recorded the highest accumulation of Pb among all the treatments. Between roots and tillers, the accumulation of Pb was remarkably higher in the tillers than in the roots among all enhanced treatments. The upper root and upper tiller for Cd+Pb+EDTA enhanced treatment as well as the upper tillers for Pb+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments accumulated considerably higher Pb compared with their different plant parts, respectively. The accumulation trend for Pb among the different types of single and mixed Pb enhanced treatments was in the order of Cd+Pb+Cu+Zn+EDTA > Cd+Pb+EDTA > Pb+EDTA >> other enhanced treatments.

Table 7.8: Concentration of Pb (mg/kg) in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Pb (mg/kg)		
	Root		
	Lower	Upper	Total
Control	17.70 ± 3.60 d	14.40 ± 2..46 d	32.10 ± 5.99 d
EDTA	1.27 ± 0.37 d	2.35 ± 0.52 e	3.63 ± 0.86 d
Cd+EDTA	12.80 ± 1.67 d	10.26 ± 2.56 de	23.06 ± 1.46 d
Pb+EDTA	78.33 ± 8.99 c	69.97 ± 12.44 c	148.30 ± 21.42 c
Cu+EDTA	9.24 ± 3.77 d	3.10 ± 0.89 de	12.34 ± 4.62 d
Zn+EDTA	15.50 ± 4.78 d	5.25 ± 0.42 de	20.75 ± 4.40 d
Cd+Pb+EDTA	184.30 ± 25.88 a	199.20 ± 10.51 a	383.50 ± 36.24 a
Cu+Zn+EDTA	10.97 ± 1.99 d	2.41 ± 0.30 e	13.37 ± 2.27 d
Cd+Pb+Cu+Zn+EDTA	120.60 ± 19.26 b	105.97 ± 7.61 b	226.57 ± 26.87 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 7.9: Concentration of Pb (mg/kg) in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Pb (mg/kg)		
	Tiller		
	Lower	Upper	Total
Control	1.93 ± 0.38 d	1.90 ± 0.54 d	3.83 ± 0.17 d
EDTA	15.47 ± 2.54 d	5.65 ± 0.67 d	21.12 ± 3.21 d
Cd+EDTA	13.83 ± 2.51 d	22.20 ± 2.82 d	36.03 ± 5.33 d
Pb+EDTA	134.23 ± 7.75 c	237.70 ± 19.22 c	371.93 ± 12.56 c
Cu+EDTA	28.70 ± 8.61 d	25.37 ± 6.74 d	54.07 ± 5.06 d
Zn+EDTA	17.70 ± 3.18 d	15.53 ± 0.86 d	33.23 ± 4.04 d
Cd+Pb+EDTA	300.17 ± 19.75 b	368.80 ± 15.09 b	668.97 ± 32.88 b
Cu+Zn+EDTA	12.20 ± 2.27 d	14.00 ± 4.10 d	26.20 ± 2.44 d
Cd+Pb+Cu+Zn+EDTA	338.33 ± 12.62 a	531.67 ± 36.19 a	870.00 ± 48.79 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 7.10: Total Pb concentration (mg/kg) in the root and tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Pb (mg/kg)		
	Total root	Total tiller	Overall total
Control	32.10 ± 5.99 d	3.83 ± 0.17 d	35.93 ± 6.10 c
EDTA	3.63 ± 0.86 d	21.12 ± 3.21 d	24.74 ± 2.46 c
Cd+EDTA	23.06 ± 1.46 d	36.03 ± 5.33 d	59.09 ± 4.65 c
Pb+EDTA	148.30 ± 21.42 c	371.93 ± 12.56 c	520.23 ± 9.86 b
Cu+EDTA	12.34 ± 4.62 d	54.07 ± 5.06 d	66.40 ± 5.08 c
Zn+EDTA	20.75 ± 4.40 d	33.23 ± 4.04 d	53.98 ± 4.75 c
Cd+Pb+EDTA	383.50 ± 36.24 a	668.97 ± 32.88 b	1052.47 ± 6.47 a
Cu+Zn+EDTA	13.37 ± 2.27 d	26.20 ± 2.44 d	39.57 ± 4.62 c
Cd+Pb+Cu+Zn+EDTA	226.57 ± 26.87 b	870.00 ± 48.79 a	1096.57 ± 75.60 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

A significantly higher (***p< 0.05***) Cu accumulation was found in both the lower and upper roots and tillers of Vetiver grass for Cu+EDTA, Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments compared to the control (Table 9.11 – Table 9.13). Similarly, the total root, total tiller and overall total Cu accumulation for Cu+EDTA, Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments demonstrated significantly higher (***p< 0.05***) Cu among all the treatments. The upper tillers for Cd+Pb+Cu+Zn+EDTA (862.40 ± 231.34 mg/kg) and Cu+Zn+EDTA (538.97 ± 41.88 mg/kg) recorded the highest accumulation of Cu. Between roots and tillers, the accumulation of Cu was substantially higher in the tillers than in the roots among all enhanced treatments. The accumulation of Cu in the lower roots and upper tillers for Cd+Pb+Cu+Zn+EDTA and Cu+Zn+EDTA enhanced treatments were reasonably greater than the different plant parts, respectively. Among the different types of single and mixed Cu enhanced treatments, the accumulation of Cu was in the order of Cd+Pb+Cu+Zn+EDTA > Cu+Zn+EDTA > Cu+EDTA >> other enhanced treatments.

Table 7.11: Concentration of Cu (mg/kg) in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Cu (mg/kg)		
	Root		
	Lower	Upper	Total
Control	15.73 ± 5.41 d	12.67 ± 1.72 c	28.40 ± 7.12 c
EDTA	2.21 ± 0.34 d	3.89 ± 1.64 c	6.10 ± 1.59 c
Cd+EDTA	3.69 ± 0.64 d	6.13 ± 1.65 c	9.82 ± 1.98 c
Pb+EDTA	1.34 ± 0.54 d	11.13 ± 1.81 c	12.47 ± 2.35 c
Cu+EDTA	253.00 ± 24.78 c	137.60 ± 28.05 b	390.60 ± 52.31 b
Zn+EDTA	3.41 ± 0.83 d	8.64 ± 0.57 c	12.06 ± 1.32 c
Cd+Pb+EDTA	5.42 ± 0.53 d	5.73 ± 2.45 c	11.15 ± 1.96 c
Cu+Zn+EDTA	393.12 ± 9.65 a	157.03 ± 32.90 b	550.15 ± 28.73 a
Cd+Pb+Cu+Zn+EDTA	365.33 ± 18.68 b	214.40 ± 18.86 a	579.73 ± 1.24 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 7.12: Concentration of Cu (mg/kg) in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Cu (mg/kg)		
	Tiller		
	Lower	Upper	Total
Control	4.53 ± 0.80 d	2.90 ± 1.40 d	7.43 ± 0.68 c
EDTA	7.55 ± 0.78 d	12.07 ± 3.05 d	19.61 ± 3.81 c
Cd+EDTA	18.53 ± 0.68 d	23.77 ± 3.99 d	42.30 ± 3.32 c
Pb+EDTA	24.70 ± 3.70 d	30.32 ± 6.38 d	55.02 ± 9.80 c
Cu+EDTA	429.20 ± 33.51 b	381.03 ± 22.66 c	810.23 ± 55.73 b
Zn+EDTA	15.87 ± 1.56 d	36.63 ± 6.19 d	52.50 ± 7.75 c
Cd+Pb+EDTA	12.73 ± 4.45 d	40.63 ± 4.12 d	53.37 ± 8.53 c
Cu+Zn+EDTA	351.53 ± 32.06 c	538.97 ± 41.88 b	890.50 ± 14.21 b
Cd+Pb+Cu+Zn+EDTA	535.33 ± 62.21 a	862.40 ± 231.34 a	1397.73 ± 293.47 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 7.13: Total Cu concentration (mg/kg) in the root and tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Cu (mg/kg)		
	Total root	Total tiller	Overall total
Control	28.40 ± 7.12 c	7.43 ± 0.68 c	35.83 ± 7.66 d
EDTA	6.10 ± 1.59 c	19.61 ± 3.81 c	25.72 ± 5.34 d
Cd+EDTA	9.82 ± 1.98 c	42.30 ± 3.32 c	52.12 ± 5.28 d
Pb+EDTA	12.47 ± 2.35 c	55.02 ± 9.80 c	67.49 ± 12.00 d
Cu+EDTA	390.60 ± 52.31 b	810.23 ± 55.73 b	1200.83 ± 108.04 c
Zn+EDTA	12.06 ± 1.32 c	52.50 ± 7.75 c	64.56 ± 8.57 d
Cd+Pb+EDTA	11.15 ± 1.96 c	53.37 ± 8.53 c	64.52 ± 6.79 d
Cu+Zn+EDTA	550.15 ± 28.73 a	890.50 ± 14.21 b	1440.65 ± 39.57 b
Cd+Pb+Cu+Zn+EDTA	579.73 ± 1.24 a	1397.73 ± 293.47 a	1977.47 ± 293.68 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

