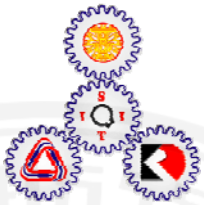


PHYTOEXTRACTION OF CADMIUM FROM CONTAMINATED SOIL

BY

NONGLAK RUNGRUANG

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
SIRINDHORN INTERNATIONAL INSTITUTE OF TECHNOLOGY
THAMMASAT UNIVERSITY
ACADEMIC YEAR 2015

PHYTOEXTRACTION OF CADMIUM FROM CONTAMINATED SOIL

BY

NONGLAK RUNGRUANG

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
SIRINDHORN INTERNATIONAL INSTITUTE OF TECHNOLOGY
THAMMASAT UNIVERSITY
ACADEMIC YEAR 2015

PHYTOEXTRACTION OF CADMIUM FROM CONTAMINATED SOIL

A Thesis Presented

By

Nonglak Rungruang

Submitted to
Sirindhorn International Institute of Technology
Thammasat University
In partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY IN TECHNOLOGY

Approved as to style and content by

Advisor and Chairperson of Thesis Committee .....
Assoc. Prof. Sandhya Babel, Ph.D.

Co-Advisor .....
Assoc. Prof. Preeda Parkpian, Ph.D.

Committee Member and
Chairperson of Examination Committee .....
Assoc. Prof. Alice Sharp, Ph.D.

Committee Member .....
Prof. Chongrak Polprasert, Ph.D.

Committee Member .....
Pichit Pongsakul, Ph.D.

External Examiner : Prof. Maleeya Kruatrachue, Ph.D.

May 2014

Acknowledgments

I would like to express my special appreciation and thanks to my advisor, Associate professor Dr. Sandhya Babel, and co-advisor, Associate professor Dr. Pereda Parkpian, for continuous supervision, encouragement, moral guidance and suggestions throughout the study. Special gratitude is extended to the committee members, Professor Dr. Chongrak Polprasert, Associate professor Dr. Alice Sharp, and Dr. Phichit Pongsakul for serving as my committee members and for their valuable guidance and suggestions; this has been a great assistance in achieving the objectives of the study.

I would like to acknowledge all staffs of Department of Environmental Health, for giving a chance for studying and many thanks to a laboratory staff at Environmental Health Laboratory, Huachiew Chalermprakiet University, in providing laboratories and facilities used in sample analysis. I would like to express my gratitude to The Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi, Thailand and Center of Excellence on Energy Technology and Environment (PERDO), Ministry of Education, Thailand for financial support. Grant support from Huachiew Chalermprakiet University is also kindly acknowledged. Also grateful thank to Thammasart University for grant support.

I would like to thank all staffs of Department of Agriculture for assistance in sample analysis. Grateful appreciation is extended to Khun Salaya Punsiri, EE Senior Lab supervisor and all technical staffs at Environmental Engineering and Management Program (EEM), Asian Institute of Technology (AIT), for assistance during experiment throughout the study. Special thank to all the staffs at Agricultural and Food Engineering, AIT, for the help in greenhouse experiment study.

A special thanks to my family. Words cannot express how grateful I am to my mother, father, sisters, brother and my beloved friend for all of the sacrifices that you have made on my behalf. I would also like to thank all of my friends who supported me in writing, and incented me to strive towards my goal. Finally, my regards to everyone who helped, supported, and encouraged me throughout my graduate years at Thammasat University.

Abstract

PHYTOEXTRACTION OF CADMIUM FROM FROM CONTAMINATED SOIL

by

Nonglak Rungruang

Bachelor of Science (Public Health), Mahidol University, 1995.
Master of Science (Environmental Technology and Management), Asian Institute of Technology, 1998.

Phytoremediation is one of the green alternative and cost effective approaches used to clean up large areas of contaminated soil. Contamination of agricultural soil by toxic heavy metals is a major environmental problem. Among these, cadmium (Cd) is one of the highly toxic heavy metals and causes chronic and acute effects on health and the environment. Cadmium in soil can accumulate in plants and can be transferred to animals through the food chain. In this study, in the first investigation, the cadmium hyperaccumulator potential of four species; marigold (*Tagetes erecta* L.), cosmos (*Cosmos sulphureus*), sunflower *(Helianthus annuus)* and Guinea grass *(Panicum maximum)* was investigated in pot culture experiments using artificially Cd spiked soil by spiking Cd solution onto heavy acid Rangsit soil. Experiments in triplicate were conducted in greenhouse and the concentration of cadmium was varied from 50 to 400 mg/kg of soil. Samples of different parts of plants after reaching flowering stage were harvested for cadmium analysis. Plant growth; flower diameter, height, leaf and stem size; were observed throughout the study period. Based on shoot Cd, translocation factor (TF), bioconcentration factor (BCF) and total uptake, marigold showed a greater ability to accumulate Cd in its biomass. The TF and BCF values of Guinea grass were lowest compared to those of other species, due to the higher biomass produced the total uptake at higher Cd concentration (200 and 400 mg/kg) was higher compared to other plants. Thus, marigold and Guinea grass were used for further experiments.

Marigold and Guinea grass selected from the first experiment were used in the second experiment to investigate the effects of Zn (Cd:Zn of 1:0, 1:10, 1:30 and 1:50), pH (5.0, 6.3, 7.0, 7.5), Cd:EDTA treatments (1:0, 1:0.5 and 1:1) on Cd uptake; under various Cd concentrations of 50, 100 and 200 mg/kg. Based on the TF, BCF, total uptake per pot, and total biomass, marigold showed higher potential to uptake Cd from the soil compared to Guinea grass. Moreover, it was noticed that marigold had a greater ability to accumulate Cd in the aboveground parts and met one of the criteria for Cd hyperaccumulation (100 mg/kg dry weight in shoot). The translocation factor and bioaccumulation factor were greater than one. The present study demonstrated that the maximum shoot Cd, total Cd in whole plant tissues, TF and BCF of marigold were highest at the pH around 5. The maximum shoot Cd, total Cd in whole plant, BCF were obtained at 1:10 and 1:0 of Cd:Zn at Cd of 50 and 100 mg/kg, respectively and then decreased as Zn concentration application was increased to 1:30-1:50 of Cd:Zn treatment.

At higher Cd concentration (100 mg/kg), the addition of Zn to soil did not promote Cd uptake by marigold. For Guinea grass, the maximum shoot Cd, Cd in whole plant, total uptake and BCF were achieved at the pH around 5.0. Shoot and root Cd, Cd in whole plant of Guinea grass increased with increasing Cd concentration in soil. EDTA application in soil promoted the transfer of Cd from roots to shoots in marigold but did not show any impact in Guinea grass. The highest Cd uptake was achieved at Cd:EDTA treatment of 100:50 mg/kg for both marigold and Guinea grass, but at the treatment of 50:25 mg/kg, only maximum Cd uptake was obtained in Guinea grass. Speciation of Cd in soil samples used for growing marigold and Guinea grass revealed the same trend that the highest fraction of Cd in soil was exchangeable fraction (F1) (72.3-83.03% in marigold and 74.54-82.01% in Guinea grass) and the lowest portion was the residual fraction (F5) (1.22-3.09% in marigold and 1.11-3.68% in Guinea grass). The Cd: EDTA of 50:25 and 100:100 mg/kg provided more bioavailable Cd fraction in the soil solution. However, the influence of the application of EDTA to the soil on the stimulation of Cd transfer from roots to shoots was only pronounced in marigold, but it did not show any effects in Guinea grass. The correlation analysis between Cd in whole plant tissues of four species (marigold, cosmos, sunflower and Guinea grass) and Cd in soil was highly positive, showing the positive linear relationship between Cd accumulation in whole plant tissues and Cd applied in the soil. The correlation between Shoot Cd of marigold and soil pH was negative, indicating negative linear relationship. Shoot Cd of marigold decreased with increasing soil pH.

Overall, marigold possesses a greater ability to accumulate Cd in plant tissues but less biomass production could lower the total uptake. In both marigold and Guinea grass, Cd treatment of 100 mg/kg provided higher uptake than that at Cd 50 mg/kg, under all pH treatments. At Cd 50 and 100 mg/kg, the pH 5.0 provided maximum Cd uptake for both species. To increase phytoremediation efficiency of Cd removal from contaminated site, the proper selection of cultivars and adjustment of its cultivation practice such as repeating growing the same crop on the soil as well as substantial soil amendment to stimulate Cd solubility and enhance plant uptake (pH around 5.0-5.5 is preferable as Cd become more solubilized) are recommended to improve the efficiency of this green technology.

Table of Contents

Table of Contents

Table of Contents

List of Abbreviations

AIT	Asian Institute of Technology
BCF	Bioconcentration Factor
CT	Control
CEC	Cation Exchange Capacity
Cd	Cadmium
C_{os}	Cosmos
DOA	Department of Agriculture
DW	Dry Weight
EC	Electrical Conductivity
EDTA	Ethylenediaminetetraacetic acid
EEC	European Economic Community
Fe	Iron
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrophotometry
ISV	An in-situ Vitrification
M_{ar}	Marigold
Mn	Manganese
MP	Maximum permissible
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PCP	Pentachlorophenol
S_{un}	Sunflower
G_{ui}	Guinea grass
Nat.Soil	Natural soil
ND	Non-detectable
Rep	Replication
SD	Standard Deviation
SRM	Standard Reference Material
SVE	Soil Vapor Extraction
TB	Total Biomass
TCE	Trichloroethylene
TNT	Trinitrotoluene
TPH	Total petroleum hydrocarbons
TF	Translocation Factor
VOCs	Volatile Organic Compounds
Zn	Zinc
WIMI	International Water Management Institute
USDA	U.S. Department of Agriculture
EPA	Environmental Protection Agency

List of Figures

List of Figures

List of Figures

List of Tables

List of Tables

Chapter 1

Introduction

1.1 General Background

There is a growing concern over the contamination of soil, water and agricultural products by heavy metals due to industrialization in Asia. The uses of heavy metals in various applications lead to their wide distribution in soil, sediment, air, waste, and wastewater. Such pollution of the environment by toxic metals and radionuclides arises as a result of many human activities, largely industrial sources.

Anthropogenic pathways by which cadmium (Cd) enters the environment are through industrial wastes from processes such as mining and smelting of metalliferous ores, electroplating, manufacturing of plastics, paint pigments, alloy preparation, and batteries that contain Cd. Household appliances, automobiles and trucks, agricultural implements, airplane parts, industrial tools, hand tools, and fasteners of all kinds (e.g., nuts, bolts, screws, nails) are commonly Cd coated. Cadmium is also used for luminescent dials, in photography, rubber curing, and as fungicides. Tobacco concentrates Cd, leading to human exposure to this carcinogenic metal through smoking (Kirkham, 2006).

In addition, mining activity is one of the industrial activities which might directly cause impact to the environment without proper management. The extraction of minerals from the earth crust under the ground surface may release toxic waste from the blasting operation, minerals haulage, ore dressing, and dump site of the waste. The waste produced from these activities can contaminate natural resources such as run-off water, soil and sediment, and many living organisms in nearby ecosystems. The transportation of the toxic substances could widely spread out in many environmental media. It can also be accumulated in the environment and vegetation and cause harmful effects, both to animals and human, if they ingest these toxicants by various pathways. Around 1977, zinc mining activities by three companies started after the Department of Mineral Resources and Ministry of Industry classified Tak province area as the richest source of zinc in Thailand. However, at present, only one company has remained in the area namely, Pha Daeng Industrial Public Company (Chusai, 2006).

Cadmium is the associated mineral in zinc mine. Along with zinc (Zn), copper (Cu), lead (Pb) and Cd may be present also. The concentration of Cd in lithosphere ranges from 0.1-0.2 μg/g of rock (Nriagu, 1980). When the minerals are extracted rapidly by human, the heavy metal can be remobilized by transportation with the run-off water and contaminate soil and plants widely. Furthermore, cadmium is a highly toxic heavy metal. It is of great concern and can cause significant environmental impact because of its toxicity to animals and humans. It is not degradable in nature and will thus, once released to the environment, remain in circulation. Cadmium can accumulate in plants and can be transferred to animals through food chain. It is toxic at very low exposure

levels and has acute and chronic effects on health. Cadmium toxicity especially affects humans rather than animals, because of their longevity and the accumulation of Cd in the organs when consuming Cd-contaminated food (Kirkham, 2006). In addition, elevated levels of Cd in humans can cause kidney damage and low levels of Cd in the diet are linked with renal dysfunction. Other diseases associated with Cd exposure are pulmonary emphysema and the notorious Itai-Itai disease.

1.2 Problem Statement

Industrial waste water, sewage sludge and solid waste have been discharged into the environment. Due to improper control or illegal discharges, these materials, including toxic metals and organic substances, contaminate irrigation systems, ground water, and even drinking water source. Irrigation water is required for rice production, especially in Thailand and other Asian countries. When polluted water is used for irrigation on paddy soils, toxic substances such as cadmium, lead and arsenic etc., may be incorporated into plants parts. These toxic substances may thus enter the food chain and possibly affect the health of animals and humans (Adriano, 1992).

The incident of cadmium contamination in Tak mining area was first addressed by the researchers from International Water Management Institute (IWMI). The study of water and soil contamination in China and other Asian countries was carried out by research team from IWMI. The study results revealed that zinc mine could lead to cadmium contamination in surrounding areas. Since 1998, the research team at IWMI conducted a study at Mae Sot district, Tak province, Thailand and found high contamination of cadmium in agricultural soils and rice grains grown in Cd contaminated soil around Mae Sot district. In addition, the rice-based agricultural system is located within Phatat Pha Daeng sub-district, Mae Sot, Tak Province. Mae Tao Creek, the upper stretches of which pass through an actively mined Zn-mineralized zone, is applied for irrigation by the eight communities with a combined resident population of 5796 and an annual combined rice production of 7592 ton/year. The total area under paddy rice for the eight villages is 2201 ha (Simmom et al. 2005). Furthermore, rice growing in the vicinity of zinc mine could lead to cadmium contamination which co-exists naturally with zinc and would inevitably cause adverse health effect. Particularly, the long-term consumption of cadmium contaminated rice has resulted in chronic and/or acute human Cd disease as manifested by Itai-Itai disease and/or chronic cadmium poisoning among the exposed population. Moreover, the impact can be vast in the area where water was naturally supplied by Mae Toa Creek in which sediment was suspected of having high contamination of cadmium.

Moreover, the study results showed that total soil cadmium levels in 154 soil samples ranged from 3.4-284 mg Cd/kg soil which was 1.13-94 times of European Economic Community (EEC) maximum permissible (MP) soil cadmium concentration of 3.0 mg Cd/kg soil and 189.3 times above Thai standard of 0.15 mg Cd/kg soil. Moreover, rice samples from 90 fields were found to be contaminated with cadmium ranging from 0.1 to 4.4 mg/kg rice while the mean background Thai rice Cd concentrations as reported by Pongsakul and Attajarusit (1999) was 0.043 ± 0.019 mg/kg rice. With this

amount of cadmium present in rice and based on Thai daily rice consumption, it was estimated that local residents would have been exposed to cadmium 14-30 times higher than the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Provisional Tolerable Weekly Intake (PTWI) of 7 μg Cd/kg body weight (BW) per week (Simmom et al., 2005: Kardkarnklai, 2007).

Cadmium is highly toxic to organisms. Use of phosphate fertilizers and sludge, mining and inputs from mining as well as smelting industries are main source of Cd contamination in vast areas of agricultural soils. Cadmium in soil can be bioavailable for plant uptake and subsequent human uptake. In humans, excessive cadmium can lead to renal failure, thus cadmium in the environment poses a significant health risk. Agricultural crops grown in Cd-polluted environments contain Cd to varying degrees. Daily consumption of Cd-contaminated foods poses a risk to human health (Wei et al. 2009b; Uraguchi et al. 2006).Techniques are required to remediate agricultural soils that have moderate and widespread metal-contamination to make food produce on these soil safe for human consumption.

Phytoremediation, the name given to a set of technologies that use plants to clean contaminated sites, is the use of plants to partially or substantially remediate selected contaminants in contaminated soil, sludge, sediment, ground water, surface water and wastewater. It utilizes a variety of plant biological processes and the physical characteristics of plants to aid in site remediation. EPA uses phytoremediation because it takes advantage of natural plant processes. It requires less equipment and labor than other methods since plants do most of the work. Trees and plants can make a site more attractive as well. The site can be cleaned up without removing polluted soil or pumping polluted groundwater. This allows workers to avoid contact with harmful chemicals. Phytoremediation has been successfully tested in many locations, and is being used at several Superfund sites (USEPA, 2001; USEPA, 2001). Phytoremediation is widely viewed as the ecologically responsible alternative to the environmentally destructive physical remediation methods currently practiced. Plants have several endogenous genetic, biochemical and physiological properties that make them ideal agents for soil and water remediation (Meagher, 2000).

Several conventional methods such as soil washing and flushing, solidification, vitrification, electrokinetic remediation etc, can be used to treat heavy metal contaminated soil. However, these technologies may destroy the biological component of the soil and can drastically alter its chemical and physical characteristics as well, creating a relatively nonviable solid waste. Thus, phytoremediation, which mainly uses hyperaccumulator and accumulator plants, can remove excess heavy metals from, contaminated soils. This is considered as a promising cost effective technology compared to conventional remediation techniques without major secondary environmental issues, especially for the remediation of large areas of contaminated soils with relatively low level of heavy metal concentration. Despite the known advantages of phytoremediation over other remediation techniques, only a few Cd hyperaccumulator have been identified, researched and documented. The two members of the Brassiscaceae family such as *Thlaspi caerulescens* and *Arabidopsis* and recently *Viola baoshanensis* and *Solanum nigrum* have been reported as Cd hyperaccumulators (Wei et al. 2009a).

Plants are considered as hyperaccumulators when they can accumulate uniquely high quantities of heavy metals. The main characteristics of a hyperaccumulator plant can be summarized as follows:

1) Critical concentration, i.e., on a dry mass basis. The suggested critical values in the shoots (stems or leaves) of a hyperaccumulator are 100 mg/kg for Cd, 1,000 mg/kg for As, Pb, Cu, Ni and Co; 10,000 mg/kg for Zn and Mn; 2) translocation factor (TF), which is the ratio of the metal concentration in the shoot to that in root, should be greater than one (metal concentrations in the shoots of a plants should be higher that those in roots); 3) enrichment factor (EF: concentration in plant/soil) is greater than one; 4) tolerance property, a hyperaccumulator should have high tolerance to toxic contaminants. The first item listed above is a unique characteristic of hyperaccumulators, while the rest three features are shared with accumulators. Although shoot concentration of heavy metals is increased by soil concentration, accumulators display consistently lower levels than hyperaccumulator. In addition, for the plants tested under experimental conditions, their aboveground biomass should not decrease significantly when growing in contaminated soil (Wei et. al. 2009b).

At present, the phytoremediation technique is not much used in practice due to long growing periods as well as low biomass of hyperaccumulators and accumulators. Thus, it is important to find species that could be regarded as (hyper) accumulator as well as yield more biomass. Screening of potential Cd accumulating cultivars locally available in Thailand is useful for the remediation of real contaminated sites, especially agricultural areas contaminated by toxic heavy metals. However, uptake of Cd can be affected due to presence of Zn in the soil and also soil pH. Zinc is one of the metals normally present with Cd in soil. Zinc and cadmium may have either a synergistic or an antagonistic effect on plant uptake that can be affected by their concentrations in soil and soil pH. Thus, it is important to study the effect of Zn and pH on Cd uptake by species that readily available at a village closed to the real contaminated site in northern Thailand, which might possess desired properties of (hyper) accumulator. Cadmium phytoextraction potential of some locally available species needs to be investigated. The results gained from these experiments could be adopted and applied as optimum operating conditions for field experiments at real contaminated agricultural area in the future.

1.3 Objectives of the Research

The main objective of the study was to evaluate the cadmium phytoextraction potential of the plants of concern; Guinea grass (*Panicum maximum*), cosmos (*Cosmos sulphureus*), marigold (*Tagetes erecta* L.) and sunflower *(Helianthus annuus)*. The followings are the specific objectives of the study:

-To investigate the best and potential accumulator for Cd uptake in pot culture experiments under various Cd soil concentrations.

-To examine the effects of soil pH, Zn concentration in soil, EDTA concentration in soil on Cd accumulation by different plant species in artificially Cd spiked soils

- To investigate relationship between dependent and independent variables of concern.

1.4 Scope of the Study

This study focused on investigation and evaluation of cadmium accumulation potential of the plants studied. Four species, namely, Guinea Grass (*Panicum maximum*), cosmos (*Cosmos sulphureus*), marigold (*Tagetes erecta* L.) and sunflower *(Helianthus annuus)* were selected. Various pot culture experiments were carried out. To evaluate the cadmium accumulation potential of the plants of concern and get better understanding of metal bioavailibity in soil for the studied plants, the factors affecting phytoavailability of Cd in contaminated soil, like plant species, soil pH, Zn concentration, and chelator applied in soil were investigated.

This study comprises of the following laboratory experiments:

(1) Screening of potential Cd accumulating plants from locally available species (four species), using artificially Cd spiked soils. For this, pot experiment was investigated to find out optimum soil Cd concentration (50, 100, 200, and 400 mg/kg), providing the best Cd uptake of the studied plants. All plants were grown until the flowering stages (75-80 days). In the final stage of each investigation, cultivar selection was based on the best accumulation for Cd and was used for the next experiments.

(2) Under optimum Cd concentration obtained from (1), effect of soil pH on Cd uptake by selected plants was carried out. Soil pH in pots was varied to four levels (approximately from 5.0, 5.5, 7.0, and 7.5)

(3) For investigation on the effect of Zn on Cd uptake by plants, various Cd:Zn ratios (1:0, 1:10, 1:30, and 1:50) under optimum pH were investigated to find the effect on Cd uptake in the presence of Zn.

(4) Investigation on the effect of chelating agents, ethylenediaminetetraacetic acid, (EDTA) in enhancing Cd uptake during phytoextraction was conducted at different treatment conditions. Soil samples was amended with EDTA in the ratio (Cd: chelator) of 1:0, 1:0.5, and 1:1, based on optimum soil Cd concentration for each species. Moreover, Sequential extraction of soil samples was carried out to determine chemical forms of Cd and Zn. Relationship between soil Cd, soil pH, Zn and EDTA applied to the soils and Cd accumulation in plant biomass were investigated using linear regression analysis.

Chapter 2

Literature Review

The environmental pollution is a matter of great concern worldwide. The contamination of environment and food chain with heavy metals has become a matter of growing concern in view of its role in human health and nutrition. Soil contamination with toxic metals is an important environmental problem around the world. Soils polluted with these heavy metals may damage human health and ecosystems. Various heavy metals, like mercury (Hg), lead (Pb), and cadmium (Cd), can be released to the environment through the improper management of waste produced from various industries (Sabir et al. 2003; Scheuhammer 1987). Among those toxic heavy metals, cadmium is produced as an inevitable by-product of Zn refining, since these metals occur naturally within the raw ore. For treating contaminated soils, the phytoremediation technology can be used to partially remove selected contaminated soil (Salt et al., 1995; Kumar et al., 1995; Klasssen, 2001).

2.1 Heavy Metals

Cadmium (Cd) is a toxic element and zinc (Zn) is toxic in high concentrations, and numerous investigations showed that the pronounced amounts of Cd and Zn were often found in arable soils**.** The processing and subsequent release of zinc to the environment is normally accompanied by cadmium environmental pollution because of zinc ores (ZnS) generally containing 0.1-5% and sometimes even higher cadmium (Adriano, 1986). Cadmium, unessential to plants, and zinc, essential to plants, are elements having similar geochemical and environmental properties. This association of cadmium and zinc in the environment and their chemical similarity can lead to interaction between cadmium and zinc during plant uptake, transport from roots to aboveground parts, or accumulation in edible parts (Das et al., 1997).

2.1.1 Cadmium

Cadmium is a widespread pollutants and one of the most toxic heavy metals in the environment due to its high mobility and toxicity at low concentration and tremendously toxic to organisms. It is a toxic heavy metal present in all soils, usually as a trace constituent. Soils are the main source of Cd in plants and plant-derived foods are main source of Cd in human diets (Wagner, 1993). Food chain contamination by Cd is the most important pathway of Cd exposure to the general population. Accumulation of Cd in food crop is subject to regulation by national and international agencies. In addition, cadmium is a natural component of the ecosystem and has a similar geochemical behavior to Zn. It is present as a constituent of several Zn minerals particularly the sulphide, sphalerite, and the carbonate, smithsonite. Where Zn mining has taken place in the past, it is common for soils to be enriched in both Zn and Cd, normally showing ratios ranging from 100:1 to 400:1 (Tron, 1996). Cadmium particles can be transported in air for a long distance and thus the ground and water could be contaminated far from the emission source. The presence in soils

of cadmium from anthropogenic sources has become one of the most significant environmental problems due to their toxicity and persistence. Furthermore, use of phosphate fertilizers and sludge, mining and inputs from mining as well as smelting industries are main source of Cd contamination in vast areas of agricultural soils. Cadmium in soil can be bioavailable for plant uptake and subsequent human uptake. Excessive cadmium in human can lead to various health effects.

1) Physical and chemical properties of cadmium

Cadmium, in its purest form, is a soft, silver white, transition metal. It is similar in appearance to zinc, but is softer than Zn. To some extent, it is used in the same way as zinc. Cadmium originates from the Latin word cadmia, which means "calamine". The Greek word "kadmeia" has similar meaning (Weast, 1986). Cadmium was found as an impurity of zinc carbonate, which upon heating changed color owing to impurities of cadmium. Cadmium does not have a defined taste or odor. Location in the periodic table is in group IIB. Atomic number is 48 and atomic mass is 112.411. Naturally occurring isotopes are 106 (1.22%), 108(0.88%), 110 (12.9%), 111 (12.75%), 112 (24.07%), 113 (12.6%), 114 (28.86%), and 116(7.5 %). Many radioactive isotopes of Cd, e.g., 109 and 115 are well recognized in experimental toxicology. Melting and boiling temperatures are 320.9° C and 765º C, respectively (Sarkar, 2002).

2) Cadmium in the Environment and its Applications

Cadmium is an element that occurs naturally with an average distribution of 0.1 mg/kg in the earth's crust. Pure cadmium is a soft, silver white metal and is similar in appearance to zinc, but is softer and is to some extent used in a similar way as zinc. Cadmium is the most important pollutant metal tremendously toxic to organisms and is widely dispersed in the environment (Alloway, 1995). Cadmium has been identified as a major toxic heavy metal reaching the food chain, directly through crop uptake and indirectly through animal transfer. Cadmium is regularly found in ores together with zinc, copper and lead. Cadmium occurs naturally in the geosystem and is not usually present in the environment as a pure metal, but as a mineral combined with other elements such as oxygen (cadmium oxide), chloride (cadmium chloride) or sulfur (cadmium sulfate, cadmium sulfide). Particularly, high cadmium concentrations occur in some sulfide ores, but many soils and rocks, coal, and mineral fertilizers contain some cadmium (Adriano, 1992).

Moreover, cadmium is often present naturally as complex sulfides, carbonates and oxides in copper, lead, and zinc ores. It is seldom present in large quantities as the sulfates and chlorides. These different forms of cadmium compounds are solids that dissolve in water to varying degrees. The sulfates and chlorides are the forms that are the most soluble in water. Furthermore, cadmium also presents in the industries as an inevitable by-product of copper, lead, and zinc extraction. After being applied, it enters the environment mainly via the ground, because it is found in pesticides and manures. Naturally, a lot of cadmium being released into the environment, around 25,000 tons per year. About fifty percent of the cadmium is released into rivers through weathering of rocks and some cadmium is released into the air through

volcano and forest fires (Hillel, 1998). The rest of the cadmium is released through human activities, such as manufacturing. The main mining areas are those associated with zinc (Sarkar, 2002). Actually, cadmium is widely used in industrial processes, e.g. as a color pigment, a stabilizer in PVC products, an anticorrosive agent, a neutron absorber in nuclear power plants. Chemical compounds, e.g. cadmium iodide and bromide, are applied in photoengraving and photography. Negative plates (electrodes) of nickel-Cd storage batteries are made of cadmium oxide (McBride, 1994 and Hillel, 1998). Moreover, cadmium is applied in plating to protect iron, copper, steel, iron, and other alloys from corrosion. Cadmium does not corrode easily. Cadmium also strengthens the copper used in electric wires and other commercial products.

In the environment, cadmium is present in air as a result of household waste incineration, via emission from industry including mining, and from production of energy based on combustion of coal (Nordberg, G.F., Nordberg, 1988). Cadmium particles can be transported in air distantly and thus the water and ground, in long distance, can be contaminated. Furthermore, anthropogenic sources of cadmium contamination in soil has become one of the most significant environmental problems because of their persistence and toxicity. Cadmium remained in water and soil can be bound to other compounds. Cadmium cannot be absent from the environment but can be altered to many other forms. Knowledge of the specific form of cadmium is very crucial for identifying potential risk of adverse health impact (Chusai, 2006).

3) Cadmium Exposures

Uptake of cadmium by human occurs mostly through food chain. Cadmium concentration contaminated in food can greatly enhance in human bodies. Example are mushroom, shell, mussels, dried seaweed, fish, and liver. In addition, low levels exposure to cadmium by human occurs due to natural processes and also human activities such as combustion of fossil fuel and industrial uses as well as mining and smelting. Cadmium can be produced as a by-product from metals production e.g. lead, copper, and zinc (Wagner, 1993). However, Cd is mostly found as chemical compounds of elements, such as sulfur, chlorine, oxygen, and fluorine. Cadmium compounds are presently mainly used in re-chargeable nickel-cadmium batteries. During the 20th century, cadmium emissions have increased dramatically, since cadmium-containing products are seldom re-cycled, but often discharged together with household waste (Jarup, 2003). Smoking is a main source of cadmium exposure and higher exposure level of cadmium can be occurred when smoking. Smoking a packet of 20 cigarettes can lead to the inhalation of around 2-4µg of cadmium, but levels may vary widely (Schnoor, 1995).

High intake of cadmium through contaminated food and water can cause vomiting and diarrhea. Godt et al. (2006) reviewed that a major source of cadmium exposure is smoking leading to lung adsorption (40-60 %) of cadmium in tobacco smoke. Cigarette smoking may cause significant increases in blood cadmium levels, the concentrations in smokers being on average 4–5 times higher than those in non-smokers (Jarup et al. 1998). In non-smoker, the most important source of cadmium exposure is contaminated food. Other high exposures can occur with people living

close to factories or hazardous waste sites that discharge cadmium into the atmosphere (Sarkar, 2002; Jarup, 2003).
Moreover, occupational exposure mostly occurs in workplace by inhalation of cadmium-containing fumes which have been reported to develop acute respiratory distress syndromes (ARDS). Exposure in metal welding and battery manufacturing is the most dominant. In the general environment, cadmium exposure occurs through consumption of contaminated food (e.g. rice and shellfish) and drinking water leading to cadmium accumulation in kidney, liver (Elinder, et al. 1983). Cadmium accumulates in kidneys damaging the filtering mechanisms leading to the excretion of essential proteins and sugars from the body and further kidney damage. It takes a very long time before cadmium that has accumulated in kidneys is excreted from a human body. Furthermore, other health impacts caused by cadmium are bone fracture or bone defects (osteomalacia, osteoporosis) in humans and animals, stomach pains and severe vomiting, diarrhea, reproductive failure and possibly even infertility, damage to the central nervous system and the immune system as well as psychological disorder and cancer development (Barbee, et al., 1999; Sarkar, 2002).

4) Cadmium Toxicity

Cadmium may cause health effects upon both acute and long-term exposure. Cadmium has long biological half-life, as a result, long term toxicity has attracted particular attention. Acute toxicity by inhalation may occur in workers welding cd-contaminated materials and after inhalation of fumes containing Cd causing pulmonary respiratory distress and pulmonary edema Cd (Sarkar, 2002 and Jarup, 1998). In addition, Cd exposure through the skin is not known to cause health impacts in human beings and animals. In human beings, long-term exposure is linked to renal dysfunction. High exposure related to obstructive lung disease and lung cancer. Only in some particular situations, general environmental pollution caused by Cd has been related to the development of human disease e.g. Itai-Itai disease in Japan and renal dysfunction and increased occurrence of osteoporosis in Belgium and in China (Schnoor, 1995).

Furthermore, among industrial workers, whose route of exposure is chiefly through inhalation, significant lung damage (emphysema) can occur. In long-term, low-level occupational exposure to cadmium, the kidneys rather than the lung are more frequently the critical organ. Typically, renal dysfunction occurs before lung damage. The first signs of kidney dysfunction are usually increased excretion of low-molecular-weight proteins into the urine. Such proteins include β-microglobulin, retinol-binding protein, albumin, transferrin, and IgG. Once absorbed, cadmium is very efficiently retained in the body, chiefly in liver and kidney, where about one half the body burdens is stored. Cadmium is stored to some extent in bone, where it exerts the severe skeletal toxicity known as "Itai-Itai" disease (Wagner, 1993). This condition is identical to osteomalacia and is often accompanied by osteoporosis. Animal studies suggest that skeletal effects precede renal effects. The primary cause of the bone lesions is not fully clear. Cadmium impairs hydroxylation of 25-hydroxyvitamin D to 1, 25-dihydroxyvitamin D3 in the kidney, reduces calcium

absorption from the gastrointestinal tract, causes a general alteration of trace element metabolism, and results in severe renal disease (Jarup et al. 1993).

5) Effects of Cadmium on the environment

Cadmium possesses long biological half-life and once absorbed by animals, plants and humans and retains for many years in environment. Cadmium contaminated waste released from industries mostly end up in soils. Cadmium contaminated waste may release to atmosphere via combustion of domestic (household) waste and fossil fuels (Kumar, 1995).

Another major source of cadmium released to environment is the phosphate fertilizers production. Application of the fertilizer on agricultural area can lead to the contamination of soil, surface and ground water. The cadmium-rich sludge can be transported over long distanced and polluted surface water and also soils. Organic matter in soil can adsorbs cadmium. Cadmium contaminated in acidic soil can be greatly dangerous, as cadmium uptake by food crops will enhance. Contamination of cadmium in food crops is a potential danger to animals depending on the plant for survival (Jorgensen, 1993).

Moreover, some soil organisms and worms necessary to soil are susceptible to cadmium poisoning. At very low concentrations, cadmium can kill these living organisms and consequently affects structure of the soil. When concentration of cadmium in soil is high, soil processes of microorganisms can be affected and threaten the whole soil ecosystem. Furthermore, in aquatic ecosystems, cadmium can be accumulated in aquatic organism and microorganisms. The sensitivity to cadmium can vary greatly among aquatic organisms. Freshwater organisms are known to be less resistant to cadmium poisoning than salt-water organisms (Jarup et al.1998).

2.1.2 Zinc

Zinc, is an inherent part of our environment and a natural component of the Earth's crust. Zinc is present not only in rock and soil, but also in air, water, and the biosphere. Zinc is a crucial element not only because it is necessary for organisms and plants but also it has been used in a wide varieties of industries. Naturally, zinc occurs in many minerals as carbonates, phosphates, sulfides, oxides, sulfates, and silicates with the principal ore being sphalerite, a zinc sulfate (Barak and Helmke, 1993). Zinc can be applied in many ways including production of zinc-based alloys such as bronze and brass, metal surface galvanization, zinc production used comprehensively in industries. Although, zinc is significant in daily lives, zinc is heavy metal presenting in highest concentration in the major wastes occurring in industrialized society. Most of the soil surface contamination by zinc is derived from anthropogenic activities (Boardman and McGuire, 1990) such as Smelting and mining, galvanizing and electroplating, over uses of municipal and industrial sludge to land, excessive application of Zn-contaminated agricultural chemicals, and a wide varieties use of zinc for industrial activities (Blaylock and Huang, 2000; Chaney, 1993).

Zinc can be bound to soil particles via a wide range of mechanisms such as specific adsorption, chelation and cation exchange (Barrow, 1993; McBride, 1994; Shuman, 1980). Generally, under alkaline soil solution, Zn is less available for plant uptake than that of acidic solution. According to McBride (1994), the cause for the decrease in availability at high pH related to the effect of H^+ concentration on the several sorption mechanisms. Under acidic conditions, the functional groups responsible for specific adsorption may become protonated and create a net positive charge on sesquioxides, amorphous clays, and organic matter, resulting in the release of Zn^{2+} into the soil solution. Hydrogen also has the ability to displace other cations such as Zn^{2+} from the cation exchange complex. Chelation of Zn^{2+} by organic matter is also influenced by soil acidity through its effect on the number of ligands involved in chelation. Monodentate bonds between Zn and the chelating ligands are weak compared with multidentate bonds. Under acidic conditions, the monodentate bonds are more prevalent, and Zn^{2+} can be more easily displaced from these chelate complexes through exchange processes. It is well understood that plants influence soil pH under non-contaminated conditions, particularly in the immediate vicinity of the roots (Marschner, 1995). Nonetheless, it is not clear how the root medium's pH is influenced by plants sensitive to Zn contamination under several fertility regimes. This data may help researchers to estimate changes in availability of Zn in soil, and may bring about more optimal practices which enhance the plant's capability to uptake Zn for phytoextraction.

The accumulation and partitioning of Zn in the plant is extremely rely on the supply in the root medium. When Zn supply is sufficient to toxic, a large portion is bound to the surface of cell walls in the root cortex (up to 90%). However, the amount of total Zn in the roots may be a function of the duration of exposure. There is evidence that the binding sites in roots for some metals, such as Pb, must become saturated before they are translocated to the shoot tissue. Zinc is also bound to vascular tissue in roots and stems. Under adequate supply, the Zn absorbed by roots is rapidly transported to the shoots. Movement in the plant is not necessarily via passive transport in the transpirational stream, as there is evidence that accumulation occurs in parts of the plant were transpiration is minimal, not where the greatest transpiration is occurring. There is little remobilization of Zn throughout the plant, particularly when present at deficient or adequate levels. (Dushenkov et al. 1995; Kumar et al. 1995).

2.2 Soil Remediation Technologies

Excavation of contaminated soil followed by transportation to and disposal in landfills is the most common practice of remediating those soils. The contaminated soil is excavated using conventional excavated using conventional excavating equipment, transported by authorizer haulers, and disposed of in a permitted landfill. Prior to disposal, the soil may require pretreatment to reduce concentrations below the land disposal restrictions stipulated by the regulations. This disposal approach is relatively simple, fast and cost-effective for small volumes under any soils and contaminant conditions (U.S. EPA, 1989). However, when the contaminated soil quantity is large and the contamination is deep, excavation and disposal in landfills may be very

expensive and impractical. In addition, the soil remediation technologies can be implemented either (1) in-situ or (2) ex-situ. In-situ remediation methods treat the contaminated soils in place; thus, no excavation of the contaminated soils is required. In-situ remediation methods are preferred over ex-situ methods because they cause fewer disturbances to the site as well as less contaminant exposure to personnel and members of the public near the site. In addition, in-situ methods are less expensive than ex-situ methods (Sharma et. al., 2004).

All ex-situ methods involve excavation of soil from the site. The treatment can take place on-site, or the soils can be transported to another location for treatment. Site constraints may place practical limits on the potential for successful application of ex-situ remediation methods because the contaminated soil must be accessible for excavation. A shallow water table, building, overhead power lines, or underground utilities may limit the potential for excavating all of the soil requiring remediation. Space requirements may also limit the on-site use of ex-situ technologies. Adequate space is required for treatment equipment and for stockpiles of excavated soil awaiting treatment and cleaned soil awaiting final disposal.

The most popular soil remediation technologies are (1) soil vapor extraction, (2) soil washing, (3) stabilization/ solidification, (4) electrokinetic remediation, (5) thermal desorption, (6) vitrification, (7) bioremediation, and (8) phytoremediation. These techniques, with some modifications, can be used for both in-situ and ex-situ remediation methods. All of these methods are based on manipulation of physical, chemical, electrical, thermal, or biological processes and aim to extract, immobilize, or detoxify the contaminants. Brief discussion of these remediation technologies will be described as follows (US. EPA, 1989, 1990):

(1) Soil vapor Extraction

Soil vapor extraction (SVE) is a technique for removing volatile organic compounds (VOCs) and motor fuels from contaminated soils. This technology is know in the industry by various names, including vacuum extraction, soil venting, aeration, in-situ volatilization, and enhanced volatilization. Figure 2.1 shows a schematic of the SVE implementation the field. It involves applying a vacuum to the contaminated soil through extraction wells, which create a negative pressure gradient that causes movement of vapors toward these wells. The contaminant-laden vapors extracted from the wells are then treated aboveground using standard air treatment techniques such as carbon filters or combustion (Sharma et. al., 2004).

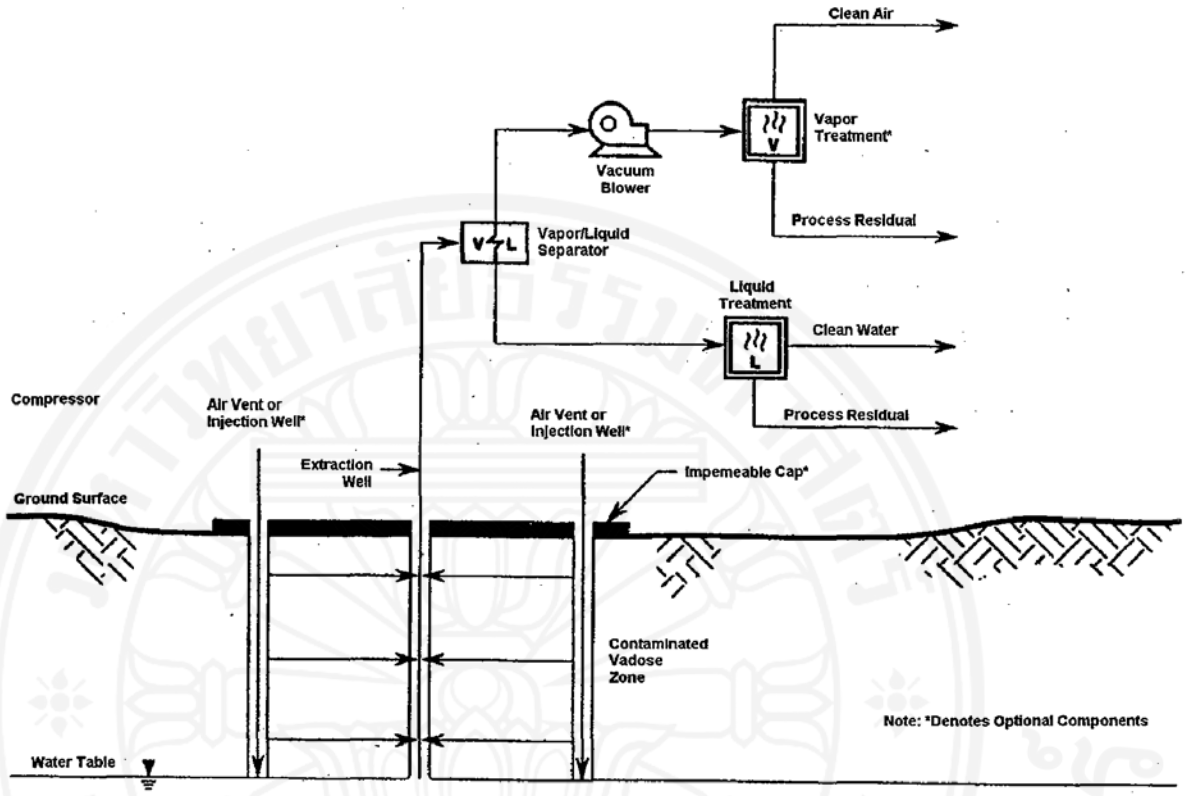


Figure 2.1 Schematic of SVE implementation in the field (Sharma et. al., 2004)

SVE is applicable when contaminants present in subsurface are volatite. This technology has been proven effective in reducing concentrations of volatile organic compounds (VOCs) and certain semivolatile organic compounds (SVOCs) found in petroleum and chlorinated products. Generally, as a simplified guideline, a compound is considered a good candidate for remediation by SVE if it has(1) a vapor pressure value greater than 0.5 mmHg and (2) a Henry's law constant greater than 0.01. The technology is applicable where soils are relatively homogeneous and highly permeable.

(2) Soil washing

Soil washing technology is used to separate contaminants from excavated soils and to decrease the soil volume requiring final treatment or disposal. The technology relies on the fact that contaminants tend to be related preferentially to organic matter and fine grained soil particles (i.e. clay and silt). Volume reduction is achieved by cleaning the coarse-grained soil fraction and leaving the contaminants in the fine grained fraction and washing fluids. The cleaned coarse fraction can then be returned to the excavation. The concentrated contaminants in the fine fraction and wash fluids are treated. A variety of soils washing processes have been developed that employ different equipment configurations and may be aimed at specific contaminants. Following excavation, the soil is screened to separate coarse debris (larger than about

2 in) such as rocks and roots (US.EPA, 1989). The remaining soils may be fluidized, or made pumpable, with the addition of water. In the scrubbing unit, a water-based washing solution is used to separate soluble contaminants and fine particles from coarser soil materials. Following washing, the soil slurry undergoes a separation step in which water, cleaned coarse material, and contaminated fines are segregated. Suspended fines may be flocculated and separated by gravity means or may be removed in a vacuum filter press. The clean soil is then returned to the excavation (US.EPA, 1990).

Soil washing can be effective for treating soils contaminated with a variety of organic and inorganic contaminants. Developmental studies indicated good to excellent applicability of the process for removal of VOCs and metals from sandy and gravelly soils. Soil washing is less likely to be effective with silt or clay soils. In general, the soil types for which this can be applied effectively are those with relatively high hydraulic conductivities. If the contaminants adsorb to the soil strongly, soil washing may not be effective.

(3) Stabilization and solidification

The stabilization and solidification (S/S) process also referred to as immobilization, fixation, or encapsulation, uses additives or processes to chemically bind and immobilize contaminants or to microencapsulate the contaminants in a matrix that physically prevents mobility. Stabilization typically refers to a chemical process that actually converts the contaminants into a less soluble, mobile, or toxic form. Solidification generally refers to a physical process where a semisolid material or sludge is treated to render it more solid. Thus, the S/S process refers to either chemically binding or physically trapping the contaminants in soils. This technology neither removes the contaminants from soils, such as soil washing, nor degrades the contaminants, such as bioremediation; rather it eliminates or impedes the mobility of contaminants. In some cases, the S/S process is used as a pretreatment to reduce soluble contaminant concentrations in the soils to below the regulatory limits (e.g., land disposal restriction limits) in order to dispose of them in a landfill (US.EPA. 1997).

The S/S process is applicable to soils contaminated with metals, radionuclides, and other inorganics as well as nonvolatile and semivolatile organic compounds. Soils contaminated solely with volatile organic compounds are not considered to be appropriate for the S/S process because they may be volatilized and released during mixing and curing operations. The S/S process is applicable to all types of soils (US.EPA. 2000).

(4) Electrokinetic Remediation

Electrokinetic remediation, also known as electrokinetics, electromigration, electrorestoration, electroremediation and electroosmosis, removes the contaminants from soils by applying an electric potential across contaminated soil through a pair of electrodes, located at the anode and the cathode. Due to a variety of processes, contaminants are transported toward the electrodes. The contaminant-laden liquids are then removed from the electrodes. The system consists of a minimum of two electrodes buried underground and connected to a power supply. The electrodes are located a certain distance apart and are encased by reservoirs or wells. The electrodes are called anodes or cathodes, the anode being the positively charged component and the cathode the negatively charged component (US.EPA. 1997; Huang et al., 2012). In simple terms the anode attracts contaminants that have a negative charge, and the cathode attracts positively charged contaminants. In remediating unsaturated soils, water is injected into electrode wells or reservoirs. Removal of contaminants can be achieved by pumping the contaminated water in the reservoirs or wells or by electroplating, precipitation, or coprecipitation at the electrodes. This technique can be used to remediate soil with high clay or humic content. It can also be used in heterogeneous soils. It can also be used on both saturated and unsaturated soils. Electrokinetics can be used to treat a wide varieties of contaminants, for example, organic contaminants, radionuclides, and heavy metals (Huang et al., 2012).

(5) Thermal desorption

Thermal desorption is a technology that treats contaminated soil by heating soils to temperatures between 200 and 1000°F. This causes contaminants with low boiling points to volatile and thus segregate from the soil. The vapors are collected by a vacuum system and transported to a treatment center. In addition, the heat used in thermal desorption does not destroy the contaminants. Instead, thermal desorption physically separates the contaminants from the soil. Subsequently, the vapors taken from the treatment are either condensed for disposal (as in higher-temperature incinerators), or they are reused. Thermal desorption can be an ex-situ or an in-situ application. In an ex-situ application the soil is excavated and brought to a facility to be processes. The facility can be located at the site or the soil can be transported to another location. In an in-situ application, the process is done completely in place. Thermal blankets are placed on the soil surface to treat any shallow contamination. Also, thermal wells are placed in the ground to treat the deeper contamination. Heat from the wells is transferred via radiation and thermal conduction to the contaminated soil. Extraction wells are added to remove the soil vapors that are produced as a part of the process (US. EPA, 1989, 1990).

Thermal desorption is effective in removing volatile and semivolatile organics from soils contaminated with oil refining wastes, coal tar wastes, wood-treating wastes, creosotes, hydrocarbons, chlorinated solvents, fuels, PCBs, mixed wastes, synthetic rubber processing waste, pesticides, and paint wastes.

(6) Vitrification

Vitrification involves the use of heat to melt and convert the contaminated soil into a stable glass or crystalline product. When the contaminated soil is melted, thermally stable inorganic contaminants are surrounded by the molten soil. As the molten soil cools, it forms a solidified mass of waste glass that incorporates these inorganic contaminants. These contaminants are incorporated into the waste glass either through chemical bonding or through encapsulation. Also during the vitrification process, the high temperatures necessary to melt the contaminated soil will cause organic contaminants either to be destroyed via pyrolysis or removed as off-gases. Vitrification can be applied as an in-situ process, a stage in-situ process, or as an ex-situ process. An in-situ vitrification (ISV) system uses a group of four graphite electrodes arranged in a square array. These four electrodes are inserted into the contaminated soil and an electric current is applied to them. A stage in-situ vitrification process involves excavating the contaminated soil and consolidating it into an on-site trench. The contaminated soil can then be vitrified in the trench using an ISV system. Ex-situ vitrification processes involve excavating the contaminated soil and transporting it to an adequately equipped facility. The contaminated soils are then fed into a furnace, which is used to heat and vitrify the contaminated soil (U.S. EPA, 1989, 1990).

Vitrification is applicable to soils contaminated with mixed contaminants that include radionuclides, metals and other inorganics, and organics. The process can be applied to all types of soils; however, soil moisture content and permeability can limit the applicability of vitrification. While high moisture content in the soil will not preclude the use of vitrification due to any technological limitations of the process, it may limit its applicability due to increased energy costs, resulting from the increased energy used to disperse the water during the vitrification process. The permeability of the soil must be low enough so that it prohibits water from recharging the vitrification zone faster than the vitrification process can dry and melt the soil. Soils with a permeability of less than 10^{-4} cm/s may require additional steps to remove moisture content prior to attempting vitrification.

(7) Bioremediation

Bioremediation is a process in which microorganism degrade organic contaminants or immobile inorganic contaminants. Under favorable conditions, microorganism can degrade organic contaminants completely into nontoxic by-products such as carbon dioxide and water or organic acids and methane. In the natural attenuation process, microorganisms occurring in the soil (yeast, fungi, or bacteria) degrade the contaminants for their survival. However, depending on the type of contaminants and its toxicity levels, specific microbes may be introduced into the soil to be remediated. In addition, for microbial survival and growth, supplies of oxygen, moisture, and nutrients may be needed. The process of bioremediation refers to enhancement of the natural process by adding microorganisms to the soil, referred t o as bioaugmentation, and/or supplying oxygen, moisture, and nutrients required for microbial survival and growth to the soil, referred to as biostimulation. Bioremediation is also called

enhanced bioremediation or engineered bioremediation in the published literature (US. EPA 2000).

Bioremediation is commonly used for the treatment of soils contaminated with organic compounds. Petroleum hydrocarbons can easily be treated using bioremediation. Other organic compounds, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), are resistant to degradation, due to their high levels of toxicity to microbial population. Specific microbes that can resist these toxicity levels are often required. Bioremediation cannot degrade inorganic contaminants such as heavy metals, but it can be applied to change the valence states of these metals, thus converting them into immobile form. For example, mobile hexavalent chromium can be converted into immobile trivalent chromium. Bioremediation can be applied in any soil type with sufficient moisture content, even though, it is not easy to provide oxygen and nutrients into low-permeability soils. It should be noticed that very high contaminants concentration may be poisonous to microorganisms and therefore may be cured by bioremediation (US. EPA 2004).

(8) Phytoremediation

Phytoremediation is the use of plants to extract, separate, and/ or detoxicate contaminants. Phytoremediation is considered as the ecologically alternative to the environmentally friendly methods presently practiced (Meagher, 2000). Plants have many endogenous genetic, biochemical, and physiological properties that make them ideal agents for water and soil treatment. Important progress has been made in recent years in improving native or genetically modified plants for the treatment of environmental pollutants. As elements are immutable, phytoremediation methods for readionuclides and heavy metal pollutants focus on hyperaccumulation above-ground. In contrast, organic pollutants can potentially be completely mineralized by plants. More detail on phytoremediation will be addressed on the following paragraphs; an overview on phytoremediation (Sharma et al., 2004).

2.3 Overview on Phytoremediation

2.3.1 Background

Contamination of water and soil pose a crucial environmental and health problems that can be partially solved by bioremediation technology which is called phytoremediation. This plant-based strategies to remediation takes advantage of plants ability to sequester compounds and elements from contaminated environment using various mechanisms. Organic contaminants and heavy metals are the main targets for phytoremediation. Several field experiment ensured the feasibility of application of plants for cleanup of the contaminated environment (Salt et al., 1998).

Phytoremediation apply the green plants to substantially remediate selected contaminants in contaminated sludge, sediment, surface water, ground water, wastewater, and soil. It applies a wide range of plant biological processes and the

physical properties of plants to assist in site remediation. Phytoremediation has also been called botano-remediation, agro-remediation, vegetative remediation, and green remediation. Phytoremediation is a continual processes, with the various processes occurring to differing degrees for various conditions, media, contaminants, and plants (Agarwal, et al., 2007). A various terms have been used in the literature to refer to these several processes. Nonetheless, it must be noted that the various processes described by these terms all tend to overlap to some degree and occur in varying proportions during phytoremediation. Phytoremediation covers a wide varieties of different strategies that can bring about contaminant degradation and removal (through accumulation or dissipation), or immobilization (US.EPA, 2001; Sharma, et al., 2004).

Moreover, phytoremediation involves removal, stabilization, or degradation of contaminants in soils by plants, The various remediation mechanism are in the root zone or in the plant itself and are reflected by process specific terminology. Plants are in contact with the contaminated soil in the root zone. Pollutants must pass through root membranes before they are absorbed by the plant, called rhizofiltration. Contaminant fate is determined by the plant's ability to metabolize the absorbed organic chemicals by plant metabolic processes, called phytodegradation, or in corporate inorganic chemical in plant tissue, called phytoaccumulation (U.S.EPA, 2000). Phytodegradation continues outside the plant through the release of root exudates (soluble organic matter, nutrients) and enzymes, which stimulate bacterial and fungal degradation of organic contaminants, called rhizodegradation**.** Contaminants enter the plant through a mechanism called phytoextraction (Sharma et al. 2004).

2.3.2 Applications

Phytoremediation of metals is being developed as a potential cost-effective remediation solution for thousands of contaminated area in the United States and abroad. Its improvement is driven by the prohibitively high cost of the available soil remediation methods, which mainly involve soil removal and burial at a price of about $1 million per acre. The metals of greatest concern as environmental contaminants and some of their regulatory limits are listed in Table 2.1. Elements in each category are ranked by Salt et al. (1998) according to their significance as environmental contaminants in the United States.

In addition, phytoremediation has gain world-wide attention as an environmentally cost-effective technique to remove metals from soil. The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a "green" sustainable process. Because most applications involve photoautrophic plants, phytoremediation is primarily solar powered and thus moresustanable, especially compare to the typical mechanical approaches to hazardous waste management. An important distinction is that vascular green plants have the marvelous ability to self-engineer or exert limited control over the rhizosphere, local biogeochemistry, availability of water and nutrients, and the local microclimate (MaCutcheon and Schnoor, 2003).

In general, the ideal plant species to remediate a heavy metal-contaminated soil should be a high biomass producing crop that can both tolerate and accumulate the contaminants of interest. The use of hyperaccumulator plant species has been suggested as a promising strategy for phytorextraction. Hyperaccumulators are defined as higher plants capable of accumulating >100 mg Cd/kg, >1000 mg Cu, Ni, and Pb/kg, and >10,000 mg Zn/kg in the dry matter (dm) of shoots when growing in their natural habitats (Baker et al, 2000).

However, removal rates of metals by plants from contaminated soils are highly dependent on soil properties, degree and bioavailability of metal contamination, and obviously metal uptake characteristics and biomass production of the plant species used for remediation. Also, bioavailable metal pools in soil decrease during phytoextraction, which leads to a decrease of metal uptake by plants and lower metal removal rates. Moreover, previous researches revealed that the major factors influencing the uptake of heavy metal by crops are soil pH, the amount of heavy metal present in the soil, and the oxidation and reduction potential of the soil affecting the bioavailability (Baker, 1982; Agarwal, et al., 2007).

Table 2.1 Concentration ranges and regulatory guidelines for important metals and in the order of relative importance (Salt et al. 1998)

Element	Concentration range	Regulatory limit
Metals	(µg/kg)	(mg/ kg)[a]
Lead	1000–6,900,000	600
Cadmium	100–345,000	100
Arsenic	100–102,000	20
Chromium	5.1–3,950,000	100
Mercury	0.1–1,800,000	270
Copper	30–550,000	600
Zinc	150–5,000,000	1500

[a]Nonresidential direct contact soil cleanup criteria. In *Cleanup Standards for Contaminated Sites*, New Jersey Department of Environmental Protection (1996)

Moreover, soil is the principal source of Cd accumulated by plants. Many characteristics such as concentration and form of metals in the soil, pH, organic matter content, clay content, concentration of Zn, other cations, complexing ligands, and fertilization practices, have been recognized as major factors that determine the bioavailability of Cd in soil (Khoshgoftar et. al., 2004).In addition, the study of Simmons et. al. (2008) revealed that the phytoavailability of Cd in paddy soil is a function of the complex interaction between soil pH, redox conditions and the presence of competing ions. Despite the known advantages of phytoremediation over

other remediation techniques, only a few Cd hyperaccumulator have been identified, researched and documented, notably two members of the Brassiscaceae family such as *Thlaspi caerulescens* and *Arobidopsis.* Recently *Viola baoshanensis* and *Solanum nigrum* L have also been report as Cd hyperaccumulators (Wei et. al., 2008a). Some evidences were provided that sunflower could phytoremediate soil polluted by Cd^{2+} in association with Pseudomonas putida and Pb-contaminated soil. Furthermore, phytoremediation applications (Table 2.2) can be classified based on the contaminant fate, containment, degradation, extraction or a combination of these. Phytoremediation applications can also be grouped based upon the related mechanisms including concentration of contaminants in plant tissue; degradation of pollutants by several biotic or abiotic processes; volatilization or transpiration of volatile pollutants from plants to the atmosphere; immobilization of pollutants in the root zone; extraction of pollutants from groundwater or soil; and control of erosion, runoff, and infiltration by vegetative covers (US.EPA, 2000). A summary of these application of above mentioned categories are as follows:

1) Degradation

Plants may increase degradation in the rhizosphere (root zone of influence). Microbial counts in rhizosphere soils can be 1 or 2 orders of magnitude greater than in nonrhizosphere soils. It is not known whether this is due to microbial or fungal symbiosis with the plant, plant exudates including enzymes, or other physical/chemical effects in the root zone. There are, however, measurable effects on certain contaminants in the root zone of planted areas. Several projects examine the interaction between plants and such contaminants as trinitrotoluene (TNT), total petroleum hydrocarbons (TPH), pentachlorophenol (PCP), and polycyclic aromatic hydrocarbons (PAH) (US.EPA, 2000).

Table 2.2 Phytoremediation applications (US.EPA, 2000)

Mechanism	Contaminant	Media	Plant	Reference
Degradation	Atrazine, nitrates	Surface Water	Poplar	Schnoor et al., 1995
Degradation	Landfill leachate	Groundwater	Poplar	US.EPA, 2000
Degradation	TCE	Groundwater	Poplar, cottonwood	US.EPA, 2000
Degradation	TNT	Wetlands	Various	US.EPA, 2000
Degradation	TPH	Soil	Grasses, crops	US.EPA, 2000
Extraction-Concentration in shoot	Lead	Soil	Indian mustard	Blaylock ,1997
Extraction-concentration in root	Uranium	Surface water	Sunflower	Dushenkov, 1997
Extraction, Volatilization	Selenium	Soil, Surface Water	Various	US.EPA, 2000

2) Extraction

Phytoextraction, or phytomining, is related to planting a crop known to accumulate the pollutants in leaves and stems (shoots) of the plant and then harvesting the corp and removing the contaminant from the contaminated area. This method provides a mass of plant and contaminant (typically metals) that must be transported for disposal or recycling. This is a concentration technology that yields a much smaller mass to be disposed of as compared to excavation and land filling (Garbisu et al., 2001).

In addition, rhizofiltration is similar to phytoextraction in that it is also a concentration technology. It differs from phytoextraction in that the mechanism is root accumulation and harvest using hydroponic (soil-less) growing techniques (Dushenkov, 1997). This is beneficial for segregating metal pollutants from water. Volatilization or transpiration through plants into the atmosphere is the mechanism for separating a contaminant from the water or soil or water of contaminated area. (Baker et al., 2000).

3) Containment and immobilization

Containment is using plants to bind the contaminants to the soil, render them nonbioavailable, or immobilize them by removing the means of transport. Physical containment of contaminants by plants can take the form of binding the contaminants within a humic molecule (humification), physical sequestration of metals as occurs in

some wetlands, or by root accumulation in non-harvestable plants. Certain trees sequester large concentrations of metals in their roots, and although harvesting and removal is difficult or impractical, the contaminants present a reduced human or environmental risk while they are bound in the roots. Moreover, Risk reduction may also be achieved by transforming the contaminant into a form that is not hazardous, or by rendering the contaminant nonbioavailable. Environmental Protection Agency (EPA) and the U.S. Department of Agriculture (USDA) have ongoing research in this area (US.EPA 2000).

Vegetative cover (evapotranspiration or water-balance cover) systems are another remediation application utilizing the natural mechanisms of plants for minimizing infiltrating water. Originally proposed in arid and semi-arid regions, vegetative covers are currently being evaluated for all geographic regions. The effectiveness in all regions and climates needs to be assessed on a site-specific basis. If there is potential for gas generation a vegetative cover may not be an option. For example, a municipal solid waste landfill can produce landfill gas that may be of concern to human health and the environment. Sites with requirements to collect and control landfill gas may not meet Federal requirements under the Clean Air Act if a vegetative cover is used (U.S.EPA, 2000).