The accumulation of Zn was significantly higher (***p*<0.05**) in the Zn+EDTA, Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments in both the lower and upper roots and tillers of Vetiver grass compared to the control (Table 7.14 – Table 7.16). The Zn+EDTA, Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments exhibited a significantly higher (***p*<0.05**) Zn in the total root, total tiller and overall total Zn accumulation among all other treatments. The upper tillers of Cd+Pb+Cu+Zn+EDTA (3504.80 ± 353.40 mg/kg) and Zn+EDTA (3399.87 ± 485.06 mg/kg) recorded the highest accumulation of Zn among all the treatments. Between roots and tillers, the accumulation of Zn was markedly greater in the tillers than the roots for all treatments. The lower roots and upper tillers for Zn+EDTA, Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments accumulated substantially higher amounts of Zn compared to the different plant parts, respectively. The accumulation trend for Zn among the different types of single and mixed Zn enhanced treatments was in the order of Zn+EDTA > Cd+Pb+Cu+Zn+EDTA > Cu+Zn+EDTA >> other enhanced treatments.

Table 7.14: Concentration of Zn (mg/kg) in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Zn (mg/kg)		
	Root		
	Lower	Upper	Total
Control	215.23 ± 35.35 c	135.30 ± 21.33 c	350.53 ± 50.59 c
EDTA	62.70 ± 13.90 c	98.85 ± 23.79 c	161.55 ± 37.45 c
Cd+EDTA	55.83 ± 14.00 c	34.07 ± 6.94 c	89.90 ± 7.64 c
Pb+EDTA	82.97 ± 10.46 c	96.83 ± 9.23 c	179.80 ± 15.53 c
Cu+EDTA	51.37 ± 17.50 c	39.13 ± 4.40 c	90.50 ± 19.58 c
Zn+EDTA	2401.57 ± 484.77 a	1305.80 ± 131.61 a	3707.37 ± 367.76 a
Cd+Pb+EDTA	47.47 ± 16.05 c	51.80 ± 10.18 c	99.27 ± 26.12 c
Cu+Zn+EDTA	1520.97 ± 71.04 b	998.00 ± 176.43 b	2518.97 ± 116.88 b
Cd+Pb+Cu+Zn+EDTA	1423.17 ± 268.01 b	946.50 ± 52.05 b	2369.67 ± 319.66 b

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 7.15: Concentration of Zn (mg/kg) in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Zn (mg/kg)		
	Tiller		
	Lower	Upper	Total
Control	49.97 ± 5.07 c	55.50 ± 15.30 c	105.47 ± 19.40 c
EDTA	102.60 ± 8.09 c	71.03 ± 15.04 c	173.63 ± 13.69 c
Cd+EDTA	222.23 ± 33.67 c	94.30 ± 4.12 c	316.53 ± 35.02 c
Pb+EDTA	244.87 ± 42.48 c	107.30 ± 17.66 c	352.17 ± 25.02 c
Cu+EDTA	256.33 ± 38.50 c	81.67 ± 5.68 c	338.00 ± 43.56 c
Zn+EDTA	1115.43 ± 168.62 b	3399.87 ± 485.06 a	4515.30 ± 649.08 b
Cd+Pb+EDTA	294.33 ± 23.79 c	151.53 ± 25.58 c	445.87 ± 3.86 c
Cu+Zn+EDTA	2575.33 ± 478.90 a	1552.70 ± 202.46 b	4128.03 ± 277.23 b
Cd+Pb+Cu+Zn+EDTA	2193.67 ± 422.23 a	3504.80 ± 353.40 a	5698.47 ± 116.93 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Table 7.16: Total Zn concentration (mg/kg) in the root and tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Concentration of Zn (mg/kg)		
	Total root	**Total tiller**	**Overall total**
Control	350.53 ± 50.59 c	105.47 ± 19.40 c	456.00 ± 42.90 c
EDTA	161.55 ± 37.45 c	173.63 ± 13.69 c	335.19 ± 49.02 c
Cd+EDTA	89.90 ± 7.64 c	316.53 ± 35.02 c	406.43 ± 40.89 c
Pb+EDTA	179.80 ± 15.53 c	352.17 ± 25.02 c	531.97 ± 12.62 c
Cu+EDTA	90.50 ± 19.58 c	338.00 ± 43.56 c	428.50 ± 60.19 c
Zn+EDTA	3707.37 ± 367.76 a	4515.30 ± 649.08 b	8222.67 ± 431.78 a
Cd+Pb+EDTA	99.27 ± 26.12 c	445.87 ± 3.86 c	545.13 ± 24.47 c
Cu+Zn+EDTA	2518.97 ± 116.88 b	4128.03 ± 277.23 b	6647.00 ± 203.87 b
Cd+Pb+Cu+Zn+EDTA	2369.67 ± 319.66 b	5698.47 ± 116.93 a	8068.13 ± 407.35 a

Mean ± standard deviation followed by the same letters is not significantly different for each treatment means at 0.05 levels of probability

Between single and mixed enhanced treatments, mixed Cd+Pb+Cu+Zn+EDTA enhanced treatment exhibited appreciably higher accumulation for Cu and Pb whilst mixed Cd+Pb+EDTA enhanced treatment was higher for Cd compared with the single enhanced treatments. On the other hand, Zn+EDTA was the only single enhanced treatment that showed much higher accumulation for Zn compared with the other mixed enhanced treatments. The findings obtained indicated that the single Zn+EDTA enhanced treatment accumulated the highest overall total amount for Zn (8068.13 ± 407.35 mg/kg) whilst the highest accumulation for Cu (1977.47 ± 293.68 mg/kg) and Pb (1096.57 ± 75.60 mg/kg) were recorded in the mixed Cd+Pb+Cu+Zn+EDTA enhanced treatment, respectively. The mixed Cd+Pb+EDTA enhanced treatment demonstrated the highest overall total amount for Cd (302.97 ± 29.44 mg/kg). Generally, the trend of heavy metal accumulation for all enhanced treatments were in the order of Zn >>> Cu > Pb >> Cd regardless of the total amount of heavy metal put into the soil.

7.3.3 Heavy metal uptake and translocation

Table 7.17 – Table 7.24 shows the plant-soil association of the different types of single and mixed enhanced heavy metals accumulation from the soils into the roots and tillers of Vetiver grass, in terms of biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and the percentage of metal uptake efficacy.

The ability for heavy metal translocation from the soil to the root of the plant is assessed using the BCF coefficient. Both lower (7.116 – 12.008) and upper (4.733 – 6.529) roots for single and mixed Zn enhanced treatment showed significantly higher (***p< 0.05***) BCF values compared with the other treatments. Despite the tolerably higher accumulation of heavy metals in the tillers than the roots, both lower and upper roots for the single and mixed Cd (1.837 – 5.980), Cu (1.376 – 3.931) and Zn (4.733 – 12.008) enhanced treatments recorded relatively high BCF values > 1, suggesting that the heavy metal uptake from soil to root was substantially greater and the roots acted as the sink for heavy metal accumulation.

Nevertheless, the BAC, TF and percentage of metal efficacy were employed to evaluate the capabilities and competency for heavy metal uptake from the roots to the tillers. Similarly, the lower and upper tillers in all the specified single and mixed heavy metal enhanced treatments showed appreciably higher BAC values > 1 compared with the other treatments. The BAC values > 1 in lower and upper tillers for single and mixed Cd (1.503 – 6.402), Pb (0.671 – 2.658), Zn (7.764 – 16.999) and Cu (3.515 – 8.624) enhanced treatments indicated that the tillers acted as the sink for heavy metal accumulation due to the fairly effective translocation of the heavy metal from the roots to the tillers.

On the other hand, despite the relatively higher accumulation of heavy metal in the tillers than the roots, TF values < 1 were recorded in the lower and upper tillers for all the single and mixed heavy metal enhanced treatments. However, the mixed Cd+Pb+Cu+Zn+EDTA enhanced treatment exhibited TF values > 1 in both the lower (1.503) and upper (2.356) tiller for Pb accumulation compared to the other treatments.

In terms of percentage of metal efficacy, the upper tiller for mixed Cd+Pb+Cu+Zn+EDTA enhanced treatment exhibited the highest metal efficacy for Cd (49.055%) and Pb (48.487%) compared with other treatments. In contrast, the lower tiller of mixed Cu+Zn+EDTA (38.637%) followed by the single Cu+EDTA (35.769%) enhanced treatment recorded the highest metal efficacy for Zn and Cu, respectively. Between single and mixed enhanced treatments, the mixed Cd+Pb+EDTA, Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments recorded considerably higher percentage of metal efficacy for Cd, Pb and Zn compared to the single enhanced treatments. However, the lower tiller of single Cu+EDTA (35.769%) enhanced treatment demonstrated reasonably higher percentage of Cu efficacy compared to the mixed enhanced treatments. Generally, the percentages of metal efficacy were remarkably higher in the upper tiller compared to the lower tiller for all the four different types of heavy metals accumulation.

Table 7.17: Biological concentration factor (BCF) of Cd accumulation in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Cd accumulation	
	BCF (Root)	
	Lower	Upper
Control	1.594 c	1.449 cd
EDTA	0.046 d	0.481 e
Cd+EDTA	5.980 a	3.005 b
Pb+EDTA	0.055 d	1.246 cd
Cu+EDTA	0.406 d	1.035 de
Zn+EDTA	0.501 d	1.223 cd
Cd+Pb+EDTA	2.472 b	4.878 a
Cu+Zn+EDTA	0.072 d	0.988 de
Cd+Pb+Cu+Zn+EDTA	2.247 bc	1.837 c

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 7.18: Biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cd accumulation in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Cd accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.652 de	0.383 d	0.203 d	0.142 d	15.151 cd	10.448 d
EDTA	2.000 abc	2.171 c	3.700 a	4.057 ab	42.099 a	46.284 b
Cd+EDTA	2.557 a	1.503 cd	0.282 cd	0.175 d	19.402 cd	11.919 cd
Pb+EDTA	1.846 abcd	2.493 c	1.460 b	2.357 bc	33.132 ab	43.214 b
Cu+EDTA	1.217 bcde	1.826 c	0.779 c	1.507 cd	26.903 bc	40.933 b
Zn+EDTA	0.907 cde	1.351 cd	0.530 cd	0.797 d	22.087 bc	31.616 bc
Cd+Pb+EDTA	2.482 a	5.317 ab	0.345 cd	0.734 d	16.156 cd	34.904 bc
Cu+Zn+EDTA	0.426 e	5.014 ab	0.381 cd	5.095 a	6.651 d	76.873 a
Cd+Pb+Cu+Zn+EDTA	2.605 a	6.402 a	0.630 cd	1.605 cd	19.806 bcd	49.055 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 7.19: Biological concentration factor (BCF) of Pb accumulation in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Pb accumulation	
	BCF (Root)	
	Lower	Upper
Control	0.544 bc	0.442 b
EDTA	0.039 f	0.072 e
Cd+EDTA	0.393 cde	0.315 c
Pb+EDTA	0.392 cde	0.350 c
Cu+EDTA	0.284 e	0.095 d
Zn+EDTA	0.476 bcd	0.161 d
Cd+Pb+EDTA	0.922 a	0.996 a
Cu+Zn+EDTA	0.337 de	0.074 de
Cd+Pb+Cu+Zn+EDTA	0.603 b	0.530 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 7.20: Biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Pb accumulation in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Pb accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.059 e	0.058 g	0.063 c	0.059 e	5.581 d	5.247 e
EDTA	0.475 cd	0.174 g	4.527 a	1.643 bc	62.257 a	22.807 d
Cd+EDTA	0.425 d	0.682 de	0.604 c	0.968 d	23.289 c	37.480 b
Pb+EDTA	0.671 c	1.189 c	0.913 c	1.637 bc	25.790 c	45.746 a
Cu+EDTA	0.882 b	0.779 d	2.794 b	2.151 ab	43.543 b	37.955 b
Zn+EDTA	0.544 cd	0.477 ef	0.891 c	0.777 d	32.797 bc	28.873 cd
Cd+Pb+EDTA	1.501 a	1.844 b	0.790 c	0.970 d	28.522 c	35.047 bc
Cu+Zn+EDTA	0.375 d	0.430 f	0.945 c	1.033 d	31.378 c	34.952 bc
Cd+Pb+Cu+Zn+EDTA	1.692 a	2.658 a	1.503 bc	2.356 a	30.898 c	48.487 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 7.21: Biological concentration factor (BCF) of Cu accumulation in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Cu accumulation	
	BCF (Root)	
	Lower	Upper
Control	1.318 c	1.061 cd
EDTA	0.185 d	0.326 f
Cd+EDTA	0.309 d	0.514 ef
Pb+EDTA	0.112 d	0.932 d
Cu+EDTA	2.530 b	1.376 bc
Zn+EDTA	0.286 d	0.724 de
Cd+Pb+EDTA	0.454 d	0.480 ef
Cu+Zn+EDTA	3.931 a	1.570 b
Cd+Pb+Cu+Zn+EDTA	3.653 a	2.144 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 7.22: Biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Cu accumulation in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Cu accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.380 g	0.243 f	0.171 d	0.099 f	13.356 e	7.818 f
EDTA	0.632 fg	1.011 ef	1.278 b	1.991 cd	29.801 bc	46.652 c
Cd+EDTA	1.552 de	1.991 de	1.955 a	2.433 bc	35.901 ab	45.385 c
Pb+EDTA	2.069 d	2.539 cde	1.997 a	2.426 bc	36.744 a	44.767 c
Cu+EDTA	4.292 b	3.810 c	1.104 bc	0.982 e	35.769 ab	31.789 e
Zn+EDTA	1.329 e	3.068 cd	1.322 b	3.043 ab	24.653 cd	56.560 b
Cd+Pb+EDTA	1.066 ef	3.403 cd	1.207 b	3.752 a	19.414 de	62.995 a
Cu+Zn+EDTA	3.515 c	5.390 b	0.642 cd	0.979 e	24.451 cd	37.378 d
Cd+Pb+Cu+Zn+EDTA	5.353 a	8.624 a	0.923 bc	1.487 de	27.156 c	43.101 c

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 7.23: Biological concentration factor (BCF) of Zn accumulation in the lower and upper root of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Zn accumulation	
	BCF (Root)	
	Lower	Upper
Control	3.574 c	2.247 c
EDTA	1.041 d	1.642 cd
Cd+EDTA	0.927 d	0.566 e
Pb+EDTA	1.378 d	1.608 cd
Cu+EDTA	0.853 d	0.650 e
Zn+EDTA	12.008 a	6.529 a
Cd+Pb+EDTA	0.788 d	0.860 de
Cu+Zn+EDTA	7.605 b	4.990 b
Cd+Pb+Cu+Zn+EDTA	7.116 b	4.733 b

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

Table 7.24: Biological accumulation coefficient (BAC), translocation factor (TF) and metal uptake efficacy (%) of Zn accumulation in the lower and upper tiller of Vetiver as influenced by different types of single and mixed heavy metal enhanced treatments