2.3.3 Phytoremediation processes

There are a number of various forms of bioremediation applied to specific types of contaminated media or pollutants and may require various types of plants. Phytoremediation covers a number of different methods leading to pollutants break down, removal or immobilization. Various forms of phytoremediation are roughly presented as follows (Baker et al., 2000; U.S.EPA, 2001).

Phytoextraction

Phytoextraction is a contaminant removal process (uptake of contaminant) by roots with subsequent accumulation in the shoot portion of a plant, normally, to be followed by harvest and ultimate disposal of the plant biomass. Phytoextraction can be applied to radionuclides (e.g., 90Sr, 137Cs, 234U, 238U), metals (e.g., Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn), metalloids (e.g., As, Se), and non-metals (e.g., B) (Salt et al., 1995; Kumar et al., 1995) as these are generally not further degraded within the plant. Phytoextraction has generally not been considered for organic or nutrient contaminants taken up by a plant, as these can be changed, volatilized, or metabolized by the crop, thus preventing accumulation of the contaminants. Phytoextraction is also known as phytoaccumulation, phytoabsorption, and phytosequestration (which can all also apply to contaminant accumulation within the roots) (US.EPA, 2001). Hyperaccumulators can accumulate a metal from metal-rich soil to a much greater degree (such as 100-fold or 1000-fold) than do other plants in that soil, and reach some specified unusually high concentration of metal in some part of the plant. These plants are generally relatively rare and found only in localized areas around the world, with less than four hundred identified species for eight heavy metals (Brown et al., 1994; Blaylock, 2000). A possible physiological reason for metals hyperaccumulation

could be as a tolerance strategy for these high soil concentrations of metals. Other potential reasons for metals hyperaccumulation include a possible competitive advantage, a means to resist drought, inadvertent metal uptake, or a defense against herbivores or pathogens such as bacteria and fungi (Brooks, 1998).

Phytostabilization

Phytostabilization is the use of vegetation to contain soil contaminants in situ, through modification of the chemical, biological, and physical conditions in the soil. Contaminant transport in soil, sediments, or sludges can be reduced through absorption and accumulation by roots; adsorption onto roots; precipitation, complexation, or metal valence reduction in soil within the root zone; or binding into humic (organic) matter through the process of humification. In addition, vegetation can reduce wind and water erosion of the soil, thus preventing dispersal of the contaminant in runoff or fugitive dust emissions, and may reduce or prevent leachate generation. Phytostabilization is also known as in-place inactivation or Phytoimmobilization. Phytostabilization research to date has generally focused on metals contamination, with lead, chromium, and mercury being identified as the top potential candidates for phytostabilization (US.EPA, 1997). However, there may be potential for phytostabilization of organic contaminants, since some organic contaminants or metabolic byproducts of these contaminants can be attached to or incorporated into plant components such as lignin. This form of phytostabilization has been called phytolignification (Cunningham et al., 1995). One difference, however, is that phytostabilization of metals is generally intended to occur in the soil, whereas phytostabilization of organic contaminants through phytolignification can occur aboveground.

Rhizofiltration

Rhizofiltration (also known as phytofiltration) is the removal by plant roots of contaminants in surface water, waste water, or extracted ground water, through adsorption or precipitation onto the roots, or absorption into the roots. The root environment or root exudates may produce biogeochemical conditions that result in precipitation of contaminants onto the roots or into the water body (Dushenkov et al., 1995). The contaminant may remain on the root, within the root, or be taken up and translocated into other portions of the plant, depending on the contaminant, its concentration, and the plant species. Rhizofiltration and phytoextraction are similar in that they each result in accumulation of the contaminant in or on the plant. However, in rhizofiltration this accumulation can occur in the roots or in the portion of the plant above water, whereas for effective phytoextraction the accumulation occurs aboveground, not in the roots. In addition, rhizofiltration differs from phytoextraction in that the contaminant is initially in water, rather than in soil (U.S. EPA, 2001).

Rhizofiltration is a contaminant removal process, in which contaminant removal from the site is accomplished by harvesting the roots and, if necessary, the above-water portion of the plant, followed by proper disposal of the contaminated plant mass. Thus, rhizofiltration differs from phytostabilization occurring in soil, in which the

contaminant remains in the root zone. Moreover, rhizofiltration is generally applicable to treating large volumes of water with low contaminant concentrations (in the ppb range). It has primarily been applied to metals (Pb, Cd, Cu, Fe, Ni, Mn, Zn, Cr (VI) (Salt et al.,1997) and radionuclides radionuclides (90Sr, 137Cs, 238U, 236U) (Dushenkov et al., 1997).

Rhizodegradation

Rhizodegradation is the enhancement of naturally-occurring biodegradation in soil through the influence of plant roots, and ideally will lead to destruction or detoxification of an organic contaminant. Other terms have been used by some authors as synonyms for rhizodegradation, such as enhanced rhizosphere biodegradation (Jordahl et al., 1997).

Organic contaminants in soil can often be broken down into daughter products or completely mineralized to inorganic products such as carbon dioxide and water by naturally occurring bacteria, fungi, and actinomycetes. The presence of plant roots will often increase the size and variety of microbial populations in the soil surrounding roots (the rhizosphere) or in mycorrhizae (associations of fungi and plant roots). Significantly higher populations of total heterotrophs, denitrifiers, pseudomonads, BTX (benzene, toluene, xylenes) degraders, and atrazine degraders were found in rhizosphere soil around hybrid poplar trees in a field plot (Populus deltoides × nigra DN-34, Imperial Carolina) than in non-rhizosphere soil (Jordahl et al., 1997). The increased microbial populations are due to stimulation by plant exudates, compounds produced by plants and released from plant roots. Plant exudates include sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavanones, enzymes, and other compounds (Shimp et al., 1993).

In addition, the increased microbial populations and activity in the rhizosphere can result in increased contaminant biodegradation in the soil, and degradation of the exudates can stimulate co metabolism of contaminants in the rhizosphere. Rhizodegradation occurs primarily in soil, although stimulation of microbial activity in the root zone of aquatic plants could potentially occur. Stimulation of soil microbes by plant root exudates can also result in alteration of the geochemical conditions in the soil, such as pH, which may result in changes in the transport of inorganic contaminants. Plants and plant roots can also affect the water content, water and nutrient transport, aeration, structure, temperature, pH, or other parameters in the soil, often creating more favorable environments for soil microorganisms, regardless of the production of exudates.

Phytodegradation

Phytodegradation is, also known as phytotransformation, the uptake, metabolizing, and degradation of contaminants within the plant, or the degradation of contaminants (a contaminant destruction process) in the soil, sediments, sludges, ground water, or surface water by enzymes produced and released by the plant (using internal and external metabolic processes driven by the plants). In addition, phytodegradation is

not dependent on microorganisms associated with the rhizosphere. Contaminants subject to phytodegradation include organic compounds such as munitions, chlorinated solvents, herbicides, and insecticides, and inorganic nutrients. In phytodegradation applications, transformation of a contaminant within the plant to a more toxic form, with subsequent release to the atmosphere through transpiration, is undesirable. The formation and release of vinyl chloride resulting from the uptake and phytodegradation of TCE has been a concern. However, although low levels of TCE metabolites have been found in plant tissue vinyl- chloride has not been reported (Newman et al., 1997).

Phytovolatilization

Phytovolatilization is the uptake of a contaminant by a plant, and the subsequent release of a volatile contaminant, a volatile degradation product of a contaminant, or a volatile form of an initially non-volatile contaminant. For effective phytoremediation, the degradation product or modified volatile form should be less toxic than the initial contaminant. Phytovolatilization is primarily a contaminant removal process, transferring the contaminant from the original medium (ground water or soil water) to the atmosphere (Jury et al., 1990). However, metabolic processes within the plant might alter the form of the contaminant, and in some cases transform it to less toxic forms. Examples include the reduction of highly toxic mercury species to less toxic elemental mercury, or transformation of toxic selenium (as selenate) to the less toxic dimethyl selenide gas (Adler, 1996). In some cases, contaminant transfer to the atmosphere allows much more effective or rapid natural degradation processes to occur, such as photodegradation. Because phytovolatilization involves transfer of contaminants to the atmosphere, a risk analysis of the impact of this transfer on the ecosystem and on human health may be necessary. In addition, phytovolatilization can occur with soluble inorganic contaminants in ground water, soil, sediment, or sludges and can also occur with organic contaminants, such as trichloroethane (TCE), generally in conjunction with other phytoremediation processes.

Constructed Wetlands

Constructed wetlands or treatment wetlands are artificial wetlands that are used for treating organic, inorganic, and nutrient contaminants in contaminated surface water, municipal waste water, domestic sewage, refinery effluents, acid mine drainage, or landfill leachate. A considerable amount of research and applied work has been conducted using constructed wetlands for these applications. Natural wetlands have also been examined for treatment of these wastes. Ground-water treatment is less common, though conceivable. Except in a few cases, constructed wetlands generally have not been used in remediation of hazardous waste sites; however, constructed and natural wetlands have been investigated for the phytodegradation of munitions-contaminated water. In the future, constructed wetlands might become an option for treatment of water extracted from hazardous waste sites, using rhizofiltration and phytodegradation. Integration of hazardous waste site phytoremediation and constructed wetland technologies might increase in the future (Hammer and Bastion, 1989).

2.3.4 Limitations of phytoremediation at hazardous waste sites

Although current research continues to explore and push the boundaries of phytoremediation applications, there are certain limitations to plant-based remediation systems.

1) Root syem**:** root contact is a primary limitation on phytoremediation applicability. Remediation with plants requires that the contaminants be in contact with the root zone of the plants. Either the plants must be able to extend roots to the contaminants, or the contaminated media must be moved to within range of the plants. This movement can be accomplished with standard agricultural equipment and practices, such as deep plowing, or by irrigating trees and grasses with contaminated groundwater or wastewater. As shown in Table 2.3, the effective root depth of plants varies by species and depends on soil and climate conditions (US. EPA, 2000).

2) Growth rate: phytoremediation is also limited by the growth rate of the plants. More time may be required to phytoremediate a site as compared with other more traditional cleanup technologies. Excavation and disposal or incineration takes weeks to months to accomplish, while phytoextraction or degradation may need several years. Therefore, for sites those pose acute risks for human and other ecological receptors, phytoremediation may not be the remediation technique of choice (US. EPA, 2000)

Table 2.3 Root depth for selected phytoremediation plants (US. EPA, 2000)

Plant	Maximum Root Depth	Target Contaminants
Indian mustard	To 12 inches	Metals
Grasses	To 48 inches	Organics
Poplar trees	To 15 feet	Metals, organics, chlorinated solvents

3) Contaminant concentration: the sites with widespread, low to medium level contamination within the root zone are the best candidates for phytoremediation method. High concentrations of contaminants may prohibits the growth of plant and therefore hinder the application on some sites. This phytotoxicity could lead to an approach in which high concentration of waste is treated by expensive ex-situ techniques that quickly decrease acute risk, while in-situ phytoremediation is applied over a longer period of time to clean the high volumes of lower contaminant concentrations (US. EPA, 2000).

4) Impacts of contaminated vegetation: some ecological exposure may occur whenever plants are used to interact with contaminants from the soil. The fate of the metals in the biomass is a concern. Although some forms of phytoremediation involve

accumulation of metals and require handling of plant material embedded with metals, most plants do not accumulate significant levels of organic contaminants. While metal accumulating plants will need to be harvested and either recycled or disposed of in compliance with applicable remediate metal contaminated soil have been used at highly contaminated sites worldwide, those methods are not applicable to large areas. These remediation methods require high energy input and expensive machinery. At the same time they destroy soil structure and decrease soil productivity. Phytoremediation, the use of plants to clean regulations. Often overlooked, however, is the possibility that natural vegetation on the site is already creating very similar (but often unrecognized) food chain exposures (US.EPA, 2000). In addition, even on currently unvegetated sites, contaminants will be entering the food chain through soil organisms. The remediation plan should identify and, if possible, quantify potential avenues of ecological exposure, and determine if and where any accumulation of toxics in the selected plants will occur (Pollard, 1996).

2.3.5 Advantages and disadvantages of phytoremediation

Although different conventional methods to soils can be a cost effective in situ alternative for low or medium contamination soils and does not adversely affect soil fertility. A number of advantages and disadvantages of phytoremediation; compared to conventional remediation methods, are briefly addressed below (U.S. EPA, 2000, U.S. EPA, 2001).

Phytoremediation offers the following advantages:

1) It is more economically viable using the same tools and supplies as agriculture.
2) Phytoremediation has been perceived to be a more environmentally-friendly "green" and low-tech alternative to more active and intrusive remedial methods. As such, public acceptance could be greater.
3) Phytoremediation can be applied in situ to remediate shallow soil and ground water, and can be used in surface water bodies.
4) Phytoremediation does not have the destructive impact on soil fertility and structure that some more vigorous conventional technologies may have, such as acid extraction and soil washing.
5) Vegetation can also reduce or prevent erosion and fugitive dust emissions (US.EPA, 2001).

Phytoremediation has the following disadvantages:

1) A significant disadvantage of phytoremediation is the depth limitation due to the generally shallow distribution of plant roots.
2) A longer time period is likely to be required for phytoremediation, as this technology is dependent on plant growth rates for establishment of an extensive root system or significant above ground biomass.
3) Plant matter that is contaminated will require either proper disposal or an analysis of risk pathways. Harvesting and proper disposal is required for plant biomass

that accumulates heavy metals or radionuclides in phytoextraction and rhizofiltration, and may be necessary for other forms of phytoremediation if contaminants accumulate within the plant.

4) High initial contaminant concentrations can be phytotoxic, and prevent plant growth. Preliminary phytotoxicity studies are likely to be necessary to screen candidate plants.
5) Plant species or varieties of one species can vary significantly in their efficacy for phytoremediation. Site specific studies may always be necessary prior to implementation.
6) Cultivation of vegetation often requires great care due to stresses of climate and pests. Under the adverse conditions of contaminated soil or ground water, successful cultivation can be much more difficult.
7) Phytoremediation might require use of a greater land area than other remedial methods. This might interfere with other remediation or site activities.
8) Amendments and cultivation practices might have unintended consequences on contaminant mobility. Potential effects of soil amendments should be understood before their use (US. EPA, 2001).

2.3.6 Phytoextraction of metals

A review of the phytoremediation literature reveals that, at present, there are two basic strategies of phytoextraction being developed: chelate-assisted phytoextraction (Figure 2.2) which is termed *induced phytoextraction*; and long-term *continuous phytoextraction* (Figure 2.3). Of the two processes, chelate-assisted phytoextraction is the more developed and is presently being implemented commercially. Continuous phytoextraction is also being studied by several groups for the removal of metals such as zinc, cadmium, and nickel and oxianionic metals such as selenium, arsenic, and chromium. Field trials have been performed using both phytoextraction strategies. The results, though encouraging, suggest that further development of these technologies is needed (Table 2.4) (Salt et al., 1998).

Induced phytoextraction

The concept of chelate-assisted phytoextraction: early studies by Jøgensen (1993) showed that application of synthetic metal chelates such as ethylenediaminetetraacetic acid (EDTA) to soils enhances lead accumulation by plants. Huang et al. (1997, 1996) and Blaylock et al (1997) were able to achieve rapid accumulation of lead in shoots to greater than 1% of shoot dry biomass. These discoveries paved the way to successful phytoremediation of lead and to defining strategies for the development of phytoextraction of other toxic metals using appropriate chelates.

The total amount of metal removed from a site is a product of metal concentration in the harvested plant material and the total harvested biomass. The observation that high biomass crop plants including Indian mustard, corn, and sunflower could be "induced" to accumulate high concentrations of lead (Blaylock et.al.1997; Huang et.al.1997; Huang et.al.1996) was another advance in the development of chelate-assisted phytoextraction. The concept of chelate-assisted phytoextraction is applicable

to other metals in addition to lead (Blaylock et.al.1997). Previous researches demonstrated the simultaneous accumulation of lead, cadmium, copper, nickel, and zinc in Indian mustard plants after application of EDTA to soil contaminated with various heavy metals. Metal accumulation efficiency in these experiments was directly related to the affinity of the applied chelate for the metal. This suggests that for efficient phytoextraction synthetic chelates having a high affinity for the metal of interest should be used; for example, EDTA for lead, EGTA for cadmium (Blaylock et.al.1997) and possibly citrate for uranium.

Based on the above information, a hypothetical protocol for the chelateassisted phytoextraction of a contaminated site can be outlined (Figure 2.2). 1) The site is evaluated and the appropriate chelate/crop combination is determined. 2) The site is prepared and planted, and the crop is cultivated. 3) Once optimal biomass is produced, the appropriate metal chelate is applied. 4. After a short metal-accumulation phase (several days or weeks), the crop is harvested. Depending on the crop and the season, the site could be replanted for further phytoextraction. Estimates suggest that plants can remove between 180 and 530 kg/ha of lead per year (Blaylock et.al.1997; Huang et.al.1996), making remediation of sites contaminated with up to 2500 mg/kg lead possible in under 10 years. Following harvest, the weight and volume of contaminated material can be further reduced by ashing or composting. Metal-enriched plant residue can be disposed of as hazardous material or, if economically feasible, used for metal recovery

Development of chelate-assisted phytoextraction

Chelate-assisted phytoextraction consists of two basic processes release of bound metals into soil solution combined with transport of metals to the harvestable shoot. The role of chelates in increasing the soluble metal concentration in the soil solution can be explained using well-established equilibrium principles. However, the mechanisms involved in metal-chelate induced plant uptake and translocation of metals are not well understood (Blaylock et al., 1997). Following EDTA application, lead accumulation in shoots is directly correlated with an accumulation of EDTA. Thus, it is likely that lead is transported within the plant as a Pb-EDTA complex (Salt et al., 1998). The presence of high levels of EDTA in plant tissues should increase soluble lead concentrations within the plant by formation of soluble Pb-EDTA, allowing its movement from roots to shoots where lead would likely accumulate as Pb-EDTA.

Table 2.4 Examples of field trials for the phytoremediation of metals (Salt et al., 1998)

Metal	Plant	Location	Method[a]	Comments
Pb	*Brassica juncea*	Trenton, N.J.	PE-CA	EDTA-enhanced uptake over one cropping season resulted in a 28% reduction in the Pb contamination area
Cd PE-C	*Thlaspi caerulescens*	Beltsville, Md.		Phytoextraction of sludge-amended soils. Cd accumulation was similar in all three species. Zn accumulation in *T. caerulescens* was 10-fold higher then in other plants
Zn	*Silene vulgaris*			
Zn,	*Brassica oleracea*	Rothamstead	PE-C	Sludge-amended soil
Cd	*Raphanus sativus*	U.K.		
Ni	*Thlaspi caerulescens*			
Cu	*Alyssum lesbiacum*			
Pb	*Alyssum murale*			
Cr	*Arabidopsis thaliana*			
Se	*Brassica juncea*	Los Ba˜nos,	PE-C	
B	*Festuca arundinacea*	Calif.	PV	was reduced between
	Hibiscus cannibus			24–52% and total Se
	Lotus corniculatus			reduced between 13–48% by all species
U	*Helianthus annus*	Asthabula, Ohio	RF	Removal of U from ground water[b]

[a]Method of phytoremediation: PE, phytoextraction; PV, phytovolatilization; RF, rhizofiltration; CA, chelateassisted phytoextraction; C, continuous phytoextraction.
[b]Phytotech Inc., personal communication.

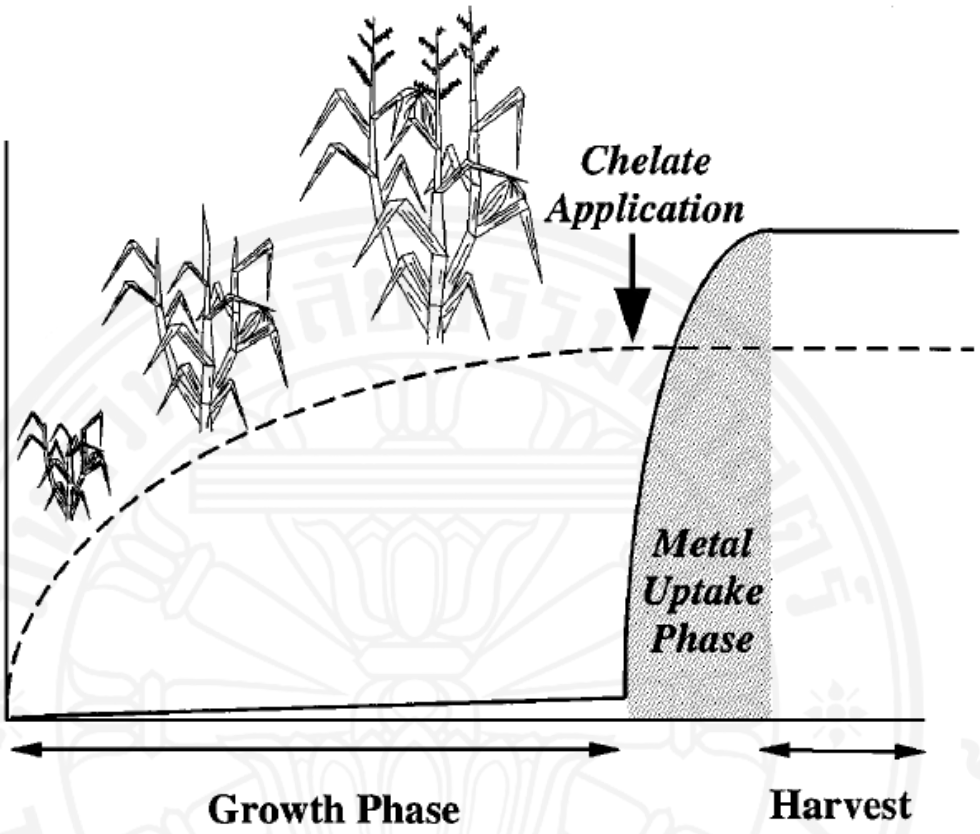


Figure 2.2 Schematic representation of chelate-assisted phytoextraction. *Solid line represents metal concentration in shoot biomass; dashed line represents shoot biomass* (Salt et al., 1998).

Continuous phytoextraction

An alternative approach to chelate-assisted metal accumulation is the reliance on the specialized physiological processes that allow plants to accumulate metals over the complete growth cycle. This type of metal uptake is epitomized by hyperaccumulating plants that grow on soils rich in heavy metals. These plants are naturally able to accumulate >1% of shoot dry biomass as Zn, Ni, Mn, or Se. Unlike induced metal uptake, continuous phytoextraction is based on the genetic and physiological capacity of specialized plants to accumulate, translocate, and resist high amounts of metals. Major disadvantages of using naturally occurring metal hyperaccumulators for continuous phytoextraction are their relatively low biomass, slow growth rates, and the lack of any hyperaccumulators for the most environmentally important metallic pollutants (e.g. lead, cadmium, arsenic, and uranium). However, understanding the biological mechanisms of hyperaccumulation may help in the development of superior plants for the phytoremediation of metals (Salt et al., 1998).

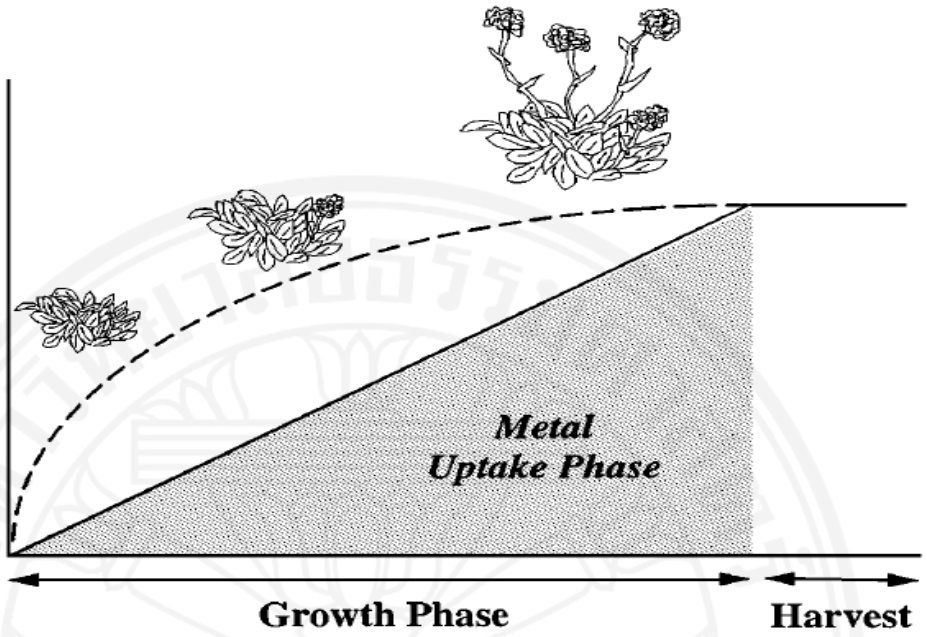


Figure 2.3 Schematic representation of continuous phytoextraction. *Solid line represents metal concentration in shoot biomass; dashed line represents shoot biomass* (Salt et al., 1998)

2.3.7 Mechanism of phytoextraction

The metal must mobilize into the soil solution, for the plants to uptake metals from soil. The bioavailability of metals in soil is enhanced via several means. Both acidification of the rhizosphere and exudation of carboxylates are considered potential targets for increasing accumulation of targeted metals in plants (Salt et al., 1998). Following mobilization, a metal has to be captured by root cells. Metals are first bound by the cell wall; it is an ion exchanger of comparatively low affinity and low selectivity. Uptake of metal ions is likely to take place through secondary transporters such as channel proteins and/or H^+- coupled carrier proteins. Once inside the plant, most metals are too insoluble to mobile freely in the vascular system, so they usually form carbonate, sulphate or phosphate precipitates immobilizing them in apoplastic (extracellular) and symplastic (intra cellular) compartments. Unless the metal ion is transported as a non-cationic metal chelate, apoplastic transport is further limited by the high cation exchange capacity of cell walls. The apoplast continuum of the root epidermis and cortex is readily permeable for solutes. Apoplastic pathway is relatively unregulated, because water and dissolved substance can flow and diffuse without having to cross a membrane. (Blaylock et al., 1997).

Generally, solutes have to be taken up into the root symplasm before they can enter the xylem. Subsequent to metal uptake into the root symplasm, three processes facilitate the movement of metals from the root into the xylem: sequestration of

metals inside root cells, symplastic transport into the stele and release into the xylem. The transport of ions into the xylem is generally a tightly controlled process mediated by membrane transport proteins. Symplastic transport of heavy metals probably takes place in the xylem after they cross the casparian strip (Blaylock et al., 1997). Most metal ions enter plant cells by an energy dependent saturable process via specific or generic metal ion carriers or channels. Non-essential heavy metals may effectively compete for the same transmembrane carriers used by essential heavy metals. Toxic heavy metals such as cadmium may effectively compete for the same transmembranic carrier as used by micronutrient heavy metal. This relative lack of selectivity in transmembrane ion transport may partially explain why non-essential heavy metals can enter cells, even against a concentration gradient. For example, kinetic data demonstrate that essential Cu^{2+} and Zn^{2+} and nonessential Ni^{2+} and Cd^{2+} compete for the same transmembrane carrier (Crowley et.al., 1991). After heavy metals have accessed to the root they are either stored in the root or translocated to the shoots. Metal ions can be actively transported across the tonoplast as free ions or as metal-chelate complexes. It is believed that in order to pass through the casparian strip, water and dissolved ions (salt and metal) require active transport, by utilizing energy (Ghosh et al., 2005). For example, Cd is actively transported across the tonoplast of oat roots as either a free ion via a Cd/H^{+} antiport. The vacuole is an important component of the metal ion storage where they are often chelated either by organic acid or phytochelatins. Insoluble precipitates may form under certain conditions. Precipitation compartmentalisation and chelating are the most likely major events that take place in resisting the damaging effects of metals. Transporters mediate uptake into the symplast, and distribution with in the leaf occurs via the apoplast or the symplast. Plants transpire water to move nutrients from the soil solution to leaves and stems, where photosynthesis occurs. Willows, hybrid poplar are also good phytoremediators, because they take up and process large volumes of soil water. For example, data show that a single willow tree, on a hot summer day, can transpire more than 19,000 liters of water (Ghosh et al., 2005).

2.3.8 Enhancing the phytoextraction process

Increasing metal availability in soil

A major factor influencing the efficiency of phytoextraction is the plants' capability to absorb large amount of metals in a short period. Hyperaccumulators accumulate large quantities of metal in their tissue regardless of the metal concentration in the soil**.** This property is unlike moderate accumulators now being used for phytoextraction where the quantity of absorbed metal is a reflection of the concentration in the soil Alloway (1995). Although the total soil metal concentration may be high, it is the fraction that is easily available in the soil solution determining the performance of metal absorption by roots of the plants. To increase the speed and quantity of metal removal by plants, some researchers advocate the use of various chemicals for enhancing the quantity of available metal for plant uptake. Chemicals suggested for this purpose include different fertilizer salts, acidifying agents, and chelating agents**.** These chemicals enhance the amount of bioavailable metal in the soil solution by liberating

or displacing metal from the solid phase of the soil or by making precipitated metal species more soluble (Crowley et al., 1991).

Soil pH is a major factor affecting the phyto-availability of elements in the soil for plant uptake. Under acidic conditions, H^+ ions displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from sesquioxides and variable-charged clays to which they have been chemisorbed (i.e. specific adsorption). The retention of metals to soil organic matter is also weaker at low pH, resulting in more available metal in the soil solution for root absorption. Many metal cations are more soluble and available in the soil solution at low pH (below 5.5) including Cd, Cu, Hg, Ni, Pb, and Zn. It is advised that phytoextraction process is improved while availability of metal to plant roots in enhanced via application of the acidifying agent to the soil (Salt et al., 199; Huang et al. (1998)).
Previous researches stated that plant roots acidify hydroponic solutions in response to NH_4 nutrition and cause solutions to become more alkaline in response to NO_3 nutrition. In addition, the acidification of soil with elemental Sulphur (S) can be used to mobilize metal cations in soil. Brown et al. (1994) acidified a Cd- and Zn-contaminated soil with elemental S and observed that accumulation of these metals by plants was greater than when the amendment was not used. Huang et al. (1998) reported that the addition of citric acid increases uranium (U) accumulation in Indian mustard (*B. juncea*) tissues more than nitric or sulfuric acid although all acids decrease soil pH by the same amount.

Enhancing metal bioavailability with synthetic chelators

As known, phytoextraction, the use of plants to extract contaminants from soils has provided great potential, but is hindered by the truth that plants need nutrient and time and have a limited metal uptake capability. Synthetic chelators, such as EDTA, have shown potential effects in increasing heavy metal extraction via phytoremediation (Blaylock 2000).

Salt et al. (1998) stated that in the process of acquiring metal ions from soil, plants have associated with several strategies for enhancing the metal phyto-availability since the high binding ability for metallic micronutrients by soil particles. The first strategy is the plants' capability to produce metal-chelating compounds (phytosiderophores) to mobilize metal compounds from soil. The second approach involves the solubilization of metals by exuduing protons from roots to acidify the rhizosphere soil (Crowley et al., 1991). Alloway (1995) also discussed that the roots possess a significant cation exchange capacity (CEC) since the presence of carbozyl groups, which might help to move ions through the outer part of the root to the plasma lemma where active absorption occurs.

For some toxic metals such as Pb, a major factor limiting the potential for phytoextraction is limited solubility and bioavailability for uptake into roots. One way to induce Pb solubility is to decrease soil pH. Following soil acidification, however, mobilized Pb can leach rapidly below the root zone. In addition, soluble ionic lead has little propensity for uptake into roots (Blaylock et al., 1997). The use of specific

chemicals, synthetic chelates, has been shown to dramatically stimulate the potential for Pb accumulation in plants. These compounds prevent Pb precipitation and keep the metal as soluble chelate-Pb complexes available for uptake into roots and transport within plants. For example, addition of EDTA (ethylene-diamine-tetraacetic acid), at a rate of 10 mmol/kg soil, increased Pb accumulation in shoots of maize up to 1.6wt% of dry biomass (Blaylock et al., 1997).

In addition to natural plant adaptation, the addition of synthetic chelators, soil acidifiers, or commercial nutrients can enhance phytoremediation. Several studies have documented the success of pH adjustments for mobilizing metals (Salt et al., 1998; Chaney et al., 1997; Huang et al., 1997). Although acidification of soil increased metal mobility, it decreased the microbial activity of the surrounding area (Salt et al., 1998). Only the addition of synthetic chelators has been shown to increase both the metal mobility within the soil and the uptake (and translocation) through the plant tissue without being irreversibly toxic to microbial activity. For instance, Cunningham (1995) tested N-(2-hydroxyethyl)-ethylenediaminetriacetic acid (HEDTA); applied at 2.0 g/kg soil contaminated with 2,500 ppm Pb; on Pb accumulation enhancement and found that one week after transplanting, the shoot Pb concentration of Indian mustard was increased from 40 to 10,600 mg/kg. In addition to shoot concentration, the shoot to root Pb content was increased from 0.2 to 1.2. Blaylock et al. (1997) indicated that, in addition to Pb, chelate-assisted phytoextraction is applicable to other metals. These authors indicated that application of EDTA also stimulated Cd, Cu, Ni, and Zn phytoaccumulation. Chelate ability to facilitate phytoextraction was shown to be directly related to its affinity for metals. For example, EGTA (ethylenebis (oxyethylenetrinitrilo) tetraacetic acid) has a high affinity for Cd^{2+}, but does not bind Zn^{2+}. EDTA, HEDTA, and DTPA (diethylene-triamine-pentaacetic acid) are selective for Zn. In fact, zinc binding by DTPA is so strong that plants cannot use Zn from this complex and potentially suffer from Zn deficiency. Huang et al. (1997) further reported that among the five chelators, Ethylenediaminetriacetic acid (EDTA) was the most efficient in increasing shoot Pb concentration in both pea and corn, followed by HEDTA. They found the order of the effectiveness in increasing Pb accumulation to be EDTA>HEDTA> (DTPA)> ethylenediaminedinitrilodiac acid (EDDTA).

The addition of chelating materials to soil, such as EDTA, HEDTA, and EDDHA, is the most effective and controversial means of liberating labile metal-contaminants in to the soil solution. Chelates complex the free metal ion in solution, allowing further dissolution of the sorbed or precipitated phases until equilibrium is reached between the complexed metal, free metal, and insoluble metal fraction. Chelates are used to enhance the phytoextraction of a number of metal contaminants including Cd, Cu, Ni, Pb, and Zn **(**Blaylock et al., 1997; Huang et al., 1997)**.** Huang et al. (1997) suggested that chelates are able to induce Pb accumulation in agronomic crops such as corn (*Zea mays* L.) and pea (*Pisum sativum* L.). These authors reported a 1000-fold increase of Pb in the soil solution after HEDTA application in comparison to soil solution of a control (no HEDTA addition). Under these conditions, Pb concentrations in the shoots of corn and pea increases from less than 500 mg/kg to more than 10,000 mg/kg within one week after HEDTA application. This chelate-assisted accumulation of toxic

quantities of metal in a non-accumulator species is termed chelate-induced hyperaccumulation (Huang et al., 1997). These researchers explained that when chelate-induced hyperaccumulation is the goal; metals on site are initially immobilized to allow for rapid establishment and growth of an agronomic crop such as corn. When the crop accumulates sufficient biomass, chelating materials are applied to the soil to result in the liberation of large quantities of metal into the soil solution. Massive amounts of metal are absorbed by plant roots and are translocated to the shoot tissue where they accumulate to toxic levels. After death, plants are harvested and removed from the site. Chelate-induced hyperaccumulation is in contrast to the normal practice of phytoextraction where plants are given a gradual exposure to non-toxic quantities of metal in solution, and accumulation occurs gradually over time as the plants grow. The controversy surrounding the use of chelates deals with the fate of the residual chelate in the soil after metal absorption occurs. The massive liberation of chelate-bound metals into the soil solution makes them subject to leaching in to deeper soil layers. Metals which migrate downward beyond the root zone of plants cannot be recovered through means of phytoremediation and may require the use of more expensive conventional remediation methods. The primary concern is that the liberated metals have the ability to migrate in to uncontaminated areas, possibly groundwater reservoirs**.** The scientific literature lacks appreciable information concerning the appropriate amount of chelate to apply under different levels of contamination and for different plant species.

Proper plant selection

As a plant-based process, the success of phytoextraction is rely on proper plant selection. Plants used for phytoextraction must be fast growing and have the capability to accumulate large quantities of important metal contaminants in their above-ground tissue (Blaylock et al., 1997). Many plant species have been screened to determine their performance for phytoextraction. Researchers initially used hyperaccumulators to clean metal polluted soils. Currently, there are almost four hundred known hyperaccumulators, but most are not appropriate for phytoextraction because of their slow growing and small size. Several researchers have screened fast-growing, high-biomass-accumulating plants, including agronomic crops, for their capability to resist and accumulate metals in their shoots (Blaylock et al., 1997; Dushenkov et al., 1995). Many metal-resistant plants, particularly grasses, avoid toxicity via an exclusion mechanism and are therefore better suitable for phytostabilization than phytoextraction. However, barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) are tolerant of metals such as Cu, Cd, and Zn, and accumulate moderate to high amounts of these metals in their tissues. Many herbaceous species, including members of the Brassicaceae, also accumulate moderate amounts of various metals in their shoots. One of the most promising, and perhaps most studied, non-hyperaccumulator plant for the extraction of heavy metals from contaminated sites is Indian Mustard (*B. juncea*) (Blaylock et al., 1997).

Many hyperaccumulators belong to the Brassica family. Once it was suspected that known hyperaccumulators were not suitable for phytoextraction, researchers looked to other high biomass-accumulating members of the Brassicaceae for plants which

accumulated large quantities of toxic metals (Dushenkov et al., 1995; Kumar et al., 1995). Kumar et al. (1995) examined many fast growing Brassicas for their ability to resist and accumulate metals, including Indian mustard (*B. juncea*), black mustard (*Brassica nigra* Koch), turnip (*Brassica campestris* L.), rape (*Brassica napus* L.), and kale (*Brassica oleracea* L). Although all Brassicas accumulated metal, *B. juncea* showed a strong ability to accumulate and translocate Cu, Cr VI, Cd, Ni, Pb, and Zn to the shoots. Kumar et al. (1995) also investigated possible genetic variation of different *B. juncea* accessions in hope of finding some that had more phytoextraction potential than others.

Soil fertilization and conditioning

Phytoremediation is an agronomic process and its success rely on agronomic practices applied at the site. The significance of using effective agronomic practices has been reported by some researchers. They examined the impact of soil acidification on Zn and Cd phytoextraction and proposed the use of $(NH_4)_2SO_4$ as a soil additive to provide nutrients (N and S) needed for high yield, and to acidify the soil for greater metal bioavailability. However, there might be some adverse side impacts related to soil acidification such as some toxic metal may be released into the groundwater, creating an additional environmental risk, since the increase of solubility of metal under acidic condition. The previous study indicated that following metal phytoextraction, soil can be limed to elevate the pH near a neutral value, so that normal farm uses or ecosystem development could be retained. However, premature liming may increase soil capacity for metal binding and limit the potential for phytoextraction (Dushenkov et al., 1995). A similar effect can be expected following the addition of organic fertilizers. In addition, the increasing of soil pH may stimulate the formation of metal hydroxy ions, such as $ZnOH^+$, which is more strongly sorbed to soil solids than the uncomplexed ions. Phosphorus (P) is a major nutrient, leading the biomass production of plants. The addition of P fertilizer, however, can also inhibit the uptake of some major metal contaminants, such as Pb, due to metal precipitation as pyromorphite and chloropyromorphite. This underlines the importance of finding new approaches for P application. Such an alternative may be foliage application. This method may lead to improvement of plant P status without inhibiting Pb mobility in soil (Lasat, 2000).

Sowing

The amount of metal extraction relies on the mass of plant biomass produced. An important factor controlling biomass production is plant density (number of plants/m^2). Density affects both yield/plant and yield/ha. In general, higher density tends to reduce yield per plant and maximize yield per hectare. Density is also likely to affect the pattern of plant growth and development. For example, at higher stand density, plants will compete more strongly for light. Thus, more resources (nutrients and energy) may be allocated for plant growth. An extended growth period may be beneficial if plant metal absorption and accumulation depend on growth processes. Furthermore, the distance between plants is likely to influence the architecture of the

root system with possible further implications on metal uptake. However, the effect of this interaction is unknown and awaits investigation (Lasat, 2000).

2.4 Total and Bioavailability of Heavy Metal in Soil

Trace amount of some heavy metals are required by living organisms, however any excess amount of these metals can be dangerous to the organisms. Nonessential heavy metals include cadmium, chromium, mercury, and lead. These metals are of great concern to soil contamination and water pollution. Heavy metals exist in colloidal, ionic, particulate and dissolved phase and have a high affinity for humic acids, organic clays, and oxides coated with organic matter. The soluble forms are generally ions or unionized organometallic chelates or complexes. The solubility of metals in groundwater and soil is controlled by pH level, organic carbon, cation exchange capacity, the oxidation state of the mineral components, and the redox potential of the system (Nobuntou, 2012).

In general, soil pH seems to have the greatest impact of any single factor on the solubility of metals in soils with a greater retention and lower solubility of metal cations occurring at high soil pH (Basta et al., 1993). Under the neutral to basic conditions typical of most soils, cationic metals are strongly captured on the clay fractions and can be adsorbed by hydrous oxides of iron, aluminium, or manganese present in soil minerals. Elevated salt concentration creates increased competition between cations and metals for binding sites. Also competitive adsorption between various metals has been observed in experiments involving various solids with oxide surfaces, in several experiments, Cd adsorption was decreased by the addition of Pb or Cu.

It is generally recognized that chemical extraction is used to assess metal fractions, which can be related to chemical species, and to potentially mobile, bioavailability or ecotoxic phases of polluted soil. The potential hazards associated with Cd are not so much related to its total concentration in the soil, but rather to its potential for entry into the food chain. To understand the risks associate with Cd uptake by plant and subsequent entry into food chain it is important to know how the cadmium is chemically distributed in the soil (Lenna and Gade, 1997). To assess the potential environmental impacts of metals contaminated soil, only total metals concentration in the soil is insufficient to understand the particular behavior of trace metals. Particular behaviors of metals, soluble in soil matrix, and their mobility capacity can be determined by their specific physiochemical forms rather than by their total concentration (Gupta, 1996).

The transport of ions within plant tissues and organs involves many processes: (1) movement in xylem, (2) movement in phloem, (3) storage, accumulation, and immobilization. The chelating ligands are most important in the control of cation translocation in plants. However, numerous other factors such as pH, the oxidation–reduction state, competing cations, hydrolysis, polymerization, and formation of insoluble salts (e.g., phosphate, oxalate, etc.) govern metal mobility within plant

tissues. The complexation of cations with organic acids (e.g., citric, malic, and amino acids) prevents their immo- bilization in the xylem and allows their transfer to the shoots. The immobilization of metals in roots due to various processes has a dominating impact on their translocation to the aboveground parts. The transport of trace elements among plant organs also depends on the electrochemical variables of elements. In general, easily transported from roots to aboveground parts are Ag, B, Li, Mo, and Se; moderately mobile are Mn, Ni, Cd, and Zn; and strongly bound in root cells are Co, Cu, Cr, Pb (Horiguchi et al. (1981).

2.5 Plant Response to Heavy Metals

Plants have three basic strategies for growth on metal contaminated soil as shown in Figure 2.4.

Metal excluders: They prevent metal from entering their aerial parts or maintain low and constant metal concentration over a broad range of metal concentration in soil, they mainly restrict metal in their roots. The plant may alter its membrane permeability, change metal binding capacity of cell walls or exude more chelating substances.

Metal indicators: Species which actively accumulate metal in their aerial tissues and generally reflect metal level in the soil. They tolerate the existing concentration level of metals by producing intracellular metal binding compounds (chelators), or alter metal compartmentalization pattern by storing metals in non-sensitive parts.

Metal accumulator plant species: They can concentrate metal in their aerial parts, to levels far exceeding than soil. Hyperaccumulators are plants that can absorb high levels of contaminants concentrated either in their roots, shoots and/or leaves. Baker and Brooks (1989) have defined metal hyperaccumulator as plants that contain more than or up to 0.1% i.e. more than (1000 mg/g) of copper, cadmium, chromium, lead, nickel cobalt or 1% (>10,000 mg/g) of zinc or manganese in the dry matter. For cadmium and other rare metals, it is > 0.01% by dry weight. Researchers (Baker and Brook, 1989; Ghosh et al., 2005) have identified hyperaccumulator species by collecting plants from the areas where soil contains greater than usual amount of metals as in case of polluted areas or geographically rich in a particular element. Approximately 400 hyperaccumulator species from 22 families have been identified. The *Brassicaceae* family contains a large number of hyper accumulating species with widest range of metals; these include 87 species from 11 genera (Ghosh et al., 2005; Marmara et al., 2001).

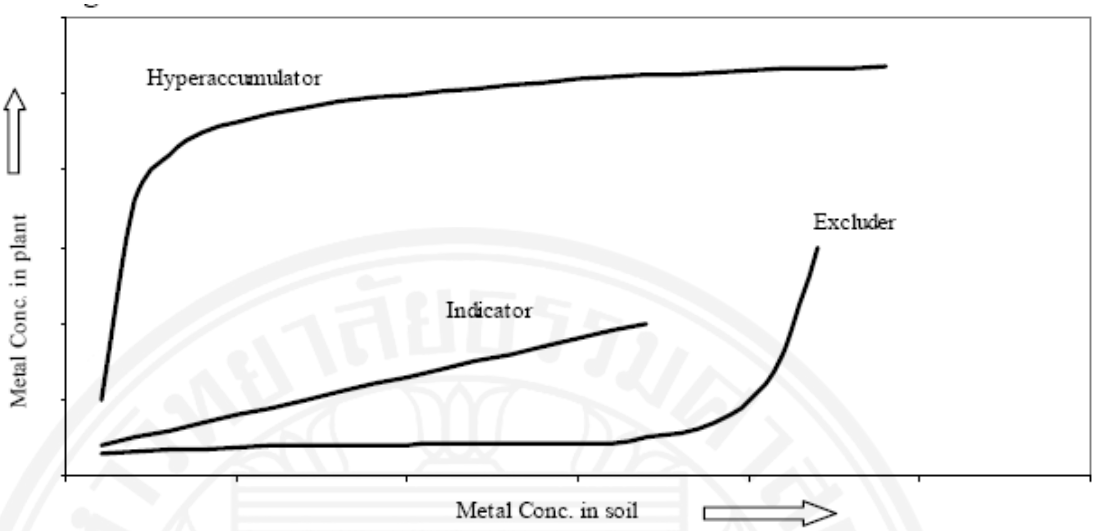


Figure 2.4 Conceptual response strategies of metal concentrations in plant tops in relation to increasing total concentrations in the soils (Ghosh, 2005).

2.6 Disposal of Harvested Plants

Several methods of contaminated crop disposal are described by different authors (Kumar et al., 1995; Salt et al., 1995, 1998; Raskin et al., 1997; Blaylock et al., 1997; Dushenkov et al., 1997; Flathman and Lanza., 1998; Blaylock and Huang, 2000; Cunningham et al., 1995; Alkorta and Garbisu, 2001. Figure2.5 shows the techniques of plant processing that have been used to phytoremediate heavy metal-contaminated sites. The composting, compaction and pyrolysis are treated as pretreatment steps, because considerable amount of contaminated material will still exist after each of the process.

2.6.1 Pretreatment-reducing volume of plant material

After successful phytoextraction, it is important to reduce the crop volume (Salt et al., 1995; Blaylock and Huang, 2000) and to remove excess water. This improves the technical parameters and reduces the cost of transportation to the treatment or disposal site. Volume reduction of contaminated plant biomass can be achieved by composting, compaction or pyrolysis. Comparison of pretreatment steps is shown in Table2.5.

Composting

Composting has been proposed as a post-harvest biomass treatment by some authors (Kumar et al., 1995; Salt et al., 1995, 1998; Raskin et al., 1997; Blaylock and Huang, 2000; Alkorta and Garbisu, 2001). Hetland et al. (2001) carried out laboratory experiments with lead-contaminated plant material (small sunflowers, grasses) obtained after induced phytoextraction. The disintegrated biomass (particles less than 0.16 cm in diameter) was composted in 125 ml borosilicate bottles with constant aeration for two months.

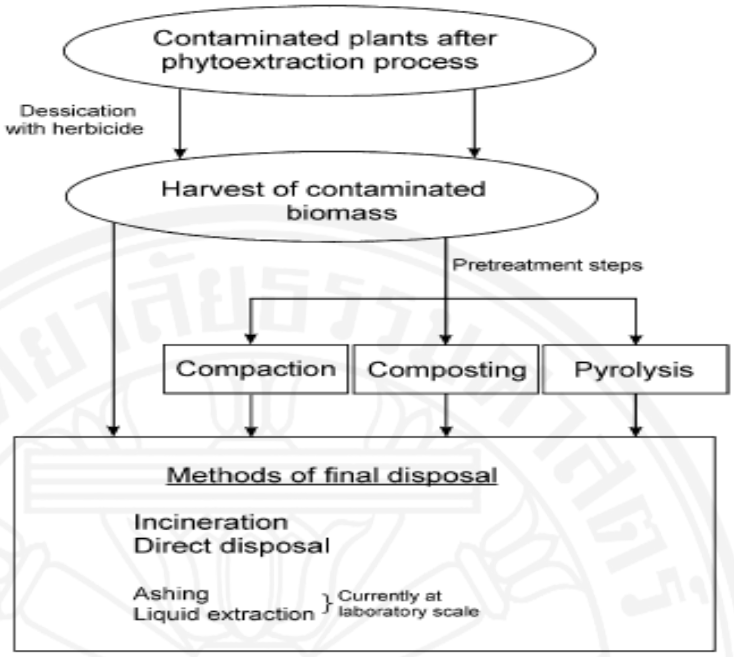


Figure 2.5 The most commonly proposed techniques of phytoextraction crop disposal (Sas-Nowosielska et al., 2004)

Total dry weight loss was about 25%. Leaching tests of the composted material showed, however, that the composting process formed soluble organic compounds that enhanced lead solubility. These results documented that composting can significantly reduce the volume of harvested biomass, however lead-contaminated plant biomass would still require treatment prior to disposal (Raskin et al., 1997; Blaylock and Huang, 2000).

EDTA is commonly used as a chelating agent for induced phytoextraction (Blaylock et al., 1997; Salt et al., 1998; Epstein et al., 1999). Vassil et al. (1998) reported that a complex of Pb-EDTA is taken up by Indian mustard plants and accumulated in the shoots. Recently, Sarret et al. (2001) have documented that metal-EDTA complexes taken up by Phaseolus vulgaris and accumulated in shoots can be totally dissociated in the case of zinc and only partly dissociated in the case of lead. These results suggest that plant biomass harvested after induced phytoextraction can contain very mobile and leachable metal–chelate complexes. Moreover Perronet et al. (2000) and Zhao et al. (2000) also showed that most of zinc within the leaves of hyperaccumulators also is present in water-soluble forms. This means that composting process should be conducted carefully in order to avoid non-desirable leachates regardless of the type of phytoextraction used. It is necessary to emphasize that the purpose of composting is to reduce the volume and weight of plant material, with no consideration to the agricultural properties of the final product. Further investigations are needed to assess the effect of the presence of chelating agents in harvested plant material, in conjunction with metals, on composting process. Furthermore, there is no information on the stability or transformation of metal–EDTA complexes during the process.

Table 2.5 Comparison of pretreatment methods (Sas-Nowosielska et al., 2004)

Process	Costs of transportation[a] in situ—no costs (€/t/km)	Costs of site preparation (€/m^2)	Costs of leachate utilization (€/t)	Costs of processing (€/t)	Advantages	Disadvantages
Composting	1-2	3-5	-	10-25	Volume and water content reduction	Time consuming (2–3 month). Special equipment is required. End-product as hazardous waste
Compaction	1–2	3–5	135	Not available	Volume reduction Recovery of metals	Special equipment is required. End-products as hazardous waste (remaining biomass, leachates)
Pyrolysis	1–2	-	-	20–30	Significant volume reduction Useful end-product (pyrolytic gas)	End-product as hazardous waste (coke breeze

Compaction

Compaction of harvested plant material was proposed by Salt et al. (1995) and Blaylock and Huang (2000) for processing metal-rich phytoextraction residue. The process of compaction uses a container equipped with a press and a leachate collection system. As discussed previously, the leachate produced by pressing contaminated plant biomass will contain high concentrations of heavy metals complexed with chelators (induced phytoextraction) or nickel, chromium and/or zinc (continuous phytoextraction) in soluble and bioavailable forms (Perronet et al., 2000; Zhao et al., 2000; Sarret et al., 2001). The leachate will need to be collected and treated appropriately. Advantages of compaction are similar as for composting.

Compaction of the same amount of biomass should be even shorter than composting, depending on efficiency of equipment (e.g. volume of container). In contrary to composting, however, there is little information on compaction. End-products of compaction (remaining contaminated biomass, leachates) should be treated as hazardous material. Research should be conducted to assess how volume and weight of fresh biomass is reduced by compaction depending on plant species. Composition and concentration of heavy metals in the leachate produced by pressing should be investigated, as well as method of recycling of metals from solutions. An experiment is also described (Sas-Nowosielska et al., 2004) where plant material was dried, and the contaminated effluents were captured in a mixture of power station fly ashes and

sawdust, to be subsequently transported to the incineration facility. The material became hard and robust and did not create any harm during handling and transporting.

Pyrolysis

Pyrolysis (Bridgwater et al., 1999) is a novel method of municipal waste treatment that might also be used for contaminated plant material. Pyrolysis decomposes material under anaerobic conditions and moderate temperatures. The capacity of a standard municipal unit is 40 tons per day, with unit costs 20–30 €/t (Table2.5). There is no emission to the air, as the process is completely hermetic. The final products are pyrolytic gas and coke breeze. Heavy metals form contaminated biomass will be contained in coke breeze. It means that this product should be treated as hazardous waste and dispose at hazardous waste dumping site. On the other hand, coke breeze could be used in a lead/zinc smelter instead of coke, and then lead or zinc might be recovered during smelting process.

The limitations in the pyrolysis of plant material would be the maximum moisture limit (30%) and the very high costs of installation and operation if used solely for plant disposal. Moisture limits might be moderated by preharvest treatment with a desiccant (e.g. glyphosate or another herbicide) or mixing together with other dryer materials. To avoid high costs connected with installation and operation, the plant material can be processed in existing facilities together with municipal wastes. That will also allow controlling the problem of excess moisture of plant material by adequate mixing with dry municipal wastes.

2.6.2. Incineration (smelting) of contaminated plant material in a lead/zinc smelter or an incineration plant

Contaminated plant material can be incinerated in a lead/zinc smelter using rotary kiln technology (e.g. the Welz process), which may tolerate the heterogeneous material resulting from phytoextraction. The process destroys organic matter, releasing metals, mainly as oxides. The liberated metals are entrained in the slag or released to the effluent gases. Modern flue gas cleaning technology assures effective capture of the metal-containing dust. Plant material also can be incinerated in an incineration plant. Cost of incineration of 1 t of hazardous material ranges from €/t 180 to 220 €/t (Table 2.6) (Sas-Nowosielska et al., 2004).

To diminish the amount of plant material to be transported to a smelter, desiccation can be conducted. Preharvest desiccation can be accomplished by treating plant shoots with herbicide such as glyphosate (Ellis et al., 1998; Bennett and Shaw, 2000). This treatment also reduces the likelihood of leachate production from the plant material during harvest and transport. Additional risks connected with the use of glyphosate are negligible, because it is commonly used herbicide, quickly degraded in soil with very low toxicity to soil organisms. In addition, concentration used for desiccation can be lower than concentration recommended by manufacturer. An advantage of this method is reducing by more than 90% of total dry weight of contaminated biomass. Considering safe, new technologies used in incineration plants and lead/zinc smelters,

this method of disposal is environmental-friendly. Part of lead and zinc might be recovered during incineration in lead/zinc smelter (Sas-Nowosielska et al., 2004).

Table 2.6 Comparison of methods of contaminated crop disposal (Sas-Nowosielska et al., 2004)

Process	Costs of transportationa (€/t/km)	Costs of processing (€/t)	Advantages	Disadvantages
Incineration	1–2	180–220	Recovery of metals Significant reduction of biomass	None
Direct disposal at a hazardous waste site	1–2	135–1136	Time effectiveness	High costs. Limitation of dumping sites. Trend towards incineration. Slow reduction of contaminated biomass
Ashing	1–2	Not available	Recovery of metals. Significant reduction of biomass	No technology
Liquid extraction	1-2	Not available	Recovery of metals	No technology

2.6.3. Ashing of harvested biomass

The weight and volume of harvested plant material also can be reduced by ashing. This manner of contaminated crop disposal is often mentioned (Kumar et al., 1995; Salt et al., 1995b, 1998; Raskin et al., 1997; Blaylock et al., 1997) but there is no data on its application. Hetland et al. (2001) investigated the possibility of co-firing plant biomass with sub-bituminous coal in a down-fired combustion system designed for laboratory experiments. They showed that co-firing plant material with coal reduced the mass of lead-contaminated plant material by over 90% and partitioned lead into the ash.

These results suggest that ashing could be a viable method of biomass reduction, but more data on combustion systems for field scale experiments and ash disposal is necessary. It may be possible to recycle the metal residue from the ash, however there are no estimates of the cost or feasibility of such a process (Dushenkov et al., 1997; Cunningham and Berti, 2000; Garbisu and Alkorta, 2001).

2.6.4. Direct disposal

Direct disposal as hazardous waste is the least complicated approach to disposal of contaminated crop material and may ultimately be the most practical. However, it is less preferable than the above-described methods. An advantage of this method is its time effectiveness. However disadvantages are more numerous. Deposition at a hazardous waste site is expensive and can reach above 1000 €/t (Table2.6). Moreover significant amounts of contaminated material will remain in the environment if deposition of contaminated plant material at hazardous waste is chosen. In addition, there is a trend towards incineration of hazardous wastes instead of deposition at dumping sites, and number of hazardous waste disposal facilities will diminish in the future (Raskin et al., 1997; (Sas-Nowosielska et al., 2004).

2.6.5. Liquid extraction

The use of leaching to extract heavy metal from harvested biomass has been described (Salt et al., 1995). Hetland et al. (2001) evaluated chelation extraction as a technique for the recovery of lead from harvested biomass. They examined two chelating agents: EDTA and N-(2-acetamido) iminodiacetic acid (ADA). They observed that at a pH of 4.5 and a 1:4.76 molar ratio of lead to EDTA, it is possible to extract 98.5% of the lead present in the biomass using two sequential batch extractions. In their opinion, this technique would be very attractive if lead could be efficiently and cost-effectively separated from the chelating agent and the chelating agent could then be recycled (Hetland et al., 2001).

The residual biomass solids would not need to be disposed of as hazardous waste, because based on data from Hetland et al. (2001) it can be calculated that plant material with 2000 mg/kg lead will have after extraction only 30 mg/kg lead in dry weight. Such materials can be disposed of as municipal wastes. Although several technologies exist which can remove metals from the solutions, a production-scale process for this type of metal recovery and recycling has yet to be demonstrated (Mulligan et al., 2001). A very thorough cost–benefit analysis should be prepared, as the cost of that type of operations could be prohibitory high.

2.7 Experimental Plants

2.7.1 Guinea grass

Guinea grass (*Panicum maximum* or Buffalo grass) is native of Africa. It was introduced to almost all tropical countries as a source of animal feed. Currently, its seeds are still sold commercially for this purpose. Their leaf is soft and contains high levels of protein (13-21%). It is an ideal forage plant as it grows well on a wide variety of soils and even under light shade of trees and bushes (and thus can be grown with other crops). It can survive long dry spells and quick-moving fires which does not harm the underground roots. It also responds quickly to fertilizer and watering. It grows from sea level up to 1,200m. The seeds are dispersed by birds. Its seeds provide food for birds such as *Munias*, and the long leaves provide nesting material for birds

like the *Baya Weaver* (*Ploceus philippinus*). They also provide shelter for smaller creatures to hide in. On the one hand, Guinea Grass is considered as a suitable plant to stop soil erosion on slopes (it has dense root mats) while providing valuable fodder. On the other hand, it is considered a dangerous exotic weed that suppresses or displaces local plants. Its resistance to drought also means it builds up a dangerous mass of plant material so when fires occur, the blaze is fiercer and native plants which have not built up fire-tolerance are wiped out. As Guinea grass can survive fires, it dominates the ground after a fire (Wisumperuma, 2007; The State of Queensland, 2014).

2.7.2 Sunflower

The sunflower (*Helianthus annuu L.*) is an annual plant in the family Asteraceae and native to the Americas, with a large flowering head (inflorescence). The stem can grow as high as 3 meters (9 3/4 ft), and the flower head can reach 30 cm (11.8 in) in diameter with the "large" seeds. The term "sunflower" is also used to refer to all plants of the genus *Helianthus*, many of which are perennial plants. In addition, sunflower is variable, erect, often unbranched, fast-growing annual plant; stems 0.7-3.5 m tall, hirsute; leaves alternate, ovate, long-petroled, lamina with 3 main veins, 10-30 cm long,5-20 cm wide, apex acute or acuminate, lower leaves a lag of 120 behind the sun's azimuth. This property has been shown to increase light interception and possibly photosynthesis. The dominant characteristic of sunflower is that the flowering heads track the sun's movement, a phenomenon known as heliptropism. Most new varieties have heads that will drop to face the ground as the plants mature which has potential of reducing bird damage and reducing some disease development that would be occurred if water collected in the seed head (Boonyapookana, 2004). Sunflower has been a popular ornamental. However, in recent years its importance as environmental crop is being increasingly recognized. De-hulled seeds are used as poultry feed (Vogel, 1979; Prusinkiewicz et al., 1990).

Sunflower growth stages

The division of growth into vegetative and reproductive stages as developed by Schneiter and Miller (1981) is shown in Table 2.7. The total time required for development of a sunflower plant and the time between the various stages of development depends on the genetic background of the plant and growing season environment. When determining the growth stage of a sunflower field, the average development of a large number of plants should be considered. This staging method can also be used for individual plants. The same system can be used for classifying either a single head or branch sunflower. In the case of branched sunflower, make determinations using only the main branch or head. In stages R-7 through R-9, use healthy, disease-free heads to determine plant development if possible, because some diseases can cause head discoloration.