Treatment	Zn accumulation					
	BAC (Tiller)		TF (Tiller)		Efficacy (Tiller)	
	Lower	Upper	Lower	Upper	Lower	Upper
Control	0.830 d	0.922 c	0.146 e	0.162 e	11.064 f	12.241 d
EDTA	1.704 cd	1.180 c	0.660 cde	0.441 de	31.115 de	21.060 bc
Cd+EDTA	3.690 bc	1.566 c	2.471 b	1.051 bc	54.495 ab	23.332 bc
Pb+EDTA	4.066 b	1.782 c	1.382 c	0.595 cde	45.933 c	20.228 c
Cu+EDTA	4.257 b	1.356 c	2.873 ab	0.923 cd	59.793 a	19.202 cd
Zn+EDTA	5.577 b	16.999 a	0.305 e	0.930 cd	13.524 f	41.262 a
Cd+Pb+EDTA	4.888 b	2.516 c	3.070 a	1.653 a	53.935 ab	27.973 b
Cu+Zn+EDTA	12.877 a	7.764 b	1.028 cd	0.615 cde	38.637 cd	23.427 bc
Cd+Pb+Cu+Zn+EDTA	10.968 a	17.524 a	0.921 cd	1.509 ab	27.066 e	43.641 a

Mean followed by the same letters are not significantly different for each treatment means at 0.05 levels of probability

7.4 Discussion

The suitability of using ethylene-diamine-tetraacetic-acid (EDTA) as an effective low cost amendment to enhance metal phytoaccumulation was presented in Chapter 6. Nonetheless, the experimental results obtained from Chapter 5 were continuously expanded to investigate the response of growth performance, accumulation trend and capability of metal uptake between different types of single and mixed Cd, Pb, Cu and Zn enhanced contaminated soil conditions with and without EDTA in both the lower and upper roots and tillers of Vetiver grass.

With reference to the results obtained in Chapter 5, the accumulation trends responded differently when EDTA was applied in all the single and mixed heavy metal enhanced treatments. In contrast to the findings obtained in Chapter 5, the accumulation of all four different heavy metals were found in both of the lower and upper parts for roots and tillers in the single and mixed heavy metal enhanced treatments. Similar effects of EDTA application were reported in Chen ***et al.*** (2004c), Zhao ***et al.*** (2011) as well as Ali and Chaudhury (2016) who observed the accumulation trends of heavy metals in both roots and tillers of the plants.

EDTA has been widely used as a common chelating agent for phytoremediation to enhance the bioavailability of heavy metals for uptake by plants in the soil (Meers ***et al.,*** 2009; Shahid ***et al.,*** 2014; Bloem ***et al.,*** 2017). The presence of EDTA molecules enhance the extraction of metals at exchange sites and subsequently form soluble metal-EDTA complexes (Hadi ***et al.,*** 2010; Leleyter ***et al.,*** 2012; Jean-Soro ***et al.,*** 2012; Dipu ***et al.,*** 2012).

Based on the findings from both Chapter 5 and 7, it was markedly indicated that the application of EDTA soil amendment managed to enhance in about 1.21 to 2.79 fold greater of the overall total accumulation for all four different types of Cd, Pb, Cu and Zn growing under both single and mixed heavy metal contaminated soil conditions. Besides, the findings of this chapter have clearly shown that soil pH became more acidic in all the single and mixed heavy metal enhanced treatments when EDTA was added compared to the control. This could affect the bioavailability of metals as a change in soil pH could conceivably affect the capability of EDTA to form complexes (Peng ***et al.,*** 2009; Bennedsen ***et al.,*** 2012). This was suggested by Sommers and Lindsay (1979) and Shahid ***et al.*** (2014) who both reported that metal-EDTA complexes are predominantly formed between pH 5.2 and 7.7 in most soil conditions due to soil acidification.

In addition to the single and mixed enhanced heavy metal treatments, this chapter included and tested separately the response of sole EDTA treatment with the control. However, the results showed no major significant findings in terms metal accumulation with the sole EDTA treatment compared to the control. Nevertheless, unlike the findings obtained in Chapter 5, the single Zn+EDTA enhanced treatment accumulated the highest Zn compared to the other mixed enhanced treatments. Furthermore, the single Zn+EDTA as well as mixed Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA treatments recorded > 1000 mg/kg of Zn accumulation in almost all of the lower and upper parts of both roots and tillers.

All of the recent studies by Antiochia ***et al.*** (2007), Danh ***et al.*** (2009) and Aksorn and Chitsomboon (2013) reported Vetiver grass to be both Pb and Zn hyperaccumulators. However, despite the high accumulation of Zn in both roots and tillers, the results of this chapter suggest the potential of Vetiver grass to be a Cd hyperaccumulator, due to its high phytoaccumulation ability in the upper tiller for both mixed Cd+Pb+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments. The fundamental concept of hyperaccumulation has been widely accepted by Baker and Brooks (1989) and Van der Ent ***et al.*** (2013) to describe any plant species which are capable of growing and bioaccumulate under extremely high concentrations of heavy metals greater than 100 mg/kg of Cd; or 1000 mg/kg of Pb and Cu; or 10000 mg/kg of Zn in its plant tissues.

Similarly, Vetiver grass can be regarded as both competent phytostabilizers and phytoextractors owing to its BCF and BAC values being > 1, as well as the high accumulation in the lower and upper parts of roots and tillers for all the four different types of heavy metals. This chapter has demonstrated that the roots and tillers act as the sink for the accumulation of all four different types of heavy metals in the presence of EDTA as the soil amendment to enhance the phytoremediation process in Vetiver grass irrespective of single and/or mixed enhanced treatments. Previous studies by Lai and Chen (2005), Wuana ***et al.*** (2016) and Luo ***et al.*** (2016a,b) using different types of plant species such as rainbow pink (***Dianthus chinensis***), castor (***Ricinus communis***) and chickpea (***Cicer arietinum***) also reported similar findings when EDTA is applied. As a continuation to Chapter 5, this chapter was also further expanded to cover separate parts of the lower and upper roots and tillers of Vetiver grass in order to provide an extensive phytoevaluation for the translocation of heavy metals from the lower root upwards till to the top of the tiller part.

On top of that, the direct use of pot assays instead of in-situ site experiments for this chapter would inevitably incur unfavourable effects such as additional increase of heavy metal accumulation due to various biotic and abiotic conditions that may influence the overall results of phytoremediation.

Despite its strong phytoaccumulation ability to enhance metal contaminants in plants, both EDTA and metal-EDTA complexes have its drawbacks as they are poorly biodegradable with high toxicity and are extremely persistent in the soils (Oviedo & Rodríguez, 2003; European Chemicals Bureau, 2004; Goel & Gautam, 2010; Zhao ***et al.,*** 2010; Mühlbachová, 2011; Bloem ***et al.,*** 2017). Additionally, this chapter has also demonstrated that there was a major significant reduction in terms of tiller number, plant height, plant survivorship and dry matter content of Vetiver grass when EDTA was applied, irrespective of both single and mixed enhanced heavy metal treatments. Thus, it is crucial to note that the application of EDTA could inhibit plant growth performance, as reported by Chen and Cutright (2001), Chen ***et al.*** (2004a,b). As a result, the appropriate management of the use of EDTA concentrations is ultimately vital to optimise metal phytoaccumulation in Vetiver grass as well as to reduce its toxicity, metal leaching and other potential risks to the environment.

7.5 Conclusions

This chapter has revealed that mixed Cd+Pb+EDTA, Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments are adequately effective to accumulate higher overall total concentration for Cd, Pb and Cu compared to the single enhanced treatments. In contrast, single Zn+EDTA enhanced treatment demonstrated the opposite trend, whereby a higher accumulation for Zn was observed in comparison to the different types of mixed heavy metal enhanced treatments. Predominantly, the inclination of heavy metal accumulation in Vetiver grass for all enhanced treatments were in the following order of Zn >>> Cu > Pb >> Cd. In terms of different plant parts, the upper roots and upper tillers of Vetiver grass showed a high tendency for the uptake of substantially larger amounts of all the four types of heavy metals, regardless of single and/or mixed enhanced treatments. As a result of the comparably higher concentration in tillers than roots and with BAC values > 1, Vetiver grass can be recommended as a potential phytoextractor for all types of heavy metals, whereby its tillers will act as the sink for heavy metal accumulation in the presence of EDTA in all enhanced treatments.

CHAPTER 8: GENERAL DISCUSSION

As has been presented in the results and discussions of the previous chapters, this study has revealed several new and interesting findings, which are invaluable in our efforts to understand phytoremediation. Nevertheless, despite these findings showing that Vetiver grass has performed well as a positive heavy metals phytoremediator, the results need to be treated with caution. This is because the entire study on the uptake of heavy metals were based upon pot trial experiments carried out under planthouse conditions using Malaysian garden soil spiked with metal salts, which can vary considerably compared to a field collection of heavy metal contaminated soil experiments. As a result, it cannot be ruled out that the experimental design employed with the application of spiked treatments using pot assays may have elevated the phytoaccumulation of heavy metals in both the soil-to-root and root-to-tiller in Vetiver grass.

Previously, Kjaer *et al*. (1998), McBride *et al*., 2009 and Hamels *et al*. (2014) also reported that lower rates and concentrations (up to 30 times difference) of heavy metals accumulation were found in plants tested in field contaminated soils, compared to the laboratory spiked soil conditions. It was suggested that plant species which were examined in an open space in field contaminated soils were freely exposed to various biotic and abiotic stressors in the environment that can relatively influence plant growth and eventually cause the plants to be insusceptible to metal uptake, thus causing lower bioavailability of heavy metals in the plants (Kjaer *et al*., 1998; McBride *et al*., 2009; John *et al*., 2012).

In addition to the above point, the method of adding 100% water soluble specific metal salt compounds to a normal garden soil to create nominal metal concentrations in a bulk soil content, as was carried out in the current research study, makes it difficult to correlate it directly to the actual status of heavy metal contamination under field contaminated soil conditions. Typically, it could take a lengthy duration (from weeks to months) to adequately mix the collected bulk soil to achieve equilibration and stabilisation of the soil before it is ready to be used for experimental studies. As a result, the incomplete stage of soil acclimatization in the prepared spiked heavy metal contaminated soils are likely to have much greater metal bioavailabilities for uptake by any tested plant (Basta *et al*., 2005; D'amore *et al*., 2005; Schwertfeger & Hendershot, 2013).

With the recognition of the existing diverse experimental limitations associated with the spiking of soil heavy metals contamination using metal salt compounds, the preliminary conditions of the tested soils included an incubation period of at least one-week, to ensure the homogeneity of the desired concentrations of soil conditions were achieved. Besides that, the initial concentrations for each individual level of heavy metal soil spiked treatments were chemically pre-tested according to the Method 3050B (US EPA, 1996) followed by the Method 7000B (US EPA, 2007a) for the elemental analysis by employing the Perkin-Elmer AAnalyst 400 flame atomic absorption spectrometer (F-AAS) prior of conducting the experimental studies.

Numerous heavy metals translocation coefficients such as translocation factor (TF), biological concentration factor (BCF), biological accumulation coefficient (BAC), metal accumulation quotient (MAQ) and percentages of metal uptake efficacy (Kabata-Pendias, 2010; Alloway, 2013; Ali *et al*., 2013) were applied throughout the entire research study to evaluate the mobility and ability to translocate heavy metals from soil-to-root and root-to-shoot of the plant species. However, the interpretation of such results tabulated by the heavy metal translocation coefficients should not be taken as clear cut or assumed to be correct as it may not truly reflect or be an accurate representation of heavy metals movement in the plant species.

Nonetheless, in order to determine the definite mobility, bioavailability, and fate of heavy metals movement in soil-plant systems, a comprehensive study into the specific cross-section of cellular and molecular plant uptake translocation mechanisms should be an essential feature, to discover new knowledge that requires further exploration in the future (Hall, 2002; Clemens, 2006; Tangahu *et al*., 2011; Jaishankar *et al*., 2014; Boros *et al*., 2014; Komal *et al*., 2015). Furthermore, the progression of ground-breaking technologies in the field of analytical bio-geochemistry have also initiated the cutting-edge techniques of sequential extraction which are able to determine the specific total contents of heavy metals and its distribution in different phases of both soil and plant components (Vodyanitskii, 2006; Arenas-Lago *et al*., 2014; Sungur *et al*., 2015).

Taking all of the above limitations of pot trial planthouse experiments, soil heavy metal spiking and metal translocation coefficients into account, this research study remains relevant, as it was managed to present extensive findings of different set-up of spiked and mixed heavy metals phytoaccumulation using Vetiver grass growing under controlled planthouse conditions. It will ultimately provide a baseline data for future investigative studies. Most importantly, the application of Vetiver grass has proven to be effective and viable to be used for heavy metals phytoaccumulation.

With regard to the adopted research methodology in the current study, it is inescapable that any tested plant species will eventually accumulate large amounts of highly bioavailable heavy metals in both the roots and shoots (tillers). Moreover, the excessive uptake of heavy metals in plants often leads to the inappropriate interpretation as hyperaccumulation which in the actual scenario, it may not necessarily to be true. The fundamental understanding of hyperaccumulation has been widely accepted by Baker & Brooks (1989) and Van der Ent *et al.* (2013) as any plant species that is capable of growing and bioaccumulate under extremely high concentrations of heavy metals greater than 100 mg/kg of Cd; or 1000 mg/kg of Pb and Cu; or 10000 mg/kg of Zn in its plant tissues.

Nevertheless, there have been several studies by Truong (1999), Greenfield (2002), Maffei (2002) and Roongtanakiat (2006) which have reported that Vetiver grass is a non-hyperaccumulator plant. However, in the case of the current findings in Chapter 7, Vetiver grass was recorded to be a potential Cd hyperaccumulator due to its high phytoaccumulation ability in the upper tiller for both mixed Cd+Pb+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments. Notwithstanding, this apparent indication of Vetiver grass as a heavy metals hyperaccumulator is comparable and supported by various past studies by Lai & Chen (2004), Chen (2004c), Antiochia *et al.* (2007), Danh *et al.* (2009) and Aksorn & Chitsomboon (2013).

Apart from the above, the current study has also indicated Vetiver grass to be a highly stress tolerant metal accumulator species with the aid of EDTA as a low cost soil amendment. However, over the years, there have been a few studies (Meeinkuirt *et al.*, 2013; Zhang *et al.*, 2014; Phusantisampan *et al.*, 2016) which have claimed Vetiver grass to be an excluder for heavy metals phytoremediation. Baker (1981) has broadly described metal accumulators as plant species which accumulated metals in the above ground plant parts from low or high soil levels, whilst metal excluders accumulate at low levels and maintained a constant amount of heavy metals over a wide range of soil concentrations (up to a critical soil value) in the shoots, not able to regulate metal uptake and have restricted transport from root to shoot in the plant species. At present, it is still a matter of argument whether Vetiver grass acts proficiently for heavy metal uptake as an accumulator and/or excluder. Nevertheless, such differences in terms of metal accumulator and/or excluder classification or definition will not conclusively influence the overall heavy metal phytoremediation performance in Vetiver grass.

On top of that, throughout the entire research study, the exposition of heavy metal concentrations accumulated in the plants were divided into two parts, mainly the roots and the shoots (tillers). In spite of that, there were two types of distributions for the different plant cross-sections conducted in the studies carried out in Chapters 3, 4 and 6, as well as in Chapters 5 and 7, respectively.

The whole tested plant species in Chapters 3, 4 and 6 were separated between the roots and shoots (tillers) as shown in Figure 8.1 below. For the latter research studies in Chapters 5 and 7 the entire harvested plant was further expanded to cover the divided parts equally among the lower and upper roots and tillers of Vetiver grass (Figure 8.2) in order to portray an inclusive phytoassessment for translocation of heavy metals from the lower root upwards to the top of the tiller. Therefore, the assumption of average concentration per unit dry weight of heavy metal present in the roots, shoots and total plant species were equitably obtained as according to the calculation formula suggested by the Method 7000B (US EPA, 2007a).