Table 2.7 Sunflower growth stages (Schneiter et al., 1981)

Stages		Description
Vegetative Emergence	VE	Seedling has emerged and the first leaf beyond the cotyledons is less than 4 cm long.
Vegetative Stages	V	These are determined by counting the number of true leaves at least 4 cm in length beginning as V-1, V-2, V-3, V-4, etc. If senescence of the lower leaves has occurred count leaf scars (excluding those where the cotyledons were attached) to determine the proper stage.
Reproductive Stages	R-1	The terminal bud forms a miniature floral head rather than a cluster of leaves. When viewed from directly above the immature bracts form a many-pointed star-like appearance.
	R-2	The immature bud elongates 0.5 to 2.0 cm above the nearest leaf attached to the stem. Disregard leaves attached directly to the back of the bud.
	R-3	The immature bud elongates more than 2.0 cm above the nearest leaf.
	R-4	The inflorescence begins to open. When viewed from directly above immature ray flowers are visible.
	R-5	This stage is the beginning of flowering. The stage can be divided into substages dependent upon the percent of the head area (disk flowers) that has completed or is in flowering. Ex. R-5.3 (30%), R-5.8 (80%) etc.
	R-6	Flowering is complete and the ray flowers are wilting.
	R-7	The back of the head has started to turn a pale yellow color.
	R-8	The back of the head is yellow but the bracts remain green.
	R-9	The bracts become yellow and brown. This stage is regarded as physiological maturity.

2.7.3 Marigold

A native of Mexico, marigolds have been grown in gardens throughout the world for hundreds of years. Marigolds are easy to grow, bloom reliably all summer, and have few insect and disease problems. Many of the commonly grown marigolds are varieties of African and French marigolds. Less known are the triploid hybrids and the signet marigolds. In addition, the two common species of marigold, both annuals, are distinguished as African, or Aztec (*T. erecta*), and French (*T. patula*) although both are native to Mexico and Guatemala. .

The African marigolds (*Tagetes erechta*) commonly have large yellow-to-orange flower heads and the strong-scented foliage typical of the genus, but an odorless kind has been developed. Flowers may measure up to 5 inches across. Plant height varies from 10 to 36 inches. African marigolds are excellent bedding plants. Tall varieties can be used as background plantings. African marigolds are also referred to as American marigolds (The Columbia Encyclopedia, 2013).

The French marigolds (*Tagetes patula*) are smaller, bushier plants with flowers up to 2 inches across. The French has smaller flower heads which may be single or double. Plant height ranges from 6 to 18 inches. The French marigolds have a longer blooming season than the African marigolds. The French marigolds also hold up better in rainy weather. French marigolds are ideal for edging flower beds and in mass plantings. They also do well in containers and window boxes (The Great Soviet Encyclopedia).

The triploid hybrids are crosses between the tall, vigorous African marigolds and the compact, free-flowering French marigolds. Triploid hybrid marigolds are unable to set seed. As a result, plants bloom repeatedly through the summer, even in hot weather. One problem with the triploids is their low seed germination rate. Average germination is around 50 percent. Since the triploid hybrids are unable to produce viable seed, they also know as mule marigolds

Breeding

Neither African or French marigolds come from Africa or France, respectively, but both *Tagetes erecta* and *Tagetes patula* are native to Mexico. Breeders have worked overtime to create cultivars in a wide range of colors, plant sizes, and flower forms. Crosses between *Tagetes erecta* and *Tagetes patula* have resulted in triploid cultivars. (www.ag.auburn.edu).

African: Hybrids of *Tagetes erecta* are larger plants than the French forms, often with fewer, larger double flowers. In the double flowering forms, there are crested doubles where the flowers appear mounded and full, and anemone doubles where the flowers appear flat and wide with the center recessed.

French: Hybrids of *Tagetes patula* are usually smaller than the African forms, 6 to 8 inches and up to 12 inches tall. Thought doubles are available, singles or semi-

doubles are more common. The single flowering forms stand-up to rain and humidity better in the south than double forms (The Columbia Encyclopedia, 2013).

Triploids (Signet hybrids): Crosses between *Tagetes erecta* and *Tagetes patula* provide the longest overall color in the landscape, often lasting through the hot weather in August and September. These plants are sterile.

Germination: Seed may be purchase as raw seed, or to facilitate sowing in automatic seeders as detailed, or coated seed. Seed germinate in 3-5 days at 75-80 ºF (stage 1). Marigold seed do not require light; therefore, cover the seed lightly with course vermiculite to retain moisture around the seed. Keep the germinating medium moist but not saturated. Germination medium pH should be 6.0-6.2 with an EC <0.75 mmhos/cm. Reduce the moisture level once the radicle emerges and reduce the temperature to 68-70 ºF in stage 2. Begin fertilization with 50-75 ppm N from calcium/potassium nitrate once the cotyledons unfold. In stages 3 and 4 temperatures should be 60-65ºF. In addition, if seed must be kept from one season to the next, store in a cool, dry environment away form insects and rodents. As a rule of thumb, store seed where the sum of the temperature and relative humidity in percent does not exceed 100, e.g. at 55 ºF, the humidity should not exceed 45%. Refrigerators dedicated for seed storage are often used (The Columbia Encyclopedia, 2013).

2.7.4 Cosmos

Cosmos belongs to that vast family of plants known as *Compositae.* Although there are 20 known species of cosmos, two annual species, *Cosmos sulphureus* and *Cosmos bipinnatus,* are most familiar to home gardeners. These two species are most easily differentiated by leaf structure and flower color. The leaves of *C. sulphureus* are long, with narrow lobes and hairy margins. The flower colors of this species are always shades of yellow, orange or red. The *C. bipinnatus* has leaves that are finely cut into threadlike segments. The foliage looks similar to ferns. The flowers are white or various shades of pink to dark rose.

Cosmos sulphureus (Yellow Cosmos): plants of yellow cosmos can range in height from 4 to 7 feet but the cultivated varieties such as 'Crest Red', 'Ladybird Dwarf Red', and 'Ladybird Dwarf Gold'. Mix are not as tall. The flower heads are composed of disc and ray flowers. The discs or center flowers are yellow: the ray, or outer petals range from pale yellow or mustard to orange-scarlet. Red is a relatively recent addition to the color range of *C. sulphureus.* The native species is golden-yellow to orange. Rich, fertile soils tend to produce unusually tall, lanky plants. Yellow cosmos requires full sun. Sow seed of *C. sulphureus* in early spring since seedlings are not winter hardy. The average planting success with this species is 80 percent. The plant height is 2 - 4 feet depending on culture and variety selected. Plants will germinate in 7 - 21 days when the soil temperature is optimum for germination at 70 - 80 degrees F. Plant seed 1/16 inch deep by raking into the soil. *C. sulphureus* plants bloom from May- November. Plants should be sheared every 30 days or whenever seed pods predominate. Large areas can be seeded at a rate of 15 pounds per acre C. sulphureus plants bloom approximately 50 - 55 days after germination. Yellow cosmos needs to

be replanted each spring for continued success (USDA, ARS, and National Genetic Resources Program).

In addition, yellow cosmos is easy to start from seed. Rich, fertile conditions are not necessary to grow yellow cosmos, but adequate drainage is. The seeds may be sown outdoors after all danger of frost is past and the soil has warmed to at least 65 degrees F. Scatter the seeds right where the yellow cosmos are to be displayed. Firm or rake seeds into a loose soil if the seed is planted too deep, germination can be affected. Keep the soil moist for 5 - 10 days after seeding. Seeds will germinate in 7 - 21 days. If the early spring has been cold, soil temperatures will remain cool also. If the soil temperature is below 65 F., seeds may not germinate as rapidly. Thinning is really not necessary. In addition, yellow cosmos is a sun-loving annual; it will not produce as many blooms if grown in the shade. Choose a location that receives at least 8 - 10 hours of direct, sunbathing sunlight. Cosmos will perform best if grown in well-drained soil. Yellow cosmos is not a heavy feeder. Excess fertilization will cause plants to produce excessive leaf growth at the expense of flower production.

Chapter 3

Methodology

This chapter discusses on how the study of phytoremediation of cadmium (Cd) contaminated soil using plants of concern was carried out. The pot culture experiments were carried out at the Environmental Research Station of the Environmental Engineering and Management Field of Study, Asian Institute of Technology (AIT), Thailand. In this study, the cadmium hyperaccumulation potential of the plants of concern; Guinea grass (*Panicum maximum*), cosmos (*Cosmos sulphureus*), marigold (*Tagetes erecta* L.) and sunflower *(Helianthus annuus)* in pot culture experiments was investigated and also the effects of soil pH, zinc concentration as well as the effectiveness of ethylenediaminetetraacetic acid (EDTA) on Cd bioavailability were investigated in artificially Cd spiked soils.

All reagents including concentrated acids and chemicals used for the experiments were analytical grades unless otherwise stated. Double de-ionized water (Milli-Q Millipore 18.2 MΩ/ cm resistivity) was used for all solutions preparation. The element standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg/L (Cd). All digestion vessels and glass wares were acid washed and rinsed with reagent water.

3.1 Soil and Plants Preparation for Pot Experiment

3.1.1 Soil preparation

Uncontaminated (natural) soil (0-20 cm) collected from Asian Institute of Technology agricultural farm was used in pot culture. Soil samples were air-dried at room temperature and crushed to pass through a 1-cm sieve to remove pieces of stone and large plant residues, and then passed through a 4-mm stainless steel sieve. Soils were then homogenized through repeated mixing and stored in closed containers before use.

3.1.2 Physicochemical properties of studied soil

Physical and chemical properties of soil were determined. Soil samples were analyzed for background concentrations of total heavy metals as well as soil pH, cation exchange capacity (CEC), electrical conductivity (EC), and soil organic matter (OM). Organic matter contents were determined by Walkey-Black titration method (wet oxidation) using potassium dichromate reagent ($K_2Cr_2O_7$) and concentrated H_2SO_4 as oxidizing agents (Walkley and Black, 1934; Nelson and Sommers, 1982). One gram of soil sample was transferred to 250 mL Erlenmeyer and 10ml of 1N $K_2Cr_2O_7$ and 10 mL of concentrated H_2SO_4 were added. After 30 minutes, 50 ml of deionized water, 3ml of concentrated H_3PO_4 and 0.5 mL of 1% defenilamina indicator were added and then, titrated slowly with 1N $FeSO_4$ solution up to a green color end point. Soil pH

was measured in a 1:1 soil: water suspension using pH meter (Rhoades, 1982; Rayment and Higginson, 1992; DOA, 2001); 20 g of sieved, air-dried soil was put into a beaker, and then 20 mL deionized water was added to the sample and stirred vigorously for 15 seconds and let stand for 30 minutes. Electrode was placed in the slurry, swirl carefully, and pH was read immediately (ensure that the electrode tip was in the slurry and not in the overlying solution). In addition, EC was determined on a 1:5 soil: water suspension; 20 of air-dry soil (2mm) was put into 250 mL flask and 100 mL deionized water was added then stirred for 30 minutes and allowing it to settle for 15 minutes. It then was filtered with Whatman filter paper No.42. The EC was measured using conductivity meter. CEC was determined using the ammonium acetate method at pH 7.0 (Chapman, 1965; Rhoades, 1982; Thomas, 1982).

Soil texture was analyzed by hydrometer method according to Allen et al. (1974); Tan (1995) and Hilled (1998); 50 g of dry sieved soil sample (2-mm sieve) was transferred into a blender cup. Distilled water was added into the blender cup to within 10 cm cup (rim) and 15 mL of calgon solution was then added into the cup. The cup was attached to a blending or stirring machine and then was blended mechanically for 15 minutes. The soil suspension was transferred into an A.S.T.M. soil testing cylinder. The remaining soil residue was washed quantitatively in the cylinder by spraying with water from a water bottle. The suspension was then mixed thoroughly by stirring with a stirring rod so that all sediment disappears from the bottom of the cylinder then the exact time was recorded, when stirring was stopped. A hydrometer was carefully placed into the suspension exactly 40 seconds after the stirring was stopped. The hydrometer reading and the temperature was recorded. The hydrometer reading and the temperature was recorded for every 2 hours. A blank was analyzed in the same manner. Percentage of sand (2.00-0.05 mm), silt (0.05-0.02 mm), and clay (<0.02 mm) was calculated from the Soil Textural Triangle. The methods used for analysis of soil properties have been shown in Table 3.1

3.1.3 Artificially cadmium spiked soil preparation and soil incubation

For preparation of artificially Cd contaminated soil, eight kilograms of ground and air-dried soils were weighed and placed in the pot and put in the container used for soil spiking. The desired amount of cadmium concentration, was prepared by dissolving analytical grade $CdCl_2 \cdot 2.5H_2O$ with deionzed water, at room temperature. For Cd soil spiking in this step, Cd stock solution with concentration of 8000 ppm was prepared. High concentration of stock solution was prepared to maintain the field capacity of the soil. This stock solution was used for spiking the soil to reach the desired concentration using deionized water. The amount of water needed for mixing with Cd stock solution to reach the desired concentrations of cadmium (50, 100, 200, and 400 mg/kg) depend on field capacity of the soils which might be varied from one place to another according to properties of the soils. After the cadmium spiking process, cadmium spiked soils in these pots were incubated for about six weeks under the shade before they were used for pot culture experiments. During this incubation period, all pots were watered with the amount of distilled water close to field capacity of soil. An uncontaminated soil was included as control treatment (CT).

Table 3.1 Analysis of soil properties

Parameters	Analysis	References
Soil texture	Hydrometer method	Hillel, 1998; Tan, 1995; Allen et al., 1974
Soil pH	pH meter (1:1 soil: water ratio)	Mclean, 1982; Rayment and Higginson, 1992; DOA, 2001
Soil organic matter	Walkley-Black titration method	Nelson and Sommers, 1982; Walkley and Black, 1934
Electrical conductivity (EC)	EC meter (1:5 soil:water ratio)	Rayment and Higginson, 1992
Cation exchange capacity (CEC)	The ammonium acetate method at pH 7.0	Chapman, 1965; Rhoades, 1982

3.1.4 Plant preparation

Seeds of marigold, cosmos and sunflower were bought from Chia Tai Co., Ltd., Thailand. Seeds of Guinea grass were bought from the Department of Livestock Development, Ministry of Agriculture and cooperatives, Thailand. For plant preparation, seeds of studied plants were germinated in sowing media, peat moss, which is mostly sterile and has high water-holding capacity, and has excellent structure for plant growth. After germination in sowing media for about 2-3 weeks, uniform and healthy seedlings of each species were selected (Figure 3.1) and were used for pot culture experiments in the greenhouse.

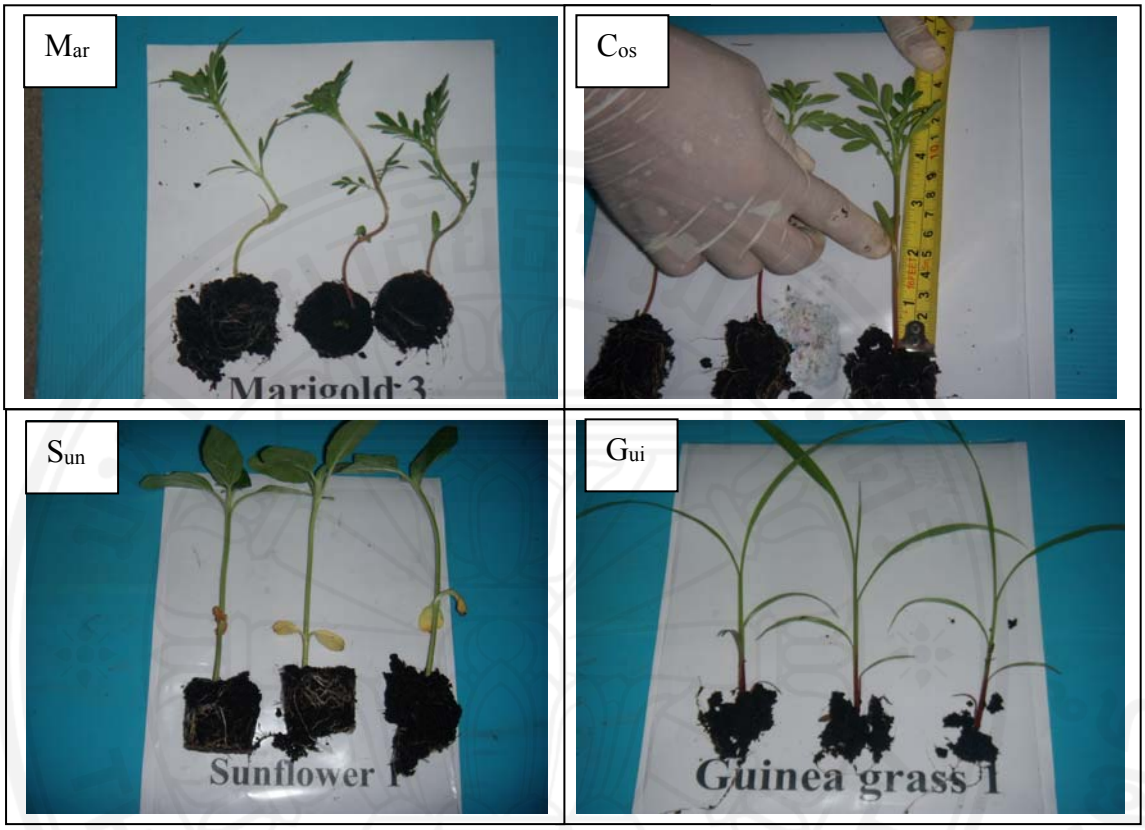


Figure 3.1 Seedlings selection for pot culture experiment
(M_{ar}: marigold, C_{os}: cosmos, S_{un}: sunflower, G_{ui}: guinea grass)

3.2 Pot Experiment

3.2.1 Screening of potential cadmium hyperaccumulator using artificially cadmium spiked soil at various concentrations

In this investigation, cadmium accumulation by studied plants to identify maximum Cd uptake using artificial Cd contaminated soil was carried out. Effect of various concentrations of soil cadmium on the uptake by studied plants was investigated. The overall framework of pot culture experiment is shown in Figure 3.2.

Effect of cadmium concentrations on cadmium uptake by plants

To find out the maximum Cd uptake by plants of concern, the cadmium solution was uniformly mixed with air-dried and homogenized soil at room temperature. A single treatment was conducted by spiking with cadmium chloride ($CdCl_2 \bullet 2.5H_2O$) solution in order to achieve levels of Cd-spiking for various concentrations (50, 100, 200, and 400 mg Cd/kg soil) in the pots. An uncontaminated soil (natural soil) was included as control treatment**.**

For this study, four species, namely, Guinea Grass (*Panicum maximum*), cosmos (*Cosmossulphureus*), marigold (*Tagetes erecta* L.) and sunflower *(Helianthus annuus)* were selected. Uniform and healthy seedlings of each species were transferred to artificially heavy metal contaminated soil pots under various cadmium concentrations. After acclimatization for a few days in the shade, all transplanted pots were placed in the greenhouse with natural sunlight and relative humidity (60-75%). Temperature throughout the experiments varied from 22-26ºC (average low temperature) to 32-37ºC (average high temperature).Three replicates were run for each treatment (three plants for each pot; so there were nine plants for each treatment) and arranged in a completely randomized design. Water loss due to evaporation was replenished using tap water. No cadmium content was found in tap water. Each pot was watered daily throughout the experiment with an equal amount of tap water to maintain soil moisture content at around 70-75 % of field capacity. Seedlings grown in uncontaminated soil (without heavy metal added) were used as control pots. Experimental diagram for screening of potential Cd hyperaccumulator under various Cd concentrations is illustrated in Figure 3.3. Plants were harvested at flowering stage (about 80-90 and days of growth). The whole plants were harvested. The harvested plants were gently removed from soils, washed with tap water and distilled water. The harvested plants were separated into roots, flowers, leaves and stems and then dried at 60-70 °C until completely dried. Dried plant samples were weighed and ground and then kept in polyethylene bag for chemical analysis. After this pot culture experiment, the two out of four species that showed high uptake and/or high biomass were selected for next experiments as mentioned in the section below.

3.2.2 Effect of cadmium concentration on plant growth

During pot culture experiment, plant growth was observed and recorded. The measurement of plant growth (height and flower diameter) was carried on the day before the plants were harvested. Flower diameter of marigold was measured by vernier caliper. The length of the plant was measured from the top layer of the soil up to the tip of the leaf (for marigold) and to the longest part of the plant leaf (for Guinea grass).

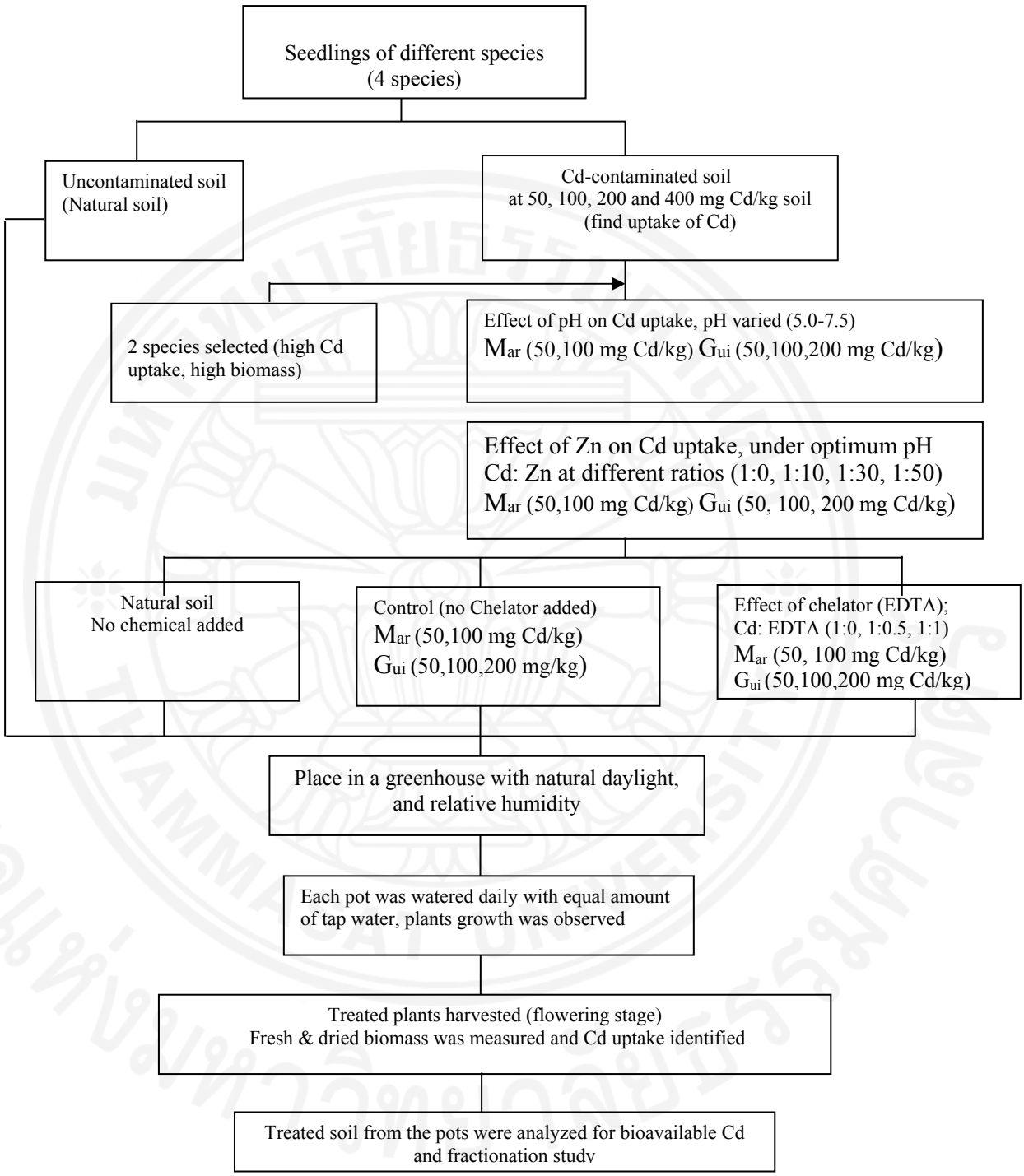
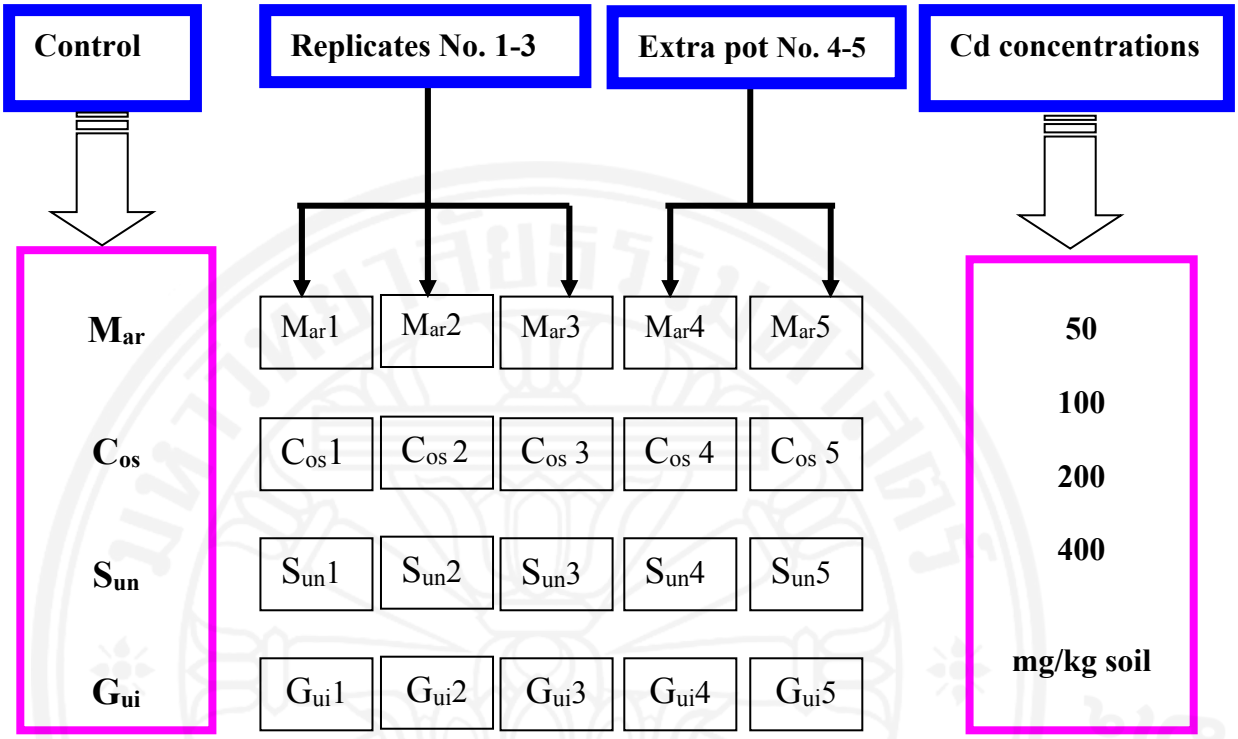


Figure 3.2 Overall frameworks of pot experiments using artificially Cd contaminated soil



Natural soil
(No Cd added)

Plant species

Mar : marigold
Cos : cosmos
Sun : sunflower
Gui : Guinea grass

Figure 3.3 Experimental diagram for screening of potential Cd hyperaccumulator under various Cd concentrations

3.2.3 Effect of different parameters on Cd uptake

From the first set of experiment, based on total cadmium accumulation in whole plant tissues, cadmium collected in plant shoots, total uptake, and TF as well as BCF values, marigold showed higher potential and possessed a greater ability to accumulate Cd in plant shoot as compared to other species. However, due to higher biomass production, the total uptake of Cd from the soil by Guinea grass could be maximized. Thus, marigold and Guinea grass were selected for the second experiment. The two species selected were used for this experiment. Effects of various factors on metal uptake were investigated to identify the optimum operating conditions.

1) Effect of pH on heavy metal uptake

In this investigation, effect of soil pH on Cd uptake by studied plants was carried out. Soil pH in the pots was varied approximately from 5-7.5 (5.0, 6.3, 7.0, 7.5) using mineral sulfur (S) or lime (CaO). Plant growth was observed and measured. Cadmium uptake of each species was identified at various pH conditions. Under this investigation, soil pH that provides the highest Cd uptake was selected. For soil pH adjustment, different amounts of elemental sulfur (S) were used to adjust pH to desired levels based on a preliminary acid incubation experiment. For this process, 300 g of soil was mixed with S and placed in plastic containers. Soil pH was monitored periodically by taking 10 g soil and measuring pH (1:1 ratio of soil:water suspension). Soil was thoroughly mixed every day to ensure equal distribution of S and to accelerate the S oxidation process. Incubation was terminated when pH did not change for 3 consecutive weeks. After reaching the final pH, deionized water was added to each pot to leach salts from soil. This procedure was repeated twice. By doing this, the excess salts, formed during S oxidation, was removed. Furthermore, to increase soil pH, calcium oxide was used for soil incubation experiment, in the same way as acid incubation experiment. After preliminary soil incubation for pH adjustment, the equations represented the relationship between S and CaO used and pH values were obtained. From the graphs showing relationships, the amount of S or CaO required to reach the desired soil pH levels can be identified; however, different soil properties would give different results. After soil pH incubation, the soil in each pot was placed in a container for soil spiking. Cadmium stock solution at desired volume was sprayed over the soil, thoroughly mixed, and the Cd spiked soil was placed into the pots, as mentioned in section 3.1.3. Cadmium dose, applied for this investigation, was the optimum dose obtained from the first set of experiments; 50 and 100 mg Cd/kg soil for marigold and 50, 100, and 200 mg Cd /kg soil for Guinea grass.

After Cd spiking, all pots were incubated in shade for about six weeks before use. In this study, the two species selected from the first experiment, namely, marigold and Guinea grass were used. For pot culture experiment, marigold seedlings were bought from AFM flower seeds (Thailand) Co., Ltd. Guinea grass seeds, bought from the Department of Livestock Development, were germinated at greenhouse nursery at AIT. The uniform and healthy seedlings of each species were transferred to artificially

heavy metal contaminated soil pots under various soil pH. All the transplanted pots were placed in shade for a few days and then transferred to greenhouse and were watered daily as previously mentioned (section 3.2.1). During and at the end of experiment, plant growth was observed and recorded. Plant and soil samples were collected and prepared for metal analysis as stated in sections 3.3.1 and 3.3.2. The flow diagram of pH variation experiment is presented in Figure 3.4 and 3.5.

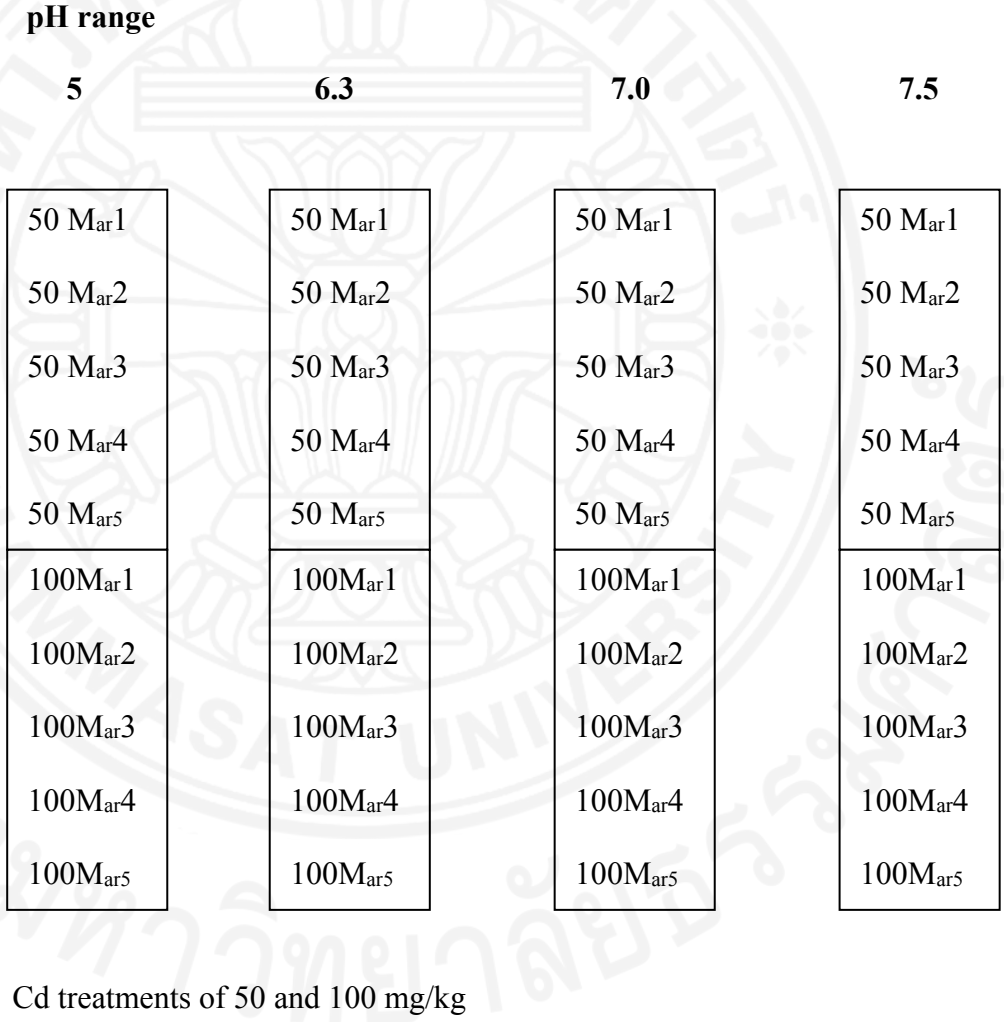


Figure 3.4 Diagram showing pot preparation to study effect of pH on Cd uptake by marigold

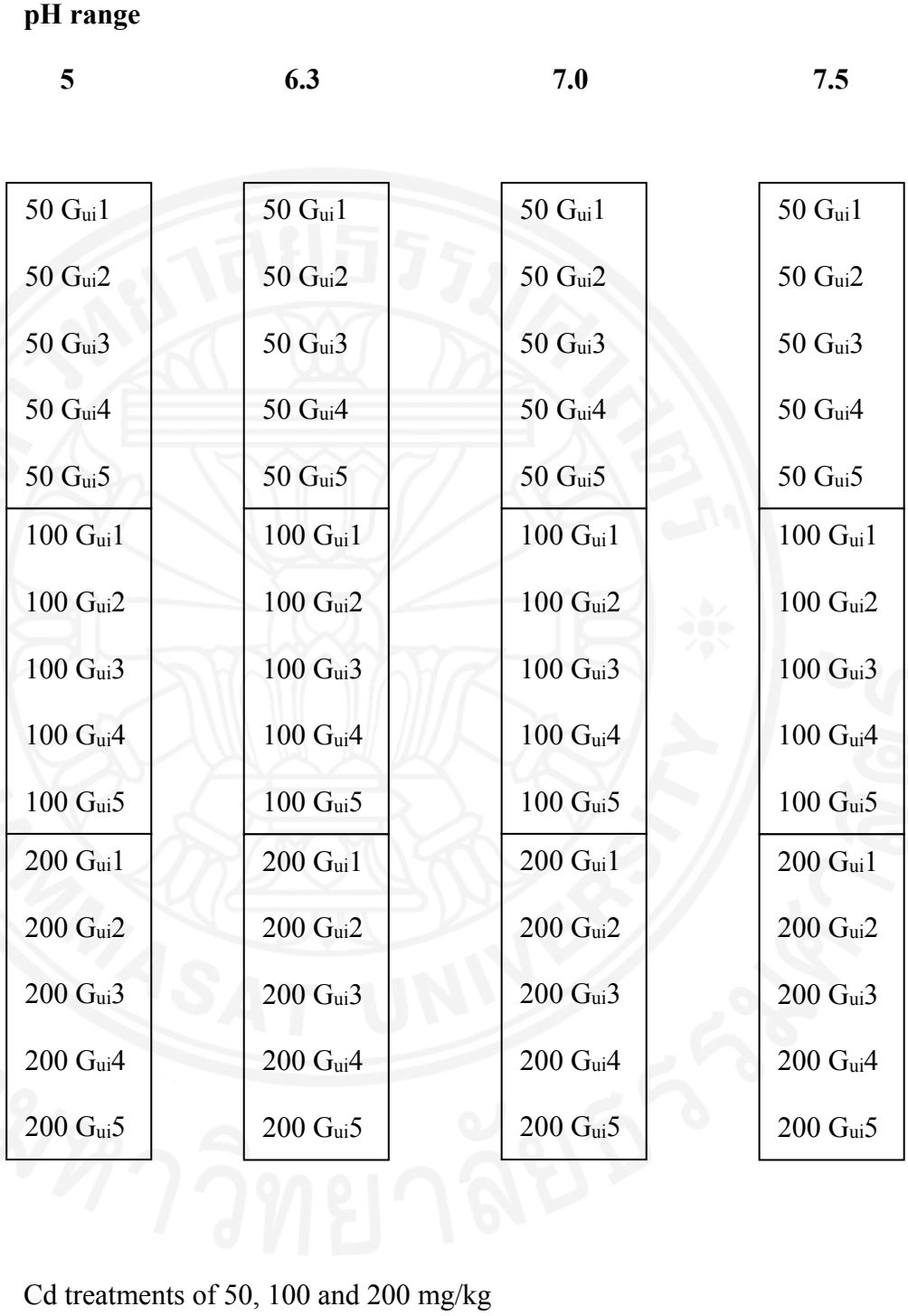


Figure 3.5 Diagram showing pot preparation to study effect of pH on Cd uptake by Guinea grass

2) Effect of zinc on cadmium uptake

In this investigation, combination of Cd and Zn was investigated at various Cd:Zn treatments (1:0, 1:10, 1:30 and 1:50) at optimum pH to find the effect on Cd uptake by plants in the presence of Zn. The control experiment was conducted simultaneously with no addition of Zn in the soil (only naturally Zn present in the soil). For preparation of Cd spiked soil, the procedures followed section 3.1.3 (artificially cadmium spiked soil preparation). After Cd spiked soil was incubated in the shade for about 4 weeks, zinc solution (prepared by diluting zinc sulfate heptahydrate, analytical grade, $ZnSO_4 \cdot 7H_2O$) was spiked to Cd spiked soil in the pots, at various concentrations to achieve the desired levels of Cd:Zn treatments of 1:0, 1:10, 1:30 and 1:50. Zn and Cd spiked soil were then incubated for another 4 weeks before use. Cd spiked soil pots without Zn addition were included as control treatment (CT). The diagram of Cd:Zn treatment for pot culture experiment is shown in Figures 3.6 and 3.7. However, some ratios were omitted as the Zn concentration in soil was much higher than that of the real contamination site in Tak province. Preparation and monitoring of pot culture experiment in greenhouse as well as sample collection were managed the same way as previously described in section 3.2.1.

3) Effect of chelating agent on cadmium uptake

In this investigation, ethylenediaminetetraacetic acid (EDTA) solution was applied to Cd contaminated soil to evaluate the effects of Cd on plant growth and Cd phytoextraction efficiency. In addition, this experiment comprised of the following treatments: (1) uncontaminated soil; (2) Cd contaminated soil without chelator, and (3) Cd contaminated soil with EDTA. In the chelator treatment, artificially Cd spiked soil samples were amended with EDTA in a 1:0, 1:0.5, and 1:1 treatment of Cd:chelator based on Cd concentration selected from the previous experiment (50 and 100 mg Cd/kg soil for marigold and 50, 100, and 200 mg Cd/kg soil for Guinea grass). The investigation on the effect of chelating agent was shown in Figures 3.8 and 3.9. Preparation and monitoring of pot culture experiment in greenhouse as well as sample collection were managed the same way as previously described in section 3.2.1.

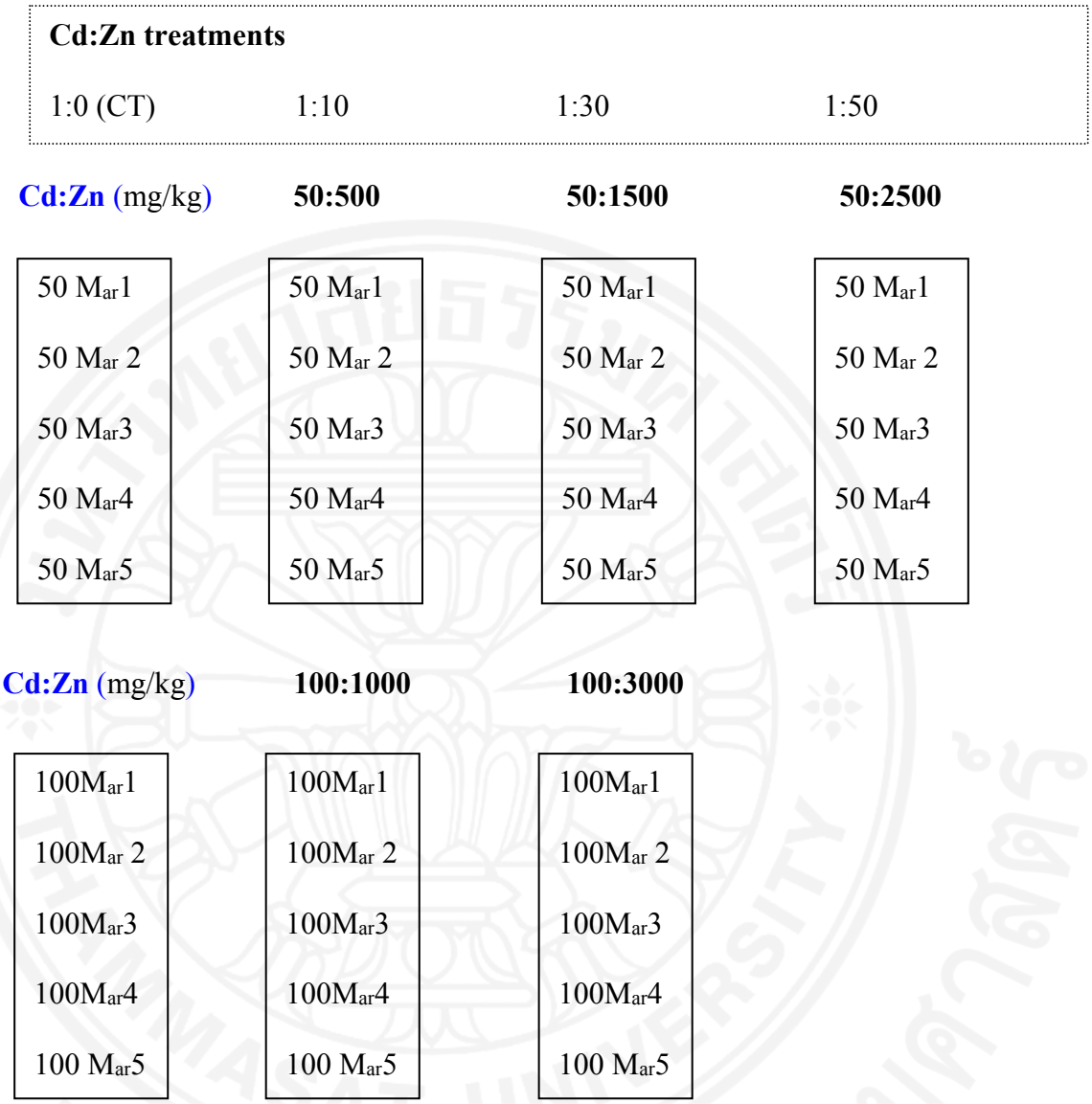


Figure 3.6 Diagram showing Cd:Zn treatments of pot culture experiment for marigold

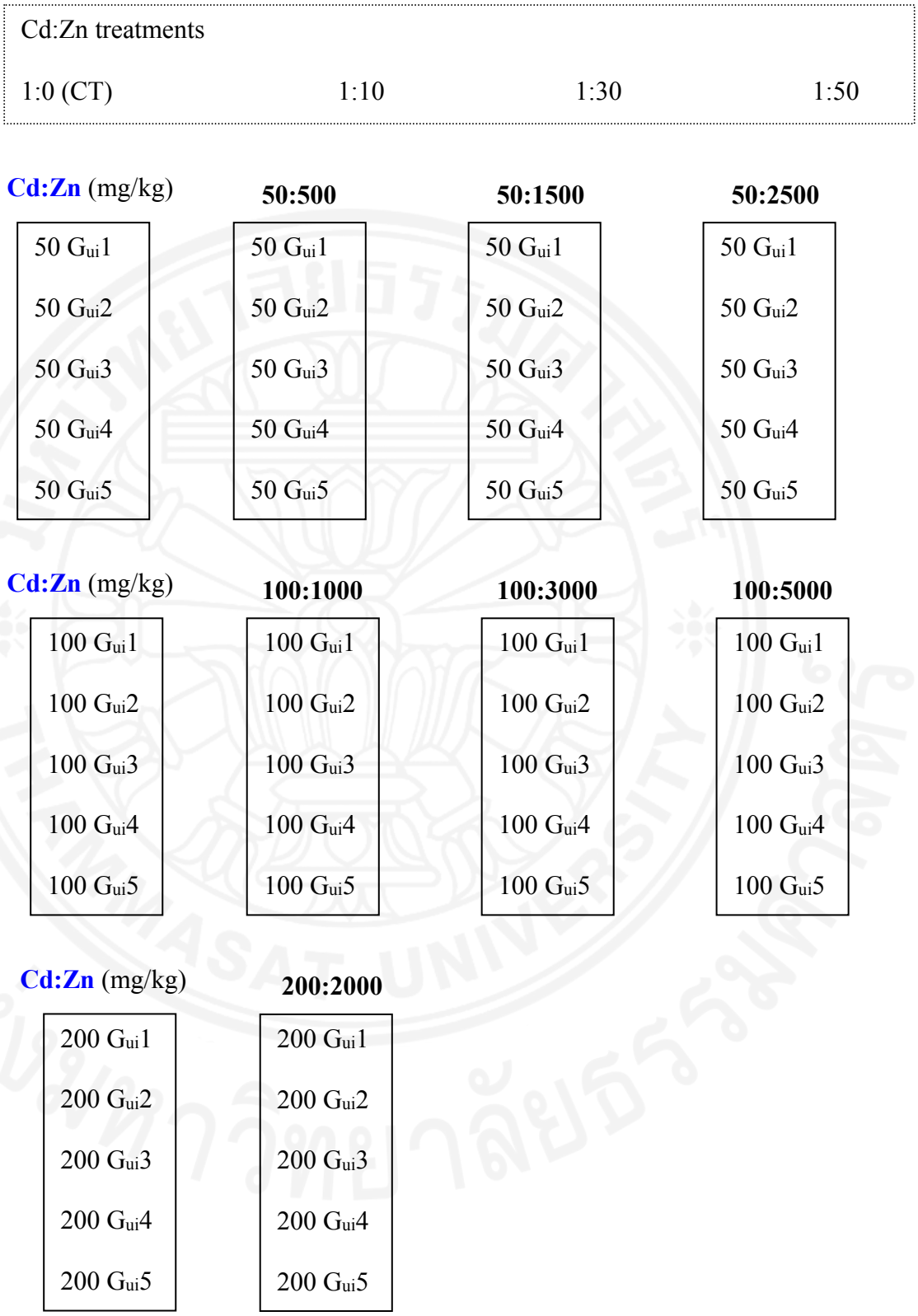


Figure 3.7 Diagram showing Cd:Zn treatments of pot culture experiment for Guinea grass

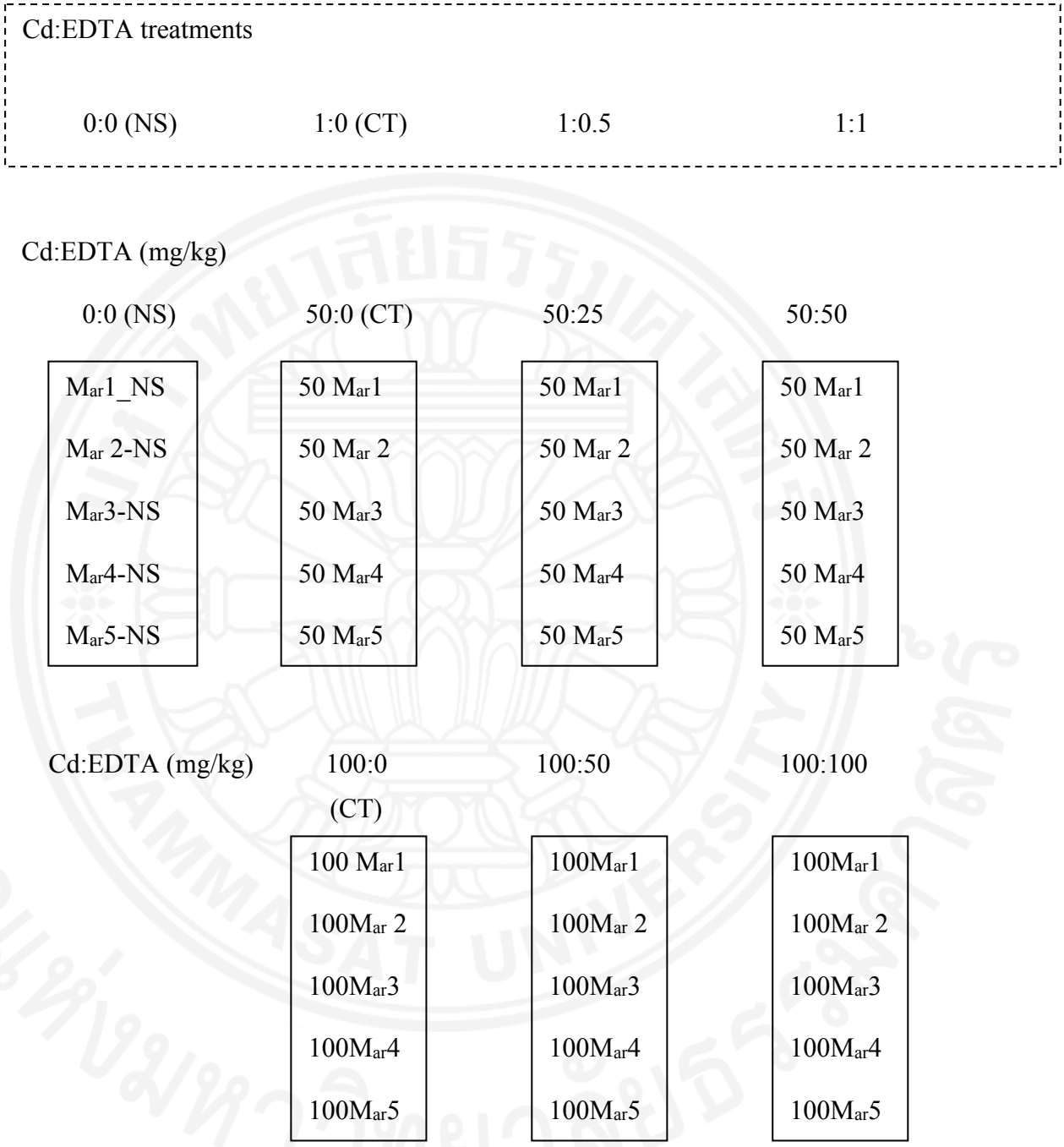
3.3 Soil and Plant Samples Analysis

3.3.1 Soil samples analysis

Before transportation of seedlings into Cd spiked soil, soil samples from each pot were collected for total heavy metal analysis for day zero concentration (initial cadmium concentration). The soil samples were air-dried and pulverized to pass through 2-mm stainless steel sieve, and kept in polyethylene bag before chemical analysis in laboratory. Furthermore, at the end of pot experiment, soil samples were also collected from the pots after harvesting the plants. After removing crop debris, soil samples were air-dried and ground to pass through a 2-mm stainless steel sieve and then used for metals analysis.

Total metal analysis

Total heavy metals concentration in soil samples were determined by digestion in aqua regia (HNO3: HCl, 1:3) according to the previous published methods (Pichtel et al., 2000; Zarcinas et al., 2004; Nobuntou, 2012; DOA, 2001). An open tube digestion was applied. One gram of soil samples was weighed into digestion tubes, including three duplicate samples and two standard reference materials, and three blank samples. Aqua regia, 5 ml, was added to each tube placed on digestion block. The temperature was adjusted approximately, not higher than 150°C. Acid solution was digested down to volume of 1 mL, then removed from digestion block and cooled down to room temperature under hood. 0.5 % nitric acid was added to make up the volume to 50 ml. This solution was thoroughly mixed using a vortex mixer, and then filtered through filter papers (Whatman No.42). Trace elements in digested samples were measured, against the appropriate mixture of set of standard, using Inductively Couple Plasma Optical Emission spectrophotometer (ICP-OES, Perkin Elmer Optima 5300 DV).



NS; Natural soil, CT; Control

Figure 3.8 Diagram for investigation on the effect of chelating agent on Cd uptake by marigold

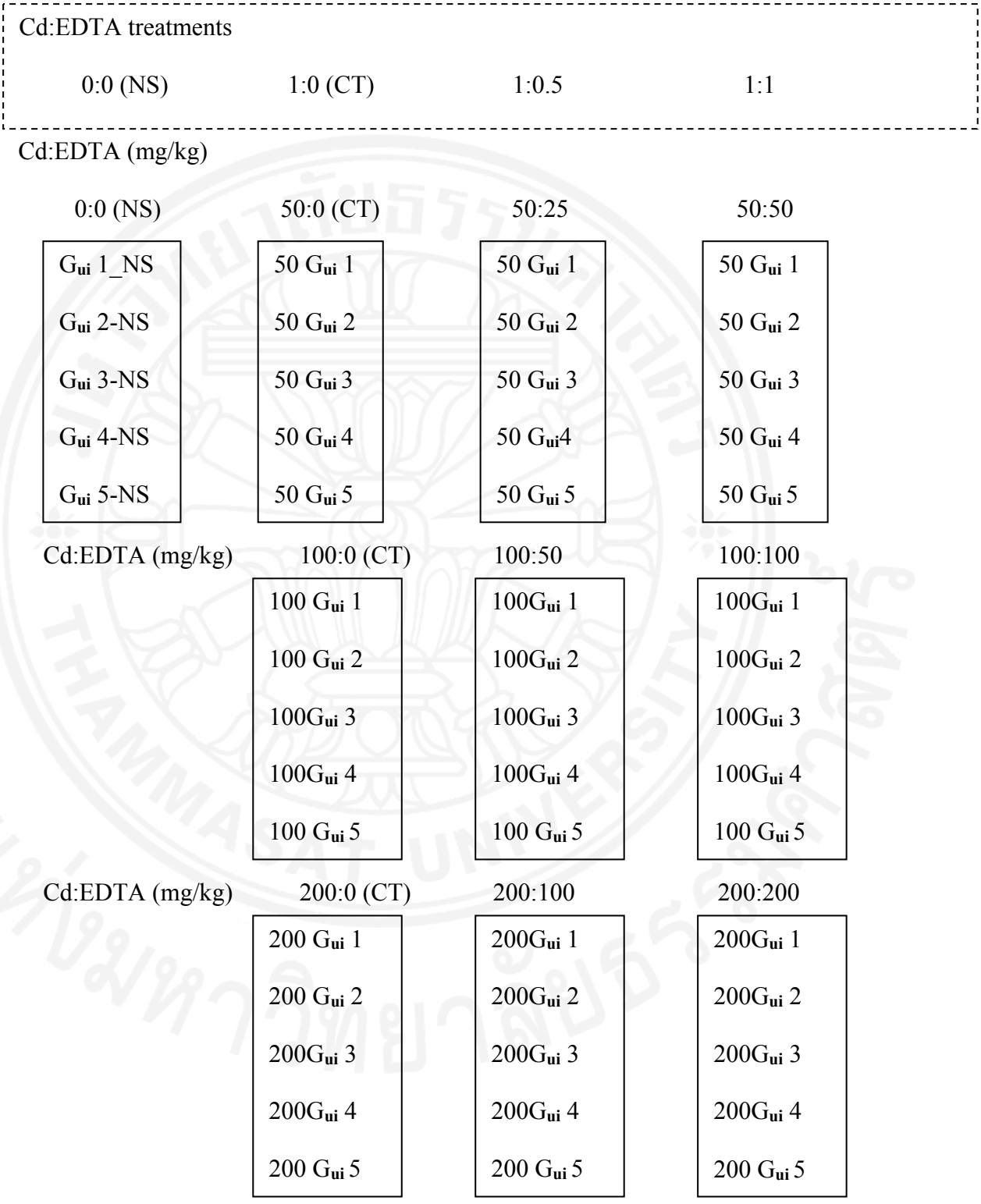


Figure 3.9 Diagram for investigation on the effect of chelating agent on Cd uptake by Guinea grass

3.3.2 Plant samples analysis

The whole plants were harvested at flowering stage (approximately 80-90 days of growth), at the end of each experiment. The harvested plants were gently removed from soil, washed with tap water thoroughly to get rid of soil attached to plants' part and then with deionized water before being divided into roots, flowers, leaves and stems, and then dried at 60-70ºC for 24-48 hours (completely dry) to obtain a constant weight. Dried plants samples were weighed and ground in a stainless steel grinder and passed through 2- mm sieve and kept in polyethylene bag for chemical analysis. For plant samples analysis, the acid digestion process according to the method of Zarcinas et al. (1987) was applied. Approximately one gram of ground plant samples was added into digestion tubes, and then 5 ml of concentrated nitric acid (HNO_3) was added, and then these tubes were placed in digestion block. Temperature was adjusted roughly but not higher than 140°C (120-140 °C) for 8 hours. The solution in the tubes was boiled until the volume was reduced to 1 ml and was then cooled to room temperature under hood. Nitric acid solution (5 %) was added to make up the volume up to 50 mL. Solution was thoroughly mixed in the tubes by a vortex mixer and filtered using filter papers (Whatman No.42). Heavy metals in the solution were measured using Inductively Couple Plasma Optical Emission Spectrophotometer (ICP-OES, Perkin Elmer Optima 5300 DV). Figure 3.10 shows schematic diagrams of plant and soil sample analysis.

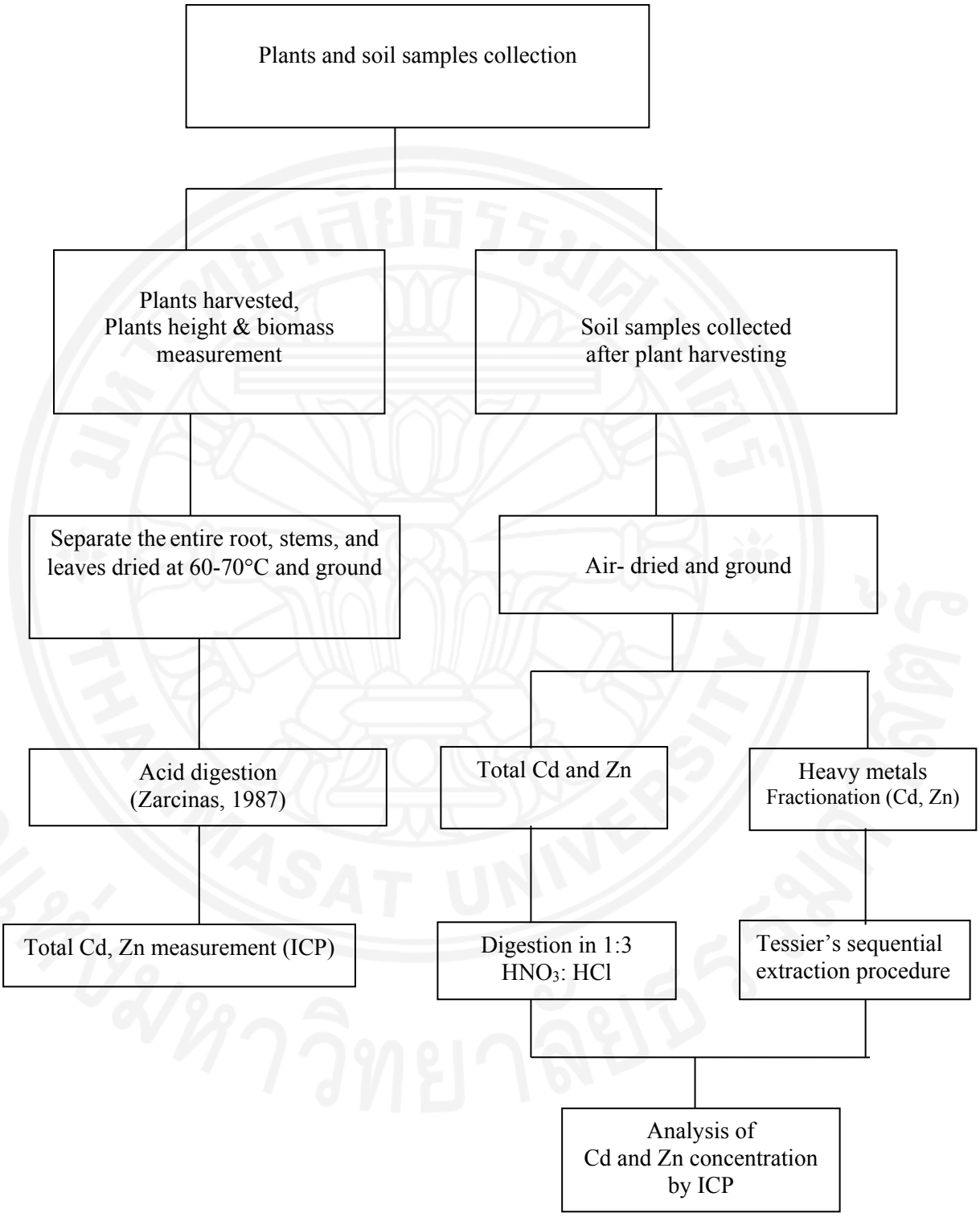


Figure 3.10 Schematic diagram of plant and soil sample analysis

3.3.3 Identifying the best Cd uptake species

All studied plants were grown until the flowering stages. At the end of the investigation, cultivar selection was based on the plant's ability to achieve the best accumulation for cadmium. The following calculation was carried out to find the best Cd accumulator (Padmavathiamma and Li, 2007; Adesodun et al., 2010):

- Translocation factor (TF), bioconcentration factor (BCF), and total metal uptake were used to evaluate plants capacity to accumulate heavy metals in plant biomass.
- The translocation factor indicated the plant's ability to translocate heavy metals from roots to the harvestable aerial part and was calculated on a dry weight basis by dividing the metal concentration in shoot ((stem or leaves) by the metal concentration in root.
- Bioconcentration factor is defined as the ratio of metal concentration in plant roots or shoots to that in the soil.
- Total metal uptake by plants was calculated by multiplying yield (dry biomass) with heavy metal concentration accumulated in plant biomass.

The potential use of phytoremediation depended on various factors like plant biomass, plant metal concentration, soil metal concentration, and the soil mass in the rooting zone. The rate of Cd removal by plant was calculated as the following equation (Zhao et al., 2003)

$$\text{Cd removal by plant (\%)} = \frac{\text{(Plant Cd concentration x Biomass) x 100}}{\text{(Soil Cd concentration x Soil mass in rooting zone)}}$$

3.4 Fractionation of Heavy Metals in Soil Sample

For fractionation of heavy metals (Cd and Zn) in soil samples collected after plant harvesting, five-step sequential extraction procedure introduced by Tessier et al (1979) was employed. The sequential extraction scheme used in this study partitioned the heavy metals in soil samples into five chemical fractions. These include exchangeable fraction (F1), carbonate bound fraction (F2), iron and manganese oxides bound fraction (F3), organic matter bound fraction (F4), and residual fraction (F5). One gram of soil sample was placed in 50 mL of polypropylene tube and was used in the following sequential extraction procedure. (1) Metals in soil sample were extracted at room temperature for 1 hour with 8 mL of 1 M $MgCl_2$ solution. (2) The residue from (1) was leached at room temperature with 8 mL of 1M NaOAc. (3) The residue from (2) was extracted with 20 mL of 0.04 M NH_2OH•HCL. (4) The residual from (3) was extracted by 0.02 M NHO_3, 30% H_2O_2, and 3.2 M NH_4OAc solution. (5) The residue from (4) was digested with mixture of HCl, HNO_3, and H_2O_2 (Tessier et al., 1979; Nobuntou, 2012). The forms of heavy metals obtained from each steps (1) to (5) are stated in the table 3.2. The sequential extraction procedure of heavy metals is summarized in Table 3.2

Table 3.2 Operating conditions for sequential extraction procedure (Modified from Tessier et al., 1979; Nobutou, 2012)

Fraction	Time	Temperature	Chemical used	Quantity (mL)
Exchangeable (F1)	1 hr	Continuous agitation	1 M $MgCl_2$, pH 7	8
Bound to Carbonate (F2)	5 hr	Continuous agitation, leached at room temperature	1 M NaOAc, pH 5	8
Bound to Iron and Manganese Oxides (F3)	6 hr	96±3ºC with occasional agitation	0.04 M NH_2OH•HCl in 25%(v/v) HOAc	20
Bound to Organic Matter (F4)	2 hr	85±2ºC with occasional agitation	0.02 M NHO_3	3
			30% H_2O_2; pH 2 with NHO_3	5
	3 hr	85±2ºC with intermittent agitation	30% H_2O_2; pH 2 with NHO_3	3
	30 min	Continuous agitation	3.2 M NH_4OAc in 20%(v/v) nitric acid, dilute to 20 mL	5
Residual (F5)		Digestion	Conc. HCl	2
			Conc. NHO_3	6
			30% H_2O_2	3

3.5 Statistical Analysis

The pot experiment was conducted in a completely randomized design. Treatment effects on cadmium uptake and plant growth were evaluated using one way analysis of variance (ANOVA). Duncan's multiple range test ($p<0.05$) was used for comparison between treatment means. Statistical analyses were performed using Microsoft Excel 2007 software program. All the values expressed are mean ± S.D (standard deviation) of the three replicates. Stepwise multiple regressions as analysis was carried out using selected soil properties.

Chapter 4

Results and Discussion

4.1 Characteristics of Studied Soil

From the results obtained, cadmium and zinc concentrations of real contaminated soil (from Mae Sot) are 43.58 and 1724.50 mg/kg, respectively (Table 4.1). The concentration of Zn in contaminated soil is about 28 times higher than that of uncontaminated soil (61.11 mg/kg). Cadmium concentration in uncontaminated soil from AIT is non-detectable (ND). Typical detection limits of ICP, the Optima 5000 DV series, are 0.1 and 0.2 µg/L for Cd and Zn, respectively. Cd concentration in sowing media is 0.16 mg/kg which is lower than the standard level of Cd for residential area (not exceed 10 mg Cd/kg soil), according to Environment Agency, UK. (2009). Soil pH of contaminated soil and uncontaminated soil are 7.2 and 6.3 respectively, and are in the optimum range for plant growth. For most plants, the optimum pH for plant growth is in the range of 5.5-7.5 (DOA, 2001). The electrical conductivity of the soil from Mae Sot and AIT are 0.120 and 0.156 dS/cm, respectively. There will be no effect on the plants if the EC of the soil is in the range of 0-2 dS/ cm (DOA, 2001). Soil texture of soil samples from Mae Sot and AIT are sandy clay loam and clay, respectively. Soil texture of AIT soil is clay with 30.5 % sand, 18.1 % silt and 51.4 % clay. Based on the real soil concentration of Cd and Zn from Mae Sot, Tak province, cadmium and zinc concentrations for spiking were selected and applied in this pot culture experiment.

4.2 Total Heavy Metal Concentration of Artificially Spiked Soil

For the first set of experiment, soil samples were collected from pots spiked with Cd and were analyzed for total heavy metal concentrations, before used for experiments in greenhouse. The results obtained show that at the desired dose of 50, 100, 200, and 400 mg Cd/ kg soil, Cd concentrations in spiked soil in the pots varied from 46.19-49.91, 108.96-113.07, 206.58-222.69, and 413.53-460.46 mg/kg, respectively. However, the total cadmium concentrations in the four control pots were non-detectable. Furthermore, zinc concentrations naturally present in studied soil varied from 49.12-75.26 mg/kg. From the results obtained, it can be seen that total Zn and Cd concentrations of artificially spiked soil varied from one pot to another as can be seen from SD values. Total heavy metal concentrations of artificially spiked soil are presented in Table 4.2. In this study, the actual concentration of elements present in the soil was used for calculation.

Table 4.1 Chemical and physical properties of studied soil

Parameters	Contaminated soil (Mae Sot)	Uncontaminated soil (AIT soil)	Sowing media	Standard *
pH	7.2	6.3	5.3	
Organic carbon (%)	1.59	1.92	43.02	
Organic matter (%)	2.75	3.31	74.18	
Electrical conductivity (EC)(dS/ cm, 25º C)	0.120	0.156	0.834	
Cation Exchange Capacity (CEC) (cmol/kg)	7.3	25.3	79.8	
Soil Texture	Sandy Clay Loam	Clay	-	
Sand (%)	62.2	30.5	-	
Silt (%)	14.2	18.1	-	
Clay (%)	23.6	51.4		
As (mg As/kg)	13.55	7.39	1.52	Not exceed 3.9
Cd (mg Cd/kg)	43.58	ND	0.16	Not exceed 10**
Zn (mg Zn/kg)	1724.50	61.11	22.32	
Cr (mg Cr/kg)	15.26	47.695	1.41	Not exceed 300[1]
Cu (mg Cu/kg)	14.35	28.11	21.69	
Hg (mg Hg/kg)	0.29	0.00	0.00	Not exceed 23[2]
Pb (mg Pb/kg)	116.60	18.94	3.60	Not exceed 400

Note: *Notification of National Environmental Board No. 25, B.E. (2004). Soil Quality Standards for Habitat and Agriculture**,** Pollution Control Department, Ministry of Natural Resources and Environment.

[1]Hexavalent chromium

[2]Mercury and compound

**Environmental Agency, UK. (2009).

ND: Non-detectable

Table 4.2 Total heavy metal concentration of artificially spiked soils (50, 100, 200, and 400 mg Cd/kg soil)

Desired Cd Concentration (mg Cd/kg soil)		Metal concentration in spiked soil (±SD)			
		M_{ar}	C_{os}	S_{un}	G_{ui}
50	Cd	46.19(±1.52)	48.62(±1.14)	49.91(±1.33)	49.91(±0.98)
	Zn*	49.83(±4.11)	49.46 (±.57)	55.15(±.43)	49.12(±3.78)
100	Cd	108.96(±6.29)	113.07 (±6.50)	110.41(±3.14)	112.06 (±3.36)
	Zn*	64.83(±2.16)	70.63(±2.89)	61.25(±5.20)	64.04(±4.81)
200	Cd	211.87(±11.32)	215.64(±7.98)	206.58(±34.65)	226.69(±12.34)
	Zn*	72.92(±13.04)	73.52(±6.40)	75.26(±5.56)	69.01(±7.07)
400	Cd	460.46(±8.37)	444.94(±19.12)	431.96(±16.82)	413.53(±5.10)
	Zn*	70.45(±1.11)	66.78(±6.19)	64.12(±0.25)	65.30(±1.43)
CT	Cd	ND	ND	ND	ND
	Zn*	65.24 (±1.52)	67.21(±1.74)	64.64 (±1.99)	56.29(±1.87)

Note: CT: Control Pot, M_{ar}: Marigold, C_{os}: Cosmos, S_{un}: Sunflower, G_{ui}: Guinea grass
ND: Non-detectable; SD: Standard deviation
Zn*: Zn that naturally present in the soil

4.3 Plant Growth under various Cadmium Concentrations

Effect of cadmium on plant growth

At flowering stage, comparison of plant growth (based on plant height) at various Cd concentrations was carried out. It was found that individual plant heights differed under various Cd treatments as shown in Table 4.3. The height of all plants, under all Cd treatments, was highest for control and differed significantly from the controls ($p<0.05$). The heights of marigold under Cd concentration of 50 mg/kg differed significantly from those at other Cd treatments (100, 200 and 400 mg/kg). Regardless of the controls, the height of all plants was highest at the Cd treatment of 50 mg/kg and then reduced with increasing Cd concentration applied to the soil. Generally, for the Cd concentration of 400 mg/kg, all of studied plants look smaller and shorter than those at the lower concentrations (50, 100, 200 mg/kg) and the control pots.

Furthermore, for marigold, cosmos and sunflower, for all of Cd treatments except the Cd concentration of 400 mg/kg, the plants started to bloom after germination about 10-11 weeks. The flowering stage of marigold, cosmos and sunflower at the highest Cd treatment of 400 mg/kg was delayed for about 2-3 weeks as compared to the control pots. For Guinea grass, under all Cd treatments, no flowering stage was observed during the time of investigation. Only the control pots showed flowering stage after germination around 12 weeks. At the end of experiment, all studied plants grew well in the control pots.

Table 4.3 Heights of studied plants under different cadmium treatments

Cd treatment (mg/kg)	Plant height (cm)			
	M_{ar}	C_{os}	S_{un}	G_{ui}*
CT	56.55 ^{c} (±1.07)	48.67^{d} (±0.34)	89.33 ^{d} (±2.40)	92.78^{d} (±2.80)
50	51.33^{b} (±1.53)	43.33^{c} (±2.33)	82.17^{c} (±0.93)	70.78^{c} (±1.83)
100	32.89^{a} (±1.50)	40.39^{c}(±1.92)	79.11^{c} (±0.70)	63.44^{bc} (±2.78)
200	31.52^{a} (±1.30)	37.45^{b} (±0.69)	67.00^{b}(±1.86)	55.89^{b} (±1.17)
400	32.11^{a} (±2.22)	31.44^{a} (±0.77)	58.33^{a} (±4.34)	46.78^{a} (±8.57)

CT: Control; M_{ar}: Marigold; C_{os}: Cosmos; S_{un}: Sunflower; G_{ui}: Guinea grass
All data are presented as means ± S.D. (n =3). Means within the column with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

The total biomass (TB) (dry weight, DW) of harvested plants from different species differed among treatments as shown in Table 4.4. For all species, the maximum TB was obtained for the controls which significantly differed from the other treatments (*$p<0.05$*). Among the four treatments, the Cd treatment of 50 mg/kg provided the maximum TB (8.93±3.77, 5.26±1.98, 10.06±0.84, 68.58±4.28 g/pot, respectively, for marigold, cosmos, sunflower and Guinea grass). Under all Cd treatments, the total biomass of Guinea grass was higher than that of the other species. However, under the higher Cd concentrations (200 and 400 mg/kg), significant decline in growth (based on plant heights and TB) of four species was observed as compared to the control. The total biomass of all plants decreased with increasing Cd concentration applied to the soil.

Table 4.4 Total biomass of studied species under different Cd treatments

Cd treatment (mg/kg)	Total biomass, g/pot, dry weight			
	M_{ar}	C_{os}	S_{un}	G_{ui}
CT	22.51^{c} (±2.37)	12.98^{c} (±0.13)	12.69^{d} (±0.34)	81.43^{d} (±3.35)
50	8.93^{b} (±3.77)	5.26^{b} (±1.98)	10.06^{c} (±0.84)	68.58^{c} (±4.28)
100	4.13^{ab} (±3.45)	2.36 ^{a} (±0.93)	5.87^{b} (±0.68)	46.57^{b} (±9.11)
200	1.31^{a} (±0.68)	1.65 ^{a} (±0.11)	4.60^{ab} (±0.42)	46.16^{b} (±4.89)
400	1.73^{a} (±0.51)	1.87 ^{a} (±0.26)	2.06^{a} (±2.19)	15.98 ^{a} (±4.59)

CT: Control; M_{ar}: Marigold; C_{os}: Cosmos; S_{un}: Sunflower; G_{ui}: Guinea grass;
All data are presented as means ±S.D. (n =3). Means within the column with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

It can be seen that plant growth (the height and TB of four species) was reduced as a result of Cd toxicity at higher concentrations. Previous researchers (Ravera, 1984; Das et al., 1997; Luo et al., 1998; Fie Wang et al., 2005) reported that Cd can reduce

plant growth, photosynthesis, and chlorophyll content, as well as induce oxidative stress and can cause various changes in biological activities. Chlorosis, leaf rolls and stunting are the main symptoms of Cd toxicity in plants. Cadmium has been shown to interfere with the uptake, transport and use of several elements and water by plants (Haghiri, 1973; Das et al., 1997). A reduction in TB and leaf size as well as stem size of marigold, cosmos and sunflower was clearly noticed at higher concentrations of 200 and 400 mg/kg, indicating phytotoxicity by cadmium. Alloway (1995) stated that an excess of both essential and non essential metals results in phytotoxicity, and also acute cadmium toxicity manifests as leaf chlorosis, wilting, and stunted growth.

Cadmium accumulation in harvested plants

In this section, the summary of dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of the 4 species studied are summarized in Table 4.5. For all Cd treatments, Cd treatment of 400 mg/kg provided the maximum Cd accumulation in whole plant tissues of all species studied (Figure 4.1). Under the same Cd treatment, the total Cd accumulation in whole plant tissues was in the order of marigold > cosmos > sunflower > Guinea grass. It can be seen that total Cd accumulation was lowest in Guinea grass as compared to the other species, even at 400 mg/kg. In general, the mean levels of Cd concentration in harvested plants increased with an increase of Cd in the soil. Based on total Cd concentration accumulated in whole plant tissues, it can be seen that marigold showed higher potential for cadmium accumulation in plant biomass.

Under all Cd treatments (50, 100, 200 and 400 mg/kg), shoot Cd in the studied plants was in the order of marigold shoots>cosmos>sunflower> Guinea grass. Marigold has a greater ability to accumulate Cd in the aboveground parts (222.19±24.04, 382.94±131.07, 366.45±78.63, 612.05±20.95 mg/kg, respectively). Under all Cd treatments, shoot Cd in marigold was greater than 100 mg/kg showing the ability to accumulate Cd in plant shoots. The maximum Cd accumulation of marigold shoots reached the threshold value, 100 mg/kg dry weight, meeting one of the criteria for hyperaccumulator, as shown in Figure 4.2. Moreover, Cd contents in shoots of marigold differed significantly from other species *($p<0.05$)*. It is noticed that Cd in shoots increased with Cd applied in soils.

Similar trend as shoot Cd was observed for the roots. The root Cd in the plants was in the order of marigold > cosmos>sunflower>Guinea grass (Figure 4.3). It can be noticed that at higher Cd treatments of 50, 100, 200 and 400 mg/kg, the root Cd in Guinea grass was higher (5.13, 5.43, 9.85 and 19.69 times higher, respectively) than that of the shoot Cd, which resulted in the lowest TF of Guinea grass.

It was illustrated that heavy metal concentrations in plants are a function of heavy metal contents in the environment. Accumulation of Cd in all species displayed the same pattern that is root > leaf-stem> flower. Concentrations of Cd in root, leaf-stem and flower of individual species increased with an increase in Cd contents in soil. Several researchers (Cataldo et al., 1981; Rauser, 1986) reported that Cd concentrations were higher in roots than that of in shoots.

Although Guinea grass accumulated the lowest Cd concentration in whole plant tissues, due to higher biomass, the total Cd uptake per pot reached maximum with guinea grass under Cd treatment of 400 mg/kg (Figure 4.4). Keller, et al. (2003) found that higher biomass producing species usually uptake low to average heavy metal concentration. This could compensate their lower Cd uptake when compared to hyperaccumulating species producing lower biomass. Due to higher biomass, Guinea grass could be used for long term remediation of the contaminated areas, where mild contaminations are observed. Moreover, Guinea grass is easy to grow and the whole plant with root can be harvested. It grows well on a wide variety of soils and even under light shade of trees and bushes (and thus can be grown with other crops). It can survive long dry spells, drought tolerant and also it is considered as a suitable plant to stop soil erosion on slopes.

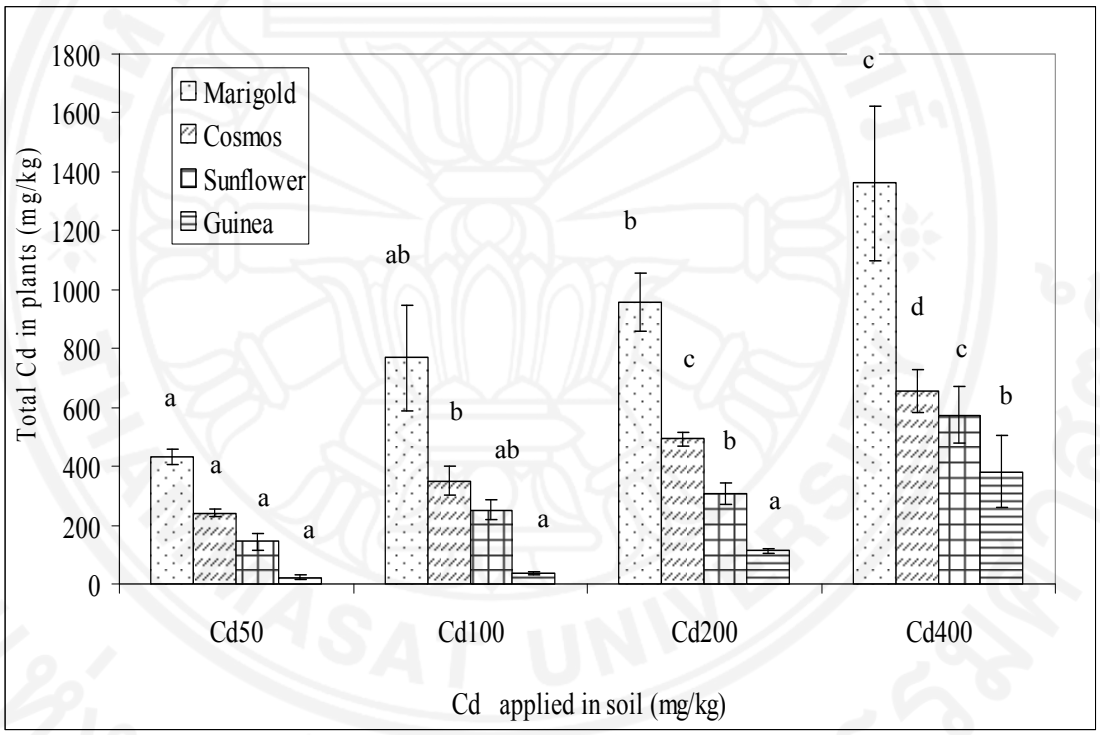


Figure 4.1 Total Cd in studied plants (whole plants) under various Cd treatments
Note: All data are mean ± S.D. (n=3). One way ANOVA (one factor: different Cd treatments) was performed for total Cd accumulation in whole plants

Table 4.5 Dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of the plants studied under different Cd treatments

Cd Treatments (mg/kg)	Dry biomass (g/pot)	Cd accumulation (mg/kg)			Cd uptake (mg/pot)	TF	BCF
		Shoot	Root	Total			
Marigold 50	8.93^{b} (±3.77)	222.19 ^{a} (±24.04)	209.00^{a} (±14.74)	431.19^{a} (±27.35)	3.86^{b} (±1.74)	1.07^{b} (±0.14)	8.86^{b} (±1.17)
100	4.13^{ab} (±3.45)	382.94 ^{b} (±131.07)	386.53^{b} (±53.49)	769.47 ^{ab} (±5.13)	2.89^{b} (±2.55)	0.97^{b} (±0.23)	6.9 ^{b} (±1.93)
200	1.31^{a} (±0.68)	366.45 ^{b} (±78.63)	591.84^{c} (±60.52)	958.29^{b} (±6.95)	1.91^{a} (±0.10)	0.62^{a} (±0.05)	4.4 ^{a} (±0.49)
400	1.73^{a} (±0.51)	612.05 ^{c} (±20.95)	933.29^{d} (±43.02)	1361.79^{c} (±43.47)	2.31^{ab} (±0.68)	0.71^{a} (±0.09)	2.96^{a} (±0.57)
Cosmos 50	5.26^{b} (±1.98)	130.02 ^{a} (±5.00)	129.83^{a} (±3.74)	241.86^{a} (±12.67)	1.26 ^{a} (±0.43)	1.00^{b} (±0.07)	4.98^{d} (±0.36)
100	2.36 ^{a} (±0.93)	192.59 ^{b} (±25.28)	157.24^{b} (±28.18)	349.83^{b} (±49.87)	0.84^{a} (±0.42)	1.26^{b} (±0.31)	3.15^{d} (±0.37)
200	1.65 ^{a} (±0.11)	169.16 ^{ab} (±9.60)	269.19^{c} (±17.56)	493.0c (±23.30)	0.82 ^{a} (±0.02)	0.86 ^{a} (±0.08)	2.18 ^{b} (±0.07)
400	1.87 ^{a} (±0.26)	271.31^{c} (±36.58)	305.56^{d} (±48.07)	657.31^{d} (±73.05)	1.23 ^{a} (±0.19)	0.82 ^{a} (±0.13)	1.48 ^{a} (±0.10)
Sunflower 50	10.06^{c} (±0.84)	80.71^{a} (±18.36)	63.94^{a} (±10.18)	144.65^{a} (±28.38)	1.44^{b} (±0.19)	1.25 ^{b} (±0.11)	3.04 ^{b} (±0.53)
100	5.87^{b} (±0.68)	116.16^{ab} (±18.53)	135.65^{b} (±15.95)	251.81 ^{ab} (±34.43)	1.47 ^{b} (±0.42)	0.86 ^{a} (±0.04)	2.29 ^{b} (±0.28)
200	4.60^{ab} (±0.42)	136.08b (±13.89)	170.76^{c} (±21.69)	306.83^{b} (±35.58)	1.42 ^{b} (±0.36)	0.80 ^{a} (±0.02)	1.45 ^{a} (±0.20)
400	2.80^{a} (±2.19)	262.84^{c} (±52.32)	325.85^{d} (±36.01)	574.85^{c} (±94.51)	1.09 ^{a} (±0.98)	0.80 ^{a} (±0.11)	1.34 ^{a} (±0.23)
Guinea grass 50	68.58^{c} (±4.28)	3.50 ^{a} (±0.77)	17.95^{a} (±7.62)	21.45^{a} (±7.95)	1.49 ^{a} (±0.61)	0.22 ^{b} (±0.11)	0.41^{a} (±0.14)
100	46.57^{b} (±9.11)	5.70 ^{a} (±0.39)	30.93^{a} (±5.13)	36.63^{a} (±5.50)	1.71 ^{a} (±0.45)	0.19 ^{b} (±0.02)	0.32 ^{a} (±0.06)
200	46.16^{b} (±4.89)	10.43 ^{b} (±0.39)	102.70^{b} (±6.95)	113.13^{a} (±6.94)	4.38 ^{b} (±2.00)	0.10 ^{ab} (±0.01)	0.48^{a} (±0.02)
400	15.98 ^{a} (±4.59)	10.43 ^{b} (±6.42)	275.5^{c} (±43.47)	381.93^{b} (±122.64)	6.47 ^{c} (±3.97)	0.05^{a} (±0.04)	0.90 ^{b} (±0.23)

All data are presented as means ±S.D. (n =3). Means within the column for individual species with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

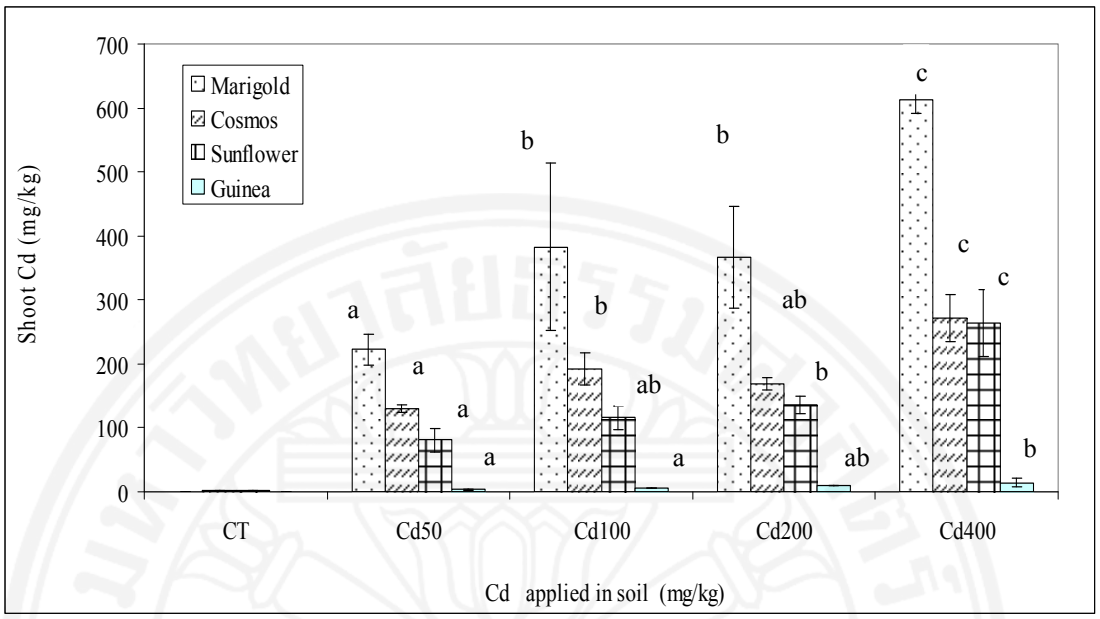


Figure 4.2 Cd concentrations in shoots of studied plants under various Cd treatments Note: All data are mean ± S.D. (n=3). One way ANOVA (one factor: different Cd treatments) was performed for total Cd accumulation in shoots. CT: Control

It can be seen from Figure 4.5, that at Cd treatment of 50 mg/kg, the maximum translocation factor (TF) was obtained by sunflower followed by marigold, cosmos and Guinea grass with TF values of 1.25±0.11, 1.07± 0.14, 1.00±0.07 and 0.22±0.11, respectively. However, no significant difference between the TF values of sunflower and marigold *(p>0.05)* and also no significant difference between TF of marigold and cosmos *(p>0.05)* were observed. Translocation factors of marigold, cosmos and sunflower were greater than one (at Cd treatment of 50 mg/kg), indicating the ability of the plants to translocate Cd from roots to shoots (Baker and Brook, 1989). It can be noticed that marigold showed the potential to be a Cd hyperaccumulator (Cd concentration in shoots is greater than 100 mg/kg). This is true for all species except for Guinea grass (Figure 4.2), based on the criteria proposed by Baker and Brook (1989). In addition, at Cd treatment of 100 mg/kg, there was no significant difference between the TF of cosmos and marigold (1.26±0.31 and 0.97±0.23), ($p>0.05$). At highest concentration (400 mg/kg), there were no significant differences in TF of Cosmos, sunflower and marigold. Higher TF values in the plants are crucial for phytoextraction of heavy metals from contaminated soil because it enables phytoremediation by harvesting only the aboveground parts of the plants.

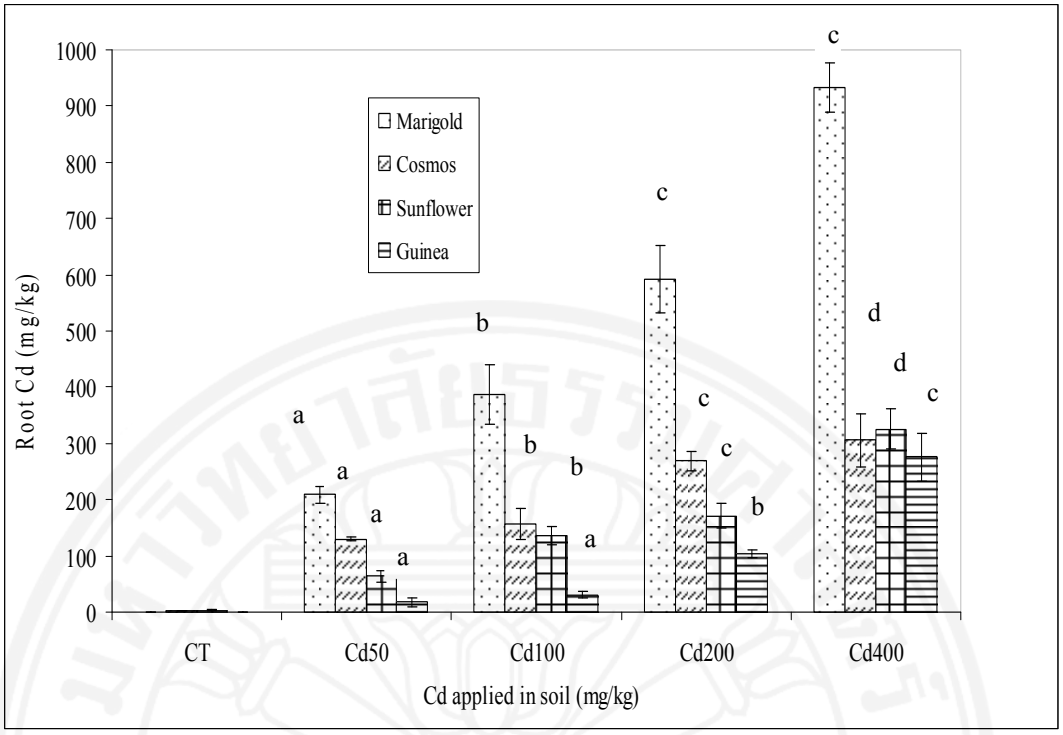


Figure 4.3 Cd concentrations in roots of studied plants under various Cd treatments
Note: All data are mean ± S.D. (n=3). One way ANOVA (one factor: different Cd treatments) was performed for total Cd accumulation in shoots. CT: Control

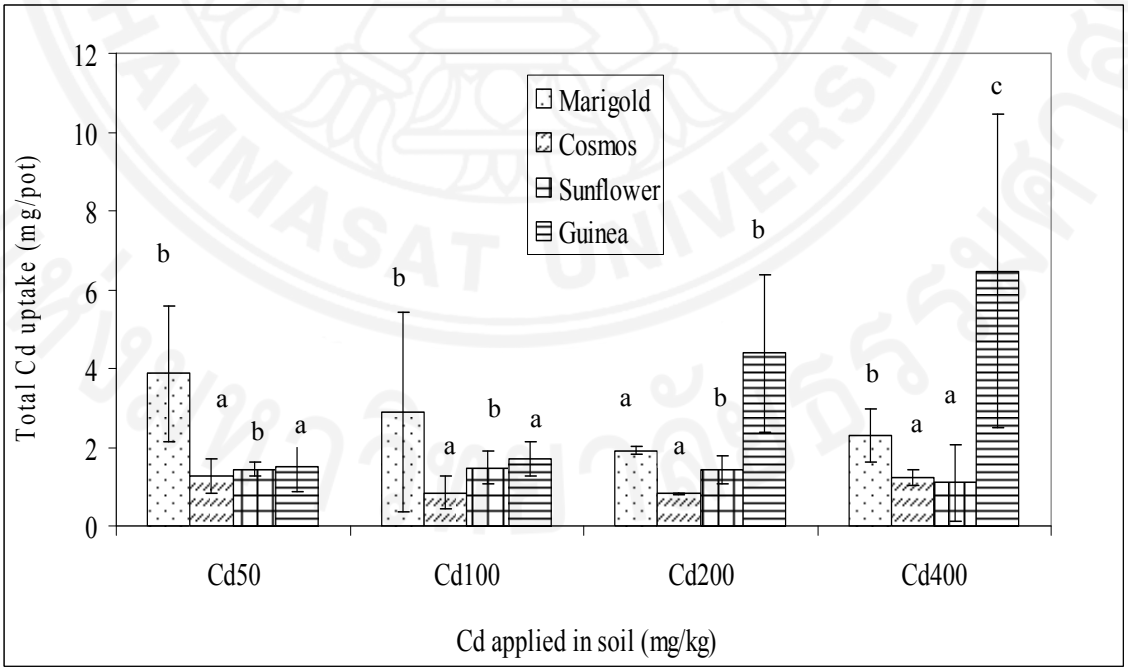


Figure 4.4 Total Cd uptake by various plants under different Cd treatments
Note: All data are means ± S.D. (n=3). One way ANOVA (one factor: different Cd treatments) was performed for total Cd uptake

The maximum bioconcentration factor (BCF) was obtained for marigold followed by cosmos, sunflower and Guinea grass under all Cd treatments, as it can be seen from Figure 4.6. It can be clearly observed that the BCF for marigold was significantly different *($p<0.05$)* from other species. The maximum BCF of 8.86±1.17, 6.96±1.93, 4.41±0.49 and 2.96±0.57 were obtained for marigold under Cd treatments of 50, 100, 200 and 400 mg/kg, respectively. At Cd treatment of 100 mg/kg, the BCF of marigold was higher than those of other species, and also showed significant difference from other species *($p<0.05$)*. However, there were no statistical difference for cosmos and sunflower *($p>0.05$)*. From the result obtained, it is noticed that under all Cd treatments, the BCF of marigold, cosmos, and sunflower were greater than one, indicating that more cadmium is accumulated in the plant biomass as compared to that in the soil.

Based on Cd accumulation in aboveground parts, marigold showed greater ability to accumulate more Cd in shoots (under all Cd treatments) as compared to other species. Regarding the TF values, although TF of sunflower (at Cd level of 50 mg/ kg) was higher than that of marigold and cosmos, there was no significant difference ($p>0.05$) among the TF of marigold and sunflower as well as the TF of marigold and cosmos.

Although, TF and BCF values of Guinea grass were lowest as compared to other species, due to higher biomass production, total uptake of Cd by Guinea grass from contaminated soil is higher, compared to other species. As a result, marigold and Guinea grass have potential as alternative plant species for phytoremediation of Cd contaminated soil. Further investigations need to be carried out for these two species. Marigold is an ornamental plant. *Tagetes erecta* L. flowers are rich sources of pigments, mainly carotenoid and flavonoid, which can be used as active ingredients in textile coloration. Jothi (2008) and Vankar et al (2009) reported that marigold flower had been shown to have good dyeing prospects. However, the potential use of marigold as a natural textile colorant on an industrial scale needs to be further investigated.

4.4 Effect of pH on Cadmium Uptake by Plants of Interest

For marigold, at Cd treatments of 50, 100 mg/kg and desired pH of 5-7.5, Cd concentrations in spiked soil varied from 48.71-59.56 mg/kg (pH varied from 4.70-7.49), and 108.43-115.21 mg/kg (pH varied from 5.13-7.58), respectively (Table 4.6). For Guinea grass, at Cd concentration of 50, 100, and 200 mg Cd/kg, Cd concentration varied from 54.06-55.41 mg/kg (pH varied from 4.62-7.41), 90.49-95.50 mg/kg (pH varied from 4.77-7.45), and 163.23-170.82 mg/kg (pH varied from 4.64-7.56), respectively. However, the total cadmium concentration in AIT soil (natural soil) is non-detectable. Total zinc concentrations naturally present in AIT soil varied from 36.67-47.20 mg/kg. From the results obtained, it can be seen that total cadmium concentrations of artificially spiked soil varied from one pot to another as can be seen from SD values, which might be due to the properties of the soil and the mixing of the soil when spiked with cadmium chloride solution. However, the calculation of relevant parameters is based on the actual concentration of cadmium in artificially spiked soil.

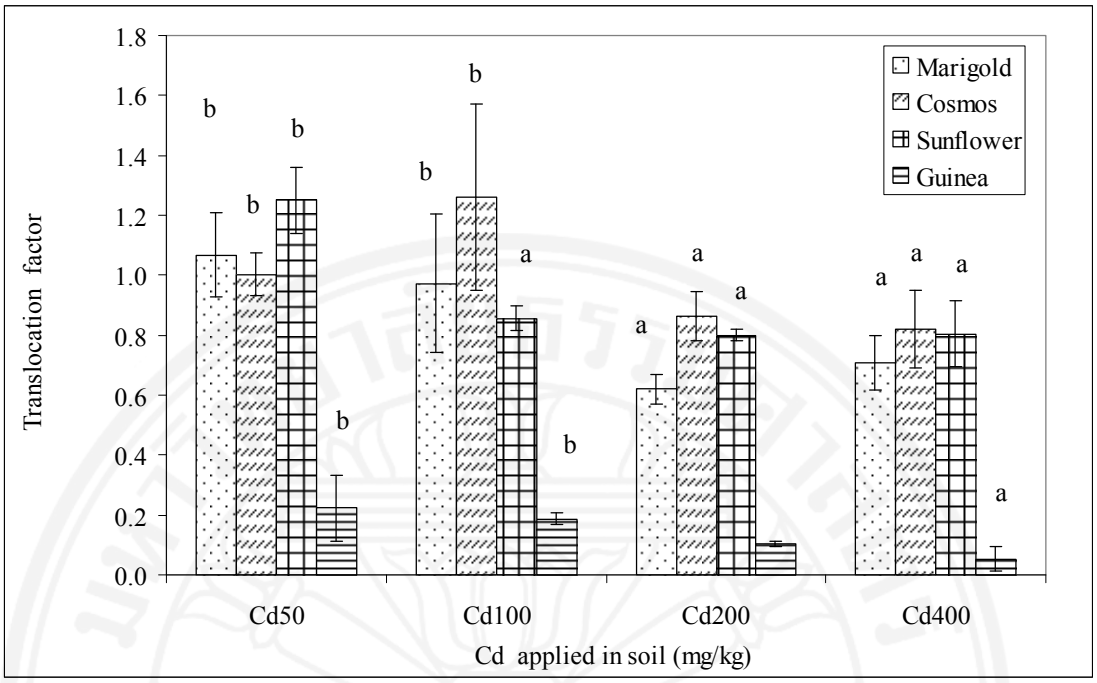


Figure 4.5 Translocation factor (TF) of harvested plants under various Cd treatments Note: All data are means ± S.D. (n=3). One way ANOVA (one factor: different Cd treatments) was performed for TF

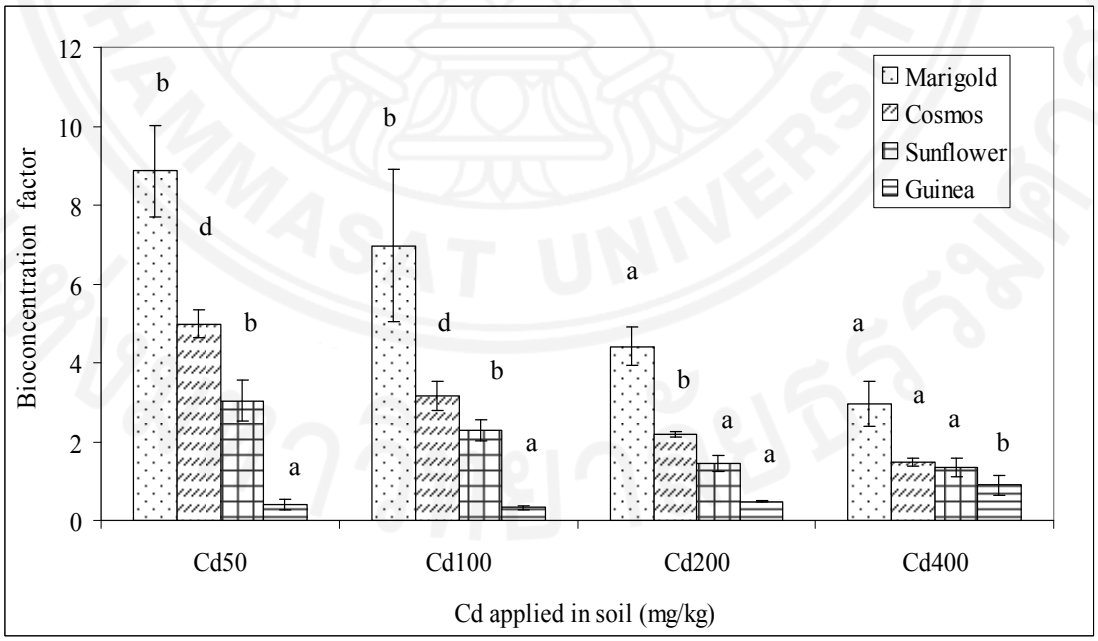


Figure 4.6 Bioconcentration factor (BCF) of harvested plants under various Cd treatments. Note: All data are means ± S.D. (n=3). One way ANOVA (one factor: different Cd treatments) was performed for BCF

Table 4.6 Cadmium concentration in artificially spiked soil and soil pH variation (desired Cd concentration of 50, 100 and 200 mg/kg, pH varied from 5-7.5)

Desired Cd concentration (mg/kg)	Marigold		Guinea grass	
	Actual pH	Actual Cd mg/kg	Actual pH	Actual Cd mg/kg
50	4.70 (±0.48)	48.71 (±1.37)	4.62(±0.27)	55.41 (±0.76)
	6.33 (±0.07) *	52.02 (±3.69)	6.33 (±0.07)	55.24 (±2.38)
	7.16 (±0.17)	59.42 (±1.52)	6.91 (±0.02)	55.53 (±2.55)
	7.49 (±0.13)	59.56 (±2.06)	7.41 (±0.41)	54.06 (±1.21)
100	5.13 (±0.16)	108.43 (±1.96)	4.77 (±0.49)	94.08 (±4.76)
	6.41 (±0.10) *	112.01(±5.16)	6.26 (±0.14)	90.49 (±0.91)
	7.14 (±0.22)	115.21(±2.58)	6.79 (±0.19)	91.19 (±1.85)
	7.58 (±0.12)	112.62 (±2.48)	7.45 (±0.21)	95.50 (±1.90)
200			4.64 (±0.47)	169.86 (±4.12)
			6.37 (±0.13)	167.76 (±4.21)
			6.78 (±0.26)	170.82 (±2.31)
			7.56 (±0.11)	163.23 (±8.00)

Note: SD: Standard deviation
* Control pot

4.4.1 Effect of soil pH on plant growth under various cadmium treatments

Generally, the plants grown in natural soil looked healthier than those grown under Cd treatments and pH variations (healthy stems, dark green leaves, longer and wider leaves were observed for control pots). After reaching flowering stage, marigold flowers grown in natural soil looked healthier than those grown under various soil pH conditions and Cd treatments. The different sizes of marigold flowers from various treatment conditions (various soil pH and Cd treatments) were observed during the time of investigation. After harvesting, the comparison of plants growth, based on plant height and total biomass (dry weight), under various soil pH conditions and different Cd treatments was carried out as shown in Tables 4.7 and 4.8 for marigold and Guinea grass, respectively. A clear trend on TB and height of marigold cannot be seen with soil pH change.

For marigold, in both 50 and 100 mg/kg treatments, the maximum total biomass was obtained at soil pH 7.0, followed by pH 6.3. Total biomass increased when pH slightly increased from 5.0 to 6.3 and then declined as soil pH was enhanced to 7.5. Total biomass at pH 6.3-7.0 was higher than that at pH 5.0 (Table 4.7). This was in agreement with the study of Singh and Myhr, (1998) who reported that barley grain yield was significantly higher at soil pH of 6.5 as compared to that at pH 5.5. Yanai, et al. (2006) stated that plant growth was drastically reduced in the soil pH of 4.4.
According the literature the pH below 5.8 should be avoided for marigold. When the soil becomes too acidic, the presence of iron and manganese increases. Uptake of too much iron and manganese in marigolds causes a nutrient disorder known as iron and manganese toxicity (Singh, A.K., (2006)).

For Guinea grass, at Cd treatment of 50 mg/kg, the maximum total biomass (213.20 g/pot) was obtained at a soil pH around 5.0 and then followed by at pH of 6.3. However, there was no significant difference *(p>0.05)* in TB among those pH (Table 4.8). It can be noticed that total biomass of Guinea grass grown in natural soil was not significantly different *(p>0.05)* from those grown in soil pH 5.0 and 6.3. The growth remained unchanged with the soil pH ranging from 6.3-7.5. Yanai, et al. (2006) observed that the growth of *Thlaspi caerulescens* was relatively stable (not significant different) in the soil pH range from 5-7.6.

Similar to the heights, total biomass, for all pH treatments, declined as Cd concentration in soil increased. Total biomass significant declined while Cd concentration in soil was increased further up to 200 mg/kg. This result showed that higher cadmium concentration in soil can significantly affect plant growth. According to the literature, Guinea grass can grow satisfactorily at soil pH from 5-8 and optimum pH for marigold growth is around 6.5-6.9. Thus, it can be noted that both pH and Cd concentration in soil influenced the total biomass and overall growth of the studied plants. It also depends on the plant species itself.

The summary of dry biomass, Cd accumulation, Cd uptake, TF and BCF values of marigold and Guinea grass under different Cd treatments and pH conditions are presented in Tables 4.9-4.10, respectively.

Table 4.7 Height, total biomass and flower diameter of marigold under various soil pH conditions at different cadmium treatments

Soil pH	Marigold					
	50 mg/kg			100 mg/kg		
	TB (g/pot)	Height (cm)	FD (mm)	TB (g/pot)	Height (cm)	FD (mm)
Nat. soil	40.15^{b} (±1.78)	51.54^{b} (±1.35)	76.97^{c} (±2.83)	40.15^{c} (±1.78)	51.54^{b} (±1.35)	76.97^{c} (±2.83)
5.0	23.67^{a} (±5.04)	45.82^{a} (± 2.06)	61.87^{a} (±1.83)	24.63^{a} (±4.53)	45.00^{a} (±0.88)	60.17^{a} (±1.00)
6.3 (CT)	32.91^{b} (±2.65)	49.78^{ab} (±2.36)	66.60^{ab} (±7.95)	27.937^{b} (±1.50)	46.76^{a} (±2.38)	63.13^{a} (±5.25)
7.0	33.27^{b} (±4.90)	50.11^{b} (±2.11)	71.70^{bc} (±2.98)	28.60^{b} (±3.85)	47.56^{a} (±1.84)	73.57^{b} (±4.57)
7.5	22.77^{a} (±2.95)	47.90^{ab} (±0.40)	64.23^{a} (±1.11)	24.67^{a} (±1.76)	46.06^{a} (±1.34)	67.77^{ab} (±6.43)

CT: Control; FD: Flower diameter; TB: Total biomass (dry weight). All data are presented as means ± S.D. (n=3). Means within the column with different letters are significantly different from each other *(p<0.05)*, where d is significantly > c > b> a.

Table 4.8 Total biomass and height of Guinea grass under various soil pH conditions at different cadmium treatments

Soil pH	Guinea grass					
	50 mg/kg		100 mg/kg		200 mg/kg	
	TB (g/pot)	Height (cm)	TB (g/pot)	Height (cm)	TB (g/pot)	Height (cm)
Nat. soil	238.70^{b} (±10.28)	128.22^{c} (±12.07)	238.70^{c} (±10.28)	128.22^{c} (±12.07)	238.70^{c} (±10.28)	128.22^{c} (±12.07)
5.0	213.2^{ab} (±11.1)	66.89^{a} (±2.22)	186.73^{b} (±8.95)	48.22^{a} (±4.40)	62.03^{a} (±8.91)	36.00^{a} (±3.06)
6.3 (CT)	206.9^{ab} (±32.5)	84.44^{b} (±3.75)	141.47^{a} (±25.46)	73.00^{b} (±6.33)	61.10^{a} (±7.06)	62.22^{b} (±4.17)
7.0	190.33^{a} (±5.8)	92.78^{bc} (±4.62)	147.83^{ab} (±30.89)	80.00^{b} (±6.49)	90.63^{b} (±15.19)	63.33^{b} (±5.69)
7.5	195.6^{a} (±12.7)	102.1^{bc} (±5.35)	127.0^{a} (±11.33)	85.44^{b} (±2.12)	79.33^{b} (±3.52)	62.00^{b} (±6.89)

CT: Control; TB: Total biomass (dry weight). All data are presented as means ± S.D. (n=3). Means within the column with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

4.4.2 Effect of soil pH on cadmium accumulation in marigold and Guinea grass under various cadmium treatments

Marigold

At treatment of 50 mg/kg, it can be noticed that the maximum total Cd (654.34±56.14 mg/kg) in whole plants was obtained at soil pH of 5.0, which is significantly different *($p<0.05$)* from those under other soil pH (Figure 4.7). Cadmium accumulation in plants was in the order of pH 5.0>6.3>7.0~7.5. Cadmium accumulation in whole plant decreased significantly ($p<0.05$) as the pH in the soil was increased. Similar results were reported by (He and Singh, 1995; Singh et al., 1995) that the concentration of Cd in oats, spinach, wheat straw and grain tissue as well as in carrot roots and leaves decreased significantly as soil pH increased from 5.5 to 6.3, but remained unchanged as pH increased further.

In Cd treatments of 100 mg/kg, the results depicted that the maximum total concentration was obtained at pH 5.0 followed by pH 6.3, where no significant difference *($p>0.05$)*, was observed at pH 5.0 and 6.3. The lowest Cd concentration was obtained at pH of 7.5. Cadmium accumulation was in the order of pH 5.0~6.3>7.0>7.5.

Table 4.9 Dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of marigold under different Cd treatments and pH

Cd Treatments (mg/kg)	Dry biomass (g/pot)	Cd accumulation (mg/kg)			Cd uptake (mg/pot)	TF	BCF
		Shoot	Root	Total			
Cd 50 mg /kg							
pH 5.0	23.67^{a} (±5.04)	454.55^{b} (±58.19)	199.79^{a} (±12.12)	654.34^{c} (±56.14)	15.66^{b} (±4.71)	2.28^{b} (±0.34)	13.46^{c} (±1.52)
6.3	32.91^{b} (±2.65)	257.49^{ab} (±6.22)	243.39^{b} (±63.74)	500.88^{b} (±61.16)	12.52^{a} (±6.69)	1.10^{a} (±0.27)	9.71^{b} (±1.84)
7.0	33.27^{b} (±4.90)	201.62^{a} (±69.29)	201.79^{a} (±1.13)	403.41^{a} (±69.17)	13.36^{ab} (±2.80)	1.00^{a} (±0.34)	6.81^{a} (±1.32)
7.5	22.77^{a} (±2.95)	221.10^{a} (±54.11)	222.39^{ab} (±22.74)	443.49^{a} (±57.74)	10.20^{a} (±2.50)	1.00^{a} (±0.29)	7.47^{a} (±1.19)
Cd 100 mg /kg							
pH 5.0	24.63^{a} (±4.53)	593.16^{c} (±32.55)	237.34^{a} (±17.07)	830.50^{c} (±47.03)	20.38^{b} (±3.24)	2.50^{b} (±0.11)	7.65^{b} (±0.37)
6.3	27.937^{b} (±1.50)	442.53^{bc} (±30.22)	425.65^{c} (±107.35)	868.18^{c} (±134.09)	19.34^{b} (±5.65)	1.08^{a} (±0.23)	7.77^{b} (±1.34)
7.0	28.60^{b} (±3.85)	390.25^{b} (±81.64)	360.11^{b} (±52.69)	750.35b^{b} (±101.77)	21.72^{b} (±5.76)	1.10^{a} (±0.24)	6.52^{ab} (±0.98)
7.5	24.67^{a} (±1.76)	283.77^{a} (±78.73)	288.94^{ab} (±15.75)	572.71^{a} (±72.83)	14.20^{a} (±2.81)	1.00^{a} (±0.29)	5.10^{a} (±0.76)

All data are presented as means ±S.D. (n =3). Means within the column for invidual species with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

Table 4.10 Dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of Guinea grass under different Cd treatments and pH

Cd Treatments (mg/kg)	Dry biomass (g/pot)	Cd accumulation (mg/kg)			Cd uptake (mg/pot)	TF	BCF
		Shoot	Root	Total			
Cd 50 mg /kg							
pH 5.0	213.2^{ab} (±11.1)	27.26^{b} (±9.31)	63.15^{b} (±5.25)	90.41^{b} (±13.37)	19.28^{b} (±3.14)	0.43^{a} (±0.13)	1.63^{b} (±0.22)
6.3	206.9^{ab} (±32.5)	10.41^{a} (±4.82)	21.23^{ab} (±2.08)	31.64^{a} (±6.59)	6.67^{a} (±2.45)	0.48^{a} (±0.18)	0.57^{a} (±0.09)
7.0	190.33^{a} (±5.8)	7.15^{a} (±0.40)	16.73^{a} (±1.36)	23.88^{a} (±1.17)	4.54^{a} (±0.19)	0.43^{a} (± 0.05)	0.43^{a} (± 0.01)
7.5	195.6^{a} (±12.7)	7.62^{a} (±2.10)	15.43^{a} (±2.03)	23.05^{a} (±3.59)	4.52^{a} (±0.90)	0.49^{b} (±0.13)	0.43^{a} (±0.06)
Cd 100 mg /kg							
pH 5.0	186.73^{b} (±8.95)	50.88^{c} (±8.90)	147.91^{c} (±32.05)	198.79^{c} (±40.94)	36.06^{b} (±4.28)	0.35^{a} (±0.01)	2.12^{b} (±0.50)
6.3	141.47^{a} (±25.46)	27.58^{b} (±1.27)	72.54^{ab} (±6.92)	100.12^{b} (±5.77)	14.12^{a} (±2.37)	0.38^{a} (±0.05)	1.11^{b} (±0.06)
7.0	147.83^{ab} (±30.89)	13.60^{a} (±1.86)	47.34^{a} (±7.67)	60.93^{a} (±8.29)	4.54^{a} (±1.98)	0.29^{a} (± .06)	0.67^{a} (±0.09)
7.5	127.0^{a} (±11.33)	14.37^{a} (±5.00)	54.78^{a} (±19.54)	69.15^{a} (±24.40)	4.52^{a} (±3.89)	0.26^{a} (±0.02)	0.73^{a} (±0.27)
Cd 200 mg /kg							
pH 5.0	62.03^{a} (±8.91)	91.49^{c} (±10.22)	229.18^{c} (±41.97)	320.67^{c} (±51.58)	19.85^{b} (±13.40)	0.40^{a} (±0.03)	1.89^{b} (±0.33)
6.3	61.10^{a} (±7.06)	75.91^{b} (±6.49)	210.81^{b} (±28.05)	286.72^{b} (±34.49)	17.68^{a} (±4.05)	0.36^{a} (±0.02)	1.71^{b} (±0.25)
7.0	90.63^{b} (±15.19)	41.83^{ab} (±9.78)	143.39^{a} (±13.59)	185.22^{a} (±18.08)	16.89^{a} (±3.92)	0.29^{a} (± 0.07)	1.08^{a} (±0.11)
7.5	79.33^{b} (±3.52)	34.26^{a} (±4.81)	147.16^{a} (±20.13)	181.42^{a} (±24.57)	13.40^{a} (±4.01)	0.23^{a} (±0.01)	1.11^{a} (±0.18)

All data are presented as means ±S.D. (n =3). Means within the column for individual species with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

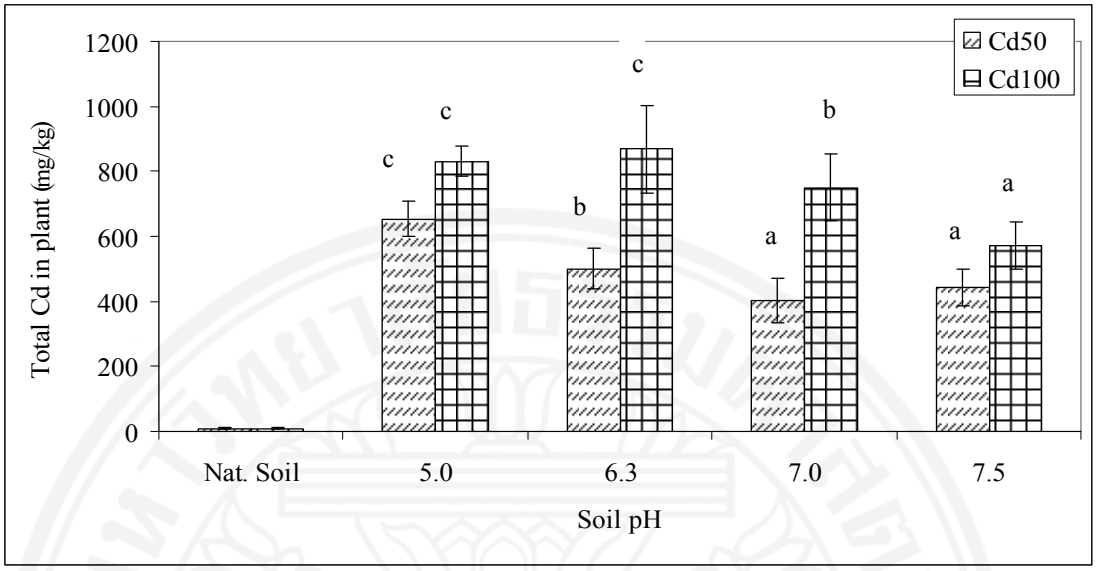


Figure 4.7 Total Cd in marigold (whole plants) under various soil pH at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications.

The maximum shoot concentrations of marigold, at Cd treatments of 50 and 100 mg/kg, were 454.55±58.19 and 593.16±32.55 mg/kg, respectively, at soil pH of 5.0 (Figure 4.8), which significantly differed from those at other soil pH conditions *($p<0.05$)*. Cadmium concentration in shoots was greater than 100 mg Cd/kg, illustrating the potential to be a Cd hyperaccumulator (Baker et al., 1970; Kirkham, 2006). The results showed that under all soil pH conditions and all Cd treatments, shoot Cd of marigold are greater than 100 mg/kg dry weight. It can be noticed that the Cd concentration accumulated in marigold shoot tissues was affected by the concentration of Cd in the soil and initial soil pH, which is in agreement with the results studied by Peralta-Videa et al. (2009).

At Cd treatment of 50 mg/kg, under various soil pH conditions, total uptake of Cd (Figure 4.9) varied from 10.20±2.50 to 15.66±4.71 mg/pot. The maximum Cd uptake was found at pH 5.0 and then declined as soil pH increased to 7.5. At Cd treatment of 100 mg/kg, the total uptake was lowest (14.20±2.81 mg/pot), at soil pH of 7.5 which was significantly different *($p<0.05$)* from that at pH of 5.0.

For translocation factor (TF) of marigold, at Cd treatments of 50 and 100 mg/kg, the maximum TF values (2.28 and 2.50, respectively) were obtained at a pH 5.0 (Figure 4.10). The TF values of marigold were greater than one, indicating the ability of the plant to translocate cadmium from roots to shoots (Baker et al., 1989). Oliver, et al., (1994) reported that TF values of wheat grain were 0.19 and 0.15 at pH of 5.5 and 6.5, respectively. Eriksson et al. (1996) also noted that TF values of carrot were 0.22 and 0.12 at pH of 4.9 and 6.4. These results illustrated that lower soil pH provided higher TF values than that at higher soil pH.

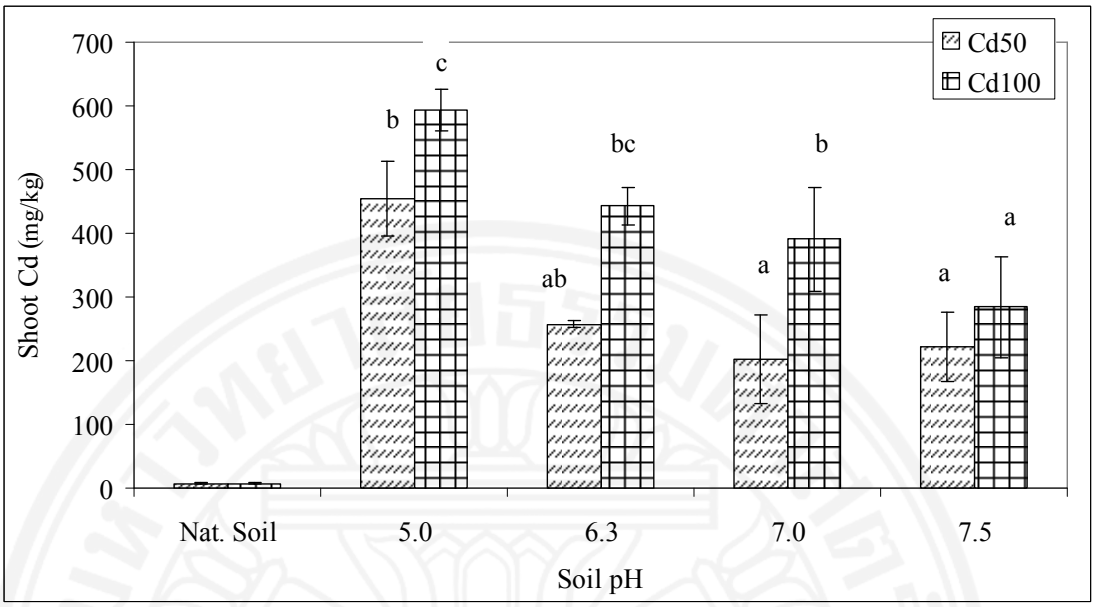


Figure 4.8 Cd concentrations in marigold shoot (dry weight) under various soil pH at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications.

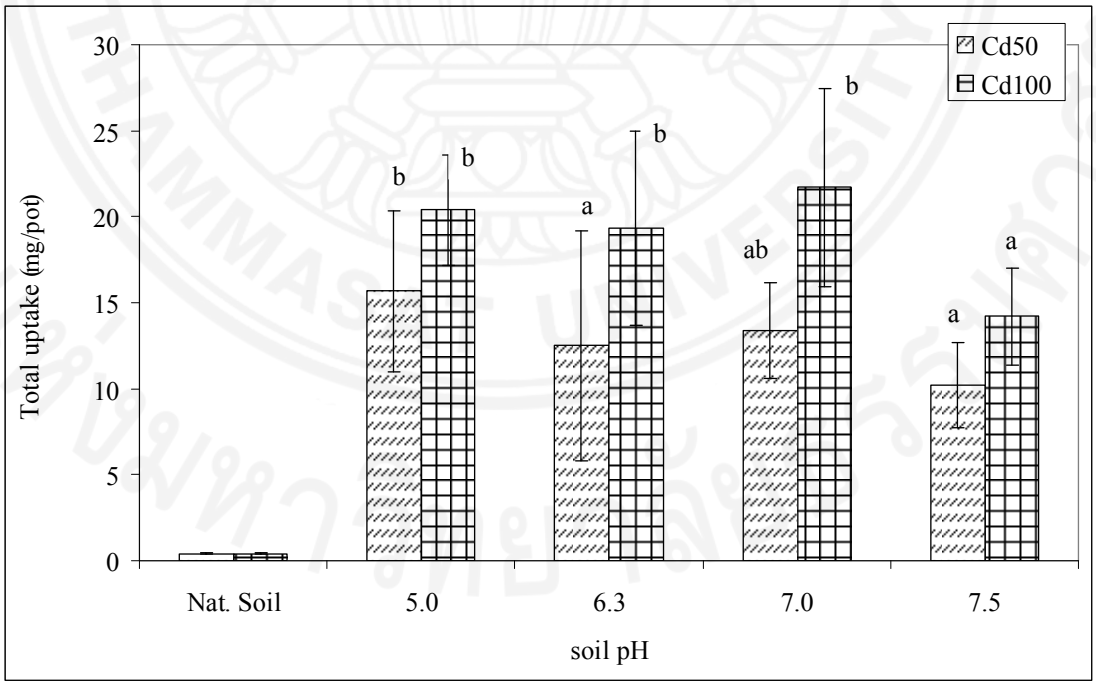


Figure 4.9 Total Cd uptake of marigold under various soil pH at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications

For BCF of marigold, at 50 and 100 mg/kg treatments, the maximum BCF values (13.46 and 7.66, respectively) were obtained at a pH 5.0 (Figure 4.11). The BCF values decreased when pH in the soil was increased further up to 7.5, providing the lowest BCF (5.10), under Cd treatment of 100 mg/kg. It demonstrated that the BCF values for both 50 and 100 mg/kg treatments were greater than one, showing that more Cd is accumulated in the plant tissues, as compared to that in the soil.