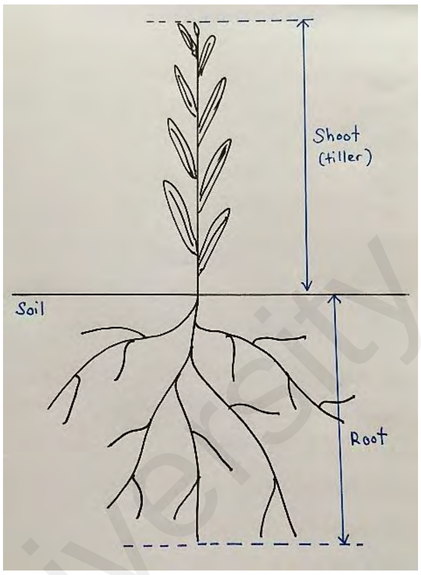

Figure 8.1: Plant cross-section between the roots and shoots (tillers) in Chapters 3, 4 and 6.

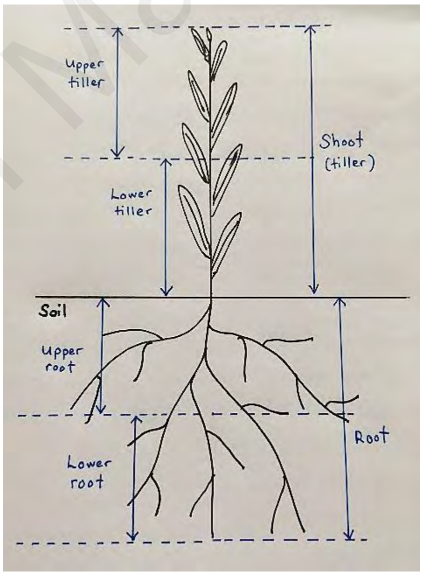

Figure 8.2: Plant cross-section for both lower and upper roots and tillers of Vetiver grass in Chapters 5 and 7.

Thus, in summation this study has reasonably shown that Vetiver grass is as a good heavy metals phytoremediator, with a promising potential to remediate heavy metals contaminated soil.

CHAPTER 9: GENERAL CONCLUSION AND RECOMMENDATIONS

9.1 General conclusion

Based on the results of the present study and discussion as described in Chapter 3 to Chapter 8, it can be concluded that all the research objectives outlined in Chapter 1 were successfully addressed. The preliminary phytoevaluation studies described in Chapter 3 has attentively confirmed the suitability and viability of Vetiver grass as a phytoremediator, compared to the other tropical plant species tested, growing under heavy metal contaminated soil conditions. The findings from these studies have shown that Vetiver grass is the most promising plant for heavy metal phytoremediation due to its positive characteristics of fast growth, resilient survivorship and ability to withstand as well as to bio-accumulate under highly contaminated heavy metal soil conditions.

Phytotolerance studies in Chapter 4 effectively contributed to an improved understanding of the accumulation trends and threshold levels for Vetiver grass grown under heavy metal contaminated soil conditions. Vetiver grass exhibited a good phytotolerance threshold for Pb, higher than 800 mg/kg Pb and for Cd not exceeding100 mg/kg as higher amounts would inhibit overall plant growth. In terms of accumulation trends for heavy metal phytoremediation, it varied considerably every single time when different plant species and experimental set up were applied.

Studies described in Chapters 5 and 7 showed the capability and phytoefficiency of Vetiver grass in both its lower and upper roots and tillers under different types of single and mixed heavy metal contaminated soil conditions. These new findings showed that mixed Cd+Pb, Cu+Zn and Cd+Pb+Cu+Zn spiked treatments were capable of accumulating higher heavy metal concentrations of all the four (Cd, Pb, Cu and Zn) different types of heavy metals, compared to the single spiked treatments in the lower roots and lower tillers of Vetiver grass. On the other hand, mixed Cd+Pb+EDTA, Cu+Zn+EDTA and Cd+Pb+Cu+Zn+EDTA enhanced treatments accumulated higher overall total concentration of Cd, Pb and Cu compared to the single enhanced treatments. In contrast, single Zn+EDTA enhanced treatment exhibited higher accumulation for Zn, in the upper roots and upper tillers of Vetiver grass.

Studies in Chapters 6 and 7 adequately examined the effects and influence of the different types of low cost soil amendments to enhance phytoavailability in Vetiver grass growing under both single and mixed heavy metal contaminated soil conditions. The results demonstrated the applicability of using EDTA as an effective metal chelator, able to increase the accumulation of heavy metals between a 1.14 to 9.24 fold margin in the upper tiller of Vetiver grass for all the four (Cd, Pb, Cu and Zn) different types of metals, under both single and mixed heavy metal contaminated soil conditions.

As an original contribution to the body of knowledge in the field of soil bioremediation and soil-plant interaction, this study has for the first time shown the phytoaccumulation, phytotolerance and translocation movement of Cd, Pb, Cu and Zn metals in the lower roots, upper roots, lower tiller and upper tillers of Vetiver grass growing under mixed heavy metal contaminated soil conditions. Furthermore, it has also shown the optimum application of different types and compositions of effective low cost soil amendments, such as EDTA, to enhance heavy metal phytoremediation across different plant parts in Vetiver grass.

In summary, it can be concluded that the results of this research study have shown that Vetiver grass is the most viable plant species for use and development as a phytoremediator, and as both a phytostabilizer and phytoextractor, for growth under single and mixed heavy metals contaminated soil conditions.

9.2 Recommendations for further studies

Inevitably, this research study has thrown up many new questions which are in need of further investigation. For instance, future research work should study the use of an actually contaminated soil, instead of artificially prepared spiked heavy metal soil treatments to assess the overall phytoremediation ability of Vetiver grass. The present study also is limited for on-site remediation strategies, as *in-situ* Vetiver grass phytoremediation remains an incomplete knowledge. As a result, the simulation impact of phytoevaluation in Vetiver grass would be more significant if it was conducted in an *in-situ* heavy metal contaminated field site, rather than in glasshouse pot assay (*ex-situ*) experiments.

Nonetheless, the association of EDTA soil amendment in Vetiver grass often give rise to many environmental concerns such as non-biodegradability, altering soil bio-physico-chemical properties and long-term persistence in soils (Oviedo & Rodríguez, 2003; Goel & Gautam, 2010; Bloem *et al.,* 2017) as well as the inhibitory effects on plant growth (Chapter 7) despite of its effective metal enhancement properties. Consequently, the application of EDTA needs to be cautiously managed and secured at all times. Hitherto the search for other alternative metal chelating agents like ethylene-diamine-N,N'-disuccinic acid (EDDS) would be necessary.

In addition, it would be worthwhile extending the scope of this soil phytoremediation study to include the myriad of different types of other hazardous heavy metals such as arsenic, mercury and chromium; soil related pollutants like hydrocarbons, pesticides and organic solvents; as well as other soil emerging contaminants. It is hoped that this work will indirectly contribute to additional information on the phytotechnology database for Vetiver grass and at the same time promote the use of phytoremediation for cleaning up various contaminated soil conditions.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

A) Scientific Peer-Reviewed Journal Articles

1) **Ng, C. C.,** Rahman, M. M., Boyce, A. N., & Abas, M. R. (2016). Heavy metals phyto-assessment in commonly grown vegetables: Water spinach (*I. aquatica*) and okra (*A. esculentus*). *SpringerPlus*, *5*(1), 469.

2) **Ng, C. C.,** Law, S.H., Boyce, A. N., Rahman, M.M, & Abas, M. R. (2016). Phyto-assessment of soil heavy metal accumulation in tropical grasses. *Journal of Animal and Plant Sciences*, *26*(3), 686-696.

3) **Ng, C. C.,** Boyce, A. N., Rahman, M. M., & Abas, M. R. (2016). Effects of different soil amendments on mixed heavy metals contamination in vetiver grass. *Bulletin of Environmental Contamination and Toxicology*, *97*(5), 695-701.

4) **Ng, C. C.,** Boyce, A. N., Rahman, M. M., & Abas, M. R. (2017). Tolerance threshold and phyto-assessment of cadmium and lead in vetiver grass, *Vetiveria zizanioides* (Linn.) Nash. *Chiang Mai Journal of Science*, 44(x), 1-12.

B) Scientific Conference Proceedings

1) **Ng, C. C.,** & Rahman, M. M. (2013). Heavy metal phyto-remediation using Vetiver grass (Oral presentation). 2013 UNESCO Youth Forum: Looking Beyond Disaster, Mercure Hotel, Padang, West Sumatra, Indonesia on 7th – 11th October 2013.