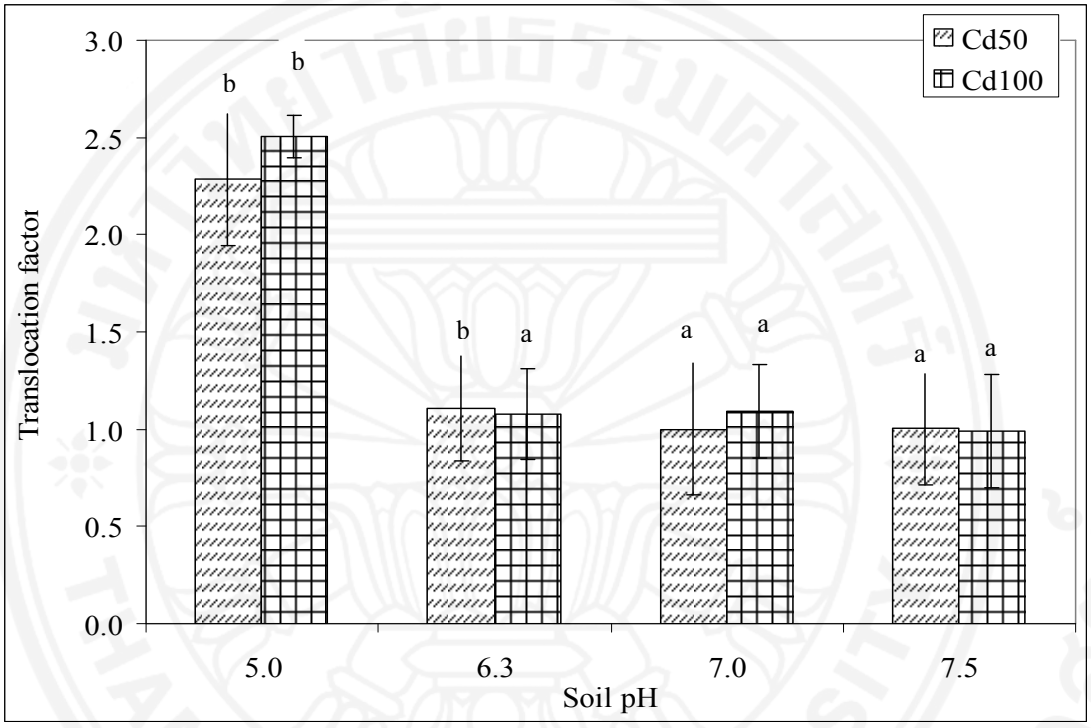


Figure 4.10 Translocation factor (TF) of marigold under various soil pH at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ± S.D of three independent replications

Percentage cadmium removal by marigold

The percentage removal of Cd by marigold was presented in Table 4.11. The results showed that for both Cd treatments of 50 and 100 mg/kg soil, the maximum removal percentages of 4.63 and 2.69 were obtained, respectively at pH around 5.0, where the percentage Cd removal was higher than that of the control (pH 6.3). Generally, the percentage of removal decreased when pH increased from 5.0 to 7.5.

At Cd treatment of 50 mg/kg, Cd uptake by marigold was 15.66 and 12.52 mg/pot per crop, respectively for pH of 5.0 and 6.3, which was equal 294.95 and 235.81 mg/ m^2 per crop, respectively (based on the surface area of each pot; 530.23×10^{-4} m^2 and one crop takes about 80-90 days). This equivalents to 2949.54 and 2358.13 g/ha per crop, respectively, at pH of 5.0 and 6.3. Under both Cd treatments, the results showed that the removal of Cd by marigold at pH of 5.0 was higher than that of pH 6.3.

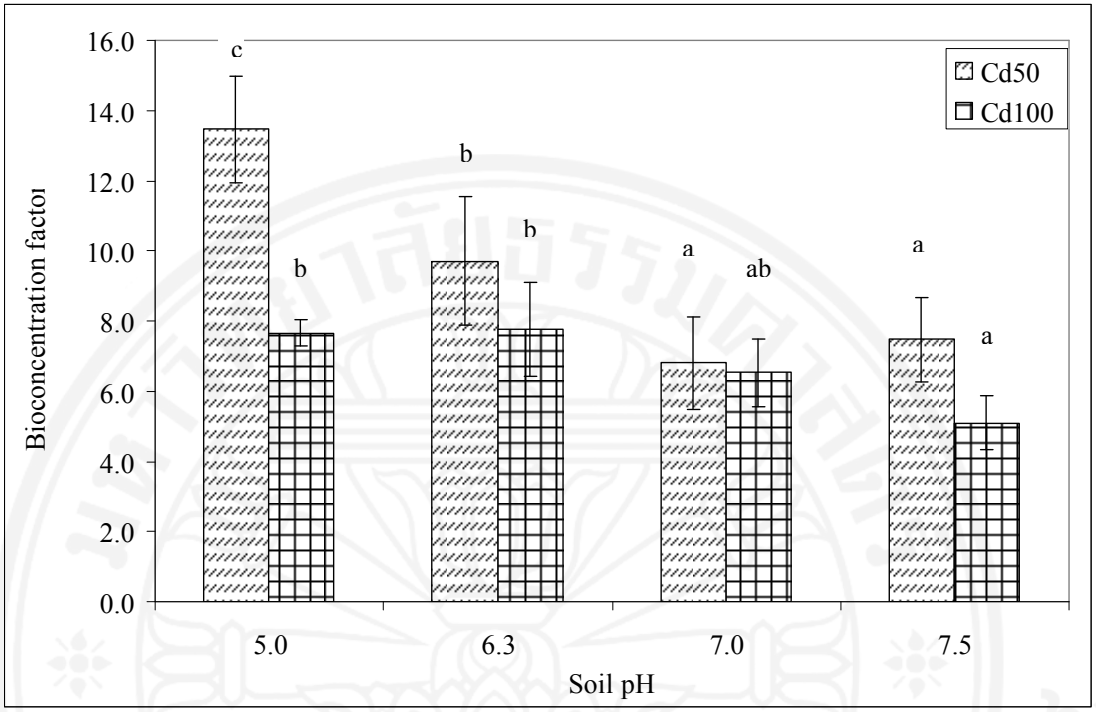


Figure 4.11 Bioconcentration factor (BCF) of marigold under various soil pH at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications

Table 4.11 Percentage removal of Cd by marigold from artificially spiked soil under various soil pH

Soil pH	Desired concentration (mg/kg)	Initial Cd applied in soil (mg/kg)	Initial Cd applied in soil/pot (mg/pot)	Final Cd in soil/pot (mg/pot)	Cd uptake by marigold (mg/pot)	% removal
5.0	50	48.71 (±1.37)	340.97 (±9.58)	325.31 (±14.23)	15.66 (±4.71)	4.63 (±1.53)
6.3	50	52.02 (±3.69)	364.16 (±25.85)	351.64 (±31.87)	12.52 (±6.69)	3.53 (±2.12)
7.0	50	59.42 (±1.52)	415.91 (±10.61)	402.55 (±12.30)	13.36 (±2.80)	3.22 (±0.72)
7.5	50	59.56 (±2.06)	416.91 (14.14)	406.71 (±16.57)	10.20 (±2.50)	2.46 (0.66)
5.0	100	108.43 (±1.96)	759.04 (±13.72)	738.66 (16.80)	20.38 (±3.24)	2.69 (±0.47)
6.3	100	112.01 (±5.16)	784.08 (±36.10)	764.74 (±33.16)	19.34 (±5.65)	2.46 (±0.65)
7.0	100	115.21 (±2.58)	806.44 (±18.05)	784.72 (±22.01)	21.72 (±5.76)	2.70 (±0.67)
7.5	100	112.62 (±2.48)	788.35 (±17.37)	774.14 (±20.15)	14.20 (±2.81)	1.81 (±0.40)

Note: 7 kg soil/ pot was used in this experiment

Guinea grass

At Cd treatment of 50 mg/kg, total Cd concentrations accumulated in Guinea grass were 90.41 ± 13.37, 31.64 ± 6.59, 23.88 ± 1.17, and 23.05 ± 3.59 mg/kg dry biomass for soil pH of 5.0, 6.3, 7.0, and 7.5, respectively (Figure 4.12). Total Cd concentration was highest at a soil pH 5.0 and then declined as soil pH was enhanced to 7.0 and remained unchanged as pH increased further up to 7.5. Similar trends were obtained for Cd treatment of 100 and 200 mg/kg. Moreover, the highest total Cd concentration in whole plant tissues was obtained at pH 5.0, under all Cd treatments. It can be noticed that Cd concentration accumulated in whole plant increased while Cd concentration in soil was enhanced further up to 200 mg/kg, but decreased as soil pH increased. However in the real contaminated site, the results obtained might vary from this study as many soil factors can influence the Cd uptake by plants.

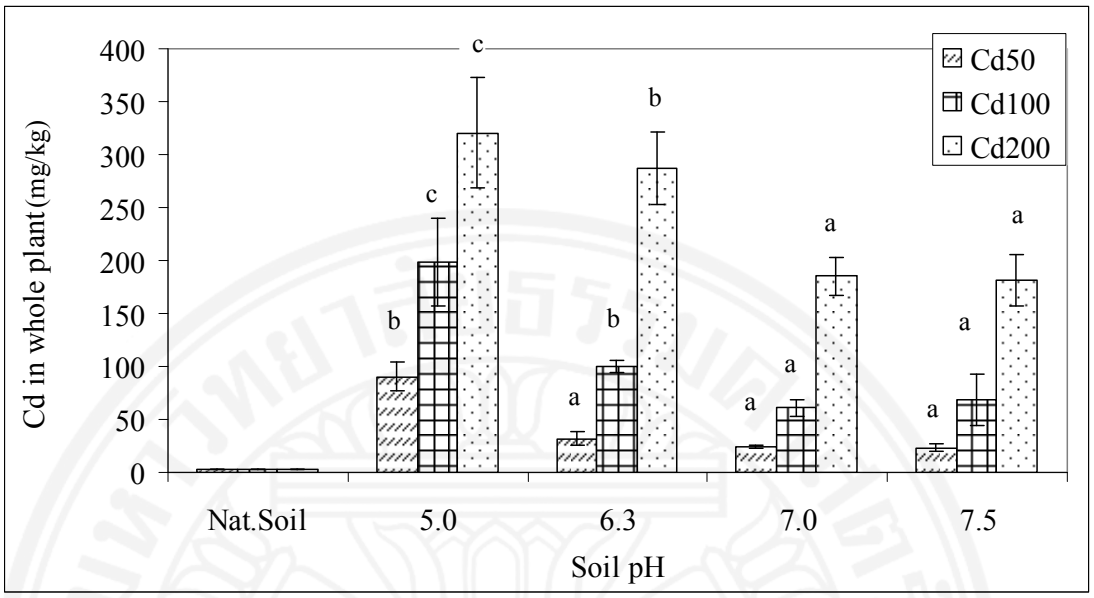


Figure 4.12 Total Cd in Guinea grass (whole plants) under various soil pH at Cd treatments of 50, 100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

The maximum Cd concentrations in shoots of Guinea grass at Cd treatments of 50, 100 and 200 mg/kg, were 27.26 ± 9.31, 50.88 ± 8.90, and 91.49 ± 10.22 mg/kg, respectively, at a soil pH of 5.0, where significant difference was observed *($p<0.05$)* from those of the other soil pH conditions (Figure 4.13). At Cd treatment of 50 mg/kg, shoot Cd significantly declined from 27.26 ± 9.31 to 7.62 ± 2.10 mg/kg, as pH in soil was increased further up to 7.5.

In all cadmium treatments, the Cd concentration accumulated in shoots increased as Cd concentration in soil increased from 50 to 200 mg/kg but reduced when pH was enhanced from 5 to 7.5. It showed that concentrations of metal in shoots increased with metal concentrations in soils but declined with increasing soil pH.

Although Cd concentrations accumulated in shoots and whole plant tissues of Guinea grass were much lower as compared to marigold, at all soil pH treatments; due to much higher biomass (dilution effect) the total uptake per pot of Guinea grass can be maximized at soil pH 5.0.

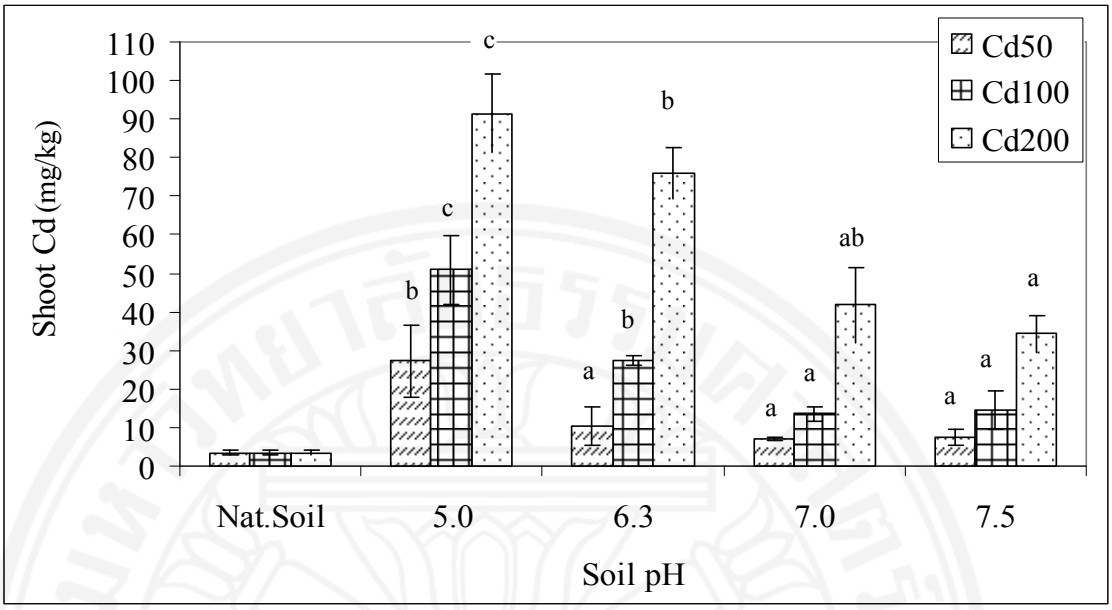


Figure 4.13 Cd concentration in shoots (dry weight) of Guinea grass under various soil pH at Cd treatments of 50,100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

At soil pH 5.0, the highest Cd uptakes were found in 50, 100 and 200 mg/kg (19.28, 36.06 and 19.85 mg/pot, respectively) (Figure 4.14). The lowest uptakes were obtained from pH 7-7.5 treatments.

It can be noticed that TF values under all soil pH conditions and all Cd treatments were less than one (Figure 4.15), indicating that Guinea grass possessed less potential to translocate Cd from roots to shoots as compared to marigold. At Cd treatments of 50 mg/kg, the highest BCF value (1.63 ± 0.22) of Guinea grass was obtained at pH of 5.0, which significantly differed *($p<0.05$)* from those of the other soil pH conditions, which no significant difference of BCF values was observed (Figure 4.16) among these pH. The same trend was observed for Cd treatments of 100 and 200 mg/kg, where the highest BCF values (2.12 ± 0.50 and 1.89 ± 0.33, respectively) was obtained at soil pH around 5.0. BCF values for all treatments declined as pH in soil increased up to 7.5.

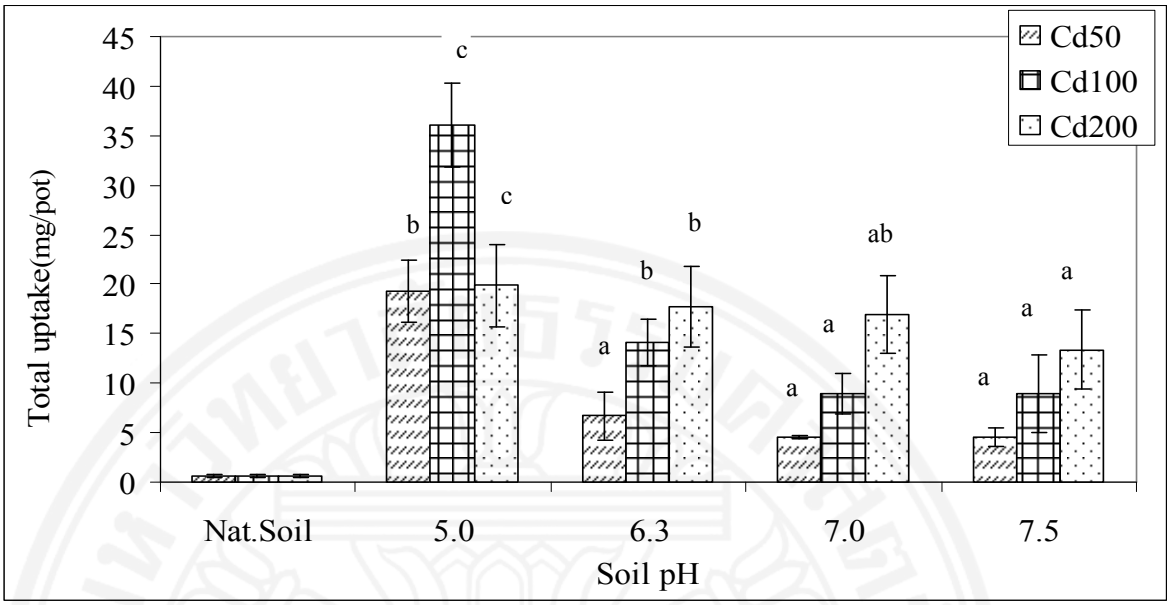


Figure 4.14 total Cd uptake by Guinea grass under various soil pH at Cd treatments of 50, 100 and 200 mg/kg. All data are presented as mean±S.D. of three independent replications.

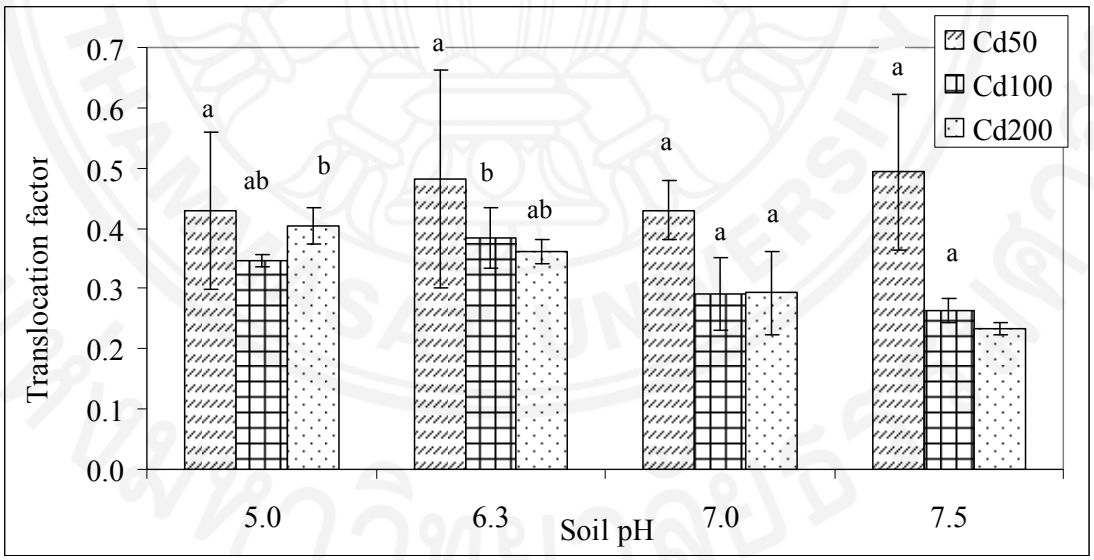


Figure 4.15 Translocation factor (TF) of Guinea grass under various soil pH at Cd treatments of 50, 100 and 200 mg/kg. All data are presented as mean ± S.D of three independent replications

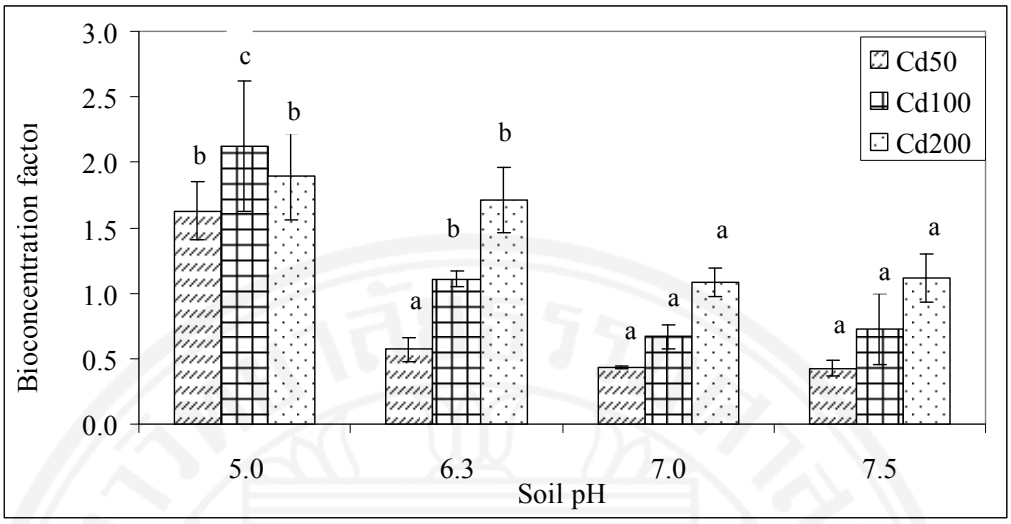


Figure 4.16 Bioconcentration factor (BCF) of Guinea grass under various soil pH at Cd treatments of 50, 100, and 200 mg/kg. All data are presented as mean ± S.D of three independent replications

Percentage cadmium removal by Guinea grass

The results showed that at pH around 5.0 for both Cd treatments of 50 and 100 mg/kg soil, the maximum removal percentages of 4.97 and 5.50 were obtained, respectively (Table 4.12). At pH 6.3, the percentage Cd removal was 1.71 and 3.00 respectively, for Cd of 50 and 100 mg/kg. The percentage Cd removal by Guinea grass significantly decreased ($p<0.05$) when pH increased from 5.0 to 6.3 and slightly declined when pH increased further to 7.5. The result depicted that lower pH (5.0) has stronger influence in Cd removal than higher soil pH (6.3). It can be noticed that at lower and higher Cd treatment (50 and 100 mg/kg), at pH of 5.0, the percentage of Cd removal by Guinea grass did not significantly differ from one another. While for marigold, at lower Cd treatment of 50 mg/kg, the percentage removal (at pH 5.0) was higher than that at higher Cd treatment of 100 mg/kg.

It can be noticed that at lower and higher Cd treatments of 50 and 100 mg/kg, at pH 5.0, the Cd uptake by Guinea grass was 1.23 and 1.77 times, respectively, higher than that of marigold. At Cd treatment of 50 mg/kg, for example, the Cd uptake by Guinea grass was 19.28 and 6.67 mg/pot, respectively, at pH 5.0 and 6.3, which were which were equivalent to 3631.37 and 1256.29 g/ha, respectively, based on the surface area of each pot (530.23×10^{-4} m^2). This illustrated that soil pH of 5.0 has a greater influence on Cd uptake by Guinea grass than that of marigold. Due to much higher biomass production, at pH around 5.0, Guinea grass provided higher than that of marigold for both Cd treatments of 50 and 100 mg/kg.

Table 4.12 Percentage removal of Cd by Guinea grass from artificially Cd spiked soil under various soil pH

Soil pH	Desired concentration (mg/kg)	Initial Cd applied in soil (mg/kg)	Initial Cd in soil/pot (mg/pot)	Final Cd in soil/pot (mg/pot)	Cd uptake by Guinea grass (mg/pot)	% removal
5.0	50	55.41 (±0.76)	387.86 (±5.35)	368.58 (±2.22)	19.28 (±3.14)	4.97 (±0.74)
6.3	50	55.24 (±2.38)	386.68 (±16.66)	380.00 (±14.21)	6.67 (±2.45)	1.71 (±0.55)
7.0	50	55.53 (±2.55)	388.74 (±17.85)	384.20 (±17.76)	4.54 (±0.19)	1.17 (±0.05)
7.5	50	54.06 (±1.21)	378.42 (±8.50)	373.90 (±8.31)	4.52 (±0.90)	1.20 (±0.24)
5.0	100	94.08 (±4.76)	658.59 (±33.33)	622.53 (±63.01)	36.06 (±4.28)	5.50 (±0.83)
6.3	100	90.49 (±0.91)	633.42 (±6.37)	619.30 (±4.08)	14.12 (±2.37)	3.00 (±1.31)
7.0	100	91.19 (±1.85)	638.33 (±12.96)	629.37 (±11.09)	8.96 (±1.98)	1.40 (±0.28)
7.5	100	95.50 (±1.90)	668.52 (±13.33)	659.55 (±16.70)	8.96 (±3.89)	1.35 (±0.61)
5.0	200	169.86 (±4.12)	1189.03 (±28.81)	1169.17 (±32.78)	19.85 (±4.20)	1.68 (±0.39)
6.3	200	167.76 (±4.21)	1174.35 (±29.74)	1156.67 (±33.43)	17.68 (±4.05)	1.51 (±0.38)
7.0	200	170.82 (±2.31)	1195.74 (±16.16)	1178.85 (±14.77)	16.89 (±3.92)	1.41 (±0.32)
7.5	200	163.23 (±8.00)	1142.63 (±55.97)	1129.23 (±57.53)	13.40 (±4.01)	1.18 (±0.38)

Note: 7 kg soil/pot was used in this experiment

Soil pH is a major factor influencing the availability of elements in the soil for plant uptake. Under acidic conditions, H^+ ions displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from sesquioxides and variable-charged clays to which they have been chemisorbed (i.e. specific adsorption). The retention of metals to soil organic matter is also weaker at low pH, resulting in more available metal in the soil solution for root absorption. Many metal cations are more soluble and available in the soil solution at low pH (below 5.5) including Cd, Cu, Hg, Ni, Pb, and Zn. It is suggested that the phytoextraction process is enhanced when metal availability to plant roots is facilitated through the addition of acidifying agents to the soil (Salt et al., 199; Huang et al. (1998)).

Peralta-Videa et al. (2009) reported that the amount of heavy metals found in *alfalfa* shoot tissues were depended on the concentration of metals in the soil and the initial soil pH. Wu et al. (2010) also reported that cadmium concentrations of shoot and root of poplar were higher when grown in acidic soil (pH 4.85), compared with those in alkaline soil (pH 8.02).

Lehoczky et al., (1998)**,** reported that generally there was a higher uptake of Cd from acidic soil than from alkaline soil. Oliver et al., (1996) reported that increasing soil pH by liming can minimize the Cd uptake by cereals. According to the results obtained from this study (under Cd treatment of 100 mg/kg), a soil pH of 7.0 provided maximum dry biomass of marigold but lower Cd accumulated in plant. At pH 5 and 6.3, Cd accumulation was higher but the biomass was lower, as compared to pH 7.0. This showed that the total uptake of Cd cannot be maximized due to limitations of biomass and/or accumulation of heavy metals. These results are in agreement with the study by Brown et al., 1994. They reported that metal concentration in plant had a tendency to increase at the lower soil pH. Bingham et al. (1980) also found that the Cd content of rice grain is highly dependent upon the soil pH and is the highest at pH 5.5. The soil pH had a profound effect on Cd solubility (Eriksson, 1989; Xue and Harrison, 1991; He and Singh, 1994). Increasing soil pH clearly reduces Cd uptake in lettuce leaves and in many other crops. Decreasing soil pH can increase plant metal uptake because metals become more readily available for plants in acidic soil (Lehoczky, 1998; Kuo, 2004; Sappin-Didier et al., 2005; Tsadukas et al., 2005; Wang et al., 2006; Yanai et al., 2006; Wu et al., 2010). As a result, the toxicity of Cd might be aggravated in acid conditions as compared to alkaline conditions. Also, some toxic elements, e.g., Mn and Al could be more available, and can possibly reach toxicity levels at low pH, which can cause reduction in plant biomass.

The results revealed that TF and BCF values of marigold were greater than one, demonstrating that cadmium is accumulated in the plant tissues, as compared to that in the soil. The maximum TF and BCF values were obtained at soil pH of 5.0 for both Cd treatment of 50 and 100 mg/kg showing that soil pH is an important factor controlling Cd availability in soil. The results were in agreement with the previous study (Ghosh and Singh, 2005), which reported that soil pH seemed to have the greatest effects of any single factor on the solubility or retention of metals in soil, with a greater retention and lower solubility of metal cations occurring at high soil pH. Wu et al. (2010) reported that translocation factor value of poplar was 1.27 in acidic soil condition (pH 4.85) and 1.09 in alkaline soil (pH 8.02). Bioconcentration factor of poplar reduced from 1.28 to 0.67 under acidic soil and alkaline soil conditions, respectively**.**

It showed that concentrations of metal in shoots of Guinea grass increased with metal concentrations in soils but declined with increasing soil pH. Similar results were observed by Xiong (1998) in *Brassica pekinensis* Rupr, who reported that heavy metal concentration in plant is a function of heavy metal contents in the environment and the soil pH. However, opposite to marigold, cadmium accumulated in shoots of Guinea grass was less than 100 mg/kg, demonstrating that Guinea grass possessed less potential to be used as a cadmium hyperaccumulator as compared to marigold.

However, because of its higher biomass production ability, Guinea grass could be considered as an alternative for remediation of mild contaminated areas.

The results obtained illustrated that the total biomass of Guinea grass was approximately 9.00 and 7.58 times higher than that of marigold under Cd treatments of 50 and 100 mg/kg, respectively, at a pH of 5.0. At this soil pH, the total Cd uptake by Guinea grass was around 1.23 and 1.77 times higher than that of marigold, respectively, at Cd treatments of 50 and 100 mg/kg. However, under Cd treatment of 200 mg/kg, at the soil pH of 5.0, although the highest Cd accumulated in whole plant was obtained (320.67 mg/kg); the total uptake (19.85 mg/pot) cannot be maximized since the biomass (62.03 g/pot) was lowest at this Cd treatment as compared to the other soil pH levels.

The maximum total biomass of Guinea grass (213.20 g/pot) was gained at Cd treatment of 50 mg/kg and at pH 5.0, but under these conditions Cd in whole plant tissues was lowest (90.41 mg/kg) as compared to at Cd treatments of 100 and 200 mg/kg, then total Cd uptake could not be increased (19.28 mg/pot). This indicated that the optimum conditions maximized the total uptake of Cd by Guinea grass were Cd treatment of 100 mg Cd/kg and soil pH around 5.

Soil pH seems to have the greatest effect on the solubility or retention of metals in soils with a greater retention and lower solubility of metal cations occurring at high soil pH. Under the neutral to basic conditions typical of most soils, cationic metals are strongly adsorbed on the clay fractions and can be adsorbed by hydrous oxides of iron, aluminium, or manganese present in soil minerals. Elevated salt concentration creates increased competition between cations and metals for binding sites. Also competitive adsorption between various metals has been observed in experiments involving various solids with oxide surfaces, in several experiments, Cd adsorption was decreased by the addition of Pb or Cu (Basta et al., 1993).

In addition, the pH is one of the factors that most affect the mobility and bioavailability of metals. Soon (1981) reported that with increasing soil pH, the surface charge become more negative, thereby increasing the adsorption of metal cations such as Cd^{2+}. The study of Bens et al (1986) revealed that the critical acidity in acid mineral soils is within the pH range of 4.0–4.5, at which a drop in pH of merely 0.2 units results in a 3–5 times increase in Cd labile pool.938. Yobouet et al (2010) reported that the pH values below 5 provided higher metals solubility as compared to at pH 5.0. Hence, at pH 5.0, cadmium was more stable and less soluble as compared to pH around 2-4. Thus, in this study, the pH of 5.0 may not cause leaching of Cd to nearby environment. However, the pH values below 5 can result in metals solubilization that could posed a major environmental hazard as mobility of heavy metals are increased. The primary concern is that the liberated metals have the ability to migrate to uncontaminated areas, possibly ground water reservoirs and also underground water closed to the contaminated area (Huang et al., 1997). The increase in soil pH enhanced the adsorption of Cd by soils and thus reduces its extractability (Chirstensen, 1984; Kuo, et al, 1985; King, 1988).

Based on Cd accumulation in aboveground parts, at all Cd treatments and various soil pH conditions, marigold showed greater ability to accumulate more Cd in the shoots (shoot Cd > 100 mg/kg), than that in roots, as compared to Guinea grass. For Guinea grass, similar trend was observed for Cd concentration accumulated in shoots and roots. The maximum Cd concentration in shoots and roots was obtained at pH around 5.0, then declined as pH in soil enhanced up to 7.0 - 7.5, which provided lower BCF value than that of pH 5. Shoot Cd in Guinea grass was less than 100 mg/kg and also TF values were less than one. This indicates that Guinea grass possessed less potential to translocate Cd from roots to shoots as compared to marigold, which failed to meet one of the criteria to be considered as Cd hyperaccumulator. The maximum BCF value was obtained at pH 5.0. Because of much higher biomass production of Guinea grass (9.1 and 7.58 times higher than that of marigold at Cd of 50 and 100 mg/kg, respectively, at pH 5.0), the total uptake by Guinea grass was higher than that of marigold at soil pH of 5.0.

Based on the shoot Cd, TF and BCF values, marigold shows higher potential to uptake Cd from contaminated soil as compared to Guinea grass. The bioavailability of Cd to plant depends on soil characteristics, and upon the plant species. Soil pH is one of the most important factors controlling Cd uptake by plants. At acidic soil condition, heavy metals tend to be easily taken up by the plants. The results of this study demonstrated that at a pH around 5, Cd concentration in marigold (whole plant) was highest (880.50 mg/kg, at Cd of 100 mg/kg). TF (2.50, at 100 mg/kg), and BCF values (13.46 at 50 mg Cd/kg) were also maximum at pH 5.0. From the results obtained, it is noticed that under all soil pH conditions and different Cd treatments, BCF values of marigold were greater than one, indicating that more cadmium is accumulated in the plants as compared to that in the soil.

Overall, for marigold, the Cd treatment of 100 mg/kg provided 1.30, 1.54 and 1.63 times higher uptake than that of Cd 50 mg/kg, at pH of 5.0, 6.3 and 7.0. At Cd treatments of 50 and 100 mg/kg, the pH 5.0 provided maximum Cd uptake (294.95 and 383.86 mg/m^2 per crop, respectively). For Guinea grass, at Cd 100 mg/kg provided 1.87, 2.12 and 1.97 times higher uptake than that at Cd of 50 mg/kg, at pH of 5.0, 6.3 and 7.0, respectively. The best Cd uptake (363.14 and 679.19 mg/m^2, respectively at Cd 50 and 100 mg/kg) was obtained at pH 5.0. This showed that at Cd treatment of 100 mg/kg and at pH 5.0 provided the maximum Cd uptake by Guinea grass (6791.87 g/ha) and marigold (3838.55 g/ha). This illustrated that soil pH of 5.0 has a greater influence on Cd uptake by Guinea grass than that of marigold. Due to much higher biomass production, at pH around 5.0, Guinea grass provided 1.77 times higher Cd uptake than that of marigold, at Cd of 100 mg/kg.

4.5 Effect of Zinc on Cadmium Uptake

After harvesting marigold and Guinea grass, effect of various Cd:Zn concentration applied in soils on cadmium uptake and accumulation in plant biomass, plant growth (based on height, total biomass) at different Cd treatments were investigated.

4.5.1 Plants Growth under various Zn concentrations applied in the soil at different cadmium treatments for marigold and Guinea grass

Flower diameter

At cadmium treatment of 50 mg/kg, the maximum diameter of marigold flower was obtained at a Cd:Zn of 50:0 mg/kg (no Zn applied to the soil) and the smallest diameter was observed when Zn applied to the soil increased to 2500 mg/kg (Table 4.13). All diameters of flowers of plants grown under all Cd:Zn treatments were significantly different *($p<0.05$)* from those grown in natural soil. While at Cd treatment of 100 mg/kg, the largest diameter (68.00±3.57 mm) was obtained at the Cd:Zn of 100:0 mg/kg. It can be noticed that flower diameters significantly declined *($p<0.05$)* as compared to the control when Zn concentration applied in the soil increased further to Cd:Zn of 50:2500 and 100:3000 mg/kg. This result showed that the flower diameter also decreased while Cd concentration in soil was enhanced.

Plant height

At Cd treatment of 50 mg/kg, the heights of marigold under all Cd:Zn concentrations applied in the soil, differed significantly from those grown in natural soil ($p<0.05$), with the maximum at a Cd:Zn of 50:0 mg/kg (Table 4.13). However, the height significantly declined from 48.33 ± 0.33 to 40.83 ± 0.50 mm when Zn applied in soil was enhanced from 0 to 2500 mg/kg. Similarly at Cd treatment of 100 mg/kg, the highest height (44.33 ± 1.45 mm**)** was observed when Zn applied to the soil at the Cd:Zn of 100:0 mg/kg and then significantly decreased *($p<0.05$)* to 37.37 ± 0.45 mm as Zn applied in soil increased further up to 3000 mg/kg (Cd:Zn of 100:3000 mg/kg). It can be noticed that the height declined with increasing Zn applied in the soil.

Similar trend was observed for Guinea grass. At Cd treatment of 50 mg/kg, the maximum height was gained at the Cd:Zn of 50:0 mg/kg and then significantly declined to 45.94 ± 2.30 mm as Zn applied in soil increased up to 2500 mg/kg. The maximum height was significantly different *($p<0.05$)* from those at other Zn concentrations (Table 4.14). While at a Cd treatment of 100 mg/kg, the maximum height (72.56 ± 51.10 mm) was observed at a Cd:Zn of 100:0 mg/kg, and then significantly decreased as Zn applied to soil was increased to 3000 mg/kg. Similarly, the maximum height at Cd treatment of 200 mg/kg, was also obtained when Zn was not applied to the soil and then significantly declined *($p<0.05$)* to 38.74±3.40 mm as Zn applied in the soil increased to 2000 mg/kg. It can be noticed that under all Cd treatments, generally, the height significantly declined, compared to the controls, with increasing Cd and Zn applied to the soils.

The results obtained in this study revealed that treatment of soil with Cd or Cd:Zn did not improve plant growth (based on height, flower diameter) as compared to plants grown in natural soil. The heights and flower diameters decreased with increasing Cd and Zn application in soil. At higher concentrations of Cd and Zn applied to the soil, plant growth was decreased to some extent.

Table 4.13 Total biomass, heights and flower diameters of marigold under various Zn concentrations applied in the soil at different cadmium treatments

Cd:Zn applied in soil (mg/kg)	Marigold					
	Cd 50 mg/kg			Cd 100 mg/kg		
	TB (g/pot)	Height (cm)	FD (mm)	TB (g/pot)	Height (cm)	FD (mm)
Natural soil	40.15^{b} (±1.78)	51.54^{b} (±1.35)	76.97^{d} (±2.83)	40.15^{c} (±1.78)	51.54^{c} (±1.35)	76.97^{c} (±2.83)
1:0 (CT)	23.47^{a} (±4.31)	48.33^{ab} (±0.33)	71.57^{c} (±1.66)	32.10^{b} (±2.36)	44.33^{b} (±1.45)	68.00^{b} (±3.57)
1:10	27.87^{a} (±5.22)	42.56^{a} ±7.31)	64.13^{b} (±4.45)	27.40^{ab} (±3.86)	42.07^{b} (±3.29)	58.13^{a} (±1.05)
1:30	27.27^{a} (±4.91)	42.88^{a} (±0.53)	62.70^{b} (±1.41)	21.30^{a} (±3.82)	37.37^{a} (±0.45)	54.2^{a} (±4.66)
1:50	1.73^{a} (±4.90)	40.83^{a} (±0.50)	55.57^{a} (±1.46)			

CT: Control (no addition of Zn solution in the soils); FD: Flower diameter; TB: Total biomass. All data are presented as means ± S.D. (n =3). Means within the column with different letters are significantly different from each other *(p<0.05)*, where d is significantly > c > b> a.

Table 4.14 Total biomass and height of Guinea grass under various Zn concentrations applied in the soil at different cadmium treatments

Cd:Zn	Guinea grass					
	Cd 50 mg/kg		Cd 100 mg/kg		Cd 200 mg/kg	
	TB (g/pot)	Height** (cm)	TB (g/pot)	Height** (cm)	TB (g/pot)	Height** (cm)
Natural soil	238.70^{d} (±10.28)	128.22^{c} (±2.07)	238.70^{d} (±10.28)	128.22^{c} (±12.07)	238.70^{c} (±10.28)	128.22^{c} (±12.07)
1:0 (CT)	83.27^{c} (±8.31)	82.28^{b} (±4.25)	90.70^{c} (±11.15)	72.56^{b} (±5.10)	53.43^{b} (±0.74)	61.67^{b} (±4.04)
1:10	88.97^{c} (±3.72)	47.28^{a} (±0.50)	48.60^{b} (±0.92)	48.67^{a} (± 0.94)	14.83^{a} (±2.32)	38.74^{a} (±3.40)
1:30	47.23^{b} (±7.77)	48.94^{a} (±2.11)	13.43^{a} (±0.93)	53.67^{a} (±1.53)		
1:50	10.43^{a} (±0.76)	45.94^{a} (±2.30)				

CT: Control; FD: Flower diameter; TB: Total biomass. All data are presented as means ± S.D. (n =3). Means within the column with different letters are significantly different from each other *(p<0.05)*, where d is significantly > c > b> a.

Total biomass

In marigold, at Cd treatment of 50 mg/kg, the maximum total biomass (27.87±5.22 g/pot) was obtained at the Cd:Zn of 50:500 mg/kg (1:10), and the lowest biomass (21.73±4.90) was obtained when higher Zn was applied to soil (Cd:Zn of 50:2500 mg/kg) (Table 4.13). No significant difference *($p>0.05$)* of total biomass among these Zn applications in soil was observed. However, the total biomass under all Zn applications significantly differed from that of natural soil *($p<0.05$).* At Cd treatment of 100 mg/kg, the maximum total biomass (32.10±2.36) was obtained at the Cd:Zn of 100:0 mg/kg and reached the lowest at 100:3000 mg/kg of Cd:Zn. The total biomass decreased as Zn applied in soil increased further above 2500 and 3000 mg Zn/kg.

In Guinea grass, at Cd treatment of 50 mg/kg, the total biomass was highest (88.97±3.72), at the Cd:Zn of 50:500 mg/kg (1:10) (Table 4.14). No significant difference of TB was observed between the Cd:Zn of 50:0 and 50:500 mg/kg. It can be noticed that TB of Guinea grass grown in natural soil significantly differed *($p<0.05$)* from those grown under other Zn concentrations applied in soil. Similar trend was observed for both Cd treatments of 100 and 200 mg/kg that the maximum total biomass was obtained at the Cd:Zn of 100:0 and 200:0 mg/kg and then the TB declined significantly as Zn applied to soil was enhanced up to the Cd:Zn of 100:3000 and 200:2000 mg/kg. Generally, the total biomass decreased while cadmium applied in soil increased up to 200 mg/kg.

From this study, the results showed that at low Cd treatment of 50 mg/kg, application of Zn in soil (500 mg/kg) increased TB of Guinea grass to some extent. However, when Zn applied to soil was enhanced further, total biomass of Guinea grass was reduced. For high Cd treatments of 100 mg/kg and 200 mg/kg, the addition of Zn in soil resulted in the declined TB of the plants.

It can be noticed that plant growth (based on the height and total biomass) was reduced as a result of Cd toxicity at higher Cd concentrations. Cadmium can reduce plant growth, photosynthesis, chlorophyll content, induce oxidative stress, and can cause various changes in biological activities (Ravera, 1984; Rosas et al., 1984; Das et al., 1997; Luo et al., 1998). Chlorosis, leaf rolls and stunting are the main symptoms of Cd toxicity in plants. Cadmium has been shown to interfere with the uptake, transport and use of several elements and water by plants (Haghiri, 1973; Das et al., 1997). This indicates that treatment of soil with Cd, or Cd-Zn did not improve plant growth as compared to the plant grown in natural soil.

Most plant species and genotypes have great tolerance to excessive amounts of Zn. Chlorosis, mainly in new leaves, and depressed plant growth is the common symptoms of Zn toxicity. An excess of Zn can be bound by phytic acid in roots of some crop plants (e.g., soybean, tomato, cabbage, and wheat). This mechanism does not work, however, when there is also an excess of Cd (Steveninck et al. (1994). In this study, for marigold, at Cd of 50 mg/kg, no significant reduction in plant height and total biomass was observed as compared to the control ($p>0.05$). However, at higher concentration of Cd (100 mg/kg) there was a significant decrease in plant height and total biomass of marigold as compared to the control. It can be seen that the height and total biomass of Guinea grass significantly declined as compared to the control at the highest Zn concentration applied to the soil for each Cd treatment.

Zinc phytotoxicity is reported relatively often, especially for acid soils. The physiology and biochemistry of the toxic effects of Zn in plants are likely to be similar to those reported for other trace metals. The toxicity limit for Zn depends on plant species and genotypes, as well as on a growth stage. Hence, Zn content at about 300 mg/kg is reported to be toxic to young barley, whereas about 400 mg/kg is toxic to oats at the beginning of tillering (David et al. (1978)). However, in root tissues, where Zn is immobilized in cell walls or complexed in non-diffusible Zn proteins, critical concentrations of Zn are much higher. Sensitive plant species are reported to be retarded in growth when their tissues contain 150–200 mg/kg Zn (Kloke et al. (1984)). Most commonly, however, the upper toxic levels range in various plants from 100 to 500 mg/kg (Macnicol et al. (1985)).

The summary of dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of marigold and Guinea grass under different Cd:Zn treatments are presented in Tables 4.15-4.16.

Table 4.15 Dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of marigold under different Cd:Zn treatments

Cd Treatments (mg/kg)	Dry biomass (g/pot)	Cd accumulation (mg/kg)			Cd uptake (mg/pot)	TF	BCF
		Shoot	Root	Total			
Cd 50 mg /kg							
Cd:Zn 1:0	23.47^{a} (±4.31)	272.39^{b} (±23.75)	204.66^{b} (±72.15)	477.05^{c} (±94.19)	11.27^{b} (±3.56)	1.41^{a} (±0.33)	7.29^{b} (±1.01)
1:10	27.87^{a} (±5.22)	382.16^{c} (±53.69)	433.70^{c} (±55.15)	815.86^{b} (±101.99)	22.39^{c} (±1.78)	1.59^{a} (±0.53)	12.77^{c} (±1.48)
1:30	27.27^{a} (±4.91)	149.26^{ab} (±11.51)	71.88^{a} (±4.36)	221.14^{a} (±12.71)	6.06^{ab} (±1.37)	2.08^{b} (±0.20)	3.54^{a} (±0.29)
1:50	21.73^{a} (±4.90)	109.59^{a} (±12.68)	77.31^{a} (±20.65)	186.90^{a} (±30.43)	3.97^{a} (±0.37)	1.48^{a} (±0.35)	2.89^{a} (±0.48)
Cd 100 mg /kg							
Cd:Zn 1:0	40.15^{c} (±1.78)	422.41^{c} (±94.09)	492.43^{c} (±92.54)	914.84^{c} (±185.50)	29.16^{c} (±4.46)	0.85^{a} (±0.05)	8.74^{c} (±1.66)
1:10	32.10^{b} (± 2.36)	199.40^{b} (±22.99)	118.98^{b} (±20.14)	318.38^{b} (±39.96)	8.70^{b} (±1.37)	1.69^{b} (±0.21)	3.06^{b} (±0.34)
1:30	27.40^{ab} (±3.86)	106.05^{a} (±24.04)	96.31^{a} (±22.23)	202.35^{a} (±46.13)	4.29^{a} (±1.02)	1.10^{a} (±0.05)	1.94^{a} (±0.45)
1:50	21.30^{a} (±3.82)						

All data are presented as means ±S.D. (n =3). Means within the column for individual species with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

Table 4.16 Dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of Guinea grass under different Cd:Zn treatments

Cd Treatments (mg/kg)	Dry biomass (g/pot)	Cd accumulation (mg/kg)			Cd uptake (mg/pot)	TF	BCF
		Shoot	Root	Total			
Cd 50 mg /kg							
Cd:Zn 1:0	83.27^{c} (±8.31)	13.21^{a} (±8.31)	24.85^{a} (±0.58)	38.07^{a} (±1.81)	3.16^{b} (±0.16)	0.53^{ab} (±0.06)	0.72^{a} (±0.05)
1:10	88.97^{c} (±3.72)	22.13^{b} (±3.72)	35.55^{a} (±10.29)	57.69^{b} (±12.78)	4.41-b^{b} (±0.72)	0.65^{b} (±0.12)	1.02^{a} (±0.21)
1:30	47.23^{b} (±7.77)	33.13^{c} (±7.77)	106.44^{c} (±26.04)	139.57^{c} (±23.78)	5.37^{c} (±1.92)	0.33^{a} (±0.12)	2.69^{b} (±0.33)
1:50	10.43^{a} (±0.76)	37.43^{c} (±0.76)	104.85^{b} (±13.96)	142.27^{c} (±16.35)	1.49^{a} (±0.23)	0.36a^{c} (±0.03)	2.61^{b} (±0.41)
Cd 100 mg /kg							
Cd:Zn 1:0	90.70^{c} (±11.15)	11.88^{a} (±2.03)	47.75^{a} (±6.48)	59.63^{a} (±8.45)	4.24^{b} (±2.74)	0.25^{a} (±0.02)	0.60^{a} (±0.07)
1:10	48.60^{b} (±0.92)	42.36^{b} (±5.45)	142.89^{b} (±49.10)	185.25^{b} (±50.18)	9.03^{c} (±2.60)	0.32^{a} (±0.09)	1.85^{b} (±0.47)
1:30	13.43^{a} (±0.93)	59.64^{c} (±12.43)	202.05^{c} (±56.11)	261.69^{c} (±68.41)	3.56^{a} (±1.15)	0.30^{a} (±0.02)	2.63^{c} (±0.69)
Cd 200 mg /kg							
Cd:Zn 1:0	53.43^{b} (±0.74)	27.40^{a} (±5.82)	103.03^{a} (±15.45)	130.43^{a} (±15.11)	6.07^{b} (±1.67)	0.27^{a} (±0.06)	0.74^{a} (±0.10)
1:10	14.83^{a} (±2.32)	53.92^{b} (±0.88)	160.67^{b} (±3.43)	214.58^{b} (±3.94)	3.19^{a} (±0.56)	0.34^{a} (±0.12)	1.21^{b} (±0.02)

All data are presented as means ±S.D. (n =3). Means within the column for individual species with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

4.5.2 Effect of Zn concentrations applied in the soil on cadmium uptake and accumulation by marigold and Guinea grass under various cadmium treatments

Marigold

At Cd treatment of 50 mg/kg, the maximum shoot Cd concentration of marigold was obtained at the Cd:Zn of 50:500 mg/kg (382.16± 53.69 mg/kg) and then decreased to 109.59±12.68 mg/kg at 50:2500 mg/kg (Figure 4.17). Under all Cd treatments and Zn application in the soil, shoot Cd was greater than 100 mg/kg, indicating the ability to accumulate Cd in aboveground tissues of the plant.

At a cadmium treatment of 100 mg/kg, shoot cadmium concentration was highest at the Cd: Zn of 100:0 mg/kg (422.41±94.09 mg/kg), and then decreased as Zn applied

to soil was increased to 3000 mg/kg (Cd:Zn of 100:3000 mg/kg). It can be seen that Cd concentrations in shoots reduced as zinc applied in the soil was enhanced further.

Similar trend (as shoot Cd) was obtained for Cd concentration in marigold root tissues (Figure 4.18)**.** At Cd treatment of 50 mg/kg, cadmium concentrations in root tissues were found to be 204.66±72.15, 433.70±55.15, 71.88±4.36, and 77.31±20.65 mg/kg dry weight, under Zn applied to soil at 0, 500, 1500 and 2500 mg/kg, respectively. Root Cd was highest at Zn of 500 mg/kg (Cd:Zn of 50:500) and then significantly reduced *($p<0.05$)* as Zn applied in the soil enhanced further to 2500 mg/kg. For Cd treatment of 100 mg/kg, the highest root Cd (492.43 mg/kg), was obtained at the Cd:Zn of 100:0 and significantly decreased to the lowest root Cd (96.31 mg/kg) with increasing Zn applied in the soil at 3000 mg/kg. When application of Zn to soil increased from 0 to 3000 mg/kg, a significant reduction in shoot and root Cd was noticed. This demonstrates that when both Cd and Zn contents increased in the soils, accumulation of Cd was reduced because of the competitive behavior of these two elements as they possess similar chemical properties.

At a Cd treatment of 50 mg/kg, total cadmium uptake by marigold was lower at Zn 0 mg/kg, as compared to the Zn applied in the soil of 500 mg/kg (Figure 4.19). This showed that total Cd uptake increased as Zn applied to soil increased from 0 to 500 mg/kg. For Cd treatment of 50 mg/kg, the maximum and optimum uptake was obtained as Zn of 500 mg/kg applied to soil. This illustrated that along with zinc, cadmium is also taken up by plants. It is noticed that at a Zn concentration of 500 mg/kg, the total uptake of Cd can be maximized. If the Zn applied to soil increased further (up to 1500 and 2500 mg/kg) the total uptake declined.

At Cd treatment of 100 mg/kg, the total uptake of Cd was in the order for Cd:Zn treatment of 1:0>1:10>1:30 (Figure 4.19). The maximum (29.16±4.46 mg/pot), was found at the Cd:Zn treatment of 100:0 mg/kg and then significantly decreased ($p<0.05$) to the lowest (4.29±1.02 mg/pot), as Zn concentration increased further up to 3000 mg/kg. As cadmium and zinc are elements having similar geochemical and environmental properties, these two species can be highly competitive for plant uptake. As a result, at higher Cd concentration in the soils, total uptake of Cd declined with increases of Zn application to the soil.

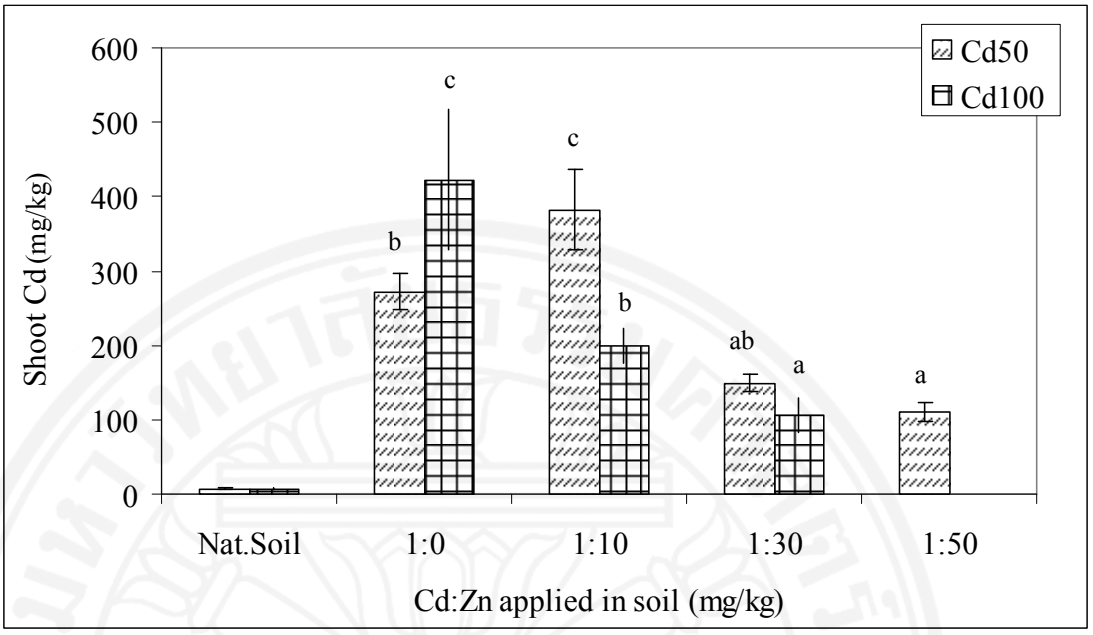


Figure 4.17 Cd concentration in shoots (dry weight) of marigold under various Zn concentrations applied in the soil at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ±S.D. of three independent replications

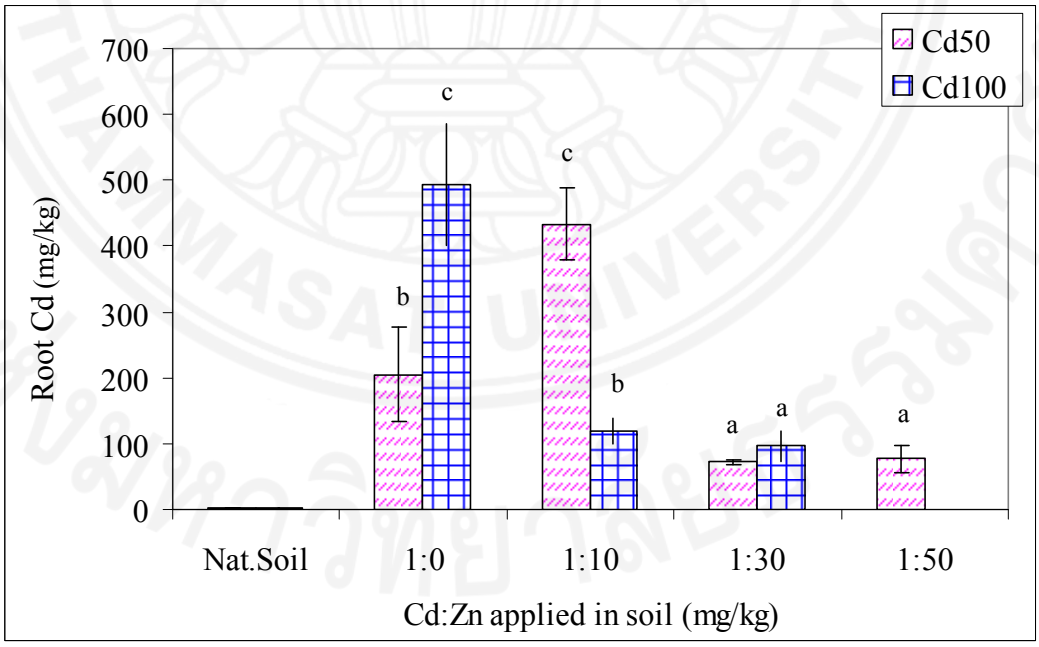


Figure 4.18 Cd concentration in roots (dry weight) of marigold under various Zn concentrations applied in the soil at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications

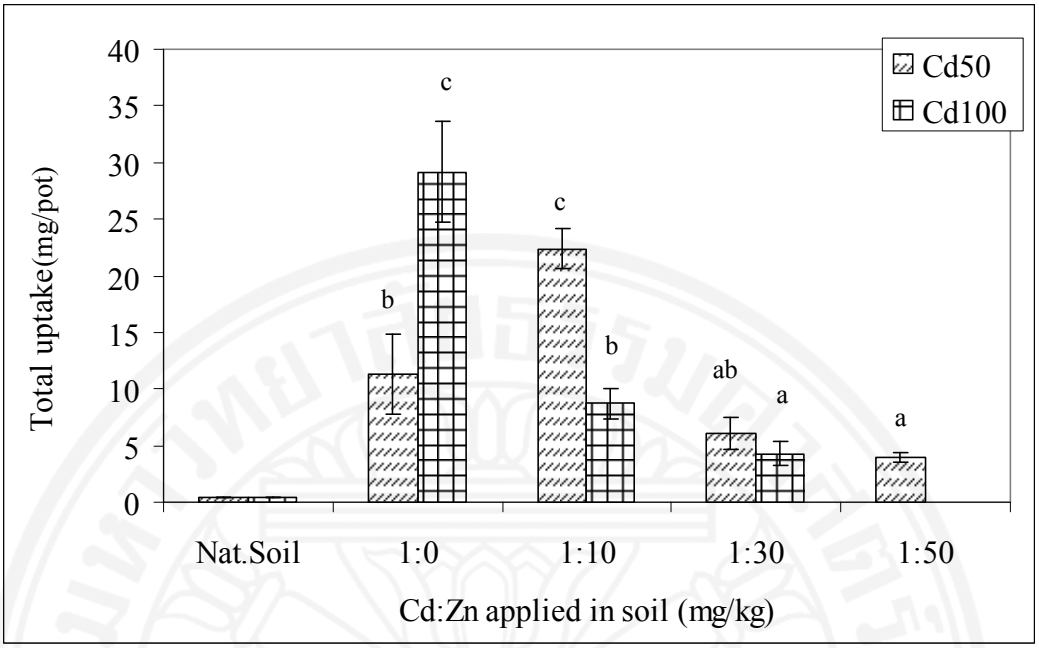


Figure 4.19 Total Cd uptake by marigold under various Zn concentrations applied in the soil at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications

The mean level of total Cd accumulated in marigold (whole plant tissues), at Cd treatment of 50 mg/kg, was highest (815.86±101.99 mg/kg) at Zn concentration of 500 mg/kg (1:50 of Cd:Zn) and then significantly declined to the lowest (186.90±30.43 mg/kg) as zinc increased further to 2500 mg/kg (1:50 of Cd:Zn). Similar to total uptake, at Cd treatment of 50 mg/kg, total Cd in whole plant tissues reduced when Zn application to soil was enhanced from 500 to 2500 mg/kg.

At a Cd treatment of 100 mg/kg, the highest Cd concentration accumulated in whole plants (914.84±185.50 mg/kg) was obtained at the Cd:Zn of 100:0 mg/kg (1:0 of Cd:Zn) and then decreased to the lowest (202.35±46.13 mg/kg), as Zn applied to soil increased to 3000 mg/kg (Figure 4.20). The Cd:Zn of 100:5000 mg/kg was not investigated as this ratio will give a very high Zn concentration which is not present in the real contaminated site at Mae Sot area, at the time of investigation**.**

The total Zn concentration in whole plant tissues of marigold significantly increased (with the values of 283.57, 3406.00, 3678.02 and 5280.09 mg/kg), when Zn concentration applied to soils increased from 0, 500, 1500 to 2500 mg/kg, respectively, (Cd:Zn of 1:0, 1:10, 1:30 and 1:50) and with the values of 283.89, 2153.44 and 5179.79 mg/kg when Zn application to soil increased from 0, 100 to 3000 mg/kg (Cd:Zn of 1:0, 1:10, and 1:30), respectively for Cd of 50 and 100 mg/kg.

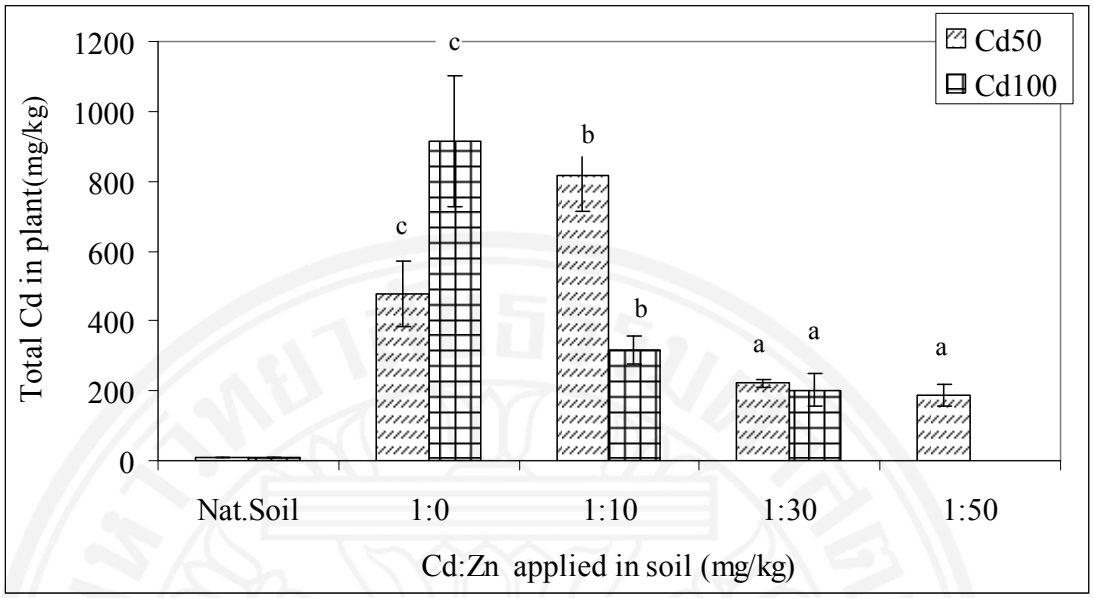


Figure 4.20 Total Cd in marigold (whole plant) under various Zn concentrations applied in the soil at Cd treatment of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications

At a Cd treatment of 50 mg/kg, the highest TF value (2.08) was obtained at the Cd:Zn of 50:1500 mg/kg (Figure 4.21). All TF values are greater than one, indicating the ability of marigold to translocate Cd from roots to shoots. For Cd treatment of 100 mg/kg, the maximum TF value (1.69) was obtained at Zn concentration of 1000 mg/kg (Cd:Zn of 100:1000 mg/kg) and then reduced as Zn in soil increased to 3000 mg/kg.

At Cd treatment of 50 mg/kg, BCF value of marigold was highest (12.77) at Cd:Zn of 50:500 mg/kg (Figure 4.22) and significantly decreased to the lowest (2.89) as Zn was increased to 2500 mg/kg. It demonstrates that zinc favors the uptake of Cd by marigold as both Cd and Zn behave similarly. However, at higher application of Zn to soil, BCF reduces and so does total Cd uptake. At a Cd treatment of 100 mg/kg, BCF value was maximum (8.74) at Cd:Zn of 100:0 mg/kg and then significantly reduced ($p<0.05$) to the minimum (1.94) as Zn applied in soil increased to 3000 mg/kg (Cd:Zn of 100:3000 mg/kg). The results show that at higher concentration of Cd in soil, when both Cd and Zn concentrations increase in the soil, total Cd uptake and total Cd in whole plant reduced significantly and so does BCF.

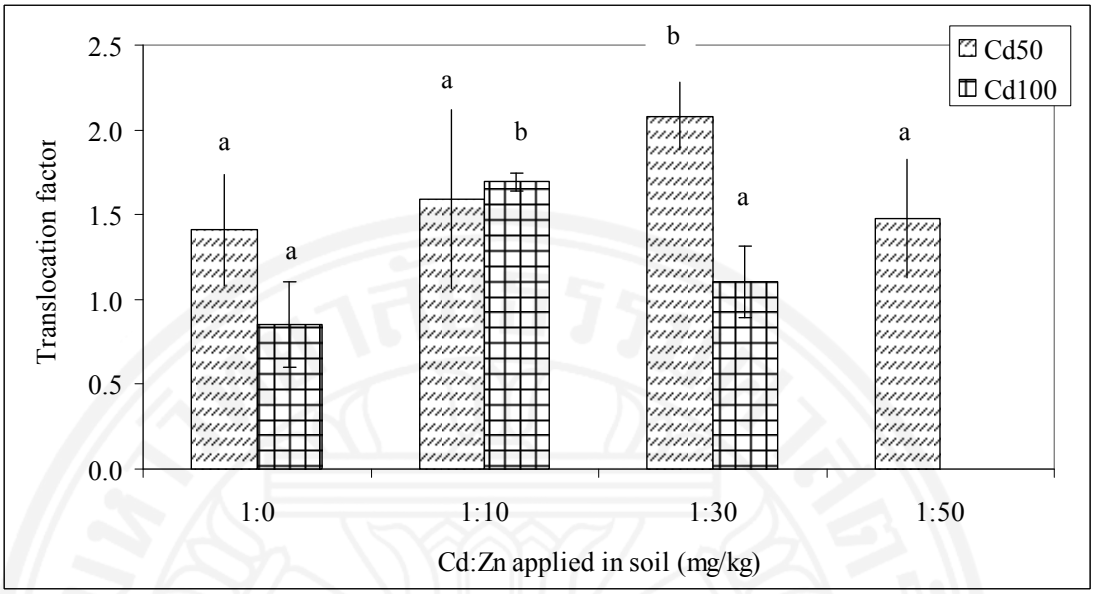


Figure 4.21 Translocation factor (TF) of marigold under various Zn concentrations applied in the soil at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications.

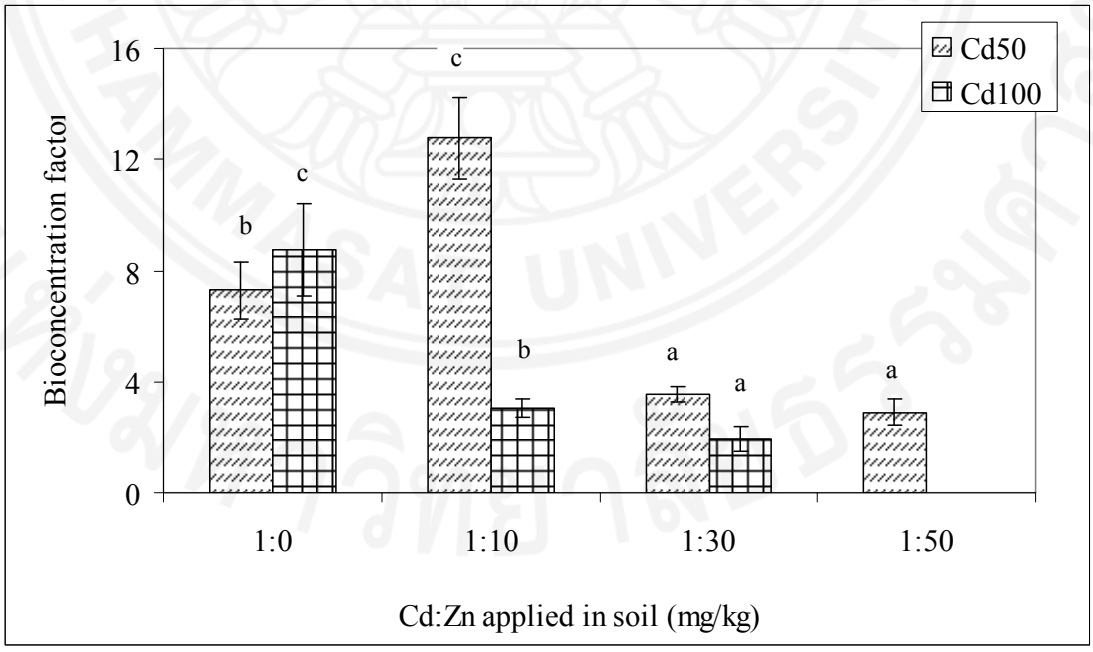


Figure 4.22 Bioconcentration factor (BCF) of marigold under various Zn concentrations applied in the soil at Cd treatments of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications.

Percentage cadmium removal by marigold

The percentage removal of Cd by marigold is present in Table 4.17. The results showed that at low Cd treatment (50 mg/kg), application of Zn in soil at concentration of 500 mg/kg promoted Cd uptake by marigold with the highest percentage removal of 5.02 and the uptake was higher than that of the control. The percentage removal declined if Zn was applied further in soil at concentration of 2500 mg/kg, which provide the lowest percentage removal of 0.88.

At high Cd treatment (100 mg/kg), application of Zn in the soil reduced the percentage of Cd removal by marigold as compared to the controls. The Cd uptake by marigold declined significantly from 29.16 to 4.29 mg/pot (which is equivalent to 5942.3 to 808 g/ha, based on the surface area of the pot) as Zn application in the soil increased to 3000 mg/kg. From this result, application of Zn or any fertilizer containing Zn to the soil was not recommended because it inhibited Cd uptake by marigold.

Guinea grass

At Cd treatment of 50 mg/kg, shoot Cd increased significantly from 50:0 mg/kg Cd:Zn treatment (13.21±1.57 mg/kg) to the maximum (37.43±2.40 mg/kg) in the treatment of 50:2500 mg/kg of Cd:Zn (Figure 4.23). For Cd treatment of 100 mg/kg, the maximum shoot Cd (59.64 ±12.43) was obtained when Zn applied to soil at 3000 mg/kg (Cd:Zn of 100:3000 mg/kg). It can be noticed that for all Cd treatments, shoot Cd increased as Zn applied in soil increased, showing opposite trend from marigold, where shoot Cd in marigold shoot declined when Zn applied in soil increased. However, shoot Cd in Guinea grass was less than 100 mg/kg dry weight, demonstrating less potential to accumulate Cd in the aboveground tissues as compared to marigold.

Table 4.17 Percentage removal of Cd by marigold from artificially Cd spiked soil under various Cd:Zn concentrations applied in the soil

Cd:Zn (mg/kg)	Desired concentration (mg/kg)	Initial Cd applied in soil (mg/kg)	Initial Cd in soil/pot (mg/pot)	Final Cd in soil/pot (mg/pot)	Cd uptake by marigold (mg/pot)	% removal
50:0	50	65.12 (±3.94)	455.84 (±27.60)	444.57 (±24.15)	11.27 (±3.56)	2.45 (±0.62)
50:500	50	63.83 (±0.55)	446.83 (±3.88)	424.44 (±5.41)	22.39 (±1.78)	5.02 (±0.43)
50:1500	50	62.55 (±1.79)	437.85 (±12.56)	431.79 (±15.81)	6.06 (±1.37)	1.39 (±0.36)
50:2500	50	64.63 (±1.12)	452.39 (±7.82)	448.42 (±8.10)	3.97 (±0.37)	0.88 (±0.09)
100:0	100	104.49 (±2.28)	731.45 (±15.93)	702.30 (±15.72)	29.16 (±4.46)	3.99 (±0.60)
100:1000	100	104.03 (±1.75)	728.19 (±12.27)	719.49 (±11.01)	8.70 (±1.37)	1.19 (±0.17)
100:3000	100	104.20 (±1.16)	729.42 (±8.11)	725.14 (±9.05)	4.29 (±1.02)	0.59 (±0.15)

Note: 7 kg soil/pot was used in this experiment

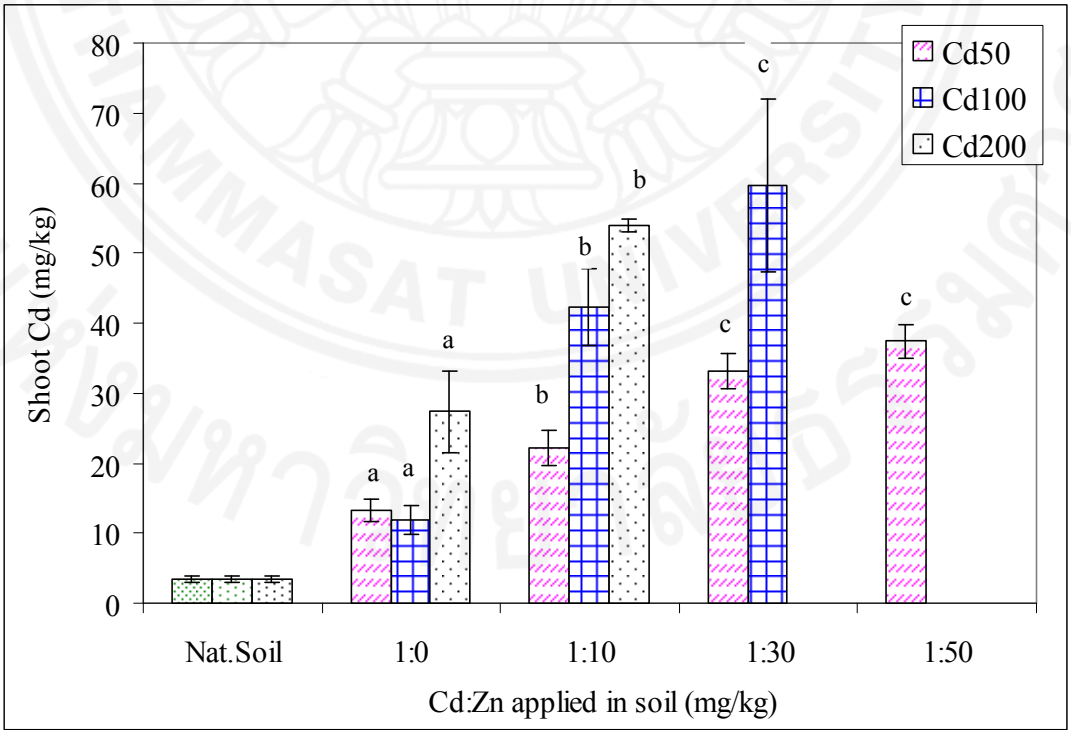


Figure 4.23 Cd concentration in shoots (dry weight) of Guinea grass under various Zn concentrations applied in the soil at Cd treatments of 50, 100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

At Cd treatment of 50 mg/kg, the maximum root Cd of Guinea grass (106.44 ± 26.04 mg/kg), was obtained at Cd:Zn of 50:1500 mg/kg, where there was no significant difference from 50:2500 mg/kg (Figure 4.24). At Cd treatment of 100 and 200 mg/kg, the highest roots Cd (202.05±56.11, and 160.67±3.43 mg/kg) were obtained, at 100:3000 and 200:2000 mg/kg of Cd:Zn, respectively. This result shows that root Cd significantly increased as Cd applied in soil increased from 50 to 200 mg/kg. Similar trend (as shoot Cd) was observed for all Cd treatments that root Cd increased when Zn applied in soil increased. However, if the application of Zn in soil increased further above 5000 mg/kg, there was a tendency that shoot and root Cd remained unchanged or might be reduced.

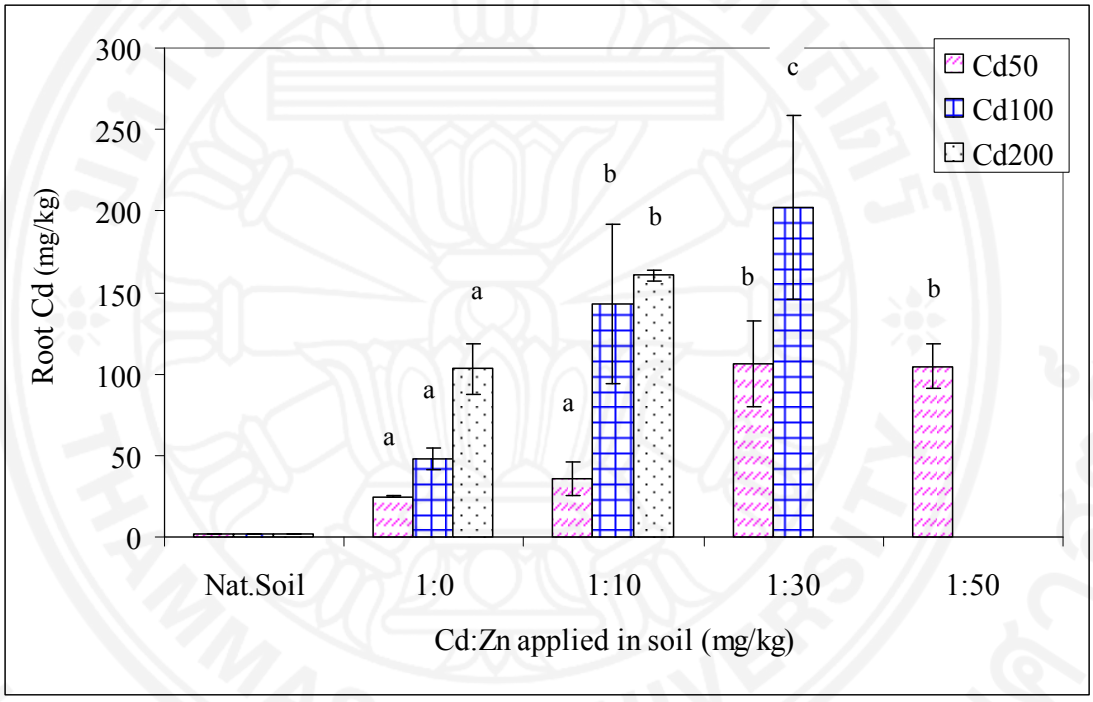


Figure 4.24 Cd concentration in roots (dry weight) of Guinea grass under various Zn concentrations applied in the soil at Cd treatments of 50,100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

At Cd treatment of 50 mg/kg, the total Cd uptake by Guinea grass (Figure 4.25) increased from 3.16±0.16 to the maximum uptake (5.37±1.92 mg/pot) as Zn application in the soil increased from 0 to 1500 mg/kg. At Cd:Zn of 50:2500 mg/kg, the total Cd in whole plant tissues was highest (142.27 mg/kg), but the total biomass was lowest (10.43 g/pot). Thus, the lowest total uptake (1.49 mg/pot) was obtained. Increasing Zn in the soil did not promote plant growth and yielded less biomass.

While at Cd treatment of 100 mg/kg, the total uptake by Guinea grass increased from 4.24 to 9.03 mg/pot when Zn applied to soil at 0 to 1000 mg/kg and then significantly declined to 3.56 mg/pot when Zn increased to 3000 mg/kg, which provided the

highest total Cd in whole plant (261.69 mg/kg), but the minimum total biomass was gained at this Zn application rate.

At higher Cd treatment of 200 mg/kg, the maximum total uptake (6.07 mg/pot) was obtained at Zn of 0 mg/kg (Cd:Zn of 200:0) then significantly declined to the lowest (3.19 mg/pot) as Zn applied to soil enhanced to 2000 mg/kg and further. This result shows that at higher Cd treatment (200 mg/kg) in the soil, Zn and Cd might be highly competitive as both elements possess similar chemical properties.

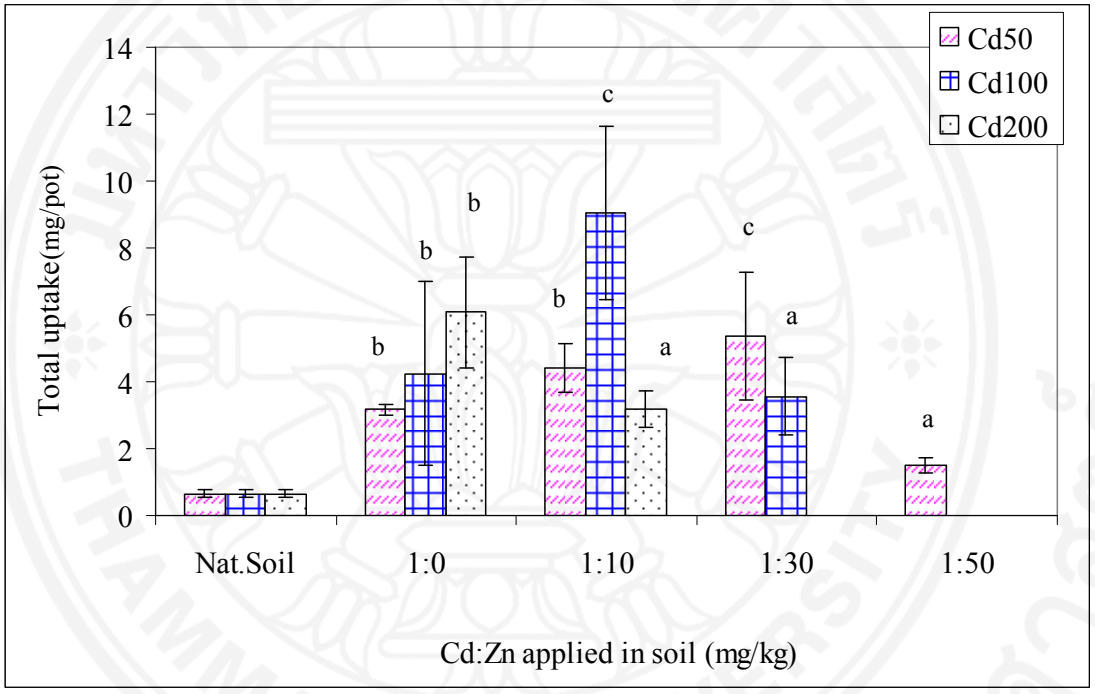


Figure 4.25 Total Cd uptake by Guinea grass under various Zn concentrations applied in the soil at Cd treatments of 50, 100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

At Cd treatment of 50 mg/kg, total Cd accumulated in Guinea grass (whole plant tissues) was highest at a Zn application of 2500 mg/kg (142.27±16.35 mg/kg), where no significant difference *($p>0.05$)* was observed between the Zn application of 2500 and 1500 mg/kg. Total Cd in whole plant increased as Zn applied to soil enhanced from 0 to 1500 mg/kg. Similar to total Cd in whole plant, total uptake also increased as Zn applied in soil enhanced from 0 to 1500 mg/kg. At higher Zn application to the soil, the higher Cd accumulation in plant tissues was obtained. However, accumulation of Cd in plant tissues became constant as Zn was around 2500 mg/kg or higher as Cd and Zn might be highly competitive under this condition. While at a Cd treatment of 100 mg/kg, total Cd in whole plant increased to the maximum (261.69±68.41mg/kg) as Zn applied to in the soil enhanced from 0 to 3000 mg/kg

(Figure 4.26). For Cd treatment of 200 mg/kg, the total Cd in whole plant increased to the maximum (214.58±3.94 mg/kg) as Zn applied in soil increased from 0 to 2000 mg/kg.

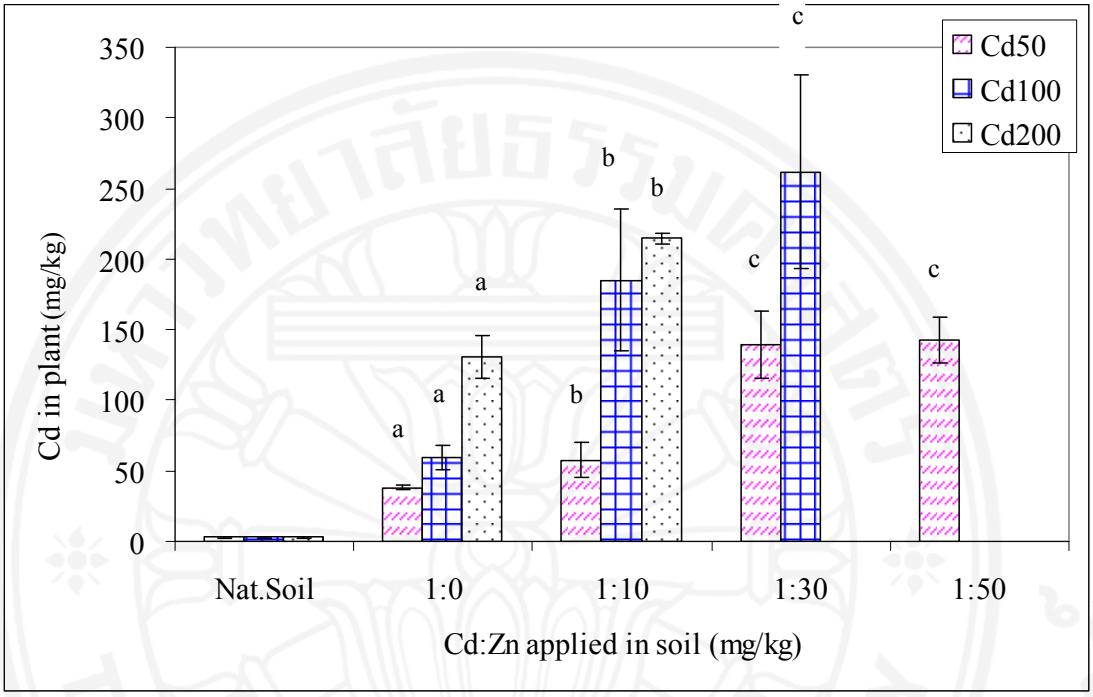


Figure 4.26 Total Cd in Guinea grass (whole plant) under various Zn concentrations applied in the soil at Cd treatments of 50, 100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

In both 50 and 100 mg/kg treatments, the maximum TF value was obtained at Cd:Zn treatment of 1:10 (Zn of 500 and 1000 mg/kg, respectively) and then slightly declined as Zn applied to soil was increased to 1500 and 3000 mg/kg and remained unchanged as Zn application was increased further (Figure 4.27).

Under Cd treatment of 50 and 100 mg/kg, BCF values increased to the maximum (2.69 and 2.3, respectively) (Figure 4.28) as Zn applied to soil increased from 0 to 1500 or 3000 mg/kg (1:30 of Cd:Zn treatment). However, BCF value remained unchanged as Zn applied to soil was increased further for Cd treatment of 50 mg/kg.

Percentage cadmium removal by Guinea grass

The results present in Table 4.18 showed that under all Cd:Zn treatments the percentage of Cd removal by Guinea grass was lower than that of marigold. At Cd treatment of 50 and 100 mg/kg, application of Zn at 500 and 1000 mg/kg (Cd:Zn of

50:500 and 100:1000), respectively, increased percentage removal of Cd to some extent as compared to the control pots. At Cd treatment of 50 mg/kg, for example, the percentage removal increased from 0.86 to 1.12 when Zn applied in soil increased from 0 to 500 mg/kg, and the total uptake increased from 3.16 to 4.41 mg/pot, respectively (which is equivalent to 595.2 to 774.1 g/ha, based on the surface area of each pot). However, at higher Cd treatment of 200 mg/kg, application of Zn to the soil did not promote percentage removal of Cd as Cd uptake reduced from 0.49 to 0.26 and total uptake reduced from 6.07 to 3.19 mg/pot.

Table 4.18 Percentage removal of Cd by Guinea grass from artificially Cd spiked soil under various Cd:Zn concentrations applied in the soil

Cd:Zn (mg/kg)	Desired concentration (mg/kg)	Initial Cd in soil (mg/kg)	Initial Cd in soil/pot (mg/pot)	Final Cd in soil/pot (mg/pot)	Cd uptake by Guinea grass (mg/pot)	% removal
50:0	50	52.71 (±1.11)	368.98 (±7.78)	365.82 (±7.69)	3.16 (±0.16)	0.86 (±0.04)
50:500	50	56.42 (±0.70)	394.97 (±4.93)	390.56 (±4.97)	4.41 (±0.72)	1.12 (±0.18)
50:1500	50	51.61 (±3.91)	361.24 (±27.38)	355.86 (±28.75)	5.37 (±1.92)	1.51 (±0.60)
50:2500	50	54.66 (±2.44)	328.64 (±17.09)	381.15 (±17.30)	1.49 (±0.23)	0.39 (±0.07)
100:0	100	98.56 (±3.35)	689.93 (±23.48)	685.69 (±23.72)	4.42 (±2.74)	0.62 (±0.40)
100:1000	100	100.08 (1.88)	700.56 (13.18)	691.53 (11.01)	9.03 (2.60)	1.29 (0.35)
100:3000	100	99.61 (±0.79)	697.30 (±5.56)	693.74 (±5.94)	3.56 (±1.15)	0.51 (±0.16)
200:0	200	176.04 (±5.76)	1232.30 (±40.30)	1226.23 (±39.12)	6.07 (±1.67)	0.49 (±0.13)
200:2000	200	176.88 (±3.69)	1238.13 (±25.81)	1234.94 (±25.39)	3.19 (±0.56)	0.26 (±0.04)

Note: 7 kg soil/pot was used in this experiment

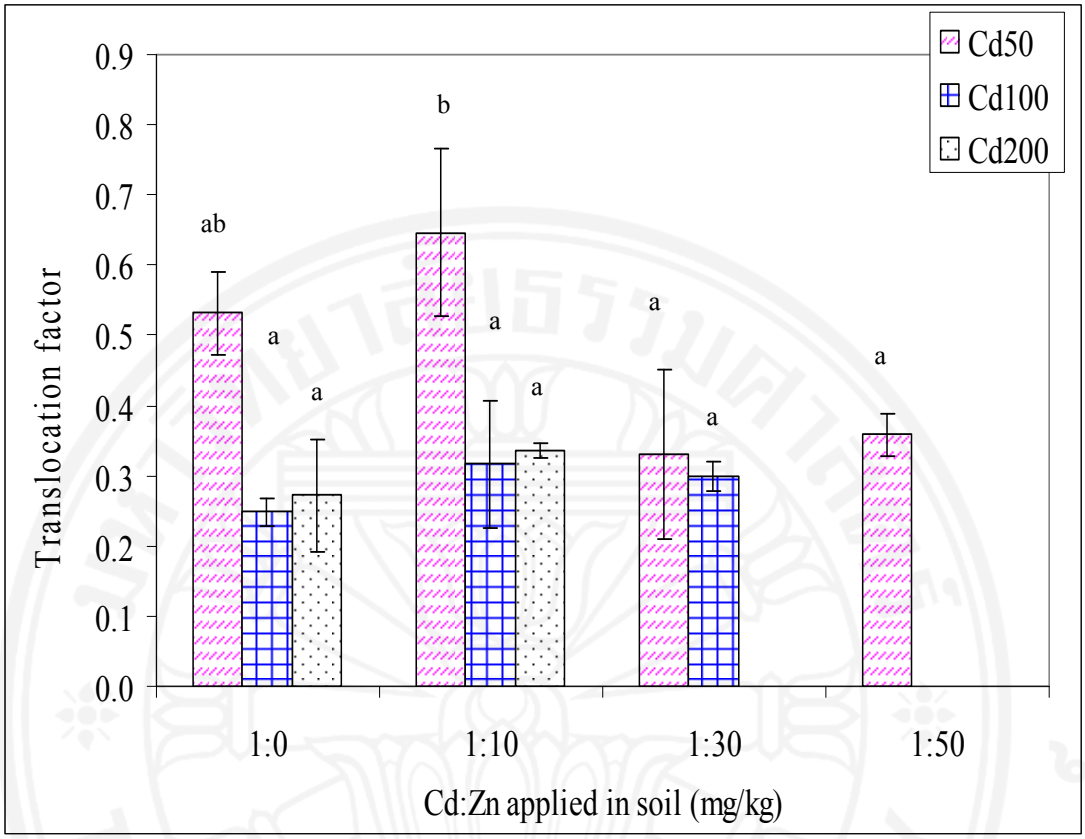


Figure 4.27 Translocation factor (TF) of Guinea grass under various Zn concentrations applied in the soil at Cd treatments of 50, 100 and 200 mg/kg. All data are presented as Mean ± S.D. of three independent replications

Cd/Zn interaction appears to be somewhat controversial, since their interaction can be both antagonism and synergism in the uptake–transport processes. Nan et al. (2002) concluded that Cd/Zn interaction is synergistic under field condition, hence increasing both metals in soils affect their increase accumulation in plants. Kitagishi and Yamane (1981) explained the observed synergism in rice plants in terms of Zn competition for the Cd sites, resulting in an increase in Cd solubility, and in Cd translocation from roots to tops. Wallace et al. (1980) reported a high Cd accumulation in roots of plants at a high Zn level and at a low pH of the solution. The earlier findings of Lagerwerff and Biersdorff (1972) showed antagonism between these cations in the uptake–transport process. It may be stated that the ratio of Cd to Zn in plant media controls the occurrence of synergism and antagonism between these cations. Papoyan et al. (2007) observed that high Zn concentrations in *Thlaspi caerulescens* increased Cd tolerance and Cd levels of a plant.

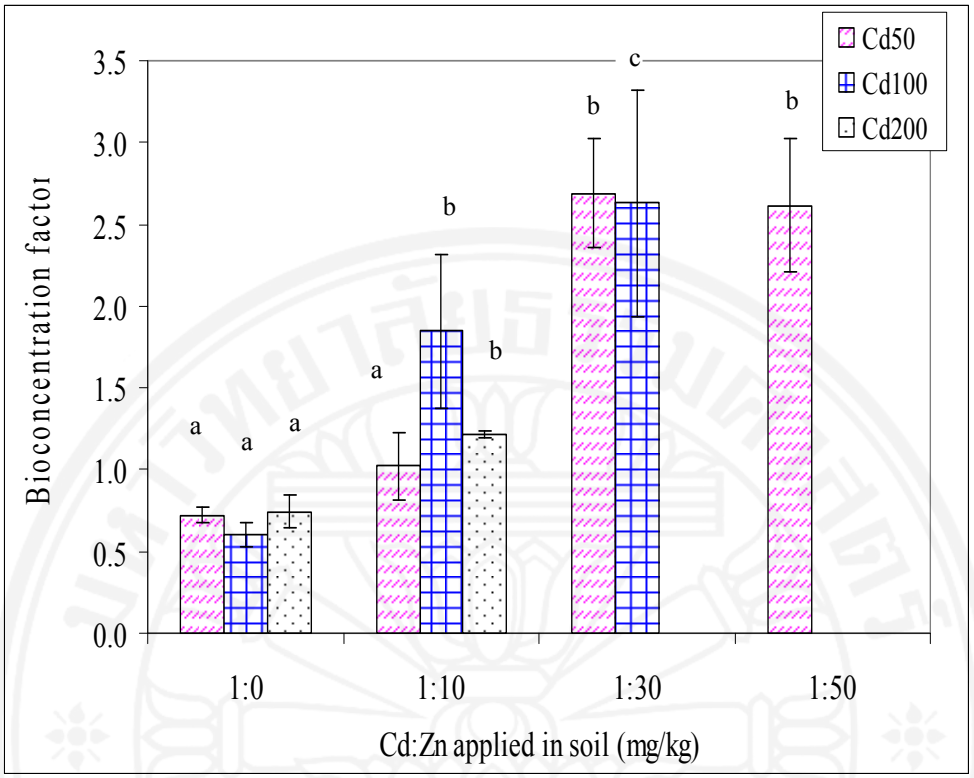


Figure 4.28 Bioconcentration factor (BCF) of Guinea grass under various Zn concentrations applied in the soil at Cd treatment of 50,100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

Kirkham (2006) also reported that cadmium and zinc are chemically similar, thus Zn is a competing ion for Cd and reduces Cd uptake. Uptake of Cd depends on the content of Zn in the soil. Plants normally take up more Cd if the Zn content is low. Zn could be added to Cd contaminated soil to reduce Cd contamination in food crops. The study by Green et al., (2003) also revealed that it might be possible to reduce Cd in wheat by adding Zn. Zn is effective in regulating Cd uptake and translocation in wheat

Yslouzilova et al. (2003) reported that the interaction between Cd and Zn is either antagonistic or synergistic. Cadmium can compete with Zn in forming protein complexes which lead to a negative association between them. However, soil Zn can induce dissociation of Cd absorbed onto the binding sites due to competition for theses sites which increases Cd in solution. They also found that Zn addition caused a higher accumulation of Cd in leaves of *Salix* spp. clones.

Long et al. (2003) and Li et al. (1990) reported that the interaction of Cd and Zn was antagonistic but others (Piotrowska et al., 1994; Salt et al., 1995; Nan et al., 2002) stated that this reaction could be synergistic as well. In wheat (*Triticumaestivum* L. and *T. turgidum* L. var. *durum*) at the level of the root cell membrane, Cd and Zn show a competitive interaction, indicating a common transport system (Hart et al.

2002). Various results have been reported concerning the interactions between the accumulation of Cd and Zn. Cadmium accumulation may or may not be influenced by increasing Zn supply. Great differences occur among species and even between different varieties of the same species (Grant and Bailey 1997). Some researchers found that Zn supply can inhibit Cd adsorption and thereby cause a high Cd concentration in plants (Adriano 1986; Nan et al. 2002).

For Cd:Zn effect on plant growth, flower diameters of marigold significantly declined *($p<0.05$)* as compared to the control when Zn concentration applied in the soil increased. The height of marigold and Guinea grass declined with increasing Zn applied in the soil. Treatment of soil with Cd or Cd:Zn did not improve plant growth (based on height, flower diameter) as compared to plants grown in the control and in natural soil. Generally, for both plants, the total biomass decreased while cadmium applied in soil increased.

The results showed that at lower concentration of Cd in soil (50 mg/kg), the total Cd in whole tissues of marigold was increased to the maximum when Zn applied to soil increased from 0 to 500 mg/kg and also the total Zn concentration in whole plant tissues increased as compared to the control showing synergistic effect between these two elements. However, when Zn concentration applied to soil increased future from 500 to 2500 mg/kg, the total Cd in whole plant tissues reduced to the lowest but total Zn in whole plant tissues increased to the maximum value. This showed antagonistic effect between these two species as they might be highly competitive for plant uptake since they are elements having similar geochemical chemical properties. For Guinea grass, enhancing Zn concentration (from 0 to 500 mg/kg) in the soil promoted Cd accumulated in plant tissues to some degree, showing synergistic interaction of Cd and Zn. Total Cd in Guinea grass remained unchanged as Zn in the soil increased further.

However, at higher concentration of Cd (100 mg/kg), these two species can possibly be highly competitive for plant uptake since they are elements having similar geochemical chemical properties. The maximum total Cd in whole plant tissues was obtained at Cd:Zn of 100:0 mg/kg then declined the to lowest with increasing Zn application to the soil, while the total Zn accumulation in whole plant tissues keep increasing from Cd:Zn 100:0 to 100:3000 mg/kg, illustrating the antagonistic effect between Cd and Zn.

For effect of Zn on accumulation of Cd in shoots and roots of marigold, for at Cd treatment of 50 mg/kg, the maximum shoot and root Cd in marigold was obtained at the Cd:Zn of 50:500 mg/kg and at treatment of 100 mg/kg, the maximum shoot and root Cd was maximum at Cd: Zn of 100:0 mg/kg. Cd concentration in shoots and roots reduced as zinc applied in the soil was enhanced further. The total Cd uptake increased as Zn applied to soil increased from 0 to 500 mg/kg, the maximum and optimum uptake was obtained as Zn of 500 mg/kg applied to soil. This illustrated that along with zinc, cadmium is also taken up by plants. At a Zn concentration of 500 mg/kg (Cd:Zn of 50:500 mg/kg), the total uptake of Cd can be maximized. If the Zn applied to soil increased further (up to 1500 and 2500 mg/kg) the total uptake was

declined. The highest TF values (2.08 and 1.69) were obtained at the Cd:Zn of 50:1500 (1:30) and 100:100 mg/kg (1:10), respectively, indicating the ability of marigold to translocate Cd from roots to shoots. For Cd treatment of 50 and 100 mg/kg, BCF values of marigold was highest (12.77 and 8.74) at Cd:Zn of 50:500 and 100:0 mg/kg, respectively. At higher application of Zn to soil, BCF reduces and so does total Cd uptake declined.

For Guinea grass, in contrast to marigold, the maximum shoot and root Cd in Guinea grass was obtained at 50:2500 and 100:300 mg/kg of Cd:Zn. For all Cd treatments, shoot Cd increased as Zn applied in soil increased, showing opposite trend from marigold, where shoot Cd in marigold shoot declined when Zn applied in soil increased. Shoot Cd in Guinea grass was less than 100 mg/kg dry weight, demonstrating less potential to accumulate Cd in the aboveground tissues as compared to marigold. In both Cd treatments of 50 and 100 mg/kg treatments, the maximum TF value was obtained at Cd:Zn treatment of 1:10. However, all of the TF values were less than one, indicating the ability of Guinea grass to translocate Cd from roots to shoots. Based on Cd accumulation in shoots and total uptake, under all Cd treatments, at various Cd:Zn treatments, marigold showed greater ability to accumulate more Cd in the shoots, which is more than 100 mg/kg, as compared to Guinea grass. However, due to higher biomass and numerous and dense roots, the total uptake of Cd from the soil by Guinea grass could be maximized.

4.6 Effect of EDTA on Cadmium Uptake by Plant of Interest

4.6.1 Plant growth under various EDTA concentrations applied in soil at different cadmium treatments

Flower diameter

In both Cd treatment of 50 and 100 mg/kg, the diameter of marigold flower was in the order of Cd:EDTA treatment 1:0>1:0.5>1:1(Table 4.19). Diameter of flowers grown under Cd:EDTA of 50:50 and 100:100 were smallest and significantly different *($p<0.05$)* from those grown in the control and natural soil. Flower diameters significantly declined to the smallest as EDTA increased and also decreased with increasing Cd concentration applied to soil.

Plant height

In both 50 and 100 mg/kg treatment, the height of marigold was in the order of Cd:EDTA treatment 1:0>1:0.5>1:1 (Table 4.19).The smallest plant (in terms of height) was significantly differed from the control *($p<0.05$)*. The results show that the height declined as EDTA and Cd concentration applied to soil increased. Treatment of soil with Cd or Cd:EDTA did not improve plant growth as compared to plants grown in the control and natural soil. There is tendency that under higher concentration of Cd and EDTA applied to the soil, plant growth is decreased to some extent. At Cd treatment of 100 mg/kg, retarded growth of marigold was observed.

Under Cd treatment of 50, 100 and 200 mg/kg, the height of Guinea grass was in the order of Cd:EDTA treatment 1:0>1:0.5>1:1 (Table 4.20). The maximum height was significantly differed from the other treatments ($p<0.05$). Heights of plants significantly reduced while Cd concentration and EDTA concentration in soil increased to Cd:EDTA of 1:1. The lowest was significantly different from the control. At the treatment of 200:200 mg/kg Cd:EDTA, the stunt growth of Guinea grass was observed.

Total biomass

For total biomass (TB), at Cd treatment of 50 and 100 mg/kg, the maximum total biomass of marigold was obtained at a Cd:EDTA of 50:0 and 100:0, respectively (Table 4.19). Total biomass declined when both Cd and EDTA application in soil enhanced. Under both 50 and 100 mg/kg Cd treatment, there was a significant reduction ($p<0.05$) in TB as compared to the control pots when EDTA was increased to 50:50 and 100:100 mg/kg of Cd:EDTA treatment. There was a slightly reduction in TB as EDTA application was increased from 50:0 to 50:25 and from 100:0 to 100:25 of Cd:EDTA. This showed that plant growth might be affected by Cd and EDTA application. The result was in agreement with the study of Hentz et al. (2012) who reporting that Cd-exposed plants illustrated a decrease in root and shoot biomass as compared to the control. There was a decrease in plant biomass as the concentration of Cd applied in soil increased.

At 50, 100 and 200 mg/kg Cd treatments, the maximum TB of Guinea grass was found at the Cd:EDTA treatment of 1:0 and followed the order of 1:0>1:0.5>1:1 (Table 4.20). The maximum TB was significantly different from those at the Cd:EDTA treatment of 50:50 and 100:100. However, at Cd:EDTA of 200:200, there was slightly reduction in TB as compared to the control. At Cd treatment of 50 and 100 mg/kg, there was a significant reduction ($p<0.05$) in TB as compared to the control when EDTA application was increased to Cd:EDTA of 50:50 and 100:100. At higher Cd treatment of 200 mg Cd/kg, total biomass slightly decreased as compared to the control with increasing EDTA application to the soil. Significant reduction in TB was observed with increasing Cd concentration applied to the soil from 50 to 200 mg/kg. This showed that the reduction in plant growth might result from both phytoxicity caused by EDTA and Cd application in soil. However, Cd toxicity at higher Cd concentration had stronger effect on the reduction in growth of studied plants which was in agreement with the study of Waterbeek et al., (1988) who reported that plant growth was declined as a result of Cd toxicity at high concentration.

Table 4.19 Total biomass, heights and flower diameters of marigold under various EDTA concentrations at different cadmium treatments of 50 and 100 mg/kg

Cd: EDTA ratio	Marigold					
	50 mg/kg			100 mg/kg		
	TB (g/pot)	Height (cm)	FD (mm)	TB (g/pot)	Height (cm)	FD (mm)
Nat. soil	117.70^{c} (±8.32)	60.52^{c} (±4.52)	73.97 ^{c} (±10.23)	117.70^{c} (±8.32)	60.52^{c} (±4.52)	73.97 ^{c} (±10.23)
1:0 (CT)	52.63^{b} (±11.42)	51.89^{b} (±10.33)	68.57 ^{b} (±12.34)	51.3^{b} (23.13)	47.75^{b} (±11.45)	62.42 ^{b} (±13.85)
1:0.5	38.17^{ab} (±13.67)	40.38^{ab} (±17.31)	61.13 ^{ab} (±19.11)	36.83 ^{ab} (±22.12)	32.12^{ab} (±13.29)	55.16 ^{ab} (±17.23)
1:1	37.70^{a} (±6.68)	34.42^{a} (±14.50)	52.72 ^{a} (±12.35)	28.83 ^{a} (±4.93)	28.53^{a} (±11.25)	45.28 ^{a} (±11.23)

Nat. Soil: Natural soil; CT: Control; FD: Flower diameter; TB: Total biomass. All data are presented as means ± S.D. (n =3). Means within the column with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

The results obtained from this study were in agreement with the study of Reinhard et al. (2008) who reported that EDTA application caused a significant decrease of plant dry shoot biomass production of *Zea mays*. *Z. mays* was affected by visible phytoxicity symptoms (wilting and plant necrosis) following EDTA treatments. Plants treated with high single concentrations of EDTA (6, 9 mmol/kg) showed visible signs of phytotoxicity within days causing death of the plants before harvest. Plant dry biomass reduction was more dominant with increasing EDTA concentration. The highest EDTA dose resulted in the highest reduction of dry biomass production. This result was in accordance with the study of Li et al. (2005) and Hovsepyan and Greipsson (2005). Chen and Cutright (2001) reported that adding EDTA led to a severe yield reduction in biomass across the treatments. Their study demonstrated that synthetic chelator addition had a significant adverse effect on plant growth and severe reduction in growth of the studied plants was attributed to the combination of heavy metal concentration and chelator addition. Liphadzi et al. (2003) also reported that dry weight of leaves decreased as the concentration of EDTA in the soil was enhanced.

The summary of dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of marigold and Guinea grass under different Cd:EDTA treatments are presented in Tables 4.21-4.22, respectively.

Table 4.20 Total biomass and height of Guinea grass under various EDTA concentrations applied in the soil at different cadmium treatments of 50, 100, and 200 mg/kg

Cd: EDTA ratio	Guinea grass					
	50 mg/kg		100 mg/kg		200 mg/kg	
	TB (g/pot)	Height (cm)	TB (g/pot)	Height (cm)	TB (g/pot)	Height (cm)
Nat. soil	373.43^{c} (±12.30)	125.86^{c} (±15.13)	373.43^{c} (±12.30)	125.86^{c} (±15.13)	373.43 ^{b} (±12.30)	125.86^{c} (±15.13)
1:0 (CT)	297.70^{b} (±16.70)	95.84^{b} (±5.67)	194.13^{b} (±16.93)	89.45^{b} (±5.10)	65.20 ^{a} (±15.24)	79.89^{b} (±19.51)
1:0.5	279.43^{b} (±19.89)	80.42^{b} (±8.41)	169.60^{a} (±30.23)	70.61^{b} (±0.94)	54.07 ^{a} (±20.71)	58.19^{a} (±14.96)
1:1	237.00^{a} (±5.25)	52.15^{a} (±7.46)	159.80^{a} (±9.31)	49.61^{a} (±1.53)	51.90 ^{a} (±16.32)	40.52^{a} (±12.75)

Nat. Soil: Natural soil; CT: Control; TB: Total biomass. All data are presented as means ± S.D. (n =3). Means within the column with different letters are significantly different from each other *(p<0.05)*, where d is significantly > c > b> a.

Table 4.21 Dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of marigold under different Cd:EDTA treatments

Cd Treatments (mg/kg)	Dry biomass (g/pot)	Cd accumulation (mg/kg)			Cd uptake (mg/pot)	TF	BCF
		Shoot	Root	Total			
Cd 50 mg /kg							
Cd:EDTA 1:0	52.63^{b} (±11.42)	200.00^{a} (±18.02)	143.88^{b} (±26.93)	344.88^{a} (±40.31)	18.21^{b} (±1.69)	1.42^{a} (±0.22)	7.88^{a} (±0.97)
1:0:5	38.17^{a} (±13.67)	277.11^{b} (±30.09)	91.85^{a} (±13.13)	368.96^{b} (±41.71)	14.08^{a} (±2.05)	3.03^{b} (±0.24)	8.07^{ab} (±1.25)
1:1	37.70^{a} (±6.68)	285.00^{b} (±18.47)	99.65^{a} (±4.18)	381.18^{b} (±50.80)	14.15^{a} (±0.84)	2.86^{ab} (±0.34)	8.65^{b} (±1.14)
Cd 100 mg /kg							
Cd:EDTA 1:0	51.3^{b} (23.13)	219.22^{a} (±5.16)	187.82^{a} (±6.76)	407.04^{a} (±2.32)	20.88^{b} (±5.39)	1.17^{a} (±0.07)	4.76^{a} (±0.16)
1:0:5	36.83 ^{ab} (±22.12)	300.53^{a} (±20.62)	224.10^{b} (±43.28)	525.24^{b} (±50.26)	23.63^{b} (±10.47)	1.97^{b} (±0.45)	6.12^{ab} (±0.20)
1:1	28.83 ^{a} (±4.93)	343.83^{b} (±8.32)	201.88^{b} (±1.73)	525.24^{b} (±7.61)	15.72^{a} (±2.54)	1.70^{ab} (±0.05)	6.48^{b} (±0.27)

All data are presented as means ±S.D. (n =3). Means within the column for individual species with different letters are significantly different from each other *(p<0.05)*, where d is significantly > c > b> a.

Table 4.22 Dry biomass, cadmium accumulation, cadmium uptake, translocation factor and bioconcentration factor values of Guinea grass under different Cd:EDTA treatments

Cd Treatments (mg/kg)	Dry biomass (g/pot)	Cd accumulation (mg/kg)			Cd uptake (mg/pot)	TF	BCF
		Shoot	Root	Total			
Cd 50 mg /kg							
Cd:EDTA 1:0	297.70^{b} (±16.70)	16.38^{a} (±1.03)	47.67^{a} (±3.72)	64.05^{a} (±3.90)	19.11 ^{a} (±2.00)	0.34^{a} (±0.08)	1.37^{a} (±0.11)
1:0:5	279.43^{b} (±19.89)	22.02^{ab} (±2.58)	57.96^{b} (±6.20)	79.98^{ab} (±5.44)	22.37^{b} (±2.40)	0.38^{a} (±0.02)	1.70^{b} (±0.18)
1:1	237.00^{a} (±5.25)	23.99^{b} (±3.84)	58.28^{b} (±7.47)	82.27^{b} (±5.36)	19.52^{a} (±1.69)	0.41^{a} (±0.03)	1.77^{b} (±0.04)
Cd 100 mg /kg							
Cd:EDTA 1:0	194.13^{b} (±16.93)	39.41^{b} (±15.76)	56.33^{a} (±16.52)	95.75^{a} (±6.50)	18.57^{a} (±1.81)	0.70^{b} (±0.08)	1.04^{a} (±0.09)
1:0:5	169.60^{a} (±30.23)	27.95 ^{a} (±1.75)	102.62^{b} (±13.70)	130.58^{ab} (±15.29)	22.16^{b} (±4.69)	0.27^{a} (±0.07)	1.41^{b} (±0.12)
1:1	159.80^{a} (±9.31)	33.57^{b} (±0.39)	102.72^{b} (±18.16)	136.29^{b} (±8.61)	21.73^{b} (±0.68)	0.33^{a} (±0.06)	1.47^{b} (±0.16)
Cd 200 mg /kg							
Cd:EDTA 1:0	65.20 ^{a} (±15.24)	30.96^{a} (±4.66)	220.61^{a} (±41.80)	251.57^{a} (±43.79)	16.33^{b} (±2.29)	0.14^{a} (±0.02)	1.44^{a} (±0.23)
1:0:5	54.07 ^{a} (±20.71)	32.19^{a} (±1.74)	221.48^{a} (±9.32)	253.67^{a} (±10.33)	13.71^{a} (±0.76)	0.15^{a} (±0.05)	1.53^{ab} (±0.14)
1:1	51.90 ^{a} (±16.32)	37.87^{b} (±8.25)	277.13^{b} (±31.30)	309.88^{b} (±17.86)	13.03^{a} (±2.35)	0.14^{a} (±0.06)	1.70^{b} (±0.08)

All data are presented as means ±S.D. (n =3). Means within the column for individual species with different letters are significantly different from each other *($p<0.05$)*, where d is significantly > c > b> a.

4.6.2 Effect of EDTA concentrations applied in soil on cadmium accumulation and uptake by marigold and Guinea grass at various cadmium treatments

Marigold

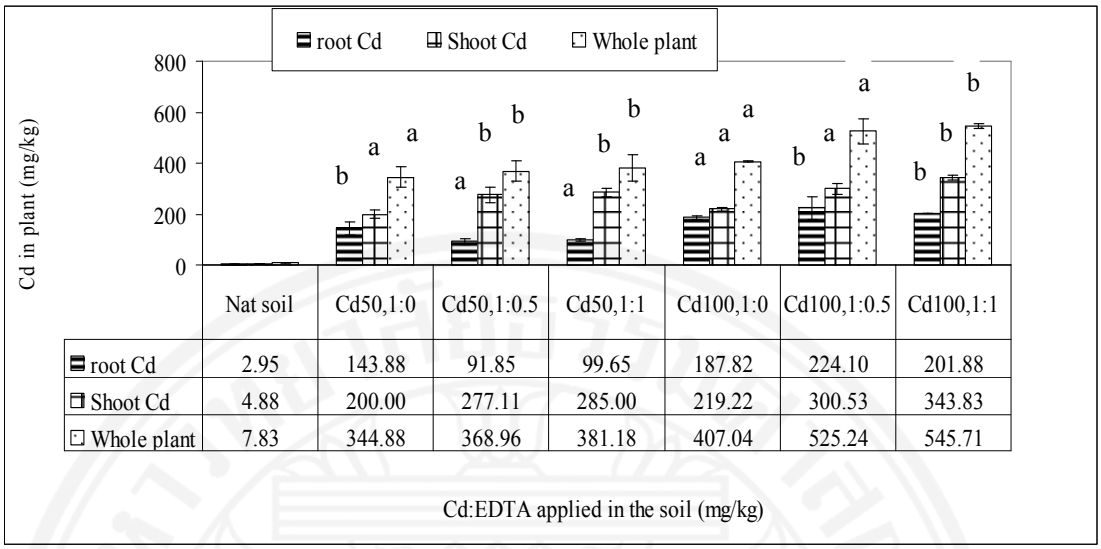
At Cd treatment of 50 mg/kg, the maximum shoot Cd in marigold was obtained at Cd:EDTA treatment of 50:50 (285.00±18.47 mg/kg) with a significant difference from the controls ($p>0.05$). Under all Cd treatments and Cd:EDTA treatments, shoot Cd concentrations were greater than 100 mg/kg, illustrating the ability of marigold to accumulate Cd in aboveground tissues (Figure 4.29). It can be seen that Cd in shoots was lowest in the treatment of 50:0 and then significantly increased (to 285 mg/kg), as EDTA applied in the soil increased to the 50:50 treatment. At Cd treatment of 100 mg/kg, shoot Cd was lowest in the treatment 100:0 and significantly increased to the highest value (343.83 mg/kg) at Cd:EDTA treatment of 100:100. This shows that shoot Cd increased with increasing EDTA application as a result of the increased mobile fraction of Cd in soil solution.

For total Cd accumulation in marigold, at Cd treatment of 50 mg/kg, the lowest concentration was found to be at the ratio of 50:0 (344.88±40.43 mg/kg) and then increased to the maximum (381.65±15.58 mg/kg) as EDTA applied to the soil was increased (Figure 4.29). At Cd treatment of 100 mg/kg, the lowest Cd concentration in whole plant tissues (407.04±2.32 mg/kg) was at the treatment of 100:0 and then significantly increased $(p<0.05)$ to the maximum of 545.71±7.61 mg/kg, when EDTA in soil increased.

At Cd treatment of 50 mg/kg, the maximum root Cd for marigold (143.88±26.93 mg/kg) was found to be in the treatment of 50:0 and then significantly reduced when EDTA in soil increased further to 50:50. The lowest root Cd was significantly different $(p<0.05)$ from that of controls. In contrast to shoot Cd, root Cd concentration decreased significantly $(p<0.05)$ after EDTA application. As a result, the root Cd was lower than that of shoot Cd.

At Cd treatment of 100 mg Cd /kg, root Cd was found to be maximum (224.10±43.28 mg/kg) at Cd:EDTA of 100:50. From the results obtained, it can be noticed that the ratio between shoot Cd and root Cd of marigold increased when EDTA applied to soil increased. The application of EDTA in soil appeared to stimulate the translocation of the metals from roots to shoots in marigold.

At Cd treatment of 50 mg/kg, the total Cd uptake by marigold was maximal at the treatment of 50:0, which provided the maximum total biomass, but total Cd accumulation in whole plant was lowest. Total Cd uptake by marigold was lowest at a 50:25 Cd:EDTA treatment (Figure 4.30). At Cd treatment of 100 mg/kg, the highest total uptake was obtained in a treatment of 100:50. Total Cd uptake by marigold significantly declined as EDTA increased (to 100:100 treatment).



	Nat soil	Cd50,1:0	Cd50,1:0.5	Cd50,1:1	Cd100,1:0	Cd100,1:0.5	Cd100,1:1
root Cd	2.95	143.88	91.85	99.65	187.82	224.10	201.88
Shoot Cd	4.88	200.00	277.11	285.00	219.22	300.53	343.83
Whole plant	7.83	344.88	368.96	381.18	407.04	525.24	545.71

Figure 4.29 Cd concentration in roots, shoots, and whole plant tissue (DW) of marigold under various EDTA concentrations applied in the soil at Cd treatment of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications

At Cd treatments of 50 and 100 mg/kg, the highest TF values of marigold (3.03 and 1.97, respectively) were obtained at a Cd:EDTA treatment 50:25 and 100:50, respectively (Figure 4.31). However, no significant difference was observed between the TF values at the ratio of 1:0.5 (50:25; 100:50) and 1:1 (50:50; 100:100). The TF values showed tendency to decline or unchanged if application of EDTA to the soil is enhanced. At Cd treatment of 100 mg/kg, the application of EDTA slightly improved the translocation of Cd from roots to shoots.

Bioconcentration factor (BCF) of marigold (7.88-8.65) under Cd treatment of 50 mg/kg, generally, was slightly higher than that of at 100 mg/kg (4.76-6.48). At treatments of 50:50 and 100:100 provided maximum BCF which significantly higher than that of the controls.