2) **Ng, C. C.,** & Rahman, M. M. (2014). Lead, zinc and copper uptake by amaranth and water spinach (Poster presentation). 18th Biological Sciences Graduate Congress (BSGC 2014), Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia on 6th – 8th January 2014.

3) **Ng, C. C.,** & Rahman, M. M. (2014). Phyto-assessment of heavy metals in water spinach and amaranth growing in contaminated soils (Poster presentation). 2014 Baku World Forum of Young Scientists – Azerbaijan National Academy of Sciences, JW Marriott Asheron Hotel, Baku, Azerbaijan on 26th – 31st May 2014.

4) **Ng, C. C.,** Boyce, A. N., & Abas, M. R. (2015). Phyto-assessment of soil heavy metal accumulation in tropical grasses (Oral presentation). 20th Biological Sciences Graduate Congress (BSGC 2015), Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand on 9th – 11th December 2015.

5) **Ng, C. C.,** Boyce, A. N., & Abas, M. R. (2016). Effects of different soil amendments on mixed heavy metals contamination in Vetiver grass (Oral presentation). 5th International Conference on Biological, Chemical and Environmental Sciences (BCES 2016), Holiday Inn Express London Heathrow T5, London, England, United Kingdom on 24th – 25th March 2016.

6) **Ng, C. C.,** Boyce, A. N., & Abas, M. R. (2016). Phyto-evaluation of mixed heavy metal contamination in Vetiver grass using different types of low cost soil amendments (Oral presentation). 21st Biological Sciences Graduate Congress (BSGC 2016), Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia on 15th – 17th December 2016.

7) **Ng, C. C.** & Boyce, A. N. (2017). Enhanced heavy metal phyto-assessment of Vetiver grass with low cost soil amendments using flame atomic absorption spectroscopy (F-AAS) (Oral presentation). 3rd Technology and Innovation International Conference (TECHON 2017), Aston Tropicana Hotel, Bandung, Indonesia on 20th – 22nd May 2017.

8) **Ng, C. C.** & Boyce, A. N. (2017). Bio-remediation of heavy metals using Vetiver grass in Malaysia (Oral presentation). 2017 International Scientific Conference: Science, Technology and Innovative Technologies in the Prosperous Epoch of the Powerful State, Academy of Sciences of Turkmenistan, Ashgabat, Turkmenistan on 12th – 13th June 2017.

C) Scientific Research and Academic Development Awards

1) 2013 K. Kumarasivam Young Environmentalist Internship Award
By the Environmental Management and Research Association of Malaysia (ENSEARCH)

2) 2014 Asia-Pacific Outstanding Academic Achievement Award
By the Golden Key International Honour Society

3) 2015 Science and Technology Research Grant Award
By the Malaysia Toray Science Foundation (MTSF)

4) 2015 Golden Key Graduate Scholar Award
By the Golden Key International Honour Society

5) 2016 BCES Session Best Paper Award (oral presentation)
By the IICBE International Conference in conjunction of the 5th International Conference on Biological, Chemical and Environmental Sciences (BCES 2016)

6) 2016 BSGC Second Place Award (oral presentation)
   In conjunction with the 21st Biological Sciences Graduate Congress (BSGC 2016)

7) 2016 IPNI Scholar Award
   By the International Plant Nutrition Institute (IPNI)

8) 2017 TECHON Best Presenter Award (oral presentation)
   In conjunction with the 3rd Technology and Innovation International Conference (TECHON 2017)

Ng *et al. SpringerPlus (2016) 5:469*
DOI 10.1186/s40064-016-2125-5

SpringerPlus

RESEARCH Open Access

Heavy metals phyto-assessment in commonly grown vegetables: water spinach (*I. aquatica*) and okra (*A. esculentus*)

Chuck Chuan Ng[1*], Md Motior Rahman[1,2], Amru Nasrulhaq Boyce[1] and Mhd Radzi Abas[3]

Abstract

The growth response, metal tolerance and phytoaccumulation properties of water spinach (*Ipomoea aquatica*) and okra (*Abelmoschus esculentus*) were assessed under different contaminated spiked metals: control, 50 mg Pb/kg soil, 50 mg Zn/kg soil and 50 mg Cu/kg soil. The availability of Pb, Zn and Cu metals in both soil and plants were detected using flame atomic absorption spectrometry. The concentration and accumulation of heavy metals from soil to roots and shoots (edible parts) were evaluated in terms of translocation factor, accumulation factor and tolerance index. Okra recorded the highest accumulation of Pb (80.20 mg/kg) in its root followed by Zn in roots (35.70 mg/kg) and shoots (34.80 mg/kg) of water spinach, respectively. Different accumulation trends were observed with, Pb > Zn > Cu in okra and Zn > Pb > Cu in water spinach. Significant differences ($p < 0.01$) of Pb, Zn and Cu accumulation were found in both water spinach and okra cultivated among tested treatments. However, only the accumulation of Pb metal in the shoots of water spinach and okra exceeded the maximum permissible levels of the national Malaysian Food Act 1983 and Food Regulations 1985 (2006) as well as the international Codex Alimentarius Commission limits. This study has shown that both water spinach and okra have good potential as Pb and Zn phytoremediators.

Keywords: Water spinach, Okra, Heavy metal accumulation, Contamination

Background

Water spinach and okra are readily available tropical vegetables found in many countries located across the equator region. Both water spinach and okra shared similar ecological vegetable properties as they are commonly grown edible plants. Both vegetables contain high amounts of vitamins and minerals such as phosphorus, magnesium, calcium, potassium and others which are required in our diet for a healthy living (Singh et al. 2010) and are often regarded as the daily staple diet for many people. Vegetables able to provide energy as it consists most of the essential nutrients, such as proteins, carbohydrates, minerals, vitamins and other trace elements (Itanna 2002). Even though vegetables are an important component of our daily diet, there is little information available as to its contamination by heavy metals. A common example of contamination includes bioaccumulation of heavy metals in vegetables. Bioaccumulation refers to the increase in concentration of a particular chemical or element in biological organisms over time and can pose a threat to the well-being of plants, animals and human beings (Sharma et al. 2006; Shi and Cai 2009). It is well documented that heavy metals inhibit many enzymes and thus able to disrupt metabolic processes, including photosynthesis in plants.

Most people assume that all vegetables are nutritious as well as safe to consume, unaware that some parts of the vegetable may be contaminated with heavy metals and other sources of contaminants. Heavy metals are non-biodegradable and can be very persistent in the environment; have the potential to accumulate in different body organs (Radwan and Salama 2006; Chailapakul et al. 2008; Qishlaqi et al. 2008). By consuming contaminated vegetables, excessive accumulation of dietary heavy metals such as cadmium, lead and chromium can lead to

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The Journal of Animal & Plant Sciences, 26(3): 2016, Page: 686-696
ISSN: 1018-7081

PHYTO-ASSESSMENT OF SOIL HEAVY METAL ACCUMULATION IN TROPICAL GRASSES

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ABSTRACT

Tropical grasses are fast growing and often used for phytoremediation. Three different types of tropical grasses: Vetiver (*V. zizanoides*), Imperata (*I. cylindrical*) and Pennisetum (*P. purpureum*) tested in different growth media of spiked heavy metal contents under the glasshouse environment of RimbaIlmu for 60-day. The growth performance, metals tolerance and phyto-assessment of cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) in shoots and roots were assessed using flame atomic absorption spectrometry (FAAS).Tolerance index (TI), translocation factor (TF), biological accumulation coefficient (BAC), biological concentration factor (BCF), and uptake efficacy was applied to evaluate the metal translocation ability among all three grasses. All three grasses showed significantly higher ($p<0.05$) accumulation of the total heavy metals in the spiked metal treatment compared with other tested treatments. Vetiver accumulated remarkably higher total concentration of Cd (93.08 ± 3.81 mg/kg) and Zn (1284.00 ± 234.83 mg/kg) than both Imperata and Pennisetum. The overall trend of heavy metals accumulation for all three grasses followed the order of Zn >Pb> Cd >Cu. The results of study suggested that both Imperata and Pennisetum are commendable and potential phytoextractors for Zn as well as phytostabilizers for Cd, Pb and Cu, respectively.