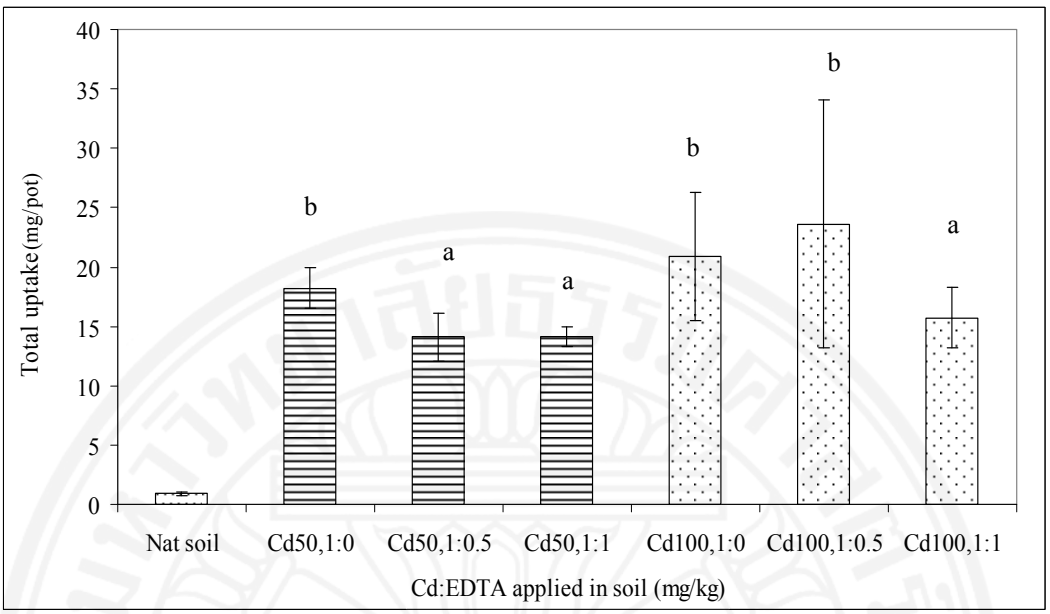


Figure 4.30 Total Cd uptake by marigold under various EDTA concentrations applied in the soil at Cd treatment of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications

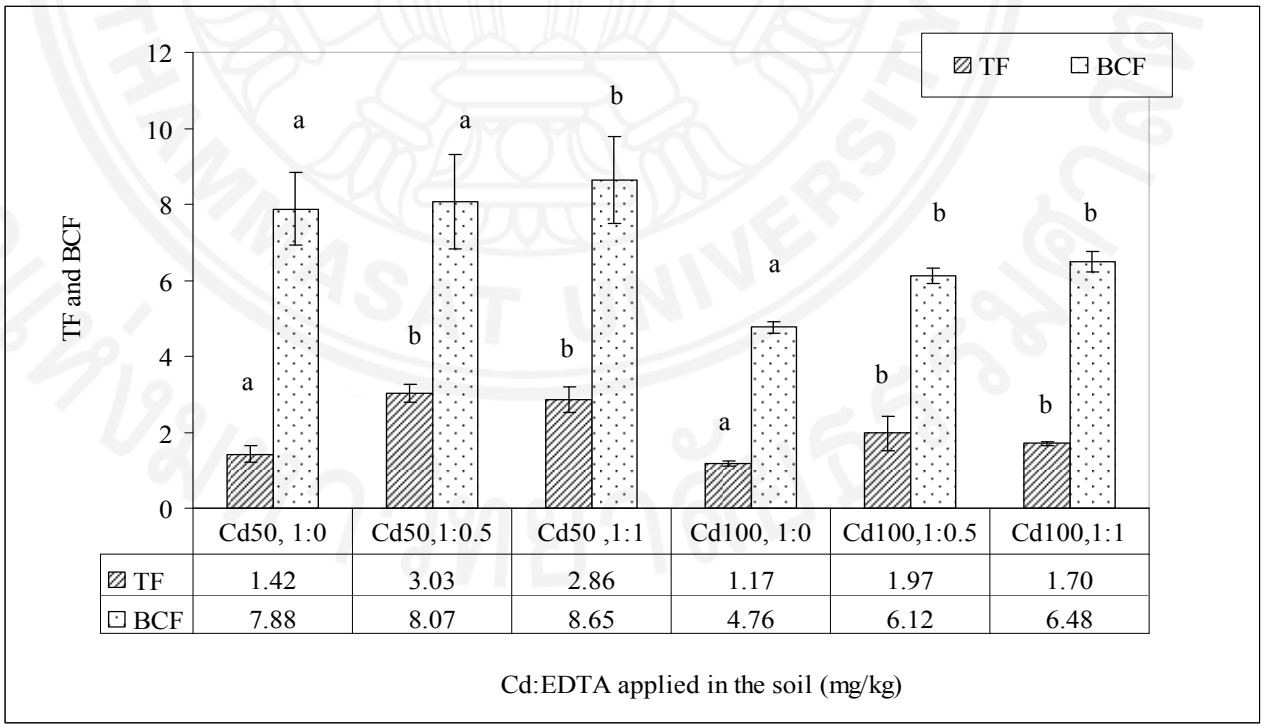


Figure 4.31 Translocation factor (TF) and Bioconcentration factor (BCF) of marigold under various EDTA concentrations applied in the soil at Cd treatment of 50 and 100 mg/kg. All data are presented as mean ± S.D. of three independent replications

Percentage cadmium removal by marigold

EDTA application slightly reduced Cd uptake as compared to the control pots. The percentage of Cd removal by marigold gradually decreased from 6.91 to 5.37 and from 4.06 to 3.12 under Cd treatment of 50 and 100 mg/kg, respectively (Table 4.23), as EDTA applied in the soil increased. At Cd of 50 mg/kg, Cd uptake after EDTA application was lower than that of the control. At Cd of 100 mg/kg, Cd uptake increased with increasing EDTA from 0 to 50 mg/kg and decreased as EDTA increased to 100 mg/kg.

Table 4.23 Percentage removal of Cd by marigold from artificially Cd spiked soil under various Cd:EDTA applied in the soil

Cd:EDTA (mg/kg)	Initial Cd applied in soil (mg/kg)	Initial Cd applied in soil/pot (mg/pot)	Final Cd in soil/pot (mg/pot)	Cd uptake by marigold (mg/pot)	% removal
50:0	43.81 (±2.13)	262.87 (±12.78)	244.75 (±12.54)	18.12 (±1.69)	6.91 (±0.66)
50:25	45.96 (±2.14)	275.74 (±12.83)	261.66 (±14.21)	14.08 (±2.05)	5.14 (±0.92)
50:50	44.06 (±0.47)	264.36 (±2.08)	250.21 (±3.62)	14.15 (±0.84)	5.37 (±0.37)
100:0	85.64 (±2.57)	513.84 (±15.42)	492.95 (±11.79)	20.88 (±5.39)	4.06 (±0.94)
100:50	85.73 (±5.39)	514.37 (±32.35)	495.08 (±31.99)	23.64 (±10.47)	4.61 (±2.09)
100:100	84. 31 (±2.71)	505.87 (±16.23)	490.16 (±17.40)	15.72 (±2.54)	3.12 (±0.54)

Note: 6 kg soil/pot was used in this experiment

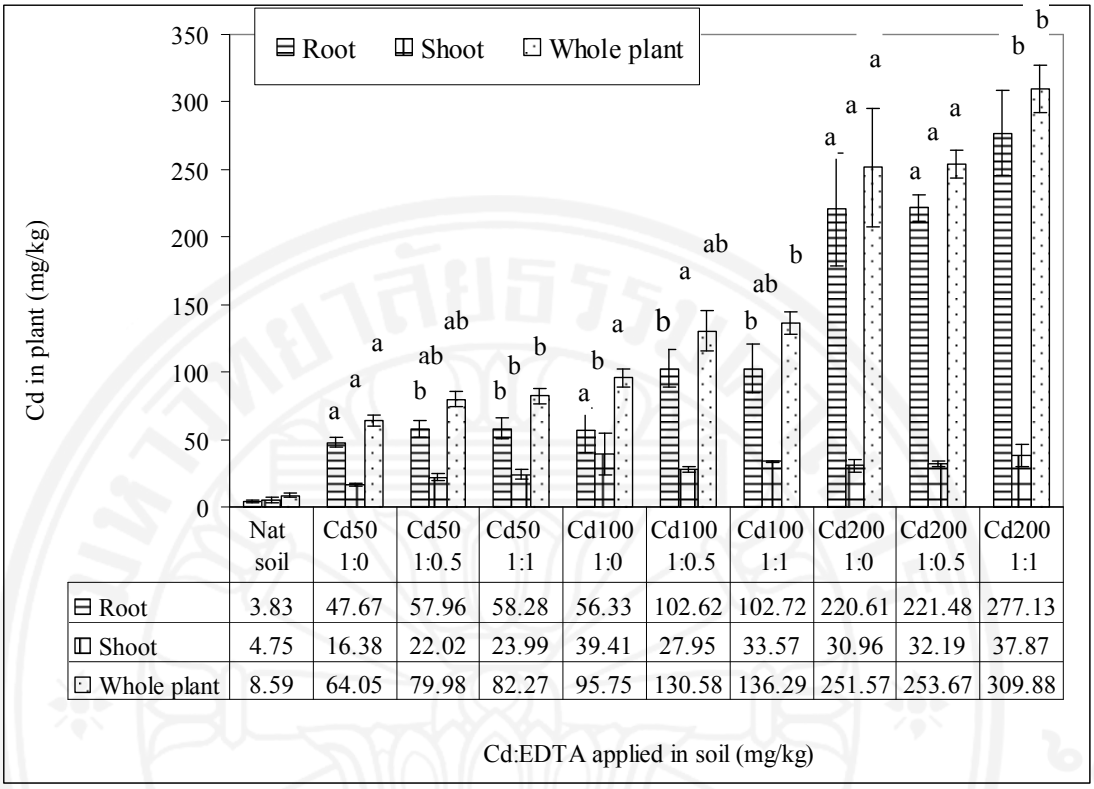
Guinea grass

At Cd treatment of 50 mg/kg, the maximum shoot Cd concentration of Guinea grass was obtained at Cd:EDTA treatment of 50:50 (23.99±3.84 mg/kg) (Figure 4.32). The shoot Cd was lowest in the treatment of 50:0 (controls), with no significant difference *($p>0.05$)* from other treatments. Shoot Cd slightly increased as EDTA applied in the soil increased. For Cd treatments of 100 mg/kg, the maximum shoot Cd was obtained in the treatment of 100:0, with no significant difference from 100:100 treatment. In 200 mg/kg Cd treatment, the maximum shoot Cd was found in 200:200 mg/kg of Cd:EDTA, which was significantly higher than that of the controls *($p<0.05$)*. Under all the Cd and EDTA treatments, shoot Cd was lower than 100 mg/kg, illustrating the less ability of Guinea grass to accumulate Cd in aboveground tissues as compared to marigold.

At Cd treatment of 50,100 and 200 mg/kg, the lowest total Cd accumulation (64.05, 95.75 and 251.57 mg/kg) were in the treatment of 50:0, 100:0 and 200:0, respectively (Figure 4.32), and then significantly increased to the maximum values as EDTA applied to soil increased to 50:50, 100:100 and 200:200 mg/kg treatments.

At Cd treatment of 50, 100 and 200 mg/kg, the lowest root Cd in Guinea grass was found to be lowest the treatment of 50:0, 100:0 and 200:0, respectively, and then significantly increased as the EDTA applied to soil increased (Figure 4.32) to Cd:EDTA of 50:50, 100:100 and 200:200. At Cd treatment of 200 mg/kg, most of Cd was retained in the roots.

In both 50 and 100 mg/kg Cd treatments, the treatments of 1:0.5 (50:25 and 100:50 mg/kg Cd:EDTA) provided highest total Cd uptake, which was significantly higher than that of the control ($p<0.05$) (Figure 4.33). At highest Cd treatment (200 mg/kg), total uptake was maximum at 200:0 treatment (control), and then declined as EDTA application increased to 200:200 treatment. This lower uptake might be possible due to retarded growth as a result of Cd toxicity at higher Cd concentration in the soil. Moreover, another reason for a lower Cd uptake by Guinea grass at higher application rate (200 mg Cd/kg) might be possible due to inhibition of plant growth caused by Cd toxicity. Application of 200 mg Cd/kg decreased Cd uptake by 20.28 % in comparison with control. This results is in agreement with the study of some researchers (Wolterbeek et al., 1988; Laurie et al., 1991), who reported that application of 40 and 80 mg Cd/kg soil decreased Cd uptake (by spinach) by 57 and 90 %, respectively, as compared to controls. It has been reported that application of EDTA to the soil lead to decrease in uptake of Cd and Zn by plants.



	Nat soil	Cd50 1:0	Cd50 1:0.5	Cd50 1:1	Cd100 1:0	Cd100 1:0.5	Cd100 1:1	Cd200 1:0	Cd200 1:0.5	Cd200 1:1
Root	3.83	47.67	57.96	58.28	56.33	102.62	102.72	220.61	221.48	277.13
Shoot	4.75	16.38	22.02	23.99	39.41	27.95	33.57	30.96	32.19	37.87
Whole plant	8.59	64.05	79.98	82.27	95.75	130.58	136.29	251.57	253.67	309.88

Figure 4.32 Cd concentration in roots, shoots, and whole plant tissue (dry biomass) of Guinea grass under various EDTA concentrations applied in the soil at Cd treatment of 50,100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

For Guinea grass, TF values under all Cd:EDTA treatments were less than one, showing the less ability to translocate Cd from roots to shoots (Figure 4.34). This result illustrated that EDTA application to the soil does not influence the translocation of Cd from roots to the shoots. Under all Cd and Cd:EDTA treatments, BCF values of Guinea grass were slightly above one (Figure 4.34), showing the less capability of the plants to transport Cd from soil to plant as compared to marigold. The transport of Cd to plant in Guinea grass could not be enhanced by EDTA.

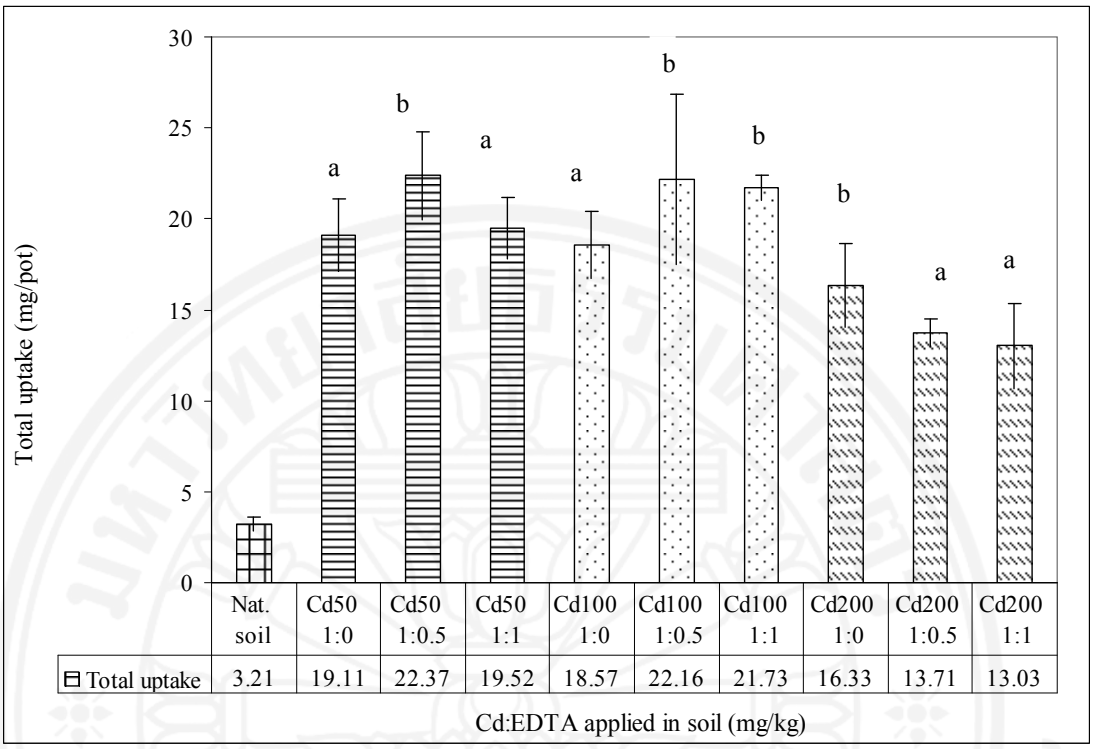
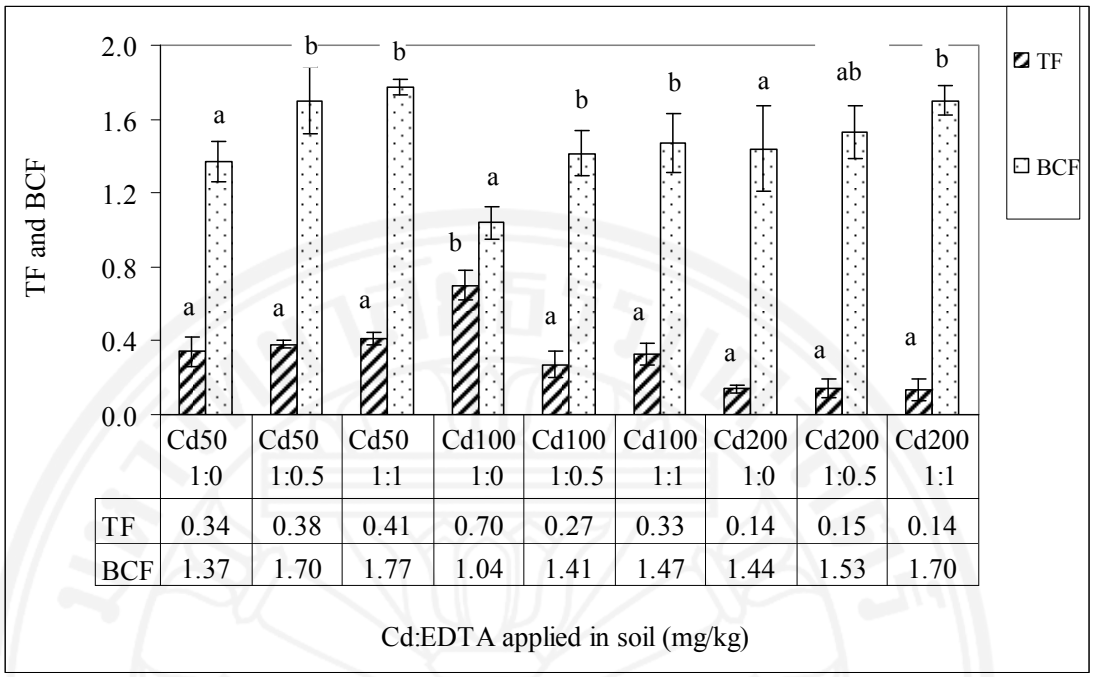


Figure 4.33 Total Cd uptake by Guinea grass under various Cd:EDTA ratios at Cd treatment of 50, 100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

Percentage cadmium removal by Guinea grass

The application of EDTA to soil at the rate of 50:25 and 100:50 mg/kg yielded maximum total Cd uptake (22.37 and 22.16 mg/pot) with the highest percentage Cd removal of 7.94 and 4.01, respectively, which is higher than that of the control pots (Table 4.24). However, at higher Cd treatment of 200 mg/kg, the maximum uptake was obtained at 200:0 of Cd:EDTA treatment. The total uptake declined as Cd:EDTA increased 200:200 mg/kg and also the percentage removal reduced to the minimum value (1.19%). This reduction in total Cd uptake by Guinea grass might be possibly due to inhibition of plant growth as a result of Cd toxicity (Wolterbeek et al., (1988)).



	Cd50 1:0	Cd50 1:0.5	Cd50 1:1	Cd100 1:0	Cd100 1:0.5	Cd100 1:1	Cd200 1:0	Cd200 1:0.5	Cd200 1:1
TF	0.34	0.38	0.41	0.70	0.27	0.33	0.14	0.15	0.14
BCF	1.37	1.70	1.77	1.04	1.41	1.47	1.44	1.53	1.70

Figure 4.34 Translocation factor (TF) and Bioconcentration factor (BCF) of Guinea grass under various EDTA concentrations applied in the soil at Cd treatment of 50,100 and 200 mg/kg. All data are presented as mean ± S.D. of three independent replications

Table 4.24 Percentage removal of Cd by Guinea grass from artificially spiked soil under various Cd:EDTA applied in the soil

Cd:EDTA (mg/kg)	Initial Cd applied in soil (mg/kg)	Initial Cd in soil/pot (mg/pot)	Final Cd in soil/pot (mg/pot)	Cd uptake by Guinea grass (mg/pot)	% removal
50:0	46.86 (±1.39)	281.14 (±8.32)	262.03 (±9.82)	19.11 (±2.2)	6.82 (±0.95)
50:25	47.27 (±2.33)	283.64 (±13.95)	261.27 (±16.33)	22.37 (±2.40)	7.94 (±1.22)
50:50	46.45 (±3.96)	278.72 (±23.76)	259.20 (±22.07)	19.52 (±1.69)	7.02 (±0.06)
100:0	92.54 (±4.04)	555.26 (±24.25)	536.69 (±25.96)	18.57 (±1.81)	3.36 (±0.46)
100:50	92.29 (±3.03)	553.76 (±18.18)	531.60 (±17.58)	22.16 (±4.69)	4.01 (±0.82)
100:100	92.78 (±4.53)	556.68 (±27.19)	534.95 (±27.15)	21.73 (±0.68)	3.92 (±0.22)
200:0	174.76 (±3.81)	1048.54 (±22.83)	1032.21 (±21.13)	16.33 (±2.29)	1.56 (±0.19)
200:100	166.49 (±17.72)	998.96 (±106.34)	985.25 (±106.75)	13.71 (±0.76)	1.39 (±0.19)
200:200	183.03 (±13.99)	1098.20 (±83.94)	1085.17 (±82.79)	13.03 (±2.35)	1.19 (±0.18)

Note: 6 kg soil/pot was used in this experiment

Turgut et al. (2004) stated that the specific cultivar, chelator source and level used yielded different uptake rate and selectivity. The cultivar source will directly affect the available biomass for storage and translocation as well as resistance and susceptibility to toxicity. The chelator type and source will affect bioavailability. It may also impact the selectivity by forming chelator-metal complexes that alters the uptake rate (Cutright et al., 2004). They found that different cultivars of *H. annuus* demonstrated different responses to EDTA treatments on Cd accumulated in plant tissues. Chen and Cutright (2001), reported that chelator enhancement is plant-and metal-specific and is also subject to the interaction and subsequent inhibitory effect when multiple heavy metals are present. Jiang et al. (2003) reported that EDTA significantly enhanced the metal concentration in plant tissues; however, they resulted in a severe biomass loss. As a result, total uptake of metals by plants was decreased. The effect of synthetic chelators on phytoremediation is subject to the influence of multiple metal interactions and specific plant species.

The results illustrated that Cd:EDTA treatments of 50:25, 50:50, 100:50 and 100:100 provided higher exchangeable fraction of Cd as compared to the control. This makes Cd readily available for plant uptake and increase the translocation of Cd from roots to shoots. However, in this study, EDTA addition to the soil may not increase Cd uptake by marigold, but appeared to stimulate translocation of Cd from roots to shoots, which is in agreement with the study of Jiang et al. (2003). They reported that EDTA treatment did not substantially enhance shoot Cd uptake compared to the control. Wolterbeek et al. (1988) stated that the application of EDTA to the soil has led to decreases in the uptake of Cd by plants. Practically, the contaminant in plant shoots is the most important parameter for phytoremediation because the harvested portions of plants at the most contaminated sites are limited to the aboveground parts (Nobuntou, 2012.) Most of metals are rapidly absorbed by roots. Translocation of the absorbed metals to shoots is the limiting step for metal accumulation in shoots. Without EDTA application to the soil, the ratio of Cd translocation to shoots was very low. However, the application of EDTA helped in mobilizing metals and enhanced translocation of Cd from soil to shoots to some extent (Blaylock and Huang., 2000).

For the effect of EDTA on plant growth, in marigold, in both Cd treatment of 50 and 100 mg/kg, the diameter of marigold flower was in the order of Cd:EDTA treatment 1:0>1:0.5>1:1. Flower diameters significantly declined as EDTA increased and also decreased with increasing Cd concentration applied to soil. In both 50 and 100 mg/kg treatment, the height of marigold was in the order of Cd:EDTA treatment 1:0 >1:0.5 > 1:1. The height declined as EDTA and Cd concentration applied to soil increased. Treatment of soil with Cd or Cd:EDTA did not improve plant growth as compared to plants grown in the control and in natural soil. Total biomass of marigold declined when both Cd and EDTA application in soil enhanced.

For Guinea grass, under Cd treatment of 50, 100 and 200 mg/kg, the height of Guinea grass was in the order of Cd:EDTA treatment at 1:0>1:0.5>1:1. The heights of plants significantly reduced while Cd concentration and EDTA concentration in soil increased. At 50, 100 and 200 mg/kg Cd treatments, the maximum TB was found at the Cd:EDTA treatment of 1:0 and followed the order of 1:0>1:0.5>1:1. There was a significant reduction ($p<0.05$) in TB as compared to the control when EDTA application was increased. The growth of studied plants was reduced as a result of Cd toxicity at higher concentrations.

For the effect of EDTA on Cd accumulation in plant, in both Cd of 50 and 100 mg/kg, total Cd in whole plant tissues of marigold and shoot Cd was maximum in 50:50 and 100:100 of Cd:EDTA treatment. Total Cd in whole plant and shoot Cd increased with increasing EDTA application as a result of the increased mobile fraction of Cd in soil solution. In contrast to shoot Cd, root Cd concentration in marigold decreased significantly *($p<0.05$)* after EDTA application. As a result, the root Cd in marigold was lower than that of shoot Cd. The application of EDTA in soil appeared to stimulate the translocation of the metals from roots to shoots in marigold. Total Cd uptake by marigold significantly declined as EDTA application in soil increased. The highest TF values of marigold were obtained at a Cd:EDTA treatment 50:25 and 100:50 mg/kg. The TF values showed tendency to decline if application of EDTA to

the soil is enhanced to 1:1 of Cd:EDTA treatment. At lower Cd concentration (50 mg/kg), EDTA application had stronger affect on translocation of Cd from roots to shoots in marigold than that of higher Cd treatment (100 mg/kg). The Cd:EDTA treatments of 50:50 and 100:100 provided maximum BCF which is significantly higher than that of the controls. Bioconcentration factor (BCF) of marigold (7.88-8.65) under Cd treatment of 50 mg/kg, was slightly higher than that of at 100 mg/kg (4.76-6.48).

The results showed that ad Cd of 50 mg/kg, shoot Cd in Guinea grass slightly increased when EDTA applied in soil enhanced from Cd:EDTA treatment of 1:0 to 1:1, the ratio of Cd in shoots and roots was not increased as the translocation of Cd from roots to shoots in Guinea grass was not increased by EDTA application. It indicates that application of EDTA in soil may not stimulate the translocation of Cd from roots to shoots in Guinea grass. This may be due to the ability of EDTA to enhance the root to shoot translocation to be metals and species specific. It also might be due to the difference in root system of the two species. Guinea grass has deep, dense and fibrous root system which can absorb more elements on the root surface and retain these elements in the root area rather than transfer it to the shoots. The fibrous root system of Guinea grass readily absorbed metal on the root surfaces. It might be due to the mechanism of rhizopshere, most of metal were retained in root area rather than transfer to the shoot portion. However, Guinea grass could perhaps be used for phytoremediation of lightly contaminated soil as the higher biomass produced by Guinea grass makes it possible to be used for phytoremediation of lightly contaminated soil. Also, Guinea grass does not require specific conditions for growth and can grow easily in adverse conditions and also can be grown under shade.

4.7 Fractionation of Cadmium in Soil

The results of soil Cd fractionation are shown in Table 4.25. Generally, the highest fraction of Cd, under all applied Cd:EDTA treatment, was exchangeable fraction (F1) (72.3-83.03%), followed by carbonate bound fraction (F2) (8.24-13.80%) and Fe-Mn oxide bound fraction (F3) (3.91-6.37%). For organic bound fraction (F4) and residual fraction (F5), the percentage of Cd ranged from 1.83-9.51 and 1.22-3.09%, respectively. The application of EDTA increased F1 of Cd in the soil and the lowest form is the residual fraction (F5), which includes mainly metals built in the crystal lattice of minerals. In natural conditions, they are practically inaccessible for living organisms and can be considered as permanently immobile. A major portion of Cd was extracted as exchangeable and carbonated fraction (Table 4.25). This might be the reason for high mobility of Cd absorbed as reported by Liang et al. (2005). Adriano (2001) reported that plants secure most of their nutrients from soluble and exchangeable forms of elements in soil, which might have reached the toxic levels for the plants, thus the plant biomass (yields) showed a tendency to decrease when EDTA was applied to the soil for mobilization of metals.

The percentage of mobile fraction (F1+F2) obtained from soil samples (used for marigold growing) at Cd:EDTA of 50:0, 50:25 and 50:50 mg/kg were 81.03, 88.87

and 91.65 %, respectively and were 89.10, 89.33 and 91.07%, respectively (Table 4.25), for 100:0, 100:50 and 100:100 mg/kg treatment. The recovery of added Cd obtained with spiked soil samples ranged from 91.19-107.02 % which is within the acceptable range. This variation may be attributed to the differences in leaching time, reagents and the total volume of extract (Ciba et al., 1999).

In both 50 and 100 mg/kg treatments, the mobile fraction of Cd increased with increasing EDTA application. The mobile fraction at 50:25 and 100:50 were significantly higher ($p<0.05$) than that of at 50:0 and 100:0. It shows that Cd:EDTA treatment of 50:25 and 100:50 mg/kg was high enough to provide more bioavailable fraction of Cd in the soil solution. As a result, the translocation of Cd from roots to shoot of marigold could be enhanced. At these two treatments (50:25 and 100:50), maximum TF values of 3.03 and 1.97 were obtained in marigold. This might be possible due to the effect of EDTA application, which enhanced mobile fraction of Cd in soil solution and then stimulated the translocation of Cd from roots to shoots in marigold.

For Guinea grass, under all Cd:EDTA treatments, the prominent form of Cd in the entire fractions is extractable fraction (74.54-82.01 %), followed by carbonate bound fraction (10.23-15 %), Fe-Mn oxide bound fraction (4.01-5.84 %), organic bound fraction (1.39-2.82 %), and residual fraction (1.11-3.68 %) (Table 4.26). The mobile fraction (F1+F2) of Cd ranged from 81.22-92.24%. Exchangeable and acid extractable fractions which are easily bioavailable can be leached during the changes in environmental conditions and pose threat to groundwater quality. Therefore, this mobile fraction can cause environmental toxicity due to mobility (Norvell, 1984).

The percentage of mobile fraction of soil sample (used for Guinea grass growing) at Cd:EDTA of 50:0, 50:25 and 50:50 mg/kg was 89.31, 89.96 and 89.50 %, respectively and 90.60, 90.00 and 89.27%, respectively (Table 4.26), for 100:0, 100:50 and 100:100 mg/kg treatments. The percent recovery of metal obtained by the summation of sequential fractions in relation to the total metal content extracted with aqua regia is in the range of 83.45-97.72 %, which is in the acceptable range.

For Cd treatment of 50 mg/kg (Table 4.26) the exchangeable fraction (F1) increased significantly from 24.54 to 35.90 mg/kg, at the Cd:EDTA of 50:0 to 50:50. A ratio of 50:25 provided significantly higher F1 ($p<0.05$) than that of the controls. Although the maximum total uptake (22.37 mg/pot) was found at this ratio, the translocation factor is less than one (0.38). This may be due to the translocation of Cd from roots to shoots in Guinea grass cannot be stimulated after EDTA application. In contrast to Guinea grass, application of EDTA in the soil promoted the transfer of Cd from roots to the shoots of marigold to some extent.

For Cd treatment of 100 mg/kg, the exchangeable fractions were slightly increased from 61.91 to 70.04 mg/kg, with no significant difference ($p>0.05$) was observed, when Cd:EDTA increased from 100:0 to 100:100 mg/kg. The exchangeable fraction gradually increased from 130.90 to 145.74 mg/kg when Cd:EDTA was applied at the rate of 200:0 to 200:200. This result is in accordance with the study of Chen and

Cutright (2001). They stated that the bioavailable forms of Cd were shown to increase with increasing EDTA application. Although the bioavailable fraction of Cd after EDTA application was obtained, the ratio between shoots to roots of Guinea grass cannot be maximized. Under all treatment conditions, TF values were less than one, indicating that Guinea grass has less capacity to translocate Cd from roots to the shoots. BCF values were slightly greater than one, illustrating the ability of Guinea grass to transfer Cd from soil to the plant biomass.

The influence of the application of EDTA on the stimulation of Cd transfer from roots to shoots in studied plants was only pronounced in marigold, but it did not show any impacts on translocation of Cd from roots to shoots in Guinea grass. Chen and Cutright (2001) reported that the effect of synthetic chelators on phytoremediation is subject to the influence of metal interactions and specific plant species**.** Liphadzi et al., (2003) also reported that EDTA did not affect uptake of Cd by poplar and uptake of metal by sunflower was little affected by EDTA.

Table 4.25 Cadmium fractions of the soil sample used for growing marigold treated with various Cd:EDTA concentrations applied in the soil

Applied Cd : EDTA (mg/kg)		Cd forms (mg/kg)					Total	Recovery (%)
Cd	EDTA	F1	F2	F3	F4	F5		
50	0	25.77^{a} (±3.56)	3.20^{a} (±0.31)	2.27^{a} (±0.42)	3.39^{c} (±0.73)	1.10^{a} (±0.09)	36.54	91.19
50	25	32.68^{b} (±2.55)	3.34^{a} (±0.46)	2.10^{a} (±0.01)	1.50^{ab} (±0.39)	0.91^{a} (0.11)	40.53	105.99
50	50	33.51^{b} (2.38)	3.48^{a} (0.33)	1.58^{a} (0.17)	0.74^{a} (0.22)	1.05^{a} (0.01)	40.36	103.35
100	0	56.16^{c} (2.59)	10.29^{b} (2.63)	4.06^{b} (0.95)	2.90^{c} (0.97)	1.31^{a} (0.61)	74.56	93.85
100	50	70.50^{d} (0.92)	10.34^{b} (1.57)	4.60^{b} (1.47)	3.76^{c} (0.31)	1.30^{a} (0.44)	90.50	107.02
100	100	76.47^{e} (2.07)	12.08^{b} (2.42)	4.94^{b} (0.81)	2.55^{bc} (0.70)	1.19^{a} (0.31)	97.23	99.69

Note: Means within column with the same letters are not significantly different at $p>0.05$ as determined by Duncan's Multiple Range, where d is significantly > c > b> a.
F1: Exchangeable fraction
F2: Carbonate bound fraction
F3: Fe-Mn oxide bound fraction
F4: Organic bound fraction
F5: Residual fraction

Sequential extraction of metals from soil, based on the Tessier's procedure, was used to identify metal mobility and bioavailability of heavy metals to plants. The contents of cadmium in the five fractions; exchangeable, bound to carbonates, bound to iron and manganese oxide, bound to organic matter and residual, were determined. Chemical forms of heavy metal determine their behavior in the environment and its mobilization ability. Ecological effects of metals (e.g., their bioavailability, ecotoxicology and risk of groundwater contamination) are related to mobile fractions rather than to the total concentration. The exchangeable and bound to carbonate species are generally bio-available as they are mobilize relative in the environment and are potentially available for plants, whereas the metals in the residual fraction are tightly bound and would not be expected to be released under natural condition. Exchangeable and acid extractable fractions, which are easily bioavailable can be leached during the changes in environmental conditions and poses threat to groundwater quality (Norvell, 1984). The fraction bound to iron and manganese oxide is sensitive to the redox potential changes and represents the fraction which can be solubilized in reducible conditions. The fraction bound to organic can be solubilized by chemical oxidation. The residual fraction includes mainly metals built in the crystal lattice of minerals. In natural conditions, they are practically inaccessible for living organisms and can therefore remain for long time in environment. Metals in the non-residual fractions are more bio-available than metals associated with the residual fraction (Konradi et al. (2005), Yobouet et al. (2010), Lena and Gade, 1997)

Enhanced metal mobility can increase the uptake into plants. The potential for movement into the groundwater is also increased. An increase in metal migration to the groundwater would have a detrimental impact on the environment. Therefore, care should be taken into consideration when selecting the final chelator for application in the field practice. The concentration used must be high enough to mobilize the metals to the root zone without being too high to cause toxicity or elevated groundwater concentrations and also the proper soil pH range needed to be carefully investigated to avoid metal leaching to the (under)ground water.

Field application of marigold and Guinea grass for phytoremediation

Based on the results, if the Cd treatment of 50 mg/kg was considered, the uptake of Cd from the soil by marigold (at soil pH around 5.0 and 6.3) was found to be 15.66 mg/pot and 12.56 mg/pot (equivalent to 294.95 and 235.81 mg/m^2 per crop) respectively, based on the surface area of the pot (530.929 x 10^{-4} m^2). If the total weight of the top soil (0-20 cm) is around 195 kg per m^2 area (based on Department of Agriculture, Bangkok, Thailand), then the total Cd concentration in the soil is around 9750 mg/m^2 when the Cd contamination is 50 mg of Cd/kg of soil. It is estimated that about 33 and 41 crops of marigold will be required and with 8.25 and 10.25 years, respectively, are needed to grow marigold for cleaning this contaminated area (assuming that the removal rate is constant and one crop takes about 90 days, and plants are grown four crops per year). This will depend also on the amount of biomass produced by the plants and other soil factors involved in the field trial.

For Guinea grass, the uptake rate of Cd from the soil by Guinea grass (at soil pH around 5.0 and 6.3) was found to be 19.28 and 6.67 mg/pot which is equivalent to 363.14 and 125.63 mg/m^2 per crop, respectively. The total Cd concentration in the soil is around 9750 mg/m^2 when the Cd contamination is 50 mg of Cd/kg of soil. It is estimated that about 27 and 78 crops of Guinea grass, respectively, will be required to clean the contaminated area. The time needed to clean this contaminated area are approximately 7.25 and 19.5 years, respectively. The results showed that lower soil pH provides good results but in the field experiment, care must be taken into consideration for acidic soil condition because leaching of metals from soil can occur.

Table 4.26 Cadmium fractions of the soil used for growing Guinea grass treated with various Cd:EDTA concentrations applied in the soil

Applied Cd : EDTA (mg/kg)		Cd forms (mg/kg)					Total	Recovery (%)
Cd	EDTA	F1	F2	F3	F4	F5		
50	0	24.54^{a} (±2.24)	4.86^{a} (±1.04)	1.65^{a} (±0.47)	0.77^{a} (±0.13)	1.10^{a} (±0.09)	32.92	83.45
50	25	33.95^{b} (±2.20)	4.85^{a} (±1.04)	1.77^{a} (±0.57)	0.91^{a} (±0.16)	1.65^{ab} (±0.39)	43.13	93.99
50	50	35.95^{b} (±2.01)	5.37^{a} (±0.94)	1.85^{ab} (±0.09)	1.30^{ab} (±0.50)	1.70^{ab} (±0.41)	46.17	86.23
100	0	61.91^{c} (±2.59)	8.03^{b} (±1.42)	3.59^{bc} (±0.01)	1.82^{bc} (±0.27)	1.85^{bc} (±0.34)	77.20	83.77
100	50	68.69^{c} (±1.13)	10.94^{c} (±0.99)	4.58^{c} (±0.11)	2.09^{bc} (±0.20)	2.18^{bc} (±0.31)	88.48	95.87
100	100	70.04^{c} (±2.01)	10.52^{c} (±0.79)	5.27^{c} (±0.79)	2.26^{cd} (±0.29)	2.15^{bc} (±0.35)	90.24	97.26
200	0	130.90^{d} (±3.38)	16.88^{d} (±1.73)	8.10^{d} (±0.43)	2.32^{cd} (±0.21)	2.29^{c} (±0.39)	160.49	86.54
200	100	137.85^{d} (±1.91)	17.19^{d} (±0.35)	8.79^{d} (±1.57)	2.39^{cd} (±0.62)	1.86^{bc} (±0.10)	168.08	90.88
200	200	145.74^{d} (±3.05)	18.67^{d} (±0.68)	9.78^{d} (±0.92)	2.48^{d} (±0.36)	2.18^{b} (±0.39)	178.85	97.72

Note: Means within column with the same letters are not significantly different at $p>0.05$ as determined by Duncan's Multiple Range, where d is significantly > c > b> a.
F1: Exchangeable fraction
F2: Carbonate bound fraction
F3: Fe-Mn oxide bound fraction
F4: Organic bound fraction
F5: Residual fraction

Based on the Cd:EDTA treatment, the total uptake of Cd from the soil by Guinea grass was 19.11 and 22.37 at Cd:EDTA treatments of 50:0 and 50:25 mg/kg, which is equal to 359.94 and 421.34 mg/ m^2 per crop, respectively (equivalent to 3599.4 and 4213.4 g/ha). Under the same conditions as mentioned above, when Cd contamination is 50 mg/kg, it is estimated that about 27 and 23 crops of Guinea grass, respectively, will be required to clean the contaminated area. If Guinea grasses are grown around 4 crops/year, then the time needed to clean this contaminated site approximately is 7 and 6 years, respectively for Cd:EDTA of 50:0 and 50:25 mg/kg.

However, under field conditions, Cd uptake by plants may be affected by many variable soil and climatic parameters. It is therefore not easy to extrapolate the results obtained from pot experiment in greenhouse studies to field conditions. The differences may be assigned to a more controlled environment and limited soil volume to which plant roots are restricted under greenhouse conditions than under field conditions, where the plant roots reach a larger soil volume. It also depends on the amount of biomass produced by the plants of concern and other soil characteristics including soil type and plant species as well as plant density grown in the contaminated areas. Moreover, root contact is a primary limitation on phytoremediation applicability. Remediation with plants requires that the contaminants be in contact with the root zone of the plants. Either the plants must be able to extend roots to some parts of sites that contained high level of contaminants. Contaminants or the contaminated media must be moved to within the range of the plants. This movement can be accomplished with standard agricultural equipment and practices, such as deep plowing, or by irrigating trees and grasses with contaminated groundwater or wastewater. Phytoremediation is also limited by the growth rate of the plants. More time may be required to phytoremediate a site as compared with other more traditional cleanup technologies. In addition to effecting root growth and distribution in soils, weather can also impact nutrient uptake. Generally, in dry season the nutrient content of crops is reduced as compared to normal or wet season. The sites with widespread, low to medium level contamination within the root zone are the best candidates for phytoremediative processes. High concentrations of contaminants may inhibit plant growth and thus may limit application on some sites. Plant species or varieties of one species can vary significantly in their efficacy for phytoremediation. Site specific studies may always be necessary prior to implementation. Cultivation of vegetation often requires great care due to stresses of climate and pests. Under the adverse conditions of contaminated soil or ground water, successful cultivation can be much more difficult.

4.8 Correlation Analysis

In this section, simple correlation analysis was carried out to determine the association between the independent (X) and dependent (Y) variable. The simple regression analysis was used to measure the degree of linear relationship between two variable X and Y. Simple correlation and regression analysis was performed to identify the simple relationship between variables of concern.

Correlation of Cd accumulation in plants and various Cd concentrations in soil

After all chemical analysis, the correlation between Cd accumulation in whole plant tissues (as dependent variable) and various Cd concentrations in soil samples (as independent variable) was investigated. Under this investigation, the natural soil pH ranged from 6.23-6.51. There was a positive linear relationship between Cd accumulation in whole plant tissues (marigold, cosmos, sunflower and Guinea grass) and Cd concentrations in soil with R^2 of 0.88, 0.87, 0.96 and 0.95, respectively (Figure 4.35).

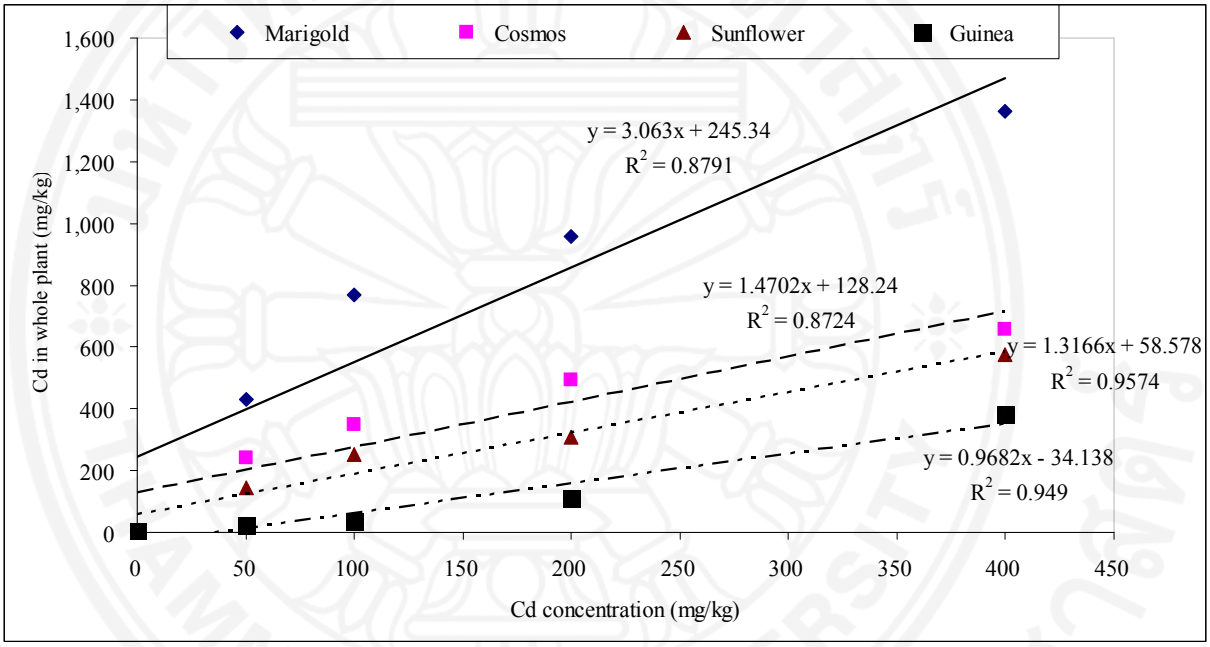


Figure 4.35 Relationship between total Cd in whole plant tissues of different species and Cd concentration applied in the soil (natural soil pH ranging from 6.23-6.51)

From the linear equations obtained, it can be seen that about 88, 87, 96 and 95 % of the variation in Cd accumulated in whole plant tissues of marigold, cosmos, sunflower and Guinea grass, respectively, were influenced by Cd concentration applied to the soils. The earlier findings of Hornburg and Bummer (1986) indicated that Cd concentrations in wheat grains increase linearly with the total Cd concentration in soils. Moreover, a worldwide experiment carried out in 30 countries with young wheat (N = 51,723) and young corn plants (N = 51,892) indicates that accumulation of Cd in plants is a function of Cd concentration in soil (Sillanpaa and Jansson, 1992)

From the linear relationship equations, if the concentration of Cd in the soil increases by 1 mg/kg, then the Cd concentration in whole plant tissues will increase by approximately 3.06, 1.47,1.32 and 0.97 mg/kg for marigold, cosmos, sunflower and Guinea grass, respectively (only under the studied conditions). The influence of soil

Cd on Cd accumulation in whole plant is greater in marigold. Hence, marigold possesses the greatest ability to accumulate Cd in whole plant tissues. For Guinea grass, although Cd accumulation in whole plant is lowest, its higher biomass production could maximize the total Cd uptake.

If the nonlinear relationship was considered (Figure 4.36), the polynomial relationship provided higher R^2 of 0.97, 0.98 and 0.97 for marigold, cosmos and sunflower, respectively. From the graph, at the lower of Cd concentration from 50 to 200 mg/kg, the slope of the graph is much increase, and Cd in whole plant tissues dramatically increased with increasing Cd concentration in the soil. However, if Cd concentration is soil increased to 400 mg/kg, Cd accumulation in whole plant tissues seem be constant and there might be tendency to decrease if Cd in soil was enhanced further above this studied concentration. This might be possible due to higher Cd concentration in the soil can leads to phytotoxicity which can result in the retarded growth of the plants.

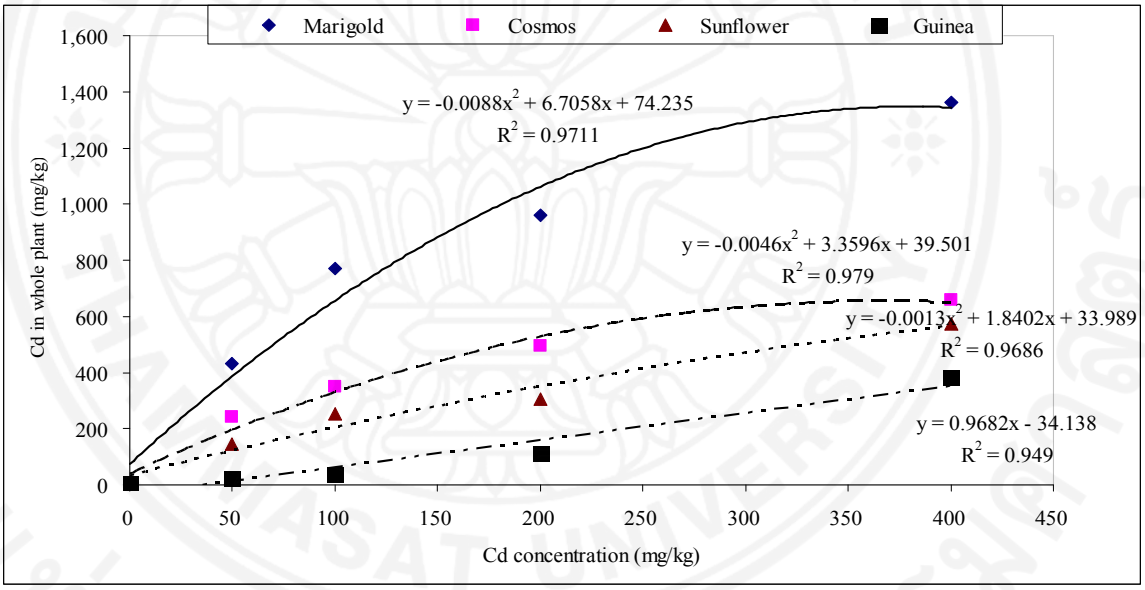


Figure 4.36 Relationship between total Cd in whole plant tissues of different species and Cd concentration applied in the soil (polynomial relationship)

Correlation of Cd accumulated in marigold under various soil pH and Zn concentration in the soil

In this section, the examination of the relationship between the shoot Cd, and Cd in whole plant tissues as dependent variables and soil pH as well as Zn applied in the soil as independent variables was investigated (only the dominant relationship was concerned and reported in this section).

For the relationship between shoot Cd and pH in soil, the results showed a negative correlation, indicating that shoot Cd decreased with increasing pH in the soil. The

linear relationship provided R^2 of 0.88 and 0.98 for Cd treatment of 50 and 100 mg/kg, respectively (Figure 4.37), showing that 88 % and 98 % of the variation of shoot Cd in marigold was influenced by pH variation in the soil (within the pH range of 5 to 7.5). The simple correlation between shoot Cd of marigold and pH in the soil was found to be negative. It revealed the positive change in one variable (soil pH) associated with a negative change in another (shoot Cd).The results obtained from this study depicted that shoot Cd decreased with increasing pH in the soil. At higher Cd concentration in the soil (100 mg/kg) the stronger influence on shoot Cd was observed as the slope of the equation is higher than that of 50 mg Cd/kg.

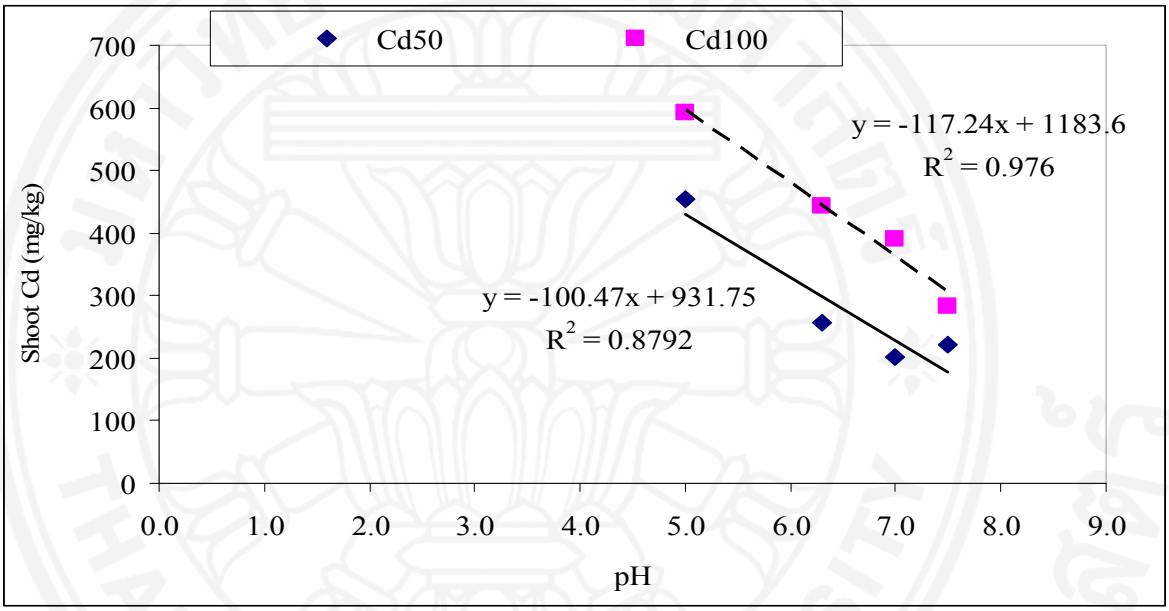


Figure 4.37 Relationship between Shoot Cd in marigold and pH in the soil

The correlation between total Cd in whole plant of marigold and soil pH was negative. At Cd treatment of 50 and 100 mg/kg, the polynomial relationship provided better R^2 of 0.95 and 0.99, showing that 95 and 99 % of variation of Cd in whole plant tissues was affected by the change in soil pH (Figure 4.38). At higher Cd concentration in the soil (100 mg/kg) the stronger influence on Cd concentration in whole plant was observed as compared to at Cd treatment of 50 mg/kg.

There was a negative association between total Cd in whole plant tissues of marigold and Zn concentration in the soil (Figure 4.39). At Cd treatment of 50 mg/kg, when Zn was added to soil, total Cd concentration in whole plant tissues of marigold significantly increased from 477.05 to 815.86 mg/kg, showing synergistic effect between Cd and Zn. However, total Cd concentration in plant significantly declined ($p<0.05$) from 815.86 to 168.90 mg/kg as Zn concentration in soil increased from 618.33 to 2708 mg/kg. This might be possibly due to the antagonistic impact when higher concentration of Zn was applied to the soil as previously mentioned. At Cd treatment of 100 mg/kg, total Cd in whole plant tissues significantly declined ($p<0.05$) from 914.84 to 202.35 mg/kg when the Zn concentration in the soil

increased from 43.08 (natural Zn concentration present in soil) to 1265.83 mg/kg, showing antagonistic effect between Cd and Zn as they both having similar chemical properties.

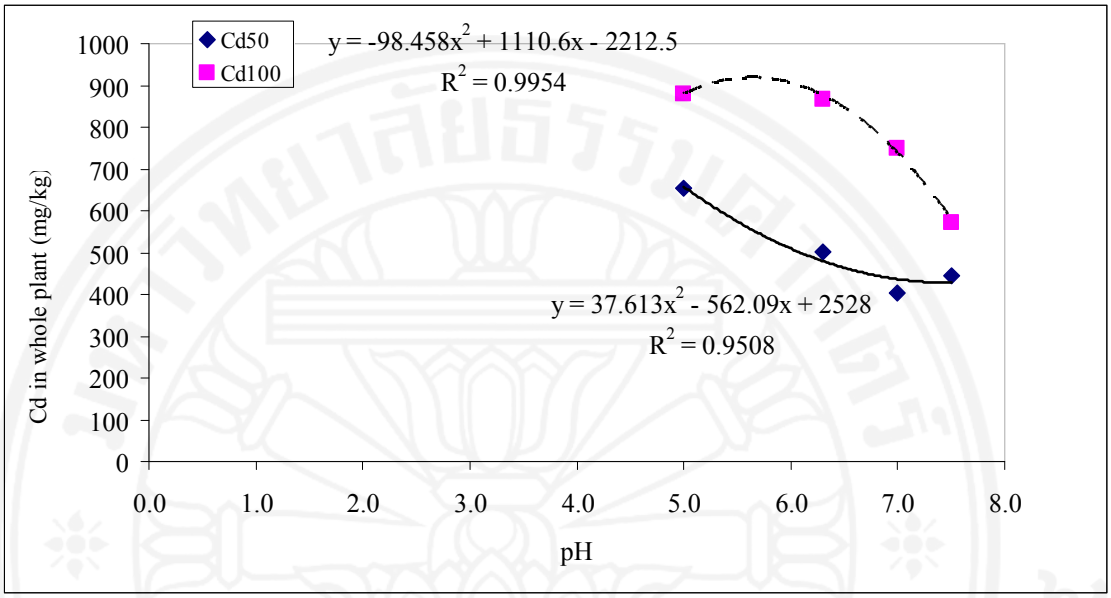


Figure 4.38 Relationship between total Cd in whole plant tissues of marigold and soil pH

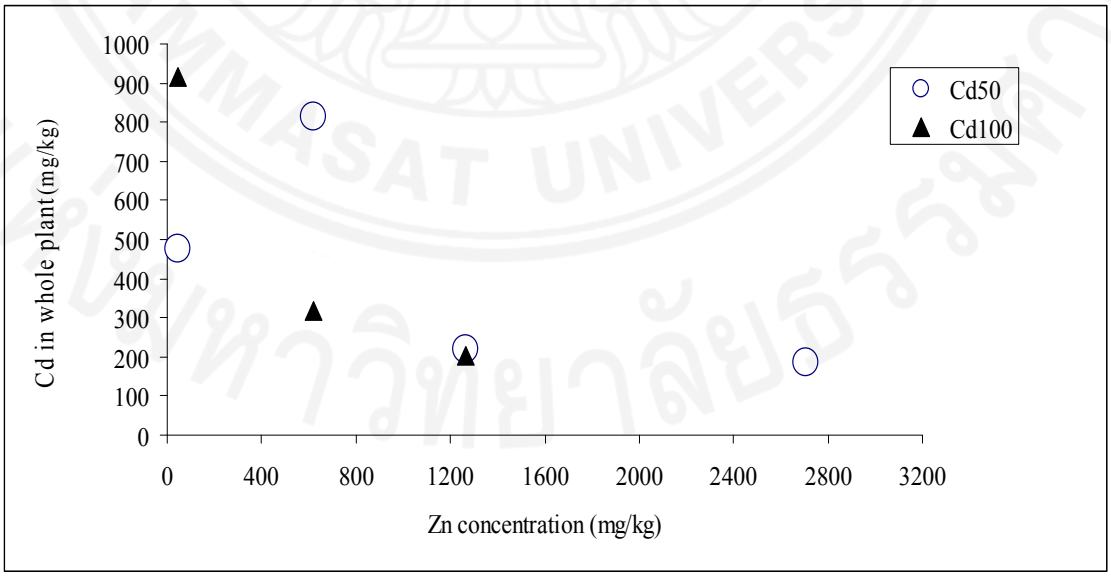


Figure 4.39 Relationship between Total Cd in whole plant tissues of marigold and Zn concentration in the soil at Cd treatment of 50 and 100 mg/kg

The correlation analysis for Cd accumulation in the four plant species showed that there was a significant positive linear relationship between Cd accumulation in whole plant tissues and Cd concentration in soil ($p<0.05$) with R^2 of 0.88, 0.87, 0.96 and 0.95 for marigold, cosmos, sunflower, and Guinea grass, respectively. This revealed that most of the variation occurred in Cd accumulation in these four species was affected by Cd concentration present in the soils. A negative correlation was found between shoot Cd in marigold and soil pH, indicating that shoot Cd declined with increasing pH in the soil. The linear relationship between the two variables depicted that 88 % and 97 % (at Cd treatment of 50 and 100 mg/kg, respectively) of the variation of shoot Cd in marigold was influenced by pH in the soil.

The results show that at higher Cd treatment (100 mg/kg), soil pH has stronger influence on shoot Cd than that of the lower one. The simple correlation between Cd in whole plant tissues of marigold and pH in the soil was found to be negative for both Cd treatments (50 and 100 mg/kg). The results depicted that Cd accumulated in whole plant tissues decreased with increasing pH in the soil. At Cd treatment of 50 mg/kg, the polynomial relationship provided R^2 of 0.0.95, showing that 95 % of variation of Cd in whole plant tissues was affected by the pH in the soil. For Cd treatment of 100 mg/kg, the polynomial relationship provided maximum R^2 of 0.99. This showed that at the beginning with low pH (5.0 to 6.3) Cd accumulation in whole plant tissues increased to some extent but when pH in soil increased further, the Cd accumulation in plant tissues reduced.

Generally, there was both negative and positive association between Zn concentration in the soil and total Cd in whole plant tissues of marigold, showing synergistic and also antagonistic effect between Cd and Zn depending on the concentration of these two elements in the soil. At higher Cd concentration in soil (100 mg/kg), total Cd in whole plant tissues decreased with increasing Zn concentration in the soil. However, at lower Cd concentration applied to soil (50 mg/kg), there was also synergistic effect between these two elements when Zn concentration increased from 43.08 to 618.33 mg/kg.

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

Based on the study, the following conclusions can be drawn:

Screening of species

For screening of the plants, the four species were used in pot culture experiments. The results indicated that height and total biomass (TB) of four species reduced with increasing Cd contents in soil. Shoot Cd in marigold was greater than 100 mg/kg, reaching the threshold value and meeting one of the criteria for hyperaccumulator; TF, BCF values were greater than one. Based on total Cd accumulated in whole plant, shoot Cd, TF and BCF, marigold showed a greater ability to accumulate Cd in plant biomass. Even though TF and BCF values of Guinea grass were lowest as compared to other species, due to higher biomass production, total Cd uptake by Guinea grass was higher, as compared to other species.

Effect of soil pH on plants growth and Cd accumulation by marigold and Guinea grass under various Cd treatments

For marigold, the maximum TB was obtained at pH around 7.0. Under both Cd of 50 and 100 mg/kg, the maximum shoot Cd, Cd in whole plant tissues, TF and BCF values were achieved at pH 5.0. Shoot Cd was greater than 100 mg/kg, illustrating that marigold showed potential to be a Cd hyperaccumulator. The shoot Cd and Cd in whole plant tissues were affected by Cd concentration in the soil and the initial soil pH. Generally, the total uptake of Cd cannot be maximized due to limitations of biomass and/or accumulation of heavy metals. The decreasing soil pH can increase plant metal uptake because metals become more readily available for plants.

For Guinea grass, the maximum TB was obtained at a soil pH around 5.0. TB declined as Cd in soil increased. Generally, TB significantly decreased while Cd concentration in soil was enhanced further up to 200 mg/kg. The higher Cd concentration in soil significantly affected plant growth. Both pH and Cd in soil influenced the TB and overall growth of plants. Maximum shoot Cd, Cd in whole plant, total uptake, and BCF values were obtained at pH around 5.0. TF values, under all pH and Cd treatments, were less than one, indicating that Guinea grass possesses less potential to translocate Cd from roots to shoots as compared to marigold.

Based on the TF and BCF values as well as shoot Cd, marigold shows higher potential to remove Cd from contaminated soil as compared to Guinea grass.

Effect of Zn concentration applied to soil on plant growth and Cd accumulation by marigold and Guinea grass under various Cd treatments

Cd:Zn application in the soil did not improve plant growth (based on height, flower diameter) as compared to plants grown in natural soil. The heights and flower diameters decreased with increasing Cd and Zn application to the soil.

For marigold, the maximum shoot-root Cd, Cd in whole plant tissues, total uptake and BCF were obtained at Cd:Zn treatments of 1:10 and 1:0 for Cd treatment of 50 and 100 mg/kg, respectively. Under all Cd and Cd:Zn treatments, shoot Cd was greater than 100 mg/kg and BCF was greater than one. Under Cd treatment of 50 mg/kg, increasing Zn in the soil (from Cd:Zn of 1:0 to 1:10) enhanced Cd in whole plant tissues of marigold, showing synergistic interaction of Cd and Zn. However, if Zn increased further (from a Cd:Zn of 1:30 to 1:50), Cd in whole plant tissues was reduced indicating antagonistic effect.

For Guinea grass, shoot and root Cd and total Cd in whole plant increased as Zn applied in soil increased, showing opposite trend from marigold. Enhancing Zn in soil (from Cd:Zn of 1:0 to 1:30) promoted Cd accumulated in plant tissues to some degree, and remained unchanged as Zn in the soil increased further. Under all Cd:Zn treatments, TF values were less than one, indicating the inability of Guinea grass to translocate Cd from roots to shoots. Shoot Cd was less than 100 mg/kg.

Effect of EDTA concentration applied to soil on plant growth and Cd accumulation by marigold and Guinea grass under various Cd treatments

For both marigold and Guinea grass, growth (based on height, TB, and flower diameter of marigold) declined with increasing EDTA application, indicating adverse effects on plant growth. Reduction in growth of studied plants was influenced by the combination of Cd concentration and EDTA applied in soil.