Key words: Vetiver; Imperata; Pennisetum; Spiked heavy metal; Heavy metal accumulation.

INTRODUCTION

Soil is commonly regarded as one of the significant natural resources that provide numerous essential elements and interrelating functionswhich include as a store for biodiversity, as a natural habitat for living organisms, food and biomass production as well as a relatively stable reservoir for the whole ecosystem. It is a limited resource that can easily deteriorateby both anthropogenicand natural changes. Soil contamination is the form of which pollutant materials present at concentrations above naturally occurring levels andare likely to cause adirectand/or long term danger to humans and the environment (DOE, 2009). Urban soil contamination has greatly affected many countries, including the United States, Germany, United Kingdom, China and India (Belluck *et al*., 2006;Meuser, 2010) meanwhile heavy metal soil contamination itself has gained a serious attention at the global perspective.

Heavy metal can be very toxic even in low concentration and are not easily degraded or destroyed. It is generally harmful to humans and other living organisms as heavy metals caneasily bio-accumulateand cause food chain contamination. Nevertheless, heavy metals often exist in small amountsin soils and plants as some of thetrace metalsplay an essential role in promoting biological growth. In general, heavy metal can be categorized into essential and non-essential. Essential heavy metals such as nickel (Ni), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) are required by living organisms in trace amounts to support their metabolic functions while non-essential heavy metals such as chromium (Cr), arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd) are not needed for the growth of living organisms (Kabata-Pendias, 2011; Cuypers *et al*., 2013). Heavy metals such as arsenic (As), chromium (Cr), mercury (Hg), cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) are hazardous and the metal toxicity can be severely hazardous if the concentration of heavy metal exceeds its threshold level (DOE, 2009; Ng *et al.*, 2016). And among all heavy metals; cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) are the most commonly foundmetals in contaminated sites (Wang *et al.*, 2009).

Many soil remediation technologies have been used over the last few decades,andphytoremediation has emerged to be one of the most cost effective and eco-friendly solution for soil metal contamination (Glass, 2000; Purakayastha and Chhonkar, 2010). In phytoremediation, plants are utilized to remove various hazardous substances present in the environment including organic compounds, inorganic ions, heavy metals and radioactive materials. As a consequence, the phytoremediation approach has gained much attention and numerous plants species have been tested for phytoremediation properties, including vegetable crops, ornamental flowers, trees, weeds and grasses.

Bull Environ Contam Toxicol (2016) 97:695–701
DOI 10.1007/s00128-016-1921-5

Effects of Different Soil Amendments on Mixed Heavy Metals Contamination in Vetiver Grass

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Received: 4 January 2016 / Accepted: 13 September 2016 / Published online: 21 September 2016

Abstract Three different types of low cost soil amendments, namely, EDTA, elemental S and N-fertilizer, were investigated with Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash growing under highly mixed Cd–Pb contamination conditions. A significant increase ($p < 0.05$) in Cd and Pb accumulation were recorded in the shoots of all EDTA and N-fertilizer assisted treatments. The accumulation of Cd in 25 mmol EDTA/kg soil and 300 mmol N/kg soil showed relatively higher translocation factor (1.72 and 2.15) and percentage metal efficacy (63.25 % and 68.22 %), respectively, compared to other treatments. However, it was observed that the increased application of elemental S may inhibit the availability of Pb translocation from soil-to-root and root-to-shoot. The study suggests that viable application of 25 mmol EDTA/kg, 300 mmol N/kg and 20 mmol S/kg soil have the potential to be used for soil amendment with Vetiver grass growing under contaminated mixed Cd–Pb soil conditions.

Keywords Mixed contamination · Soil amendment · EDTA · Elemental S · N-fertilizer · Vetiver grass

Any opinions, findings, conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Malaysia Toray Science Foundation.

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Soil contamination has become an increasingly important environmental issue in both developed and developing nations. In most cases, soil contamination has been brought about by anthropogenic factors, with humans being the culprit, continuously contaminating the soil in the past and present via industrial and domestic activities. Of these, heavy metal contamination is one of the major types of inorganic soil contamination in the environment. The major contributing factors to anthropogenic heavy metal contamination in soils and the environment include the improper management of agricultural leaching, metalliferous mining and smelting, disposal of metallurgical and electronic commodities, sewage sludge and other chemical manufacturing waste materials (Bradl 2005; Alloway 2013).

Many remediation technologies, such as landfilling, soil washing, bioleaching and excavation have been attempted to resolve soils with contaminated heavy metals. However, all of these strategies are not cost-effective, extremely complicated and are not economically viable in addition to being intrusive to the environment. As a consequence, phytoremediation has emerged to be the green plant based cleanup solution that is able to remove, metabolize and degrade a wide range of hazardous soil heavy metal contaminants with minimum cost required and are non-destructive to the natural ecosystem (Ali et al. 2013). Numerous plants have being studied over the years, with reports suggesting Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash to be one of the most promising plants, with a fast growth rate, and the ability to adapt to many environmental conditions and stress, in addition to being able to tolerate a wide range of extreme heavy metal contamination in soils (Truong et al. 2008; Truong and Danh 2015; Ng et al. 2016a).

Recent studies by Chen et al. (2012), Prasad et al. (2014) and Singh et al. (2015) have solely focused on the phytoassessment of a single metal accumulation. However, there

Chiang Mai J. Sci. 2017; 44(x) : 1-12
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Tolerance Threshold and Phyto-assessment of Cadmium and Lead in Vetiver Grass, *Vetiveria zizanioides* (Linn.) Nash

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Received: 29 April 2016
Accepted: 8 July 2016

ABSTRACT

Various types of plant species have been extensively used for heavy metals phyto-remediation without taking into consideration its tolerance threshold. In this study, Vetiver grass, *Vetiveria zizanioides* (Linn.) Nash was evaluated under five different sets of contaminated spiked cadmium (5Cd, 10Cd, 50Cd, 100Cd and 150Cd mg/kg) and lead (50Pb, 100Pb, 200Pb, 400Pb and 800Pb mg/kg) concentration levels in soil. The growth performance, metal tolerance and phyto-assessment of Cd and Pb in the roots and tillers were assessed using flame atomic absorption spectrometry (FAAS). Tolerance index (TI), translocation factor (TF), biological transfer factor (BTF), biological accumulation coefficient (BAC) and metal uptake efficacy were used to determine the Cd and Pb translocation capability in Vetiver grass. Significantly higher ($p<0.05$) accumulation of Cd and Pb was recorded in the roots of all spiked treatments. Furthermore, strong and significantly positive correlations were exhibited between the increased levels of spiked heavy metal concentrations with both Cd ($r=0.975$) and Pb ($r=0.952$) accumulations. The results of this study showed Vetiver grass as an effective phyto-stabilizer for both Cd and Pb. Nevertheless, the growth of Vetiver grass was restricted when the tolerance threshold of 100 mg/kg (dry weight basis) Cd was exceeded in the contaminated soil.

Keywords: Heavy metal, Spiked metal, Phyto-stablizer, Vetiver grass, Threshold

1. INTRODUCTION

Over the years, soil contamination has attracted much global attention as it instigates considerable risks to human health and the environment. Anthropogenic sources of soil contaminants such as heavy metals released by human activities via industrial and agricultural practices, urban activities and transportation have caused serious threats to the environment [1, 2]. The term heavy metal is widely used to describe a large group of

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APPENDIX

Figure 10.1: Conducting hydrotoxic soil-leachate experiment with acacia (Chapter 3)

Figure 10.2: Conducting hydrotoxic soil-leachate experiment with mucuna (Chapter 3)

Figure 10.3: Undergoing Cd and Pb phytotolerance experiment with Vetiver grass (Chapter 4)

Figure 11.4: Pre-harvest of Cd and Pb phytotolerance experiment with Vetiver grass (Chapter 4)

Figure 11.5: Preparation of single and mixed heavy metal spiked treatments with Vetiver grass (Chapter 5)

Figure 11.6: Undergoing experiment using different types of low cost soil amendments with Vetiver grass (Chapter 6)

Figure 11.7: Undergoing single and mixed heavy metal enhanced EDTA treatments with Vetiver grass (Chapter 7)

Figure 11.8: Root condition of a harvested Vetiver grass (Chapter 7)