For marigold, shoot Cd and Cd in whole plant tissues increased with increasing EDTA concentration and the maximum values for both were obtained at the Cd:EDTA treatment of 50:50 and 100:100 mg/kg. At Cd 50 mg/kg, root Cd significantly declined as EDTA applied in soil increased, but at higher concentration of Cd at 100 mg/kg, root Cd slightly increased as EDTA applied in soil was increased. Application of EDTA in soil promoted translocation of Cd from roots to the shoots of marigold but showed insignificant influence in Guinea grass. Under all Cd:EDTA treatments, shoot Cd of marigold was greater than 100 mg/kg, indicating the ability of phytoextraction of Cd.

For Guinea grass, shoot Cd and total Cd in whole plant tissues slightly increased with increasing EDTA concentration and the maximum value was achieved at the Cd:EDTA treatment of 50:50, 100:100 and 200:200 mg/kg. The increment of root Cd was higher than that of shoot Cd. Thus, the translocation of Cd from roots to shoots might be less effective as compared to marigold and TF value was less than one. Due to the fibrous root system of Guinea grass, more mobilized Cd was retained in the root

zone of Guinea grass. Application of EDTA to soil has no effect on translocation of Cd from roots to shoots.

Fractionation of cadmium in soil

Soil Cd fractionation illustrated that the highest fraction of Cd, under all Cd:EDTA treatments, is exchangeable fraction (F1), followed by Carbonate bound fraction (F2). The residual fraction (F5) is lowest. Application of EDTA increased F1 in the soil. Although the higher mobile fraction after EDTA application to the soil was obtained, the ratio between shoot Cd to root Cd of Guinea grass cannot be maximized. Under all treatment conditions, TF values were less than one, indicating that Guinea grass has lower capacity to translocate Cd from roots to shoots compared to marigold.

The Cd: EDTA treatments of 50:25 and 100:100 mg/kg provided more bioavailable Cd fraction in the soil solution. However, the influence of the application of EDTA to the soil on the stimulation of Cd transfer from roots to the shoots was only pronounced in marigold, but it did not show significant impacts in Guinea grass.

Correlation analysis

The correlation analysis between Cd in whole plant tissues of four species (marigold, cosmos, sunflower and Guinea grass) and Cd in soil showed that there was a positive linear relationship between Cd accumulation in whole plant tissues and Cd concentration in soil with R^2 of 0.88, 0.87, 0.96 and 0.95, respectively, indicating that most of the variations in Cd accumulation in these four species were affected by Cd concentration in the soils. Generally, the correlation analysis between soil pH (independent variable) and shoot Cd, Cd in whole plant (dependent variables) showed a negative association. This indicated that the dependent variables decreased with increasing pH in the soil.

Overall, marigold possesses a greater ability to accumulated Cd in plant biomass as compared to Guinea grass. Generally, shoot Cd was greater than 100 mg/kg. Based on TF, BCF, shoot Cd, total uptake, marigold has a higher potential to be used as Cd (hyper)accumulator but field trial experiment needs to be carried out to investigate the feasibility of using marigold for phytoextraction of Cd from contaminated soil. Although lowering soil pH promoted the Cd accumulation in plant biomass and higher uptake in some cases, care must be taken into consideration when using phytoremediation in acidic soil condition. The results showed that at low Cd contamination in soils, application of Zn in the soil promoted uptake of Cd by marigold. However, at higher Cd contamination, application of Zn to soil decreased Cd uptake by plant. Application of EDTA in the soil stimulated translocation of Cd from roots to shoots in marigold but did not affect Guinea grass. Risk of metal leaching to underground water and environment needs to be concerned if phytoextraction involves acidic soil condition (if pH below 5). Marigold possesses a greater ability to accumulate Cd in plant tissues but has less biomass production which lowers total uptake. To increase phytoremediation efficiency of Cd removal from contaminated site, the proper selection of cultivars and adjustment of its

cultivation practice such as more crop cycles being applied as well as substantial soil amendment are recommended to improve this green technology. However, soil amendments and cultivation practices might have unintended consequences on contaminant mobility. Potential effects of soil amendments should be understood before their use.

5.2 Recommendations

Based on the results of the study, the following recommendations for future research are presented.

The present study aimed to investigate the potential of marigold and Guinea grass to be used as Cd accumulator in pot experiments. In further experiment, the factorial experimental designed should be applied to examine interactions between various independent variables and interested dependent variable simultaneously. The impact of each independent variable can be clearly stated using factorial design.

The replicate of pot experiments should be increased up to five replicates or more, which will help in statistically analysis. The Cd dose treatments for marigold should be varied at least for three concentrations. This will be useful in the statistical analysis. If there are only two Cd doses, some statistically analysis procedure cannot be carried out. If modeling study needed to be investigated, a number of data sets of the samples analysis are necessary.

The results from pot experiments might give different results if employed in the field trial. The application in field experiment should be carried out to find out the optimal conditions to be adapted to the real contaminated area and also to better assess the feasibility of the phytoextraction process using marigold and Guinea grass.

Care must be taken into consideration when EDTA is applied to promote the metal uptake, as EDTA has low biodegradability in environment. Consequently, there is a potential risk of Cd being leached to groundwater. The specific amount of EDTA used in the field trail must be carefully calculated to avoid the risk of leaching of the chelator to underground water or nearby ground water when acidic condition in soil is involved. The biodegradable chelators should be introduced in the field trail study as they are readily biodegradable with low risk of contamination to surrounding environments.

The application of harvested plants (e.g. marigold) should be studied, to investigate if marigold can be used for pigment extraction for using as dye in textile industry. The heat value of Guinea grass should be investigated to identify if it can be used as an energy source.

References

Adler, T. (1996). Botanical cleanup crews. *Sci. News*, 150, 42-43.

Adesodun, J.K., Atayese, M.O., Agbaje, T.A., Osadiaye, B.A., & Soretire, A.A. (2010). Phytoremediation potentials of sunflowers (*Tithonia diversifolia and Helianthus annus*) for metals in soils contaminated with zinc and lead nitrates. *Water, Air and Soil Pollution*, 207, 195-201.

Adriano, D.C. (1986). *Trace elements in the terrestrial environment.* New York: Springer-Verlag. p. 106-149.

Adriano, D. C. (1992). *Biogeochemistry of Trace Metals*. CRC Press, Boca Raton, FA, p. 513.

Agarwal, H., & Goyal, D. (2007). Phytoremediation of some heavy metals by agronomic crops. *Developments in Environmental Science*, 5, 79-98.

Alkorta, I., & Garbisu, C. (2001). Phytoremediation of organic contaminants. *Bioresource Techno,* 79, 273–276.

Allen, S. E., Grimshaw, H. M., Parkinson, H. M., & Quarmby, J.A. (1974). *Chemical analysis of ecological materials* Oxford, UK: Blackwell.

Alloway, B.J. (1995). Cadmium. In *Heavy Metals in Soils* (2nd Ed). London: Blackie Academic & Professional.

Baker, A. J. M., & Proctor, J. (1970). The influence of cadmium, copper, lead, and zinc on the distribution and evolution of metallophytes in the British Isles. *Plant System*, 173, 91-108.

Baker, A. J. M., & Brooks, R. R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. *Biorecovery*, 1, 81-126.

Baker, A.J.M., McGrath, S.P., Reeves, R.D., & Smith, J.A.C. (2000). Metal hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource for phytoremediation of metal-polluted soils. In: N. Terry, G. Bauelos, (Eds.) *Phytoremediation of contaminated soil and water* (pp. 85-107). Florida, USA: Lewis Publishers.

Baker, A. J. M,, McGrath, S. P., Sidoli, C. M. D., & Reeves, R. D. (1994). The possibility of *in situ* heavy metals decomposition of polluted soils using crops of metal-accumulating plants. Resour. *Conserv. Recyl*, 11, 41-49.

Baker, A. J. M., & Proctor, J. (1970). The influence of cadmium, copper, lead, and zinc on the distribution and evolution of metallophytes in the British Isles. *Plant*

System, 173, 91-108.

Baker, D.E., & Amacher, M.C. (1982). Nickel, copper, zinc and cadmium. In: *Methods of soil analysis*. A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Part 2. Madison, WI: American Society of Agronomy, p. 323-334.

Barak, P., & Helmke, P.A. (1993). The chemistry of zinc. In: A. Robson (Ed), *Zinc in Soils and Plant.* Proc., Internat. Symp. on "Zinc in Soils and Plants" (pp. 1-13). Perth, Australia. Kluewer Acad. Press, Dordrecht.

Barrow, N. J. (1993). Effect of surface heterogeneity on ion adsorption by metal oxides and by soils. *Langmuir*, 9, 2606-2611.

Basta, N.T., Pantone, D.J., & Tabatabai, M.A., (1993). Path analysis of heavy metal adsorption by soil. *Agron. J*, 85, 1054-1057.

Bennet, A.C., & Shaw, D.R. (2000). Effect of preharvest desiccants on Group IV Glycine max seed viability. *Weed Sci*, 48, 426-430.

Benˇs, S. and Pabianova, J. (1986). Background Contents and Classifi cation of Trace Elements in Soils, VˇSZ, Praha, 203.

Bingham, F.T., Page, A.L., & Strong, J.E. (1980). Yield and cadmium content of rice grain in relation to addition rates of cadmium, copper, nickel, and zinc with sewage sludge and liming. *Soil Sci*, 130, 32.

Blaylock, M.J., & Huang, J.W. (2000). Phytoextraction of metals. In: I. Raskin, B.D. Ensley (Eds.), *Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment* (pp. 53–70). New York, USA: John Wiley and Sons,

Blaylock, M.J, Salt, D.E., Dushenkov, S., Zakharova, O., & Gussman, C. (1997). Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents. *Environ. Sci. Techno,* 31, 860-865.

Brooks, R.R. (1998a). General introduction. In: R.R. Brooks (Ed). *Plants that hyperaccumulate Heavy Metals* (pp. 1-14). New York, NY: CAB International.

Brown, S. L., Chaney, R. L., Angle, J.S., & Baker, A. J. M. (1994). Phytoextraction potential of *Thlaspi caerulescens* and Bladder Campion for zinc and cadmium-contaminated soil. *J. Environ. Qual*, *23*, 1151-1157.

Boardman, R., & McGuire, D.O. (1990). The role of zinc in forestry. I. Zinc in forest environments, ecosystems and tree nutrition. *For. Ecol. Manag*, 37, 167-205.

Boonyapookana, B. (2004). Phytoremediation techniques for a lead contaminated site focusing on hyperaccumulating plants. Doctoral dissertation. No.Ev-04-5, Bangkok: Asian Institute of Technology.

Bridgwater, A.V., Meier, D., & Radlein, D. (1999). An overview of fast pyrolysis of biomass. *Org. Geochem*, 30, 1479–1493.

Brown, S. L., Chaney, R. L., Angle, J.S., and Baker, A. J. M. (1994). Phytoextraction potential of *Thlaspi caerulescens* and Bladder Campion for zinc and cadmium contaminated soil. *J. Environ. Qual*, 23, 1151-1157.

Bryan, G.w., Langston, W.J. (1992). Bioavailability, accumulation and effects of heavy metals in sediment with special reference to United Kingdom estuaries. Rev. *Environ. Pollut*, 76, 89-131.

Cataldo, D.A., Garland, T.R., & Wildung, R.E. (1983). Cadmium uptake kinetics in intact soybean plants. *Plant Physiology*, 73 (3), 844-848.

Ciba, J., Korolewicz, T., & Turek, M. (1999). The occurrence of metals in composted municipal wastes and their removal. *Water Air Soil Pollut*, 111, 159-170.

Chapman, H.D. (1965). Cation exchange capacity. *In:* C.A. Black (Ed). *Methods of soil Analysis Part 2-Chemical and microbiological properties* (pp. 891-901). Agronomy 9, 2nd ed. Madison, Wisconsin: American society of Agronomy.

Chaney, R.L. (1993). Risk associated with the use of sewage sludge in agriculture. In *Proc. 12th Federal Convention. Vol 1*. Queensland, Australia: Australian Water and Wastewater Association.

Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Brewer, E.P., Angle, J.S., & Baker, A.J.M. (1997). Phytoremediation of soil metals. *Current Opin. Biotechnol*, 8, 279-284.

Chaney, R.L., Ryan, J.A., Li, Y.M., & Brown, S.L. (1999). Soil cadmium as a threat to human health. In: M.J. McLaughlin, & B.R. Singh (Eds.). *Cadmium in soils and plants*, p. 219-246.

Chen H., and Cutright, T. (2001). EDTA and HEDTA effects on Cd, Cr and Ni uptake by *Helianthus annus*. *Chemosphere*, 45: 21–28.

Christensen, T.H. (1984). Cadmium soil sorption at low concentrations, I. Effect of time, Cd load, pH and calcium. *Water Air Soil Pollut,* 21, 105-114.

Chusai, C. (2006). *Cadmium remobilization from contaminated soil: A case study in Maesot district, Tak province, Thailand.* Master's thesis, Pathumthani, Thailand: Asian Institute of Technology.

Coughtrey. P.J., Jones, C.H., Martin, M.H., & Shales, S.W. (1979). Litter accumulation in woodlands contaminated by Pb, Zn, Cd and Cu. *Oecologia*, 39, 51-60.

Crowley, D.E., Wang, Y.C., Reid, C.P.P., & Szaniszlo, P.J. (1991). Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant Soil*, 130, 179-198.

Cunningham, S.D., & Berti, W.R. (2000). Phytoextraction and phytostabilization: technical, economic, and regulatory considerations of the soil-lead issue. In: N. Terry, & G. Banuelos (Eds). *Phytoremediation of Contaminated Soil and Water* (pp.359-376). USA: Lewis Publisher, CRC Press.

Cunningham, S.D., Berti, W.R., & Huang, J.W. (1995). Phytoremediation of Contaminated Soils. *Trends in Biotechnology*, 13, 393-397.

Das, P., Samantaray, S., Rout, G.R. (1997). Studies on cadmium toxicity in plants: a review. *Environ. Pollut,* 98, 29-36.

Davis, R. D., Beckett, P. H. T., & Wollan, E. (1978). Critical levels of twenty potentially toxic elements in young spring barley. *Plant Soil*, 49, 395.

Department of Agriculture (DOA) (2001). Soil and Plants analysis Manual. Bangkok, Thailand: Soil department.

Di, Toppi, L. S., & Gabbrielli, R. (1999). Response to cadmium in higher plants. *Environ. Exp. Bot*, 41, 105-130.

Dushenkov, V., Nanda Kumar, P.B.A., Motto, H., & Raskin, I. (1995). Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. *Environ. Sci. Technol,* 29, 1239-1245.

Dushenkov, S., Vasudev, D., Kapulnik, Y., Gleba, D., Fleisher, D., Ting, K.C., & Ensley, B. (1997). Removal of uranium from water using terrestrial plants. *Environ. Sci. Technol,* 31(12), 3468-3474.

Ellis, J.M., Shaw, D.R., & Barrentine, W.L. (1998). Herbicide combinations for preharvest weed desiccation in early maturing soybean (Glycine max). *Weed Technol*, 12, 157-165.

Environemental Agency. (2009). *Updated technical background to the CLEA model*. Science Report SC050021/SR3. Environmental Agency, UK.

Epstein, A.L., Gussman, C.D., Blaylock, M.J., Yermiyahu, U., Huang, J.W., Kapulnik, Y., & Orser, C.S. (1999). EDTA and Pb-EDTA accumulation in Brassica juncea grown in Pb-amended soil. *Plant Soil*, 208, 87-94.

Eriksson, J.E. (1989). The influence of pH, soil type and time on adsorption and uptake by plants of Cd added to the soil. *Water Air Soil Pollut*, 48, 317-335.

Eriksson, J., Öborn, I., Jansson, G., and Andersson, A. (1996). Factors influencing Cd- content in crops. Results from Swedish field investigations. *Swed. J Agric. Res*, 26:125-33.

Farmer, A.A., & Farmer, A.M. (2000). Concentration of cadmium, lead, and zinc in livestock feed and organs around a metal production center in eastern Kazakhstan. *Sci Total Environ,* 257, 53-60.

Fei Wang, X., & Xing zhou, Q. (2005). Ecotoxicological effects of cadmium on three ornamental plants. *Chemosphere*, 60, 16-21.

Flathman, P.E., & Lanza, G.R. (1998). Phytoremediation: current views on an emerging green technology. *J. Soil Contam*, 7, 415-432.

Garbisu, C., & Alkorta, I. (2001). Phytoextraction: a cost-effective plant based technology for the removal of metals from the environment. *Biores. Technol*, 77, 229-236.

Garbisu, C., & Alkorta, I. (2003). Basic concepts on heavy metal soil bioremediation. *Eur. J. Min. Proc. Environ. Protect*, 13, 58-66.

Ghosh, M., & Singh, S.P.A. (2005). Review on phytoremediation of heavy metals and utilization of it's by products. *Appl. Ecology and Environmental Research*, 3(1), 1-18.

Grant, C.A., & Bailey, L.D. (1997). Effects of phosphorous and zinc fertilizer management on cadmium accumulation in flaxseed. *J. Sci. Food and Agric*, 73, 307-314.

Green, C.E., Chaney, R.L., & Bouwkamp, J. (2003). Interactions between cadmium uptake and phytoxic levels of zinc in hard red spring wheat. *J. Plant Nutrition*, 26(2), 417-430.

Godt, J., Scheidig, F., Siestrup, C., Esche, V., Brandenburg, P., Reich, A., David, A., & Groneberg, D.A. (2006). The toxicity of cadmium and resulting hazards for human health. *J. of Occupational Medicine and Toxicology*, 1, 22.

Gupta, S.K., Vollmer, M.K., & Krebs, R. (1996). The importance of mobile, mobilisable and pseudo total heavy metal fractions in soil for three-level risk assessment and risk management. *Sci. Total Environ*, 178, 11-20.

Haghiri, F. (1973). Plant uptake of cadmium as influenced by cation exchange capacity, organic matter, zinc and soil temperature. *J. Environ. Qual*, 2, 93-96.

Hammer, D.A. (Ed.) (1989). *Constructed wetlands for wastewater treatment*. Chelsea, Michigan: Lewis publishers.

Hammer, D.A.; & Bastion, R.K. (1989). *Wetlands ecosystems: Natural water purifiers*. In D.A. Hammer (Ed), pp. 5–20.

Hart, J.J., Welch, R.M., Norvell, W.A., & Kochian, L.V. (2002). Transport interaction between Cd and Zn in roots of bread wheat and durum wheat seedlings. *Physiol Plant*, 116(1), 73-78.

He, Q.B., & Singh, B.R. (1994). Crop uptake of cadmium from phosphorus fertilizers, I. Yield and cadmium content. *Water Air Soil Pollut*, 74, 251-265.

He, Q.B., & Singh, B.R. (1995). Cadmium availability to plants as affected by repeated applications of phosphorus fertilizers. Acta Agric Scand. Sect. B. *Soil Plant Sci*, 45, 22-31.

Hentz, S., McComb, J., Miller, G., Begonia, M., & Begonia, G. (2012). Cadmium Uptake, Growth and Phytochelatin Contents of *Triticum Aestivum* in Response to Various Concentrations of Cadmium. *World Environment*, 2(3), 44-50.

Hetland, M.D., Gallagher, J.R., Daly, D.J., Hassett, D.J., & Heebink, L.V. (2001). Processing of plants used to phytoremediate lead-contaminated sites. In: A. Leeson, E.A. Foote, M.K. Banks, & V.S. Magar (Eds.). Phytoremediation, Wetlands, and Sediments*, The Sixth International in situ and on-site Bioremediation Symposium,* San Diego, California.

Hillel, D. (1998). *Environmental Soil Physics*. San Diego, CA, USA: Academic Press.

Horiguchi, T. & Nishihara, T. (1981). Heavy metals associated with the major constituents of potato tubers and peanut seeds, *Mem. Fac. Agr. Kagoshima Univ*, 17, 95.

Hornburg, V. & Brummer, G. W.(1986), Cadmium availability in soils and content of wheat grain, In M. Anke, H. Braunlich, Chr. Bruckner, & B. Groppel (Eds.), *5thSpurenelement Symposium on Iodine and other Trace Elements* (pp. 916). F. Schiller University, Jena.

Hovsepyan, A., & Greipsson, S. (2005). EDTA-enhanced phytoremediation of lead contaminated soil by corn. *Journal of Plant Nutrition*, 28: 2037-2048.

Huang J.W., Blaylock M.J., Kapulnik Y., & Ensley B.D. (1998). Phytoremediation of uranium-contaminated soils: Role of organic acids in triggering uranium hyperaccumulation in plants. *Env. Sc. and Technol*, *32*, 2004-2008.

Huang, J.W., Chen, J., & Cunningham, S.D. (1997). Phytoextraction of lead from contaminated soil. In K.L. Kruger (Ed.), *Phytoremediation of soils and water contaminants* (pp. 83-298), Washington DC, USA: American Chemical Society.

Huang, J.W., & Cunningham, S.D. (1996). Lead phytoextraction: species variation in lead uptake and translocation. *New Phytol,* 134, 75-84.

Huang, D., Xu, Q., Cheng, J. Lu, X and Zhang, H. (2012). Electrokinetic Remediation and Its Combined Technologies for Removal of Organic Pollutants from Contaminated Soils. International J. of Electrochemical Science. 7: 4528-4544.

Järup, L. (2003). Hazards of heavy metal contamination**.** Oxford Journals Medicine British Medical Bulletin. 68(1):167-182.

Järup, L., Berglund, M., Elinder, C.G., Nordberg, G., & Vahter M. (1998). Health effects of cadmium exposure-a review of the literature and a risk estimate. *Scand. J. Work Environ. Health*, 24 (1):1-51.

Jiang, X.J., Luo, Y.M., Zhao, Q.G., Baker, A.J.M., Christie, P., & Wong, M.H. (2003). Soil Cd availability to Indian mustard and environmental risk following EDTA addition to Cd-contaminated soil. *Chemosphere*, 50:813-818.

Jordahl, J.L., Foster, L., Schnoor, J.L., & Alvarez P.J.J. (1997). Effect of hybrid poplar trees on microbial populations important to hazardous waste bioremediation. *Environ. Toxicol. Chem,* 16(6):1318-1321.

Jørgensen, S.E. (1993). Removal of heavy metals from compost and soil by ecotechnological methods. *Ecological Engineering*. 2: 89-100.

Jothi, D. (2008). Extraction of Natural Dyes from African Marigold Flower (Tagates Ereectal) for Textile Coloration. *AUTEX Research Journal*, 8(2):49-53.

Jury, W.A., Russo, D., Streile, G., & El Abd, H. (1990). Evaluation of volatilization by organic chemicals residing below the soil surface. *Water Resour. Res*. 26:13-20.

Kabata-Pendias, A. (2001). Trace elements in soil and plants. FL, USA: CRC press, Taylor and Francis Group.

Kalra, Y.P. (1995). Determination of pH of soils by different methods: Collaborative study. *J. AOAC Int,* 78: 310-321.

Kidder, G., W.E. Sabbe, & C.L. Parks. (1988). Procedures and practices followed by southern state soil testing laboratories for making liming recommendations. Southern Cooperative Series bulletin no. 332, Univ. of Florida.

Klasssen, C.D. (2001). *Toxicology*, 6th edition, New York, USA; the McGraw-Hill.

Keller, C., Hammer, D., Kayser, A., Richner, W., Brodbeck, M., & Sennhauser, M. (2003). Root development and heavy metal phytoextraction efficiency: comparison of different plant species. *Plant and Soil*, 249: 67-81.

King, L.D. (1988). Retention of cadmium by several soils of the southeastern United State. *J. Environ. Qual*, 17:246-250.

Kirkham, M. B. (2006). Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments. *Geoderma*, 137: 19-332.

Kitagishi, K. & Yamane, I., (Eds.) (1981), *Heavy Metal Pollution in Soils of Japan*, Tokyo: Japan Science Society Press.

Kloke, A., Sauerbeck, D. R., & Vetter, H. (1984). The contamination of plants and soils with heavy metals and the transport of metals in terrestrial food chains, in *Changing Metal Cycles and Human Health*, In J.O. Nriagu, (Ed.)., Berlin: Dahlem, Konferenzen, Springer-Verlag.

Konradi, E.A., Frentiu, T., Ponta, M., & Cordos, E. (2005). Use of sequential Extraction to assess metal fractionation in soils from Bozanta Mare, Romania. *Seria F. Chemia*, 8: 5-12.

Kumar, P. B. A. N., Dushenkov, V., Motto, H., & Raskin, I. (1995). Phytoextraction: The use of plants to remove heavy metals from soils. *Environ. Sci. Technol*, 29(5):1232-1238.

Kuo, S., Jellum, J., & Baker, A.S. (1985). Effects of soil type, liming, and sludge application on zinc and cadmium availability to Swiss chard. *Soil Sci*, 139:122-130.

Kuo, S., Huang, B., & Bembenek, R. (2004). The availability to lettuce of zinc and cadmium in a zinc fertilizer. *Soil Science*, 169: 363-373.

Lagerwerff, J. V. & Biersdorff, G. T. (1972) Interaction of zinc with uptake and translocation of cadmium in radish. In D.D. Hemphill (Ed.), *Trace Subst. Environ. Health*, Vol. 5, Hemphill, D. D., (Ed.), University of Missouri, Columbia, MO.

Lasat, M.M. (2000). Phytoextraction of metals from contaminated soil: A review of plant/soil/metal interaction and assessment of pertinent agronomic issues. *J. Hazardous Substance Research*. 5(2): 1-25.

Laurie, S.H., Tancook, N.P., McGrath, S.P., & Sanders, J.R. (1991). Influence of complexation on the uptake by plants of iron, manganese, copper and zinc. I. Effect of EDTA in a multi-metal and computer-stimulation study. *J. Exp. Bot*. 42-237; 509-513.

Lehoczky, E., Szabo, L., & Horvath. S. Z. (1998). Cadmium uptake by lettuce in different soils. *Commun. Soil Sci. Plant Anal*. 29: 903-12.

Lena, Q. M., & Gade, N. R. (1997). *Chemical fractionation of Cadmium, Copper, Nickel and Zinc in contaminated soils*. *Journal of Environmental Quality*. 26(1): 259-264.

Li, H., Wang, Q., Cui, Y., Dong, Y., & Christie, P. (2005). Slow release chelate enhancement of lead phytoextraction by corn (*Zea mays* L.) from contaminated soil-a preliminary study. *Science of the Total Environement*. 339: 179-187.

Li, S.L., Wang, H.X., & Wu, Y.S. (1990). Antagonistic effect of zinc on cadmium in water hyacinth. *Acta Scientiae Circumstantiae*. 10: 244-249.

Li, W.O., Cui, Y., & Christie, P. (2005). Slow release chelate enhancement of lead phytoextraction by corn (*Zea mays* L.) from contaminated soil- a preliminarystudy. Science of the Total Environment 339, 179-187.

Liang, Y.C., & Wong, J. (2005). Silicon-mediated enhancement of cadmium tolerance in maize (*Zea mays* L.) grown in cadmium contaminated soil. *Chemosphere*. 58, 475-452.

Liphadzi, M.S. (2002). Chelate-assisted phytoremediation of soil with heavy metals in bioslids. Ph.D. Thesis. Kansas State University. Manhattan, Kansas. p.243.

Liphadzi, M.S., Kirkham, M.B., Mankin, K.R., & Paulsen, G.M. (2003). EDTA-assisted heavy-metal uptake by poplar and sunflower grown at a long-term sewage-sludge farm. *Plant and Soil*. 257: 171-182.

Long, X.X., Yang, X.E., Ni, W.Z., Ye, Z.Q., He, Z.L., Calvert, D.V., & Stoffela, J.P. (2003). Assesing zinc thresholds for phytotoxicity. *Commun. Soil Sci. Plant Anal.* 34: 1421-1434.

Luo, L.X., Sun, T. H., & Jin, Y. H. (1998). Accumulation of superoxide radical in wheat leaves under cadmium stress. *Acta Sci. Circum.* 18: 495-499.

Marmara, A.R., Aktoprakligil, D., Ozdemir, A., & Vertii, A. (2001). Heavy metal accumulation and detoxification mechanisms in plants. *Turk. J. Bot*. 25: 111-121.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. 2nd Ed. Academic Press. London. p. 889.

McBride, M.B. (1994). Environmental chemistry of soils. *Environmental Chemistry of Soils* vii, Oxford University Press. P. 406.

McCutcheon, S.C., & Schnoor, J.L. (2003). *Phytoremediation*: Transformation and Control of Contaminants. Hoboken, NJ. Wiley-Interscience, Inc.

Macnicol, R. D. & Beckett, P. H. T. (1985). Critical tissue concentrations of potentially toxic elements. *Plant Soil*, 85:107-129.
Meagher, R.B. (2000). Phytoremediation of toxic elemental and organic pollutants. *CurrentOpinion in Plant Biology.* 3:153-162.

McLean, E. O. (1982). Soil pH and lime requirement. In: A. L. Page, R. H. Miller, &

D. R. Keeney (Eds.), *Chemical and Microbiological Properties,* 2nd edition. Methods of Soil Analysis Part 2 (pp.199-224). Madison: WI, USA. American Society of Agronomy.

McLaughlin, M.J, & Singh, B.R. (1999). *Cadmium in soils and plants*. Kluwer Academic Publishers: Dordrecht, the Netherlands. p. 257-267.

Miller, R. H., & Keeney, D. R. (Eds.) (1982). American Society of Agronomy, Madison: WI, USA. P. 59-166.

Moraghan, J.T. (1993). Accumulation of cadmium and selected elements in flax seed grown on a calcareous soil. *Plant and Soil*. 150: 61–68.

Nan, Z., Li, J., Zhang, J., & Cheng, G. (2002). Cadmium and zinc interactions and their transfer in soil-crop system under actual field conditions. *Sci. Total Environ.* 285: 187–195.

Nelson, D.W., & Sommers, L.E. (1982). *Total carbon, Organic carbon, and organic matter*. In: A.L. Page, R.H. Miller, & D.R. Keeney, (Eds.), Methods of Soil Analysis. Part 2. *Chemical and Microbiological Properties*, 2nd edition (pp.539-580). Agronomy Monograph 9, Madison, WI, USA: American Society of Agronomy.

Newman, L.A., Strand, S.E., Choe, N., Duffy, J., Ekuan, G., Ruszaj, M., Shurtleff, B.B., Wilmoth, J., Heilman, P., & Gordon, M.P. (1997). Uptake and biotransformation of trichloroethylene by hybrid poplars. *Environ. Sci. Technol*. 31:1062-1067.

Nobuntou, W. (2012). Assessing the potential toxicity of trace elements in agricultural soils with focusing on lead (Pb) and remediation options for clean up of a contaminated site. Mulligan, C.N., Yong, R.N., Gibbs, B.F., 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Eng. Geol*. 60: 193–207.

Nordberg, G.F., Nordberg, M. (1988). Biological monitoring of cadmium. In: T.W. Clarkson, L. Priberg, G.F. Nordber, P.R. Sager, (Eds.), Biological Monitoring of Toxic Metals (pp. 151-168). NY.

Notification of National Environmental Board No. 25, B.E. (2004). *Soil Quality Standards for Habitat and Agriculture*, Pollution Control Department, Ministry of Natural Resources and Environment.

Nriagu, J. O. (1980). Human influence on the global cadmium cycle. In: J. O. Nriagu, (Ed.). *Cadmium in the Environment. Part I: Ecological Cycling* (pp.2-12). New York: Wiley-lnterscience.

Oliver, D.P., Hannam, R., Tiller, K.G., Wilhelm, N.S., Merry, R.H., & Cozens, G.D. (1994). The effects of zinc fertilization on cadmium concentration in wheat grain. *J. Environ. Qual*. 23: 705-711.

Padmavathiamma, P.K., & Li, L.Y., 2007. Phytoremediation technology: Hyperaccumulation metals in plants. Water, Air and Soil Pollution. 184:105-126.

Papoyan A., Pineros N., Kochian L.V. (2007). Plant Cd^{2+} and Zn^{2+} status effects on root and shoot heavy metal accumulation in *Thlaspi caerulescens. New Phytol*. 175:51–58.

Peralta-Videa, J.R., Gardea-Torresdey, J.L., Gomez, E., Tiemann, K.J., Parsons J.G., and Carrillo, G. (2009). Effect of mixed cadmium, copper, nickel and zinc at different pH upon alfalfa growth and heavy metal uptake. *Environmental Pollution*, 119: 291-301.

Perronnet, K., Schwartz, C., Ge´rard, E., Morel, J.L. (2000). Availability of cadmium and zinc accumulated in the leaves of Thalspi caerulescens incorporated into soil. *Plant Soil*. 227: 257–263.

Perronnet, K., Schwartz, C., and Morel, J.L. (2003). Distribution of cadmium and zinc in the hyperaccumulator *Thlaspi caerulescens* grown on multi-contaminated soil. *Plant Soil.* 249: 19–25.

Piotrowska, M., Dudka, S., and Chilopecka, A. (1994). Effect of elevated concentrations of Cd and Zn in soil on spring wheat yield and metal contents of plants. *Water Air Soil Pollut.* 76: 333-341.

Pichtel, J., Kuroiwas, K., and Sawyerr, H.T. (2000). Distribution of Pb, CD, and Ba in soils and plants of two contaminated sites. Enviromental Pollution. 110:171-178.

Pongsakul, P., and Attajarusit, S. (1999). Assessment of heavy metal contaminations in soils. *Thai Journal of Soils and Fertilizers.* 21: 71–82.

Pollard, A.J., and Baker, A.J.M. (1996). The quantitative tools for hyperaccumulation in *Thlaspi caerulescens*. *New Phytol*. 132:113-118.

Prusinkiewicz, P., Lindenmayer, A. (1990). *The Algorithmic Beauty of Plants*. Springer-Verlag. pp. 101–107.

Raskin, I., Smith, R.D., Salt, D.E. (1997). Phytoremediation of metals using plants to remove pollutants from the environment. *Curr. Opin. Biotechnol*. 8: 221-226.

Rauser, W.E. (1986). The amount of cadmium associated with Cd-binding protein in roots of Agrostis gigantean, maize and tornato. *Plant Science*. 43:85-91.

Ravera, O. (1984). Cadmium in freshwater ecosystems. *Experientia*. 40: 1-14.
Rayment, G.E., & Higginson, F.R. (1992). Electrical conductivity. In: *Australian Laboratory Handbook of Soil and Water Chemical Methods*. Inkata Press, Melbourne. p. 15-16.

Reinhard, W. N., Pavel. T., Michael. K., Jirina. S. (2008). Phytoextraction of Pb and Cd from a contaminated agricultural soil using different EDTA applicatioin regimes: Laboratory versus field scale measures of efficiency. *Geoderma* 144, 446-454.

Rhoades, J.K. (1982). Cation exchange capacity. In: A.L. Page, R.H. Miller, & D.R. Keeney, (Eds.), *Methods of Soil Analysis. Part 2*. Chemical and Microbiological Properties, 2nd edition (pp.149-158). Agronomy Monograph 9, Madison, WI, USA: American Society of Agronomy.

Rhoades, J.D. (1982). Reclamation and management of salt afflicted soils after drainage. In: Proceedings of the First Annual Western Provincial Conference. *Rationalization of Water and Soil Research Management*. p. 123–197.

Rungruang, N., Babel, S., & Parkpian, P. (2011). Screening of potential hyperaccumulator for cadmium from contaminated soil. *Desalination and Water Treatment.* 32: 19-26.

Sabir, S.M., Khan, S.W., & Hayat, I. (2003). Effect of environmental pollution on quality of meat in district Bagh, Azad Kashmir. Pak. J. Nutr. 2:98-101.

Salt, D.E., Blaylock, M., & Kumar, N.P.B. (1995). Phytoremediation: a novel strategy for the removal of toxic elements from the environment using plant. *Biotechnol.* 13, 468-474.

Salt, D.E., Pickering, I.J., Prince, R.C., Gleba, D., Dushenkov S., Smith, R.D., & Raskin, I. (1997). Metal accumulation by aquacultured seedlings of Indian Mustard. *Environ. Sci. Technol*. 31(6):1636-1644.

Salt, D.E., Prince, R.C., Pickering, I.J., & Raskin, I. (1995a). Mechanism of cadmium mobility and accumulation in Indian mustard. *Plant Physiol*. 109: 1427–1433.

Salt, D.E., Smith, R.D., & Raskin, I. (1998). Phytoremediation. Annu. Rev. *Plant Physiol. Plant Mol. Biol.* 49, 643-668.

Sappin-Didier, V., Vansuyts, G., Mench, M., & Mriat, J. F. (2005). Cadmium availability at different soil pH to transgenic tobacco overexpresing ferritin. *Plant and Soil*. 270: 189-197.

Sarkar, B., 2002. Heavy Metals in the Environment. NY: Marcel Dekker, Inc.

Sarret, G., Vangronsveld, J., Manceau, A., Musso, M., D'Haen, J., Menthonnex, J.J., & Hazemann, J.L. (2001). Accumulation forms of Zn and Pb in Phaseolus vulgaris in the presence and absence of EDTA. *Environ. Sci. Technol*. 35: 2854–2859.

Sas-Nowosielska, A., Kucharski, R., Malkowski, E., Pogrzeba, M., Kuperberg, J.M., & Krynski, K. (2004). Phytoextraction crop disposal - an unsolved problem. *Environ. Pollut.* 128:373–379.

Singh, b.R., & Myhr, K. (1998). Cadmium uptake by barley as affected by Cd sources and pH levels. *Geomerma*. 84:185-194.

Scheuhammer, A.M. (1987). The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: A review. Environ. Pollution. 46:263–295.

Schneiter, A.A., & Miller, J.F. (1981). *Description of Sunflower Growth Stages. Crop Sci.* 21:901-903.

Schnoor, J.L., Licht, L. A., McCutcheon, S.C., Wolfe, N.L., & Carreira, L.H. (1995). Phytoremediation of Organic and Nutrient Contaminants, *Environ. Sci. Technol.* 29 (7): 318A-323A.

Sharma, D.H., & Reddy, R.K. (2004). Geoenvironmental engineering: Site Remediation, Waste Containment, and Emerging Waste Management Technologies. USA: John Wiley and Sons, Inc.

Shimp, J.F., Tracy, L.C. Davis, E. Lee, W. Huang, L.E. Erickson, & Schnoor, J.L. (1993). Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. *Crit. Rev. Environ. Sci. Technol*. 23:41-77.

Shuman, L. M., 1980. Effect of soil temperature, moisture and air-drying on extractable manganese, iron, copper and zinc. *Soil Science*. 130: 336-43.

Sillanpaa, M., & Jansson, H. (1992). Status of cadmium, lead, cobalt and selenium in soils and plants of thirty countries, *FAO Soil Bull.*, Rome, 65:195.

Singh, A.K. (2006). *Flower Crops: Cultivation and Management*. Delhi, INDIA: New India Publishing Agency.

Singh, B. R., & Myhr, K. (1997). Cadmium uptake by barley as affected by Cd sources and pH levels. Geoderma. 84; 185-194.

Singh, B. R., Narwal, R. P., Jeng, A. S., & Almas, A. (1995). Crop uptake and extractability of cadmium in soil naturally high in metal at different pH levels. *Commun. Soil Sci*. Plant *Anal*. 26: 2123-42.

Simmon, R.W., Noble, A.D., Pongsakul, P., Sukreeyapongse, O., & Chinabut, N. (2008). Analysis of field-moist Cd contaminated paddy soils during rice grain fill allows reliable prediction of grain Cd levels. *Plant soil.* 302:125-137.

Soon, Y. K. (1981). Solubility and sorption of cadmium in soils amended with sewage-sludge. *J. Soil Sci.* 32, 85–95.

Steveninck, van R. F. M., Barbare, A., and Fernando, D. R., and van Steveninck, M. E. (1994). The binding of zinc, but not cadmium, by phytic acid in roots of crop plants, *Plant Soil*, 167, 157.

Tan, K.H. (1995). *Soil Sampling, Preparation, and Analysis*. Marcel Dekker, New York.

The State of Queensland, Department of Agriculture, Fisheries and Forestry, 2014. Fact sheet, pest plant.

Thomas, G.W. (1982). Exchangeable cation. In: A.L. Page, R.H. Miller, & D.R. Keeney, (Eds.), *Methods of Soil Analysis. Part 2*. Chemical and Microbiological Properties, 2nd edition (pp.159-166). Agronomy Monograph 9, Madison, WI, USA: American Society of Agronomy.

Tissier, A., Campbell, P.G. C., & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem*. 51, 844-851.

Tron, I. (1996). Impacts of mining on the environment; some local, regional and global issues. *Applied geochemistry*. 11: 355-361.

Tsadilas, C. D., Karaivazoglou, N. A., Tsotsolis, N. C., Stamatiadis, S., & Samaras, V. (2005). Cadmium uptake by tobacco as affected by liming, N form, and year of cultivation. *Environ. Pollut*. 134(2), 239-246.

Turgut, C., Pepe, M.K., & Cutrigh, T.J. (2004). The effect of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using *Helianthus annuus*. *Environ. Pollut*. 131,147-154.

US.EPA. (1989). *Innovative Technology: Soil Washing*, OSWER Directive 9200.5-250FS.

US.EPA. (1990). *Soil Washing Treatment*, Engineering Bulletin, EPA, OERR, Washington, DC, EPA/540/2-90/017. Available from NTIS, Springfield, VA, Order No. PB91-228056. EPA 542-B-97-007

US.EPA. (1997). Innovative Site Remediation Design and Application, Volume 4: Stabilization/Solidification

US.EPA. (1997). Status of in situ phytoremediation technology. In *Recent developments for in situ treatment of metal contaminated soils*. March. EPA-542-R-97-004. p. 31-42.

US.EPA. (2000). *Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents:* Fundamentals and Field Applications EPA 542-R-00-008.

US.EPA. (2000). *Introduction to Phytoremediation*, EPA/600/R-99/107, National Risk Management Research Laboratory Office of Research and Development, USEPA, Washington, DC.

US.EPA. (2000). Solidification/Stabilization Use at Superfund Sites. EPA 542-R-00-010.

US.EPA. (2001). *Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites*, EPA/600/R-99/107, Office of Solid Waste and Emergency Response, Technology Innovation Office. USEPA, Washington, DC.

US.EPA 2004. In-Situ Groundwater Bioremediation. Chapter 10 in How to Evaluate Alternative Cleanup Technologies for Underground Storage Tank Sites: A Guide for Corrective Action Plan Reviewers. EPA 510-R-04-002.

Vankar, P.S., Shankar, R., & Wijayapala, S., 2009. Dyeing Cotton, Silk and Wool Yarn with Extract of Garnicia Mangostana Pericarp. *J. TATM*. 6(1):1-10.

Vassil A. D., Kapulnik Y., Raskin I., & Salt D. E., 1998. The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiol.* 117:447-453.

Violantel, A., Cozzolinol, V., Perelomov, L., Caparale, A.G., & Pignal, M. (2010). Mobility and bioavailability of HM and metalloids in the soil. *J. Soil. Sci. Plant Nutr.* 10(3), 268-292.

Vyslouzilova, M., Tlustos, P., Szakova, J., & Pavlikova, D., 2003. As, Cd, Pb and Zn uptake by *Salix* spp. Ckibes grown in soils enriched by high loads of these elements. *Plant Soil Environ.* 49: 191-196.

Wallace, A., Mueller, R. T., and Wood, R. A., 1980. Arsenic phytotoxicity and interactions in bush bean plants grown in solution culture, *J. Plant Nutr.* 2:111.

Wang, A.S., Angle, J.S., Chaney, R.L., Delorme, A.T., and Reeves, R.D., 2006. Soil pH effects on uptake of Cd and Zn by *Thlaspi caerulescens*. *Plant and soil*. 281: 325-337.

Wagner, G.J., 1993. Accumulation of cadmium in crop plants and its consequences to human health. *Advances in Agronomy*. 51: 173-212.

Weast, R.C., 1986. Handbook of Chemistry and Physics, 67th ed. Boca Raton, FL, CRC Press.

Wei, S., Zhou, Q., Saha U.K, Xiao, H., Hu, Y., Ren, L., & Ping, G. (2009a). Identification of a Cd accumulator *Conyza Canadensis*. *J. of Hazard. Mater*, 163(1):32-5.

Wei, S., Zhou, Q., Srivastava, M., Xiao, H., Yang, C., & Zhnag, Q. (2009b). Kalimeris integrifolia Turcz.ex.DC: An accumulator of Cd. *J. Hazard. Mater*, 162(2-3):1571-3.

Wolterbeek, H.T., Meer, A. Van der, & Bruin, M.D. (1988). The uptake and distribution of cadmium in tomato plants as affected by ethylenediamine tetraacetic acid and 2, 4-dini-tropenol. *Environ. Pollut*. 55, 301-315.

Wu, F., Yang, W., Zhang, J., and Zhou, L. (2010). Cadmium accumulation and growth responses of a poplar (*Populus deltoids x Polulus nigra)* in cadmium contaminated purple soil and alluvial soil. *J. Hazard. Mater*, 177: 268-273.

Xiong, Z.T. (1998). Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumulator *Brassica pekinensis* Rupr. *Bull. Environ. Contam. Toxicol*, 60: 285-291.

Xue, Q., and Harrison, H. C. (1991). Effect of soil zinc, pH, and cultivar on cadmium uptake in leaf lettuce (*Lactuca sativa* L. crispa). *Commun. Soil Sci. Plant Anal,* 22: 975-991.

Yanai, J., Zhao, F. J., Mcgrath, S. P., & Kosaki, T. (2006). Effect of soil characteristics on Cd uptake by the hyperaccumulator Thlaspi caerulescens. *Environ. Pollut*, 139: 167- 175.

Yobouet, Y. A., Adouby, K., Trokourey, A., & Yao, B. (2010). Cadmium, Copper, Lead and Zinc speciation in contaminated soils. *International Journal of Engineering Science and Technology*, 2 (5): 802-812.

Zarcinas, B.A., Cartwright, B., & Spouncer, L.R. (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun. Soil. Sci. Plant. Anal,* 18: 131-146.

Zarcinas, B.A., Pongsakul, P., McLaughlin, M.J., & Cozens, G.D. (2004). Heavy metals in soils and crops in Southeast Asia.2., Thailand. *Environ. Geochemistry and Health,* 26: 359-371.

Zaurov, D.E., Perdomo, P., & Raskin, I. (1999). Optimizing soil fertility and pH to maximize cadmium removed by Indian mustard from contaminated soils. *J. Plant Nutr*, 22(6):977-986.

Zhao, F.J., Lombi, E., Breedon, T., & McGrath, S.P. (2000). Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri*. *Plant Cell Environ*, 23:507-514.

Zhao, F.J., Lombi, E., & McGrath, S.P. (2003). Assessing the potential of zinc and cadmium phytoremediation with the hyperaccumulator Thlaspi caerulescens. *Plant soil,* 249: 37-43.

The Columbia Encyclopedia, 6th ed. 2013. *Encyclopedia.com.* (April 8, 2014).http://www.encyclopedia.com/doc/1E1-marigold.html"marigold."

The Great Soviet Encyclopedia, 3rd ed. (1970-1979). (April 8, 2014).http://encyclopedia2.thefreedictionary.com/French+marigold

USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network - (GRIN)* [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland.Cosmos-URL:http://www.ars-grin.gov/cgi-bin/npgs/html/genus.pl?2986 (8 April 2014)

APPENDICES

APPENDIX A : Method of analysis

Appendix A-1

Analysis method for Cation Exchange Capacity
CEC at pH 7 with Ammonium Acetate
(Chapman, 1965; Rhoades, 1982)

Cation Exchange Capacity (CEC) can be determined using different methods. However, in all methods the adsorbed cations must be replaced by a single exchanger cation such as Ba^{2+}, NH^{4+}or Sr^{2+} and then the CEC is calculated either from the amount of the exchanger cations used for replacement or from the amounts of each of the replaced cations originally held on the soil exchange sites (usually Ca^{2+}, Al^{3+}, Mg^{2+}, K^{+} and Na^{+}). Most laboratories determine CEC in a buffered solution using one M NH_4Cl at pH 8.5 in 60 per cent ethanol, or ammonium as the exchanger cation at pH 7.0, or barium as the exchanger cation at pH 8.2. If the soil pH is less than the pH of the buffered solution then the pH-dependent exchange sites that would become negatively charged at pH 7.0 or 8.2 will be measured too.

The CEC of a given soil is determined by the relative amount of different colloids in that soil and by the CEC of each of these colloids. The major soil colloids are clay and organic matter. Silt may contribute a little to soil CEC, while the contribution of sand is very small and in most cases negligible. In sandy soils most of the CEC comes from the clay and organic fractions of the soil. The method for measuring CEC is present as following:

Equipments:
1. Buchner funnel filtration apparatus.
2. Balance.
3. 250 and 500 mL Erlenmeyer flasks.
4. Apparatus for ammonium determination (steam distillation or colorimetric).

Reagents:
1. 1 *M* ammonium acetate (NH_4OAc) saturating solution: Dilute, in a chemical hood, 57 mLs glacial acetic acid (99.5%) with ~800 mL of distilled H_2O in a 1 L volumetric flask. Add 68 mL of concentrated NH4OH, mix and cool. Adjust pH to 7.0 with NH_4OH if needed and dilute to 1 L.
2. 1 *M* KCl replacing solution: Completely dissolve 74.5 g KCl in distilled water and dilute to a final volume of 1 L.
3. Ethanol, 95%.

Procedure:
1. Add 25.0 g of soil to a 500 mL Erlenmeyer flask.
2. Add 125 mL of the 1 *M* NH_4OAc, shake thoroughly and allow to stand 16 hours (or overnight).
3. Fit a 5.5 cm Buchner funnel with retentive filter paper, moisten the paper, apply light suction, and transfer the soil. If the filtrate is not clear, re-filter through the soil.

4. Gently wash the soil four times with 25 mL additions of the NH_4OAc, allowing each addition to filter through but not allowing the soil to crack or dry. Apply suction only as needed to ensure slow filtering. Discard the leachate, unless exchangeable cations are to be determined.

Note: Exchangeable cations can be determined on the leachate after diluting it to 250 mL.

5. Wash the soil with eight separate additions of 95% ethanol to remove excess saturating solution. Only add enough to cover the soil surface, and allow each addition to filter through before adding more. Discard the leachate and clean the receiving flask.

6. Extract the adsorbed NH4 by leaching the soil with eight separate 25 mL additions of 1 *M* KCl, leaching slowly and completely as above. Discard the soil and transfer the leachate to a 250 mL volumetric. Dilute to volume with additional KCl.

7. Determine the concentration of NH_4-N in the KCl extract by distillation or colorimetry. Also determine NH_4-N in the original KCl extracting solution (blank) to adjust for possible NH_4-N contamination in this reagent.

Calculations:

Where NH_4-N is reported in mg N/L:

CEC (cmolc/kg) = (NH_4-N in extract - NH_4-N in blank) / 14

Where NH_4-N is reported in mg NH4/L:

CEC (cmolc/kg) = (NH_4-N in extract - NH_4-N in blank) / 18

Appendix A-2

Particle Size Analysis
(Hydrometer Method)
(Bouyoucos, 1962; Tan, 1995: Hillel, 1998)

1. Application

The percentage of sand, silt and clay in the inorganic fraction of soil is measured in this procedure. The method is based on Stoke's law governing the rate of sedimentation of particles suspended in water.

2. Summary of Methods

The sample is treated with sodium hexametaphosphate to complex Ca^{2+}, Al^{3+}, Fe^{3+}, and other cations that bind clay and silt particles into aggregates. Organic matter is suspended in this solution. The density of the soil suspension is determined with a hydrometer calibrated to read in grams of solids per liter after the sand settles out and again after the silt settles. Corrections are made for the density and temperature of the dispersing solution.

3. Interferences

The principal source of error in this procedure is the incomplete dispersion of soil clays. These clays are cemented by various chemical agents and organic matter into aggregates of larger size. Failure to effect complete dispersion results in low values for clay and high values for silt and sand. The rate of sedimentation also is affected by temperature and the density of the dispersing solution.

4. Apparatus and Materials

4.1 Glass cylinders, 1000-ml capacity
4.2 Thermometer, Fahrenheit
4.3 Hydrometer, Bouyoucos (Fisherbrand Model # 14-331-5c)
4.4 Electric mixer with dispersing cup
4.5 Plunger
4.6 Balance sensitive to±0.01g

5. Reagents

Dispersing solution, 5%: Dissolve 50 g of sodium hexametaphosphate, $Na_6 (PO_3)_6$ in deionized water and dilute to 1 liter.

6. Methods

6.1 Mix 100 ml of the 5% dispersing solution and 880 ml of deionized water in a 1000 ml cylinder. This mixture is the blank. (Note: 100 ml + 880 ml = 980 ml. This blank is not diluted to 1000 ml; the other 20 ml is the volume occupied by 50 g of soil.).
6.2 Weigh 25-50 g of soil and transfer r to a dispersing cup. Record weight to to±0.01g
6.3 Add 100-ml of 5% dispersing solution.

6.4 Attach dispersing cup to mixer and mix the sample for 30 – 60 sec.

6.5 Transfer the suspension quantitatively from the dispersing cup to a 1000 ml cylinder.
6.6 Fill to the 1000- ml mark with deionized water equilibrated to room temperature, or allow to stand overnight to equilibrate.
6.7 At the beginning of each set, record the temperature, and the hydrometer reading of the blank, using the procedure described below.
6.8 To determine the density insert plunger into suspension, and carefully mix for 30 sec. until a uniform suspension is obtained. Remove plunger (begin 40 second timer) and gently insert the hydrometer into the suspension.
6.9 Record the hydrometer reading at 40 sec. This is the amount of silt plus clay suspended. The sand has settled to the bottom of the cylinder by this time. (Repeat 7.8 – 7.9 for each sample)
6.10 Record the hydrometer reading again after 6 hours, 52 minutes. This is the amount of clay in suspension. The silt has settled to the bottom of the cylinder by this time.

7. Calculations
7.1 Temperature and density corrections:
- add 0.2 unit to the readings of the samples for every 1°F above 67°F, and subtract 0.2 unit for every 1 °F below 67°F.
- subtract the density of the blank at each reading, from the corresponding density readings for the samples.

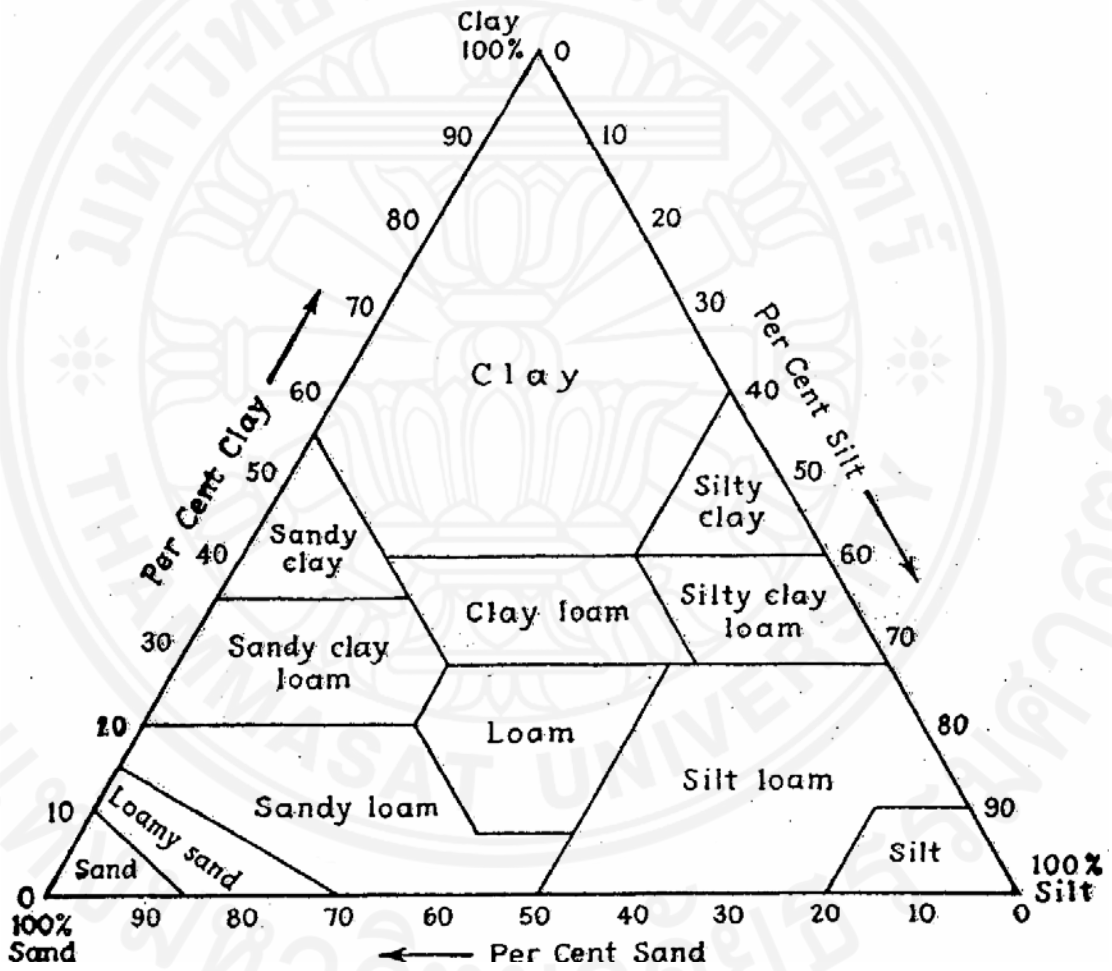
7.2 Percent clay:
% clay = corrected hydrometer reading at 6 hrs, 52 min. x 100/ wt. of sample

7.3 Percent silt:
% silt = corrected hydrometer reading at 40 sec. x 100/ wt. of sample - % clay
7.4 Percent sand:
% sand = 100% - % silt - % clay

8. Quality Control
Standard soil - a standard soil of known particle size content is analyzed with each batch of samples to check for instrument calibration and procedural accuracy.

9. Reporting
Results are reported as percentages of the mineral fraction, % sand, % silt, and % clay.
Results are reported as percentages of the mineral fraction, % sand, % silt, and % clay.
Soil texture is based on the USDA textural triangle. (see chart below)

Textural Triangle



Appendix A-3

Walkley-Black Method
(Walkley and Black, 1934)

Equipments:
1. 500-mL Erlenmeyer flasks.
2. 10-mL pipette.
3. 10-and 20-mL dispensers.
4. 50-mL burette.
5. Analytical balance.
6. Magnetic stirrer.
7. Incandescent lamp.

Reagents:
1. H_3PO_4, 85%.
2. H_2SO_4, concentrated (96%).
3. NaF, solid.
4. Standard 0.167*M* $K_2Cr_2O_7$: Dissolve 49.04 g of dried (105 °C) $K_2Cr_2O_7$ in water and dilute to 1 L.
5. 0.5*M* Fe^{2+} solution: Dissolve 196.1 g of $Fe(NH_4)_2(SO_4)$•6H2O in 800 mL of water containing 20 mL of concentrated H_2SO_4 and dilute to 1 L. The Fe^{2+} in this solution oxidizes slowly on exposure to air so it must be standardized against the dichromate daily.
6. Ferroin indicator: Slowly dissolve 3.71 g of o-phenanthroline and 1.74 g of $FeSO_4$•$7H_2O$ in 250 mL of water.

Procedure:
1. Weigh out 0.10 to 2.00 g dried soil (ground to <60 mesh) and transfer to a 500-mL Erlenmeyer flask. The sample should contain 10 to 25 mg of organic C (17 to 43 mg organic matter). For a 1 g soil sample, this would be 1.2 to 4.3% organic matter. Use up to 2.0 g of sample for light colored soils and 0.1 g for organic soils.

2. Add 10 mL of 0.167 *M* $K_2Cr_2O_7$ by means of a pipette.

3. Add 20 mL of concentrated H_2SO_4 by means of dispenser and swirl gently to mix. Avoid excessive swirling that would result in organic particles adhering to the sides of the flask out of the solution.

4. Allow to stand 30 minutes. The flasks should be placed on an insulation pad during this time to avoid rapid heat loss.

5. Dilute the suspension with about 200 mL of water to provide a clearer suspension for viewing the endpoint.

6. Add 10 mL of 85% H_3PO_4, using a suitable dispenser, and 0.2 g of NaF. The H_3PO_4 and NaF are added to complex Fe^{3+} which would interfere with the titration endpoint.

7. Add 10 drops of ferroin indicator. The indicator should be added just prior to titration to avoid deactivation by adsorption onto clay surfaces.

8. Titrate with 0.5 *M* Fe^{2+} to a burgundy endpoint. The color of the solution at the beginning is yellow-orange to dark green, depending on the amount of unreacted $Cr_2O_7^{2-}$ remaining, which shifts to a turbid gray before the endpoint and then changes sharply to a wine red at the endpoint. Use of a magnetic stirrer with an incandescent light makes the endpoint easier to see in the turbid system (fluorescent lighting gives a different endpoint color). Alternatively use a Pt electrode to determine the endpoint after step 5 above. This will eliminate uncertainty in determining the endpoint by color change. If less than 5 mL of Fe2+ solution was required to backtitrate the excess $Cr_2O_7^{2-}$ there was insufficient $Cr_2O_7^{2-}$ present, and the analysis should be repeated either by using a smaller sample size or doubling the amount of $K_2Cr_2O_7$ and H_2SO_4

9. Run a reagent blank using the above procedure without soil. The blank is used to standardize the Fe^{2+} solution daily.

10. Calculate % C and % organic matter:

a. *% Easily Oxidizable Organic C*

$$\% C = [(B-S) \times M \text{ of } Fe2+ \times 12 \times 100] / g \text{ of soil} \times 4000$$

where:

B = mL of Fe^{2+} solution used to titrate blank
S = mL of Fe^{2+} solution used to titrate sample
12/4000 = milliequivalent weight of C in g.

To convert easily oxidizable organic C to total C, divide by 0.77 (or multiply by 1.30) or other experimentally determined correction factor. To convert total organic C to organic matter use the following equation:

b. % *Organic Matter*

$$\% OM = \% \text{ (total C} \times 1.72)/0.58$$

Appendix A-4

Soil pH Measurement

(Mclean, 1982; Kidder, G., et al., 1988;
Rayment and Higginson, 1992; Kalra, Y.P. 1995)

Application and Principle

Soil pH is an important measurement to assess potential availability of beneficial nutrients and toxic elements to plants. Soil pH is determined with a H^+ ion-selective glass electrode and is a measurement that can be performed for all soils.

A common method of measuring soil pH is performed by placing a glass electrode in a mixture of soil and deionized water. Most plants grow optimally with a soil-water pH from 5.7 to 7. Various modifications exist for determining soil pH. The most common ratio used for soil-water pH is 1:1 soil:water. Some laboratories measure pH in a 1:2 ratio of soil to deionized to improve the fluidity of the slurry; particularly for soils with high organic matter and clay concentrations that can absorb a significant volume of water. Electrolyte solutions, such as 0.01 M $CaCl_2$ or 1 M KCl, can be added to soil rather than deionized water with the resultant pH referred to as salt pH. Use of electrolyte solutions avoids variable soil-water pH due to varying background salt levels in different soils and improves electrical conductivity in the electrical circuit for pH measurement (Miller and Kissel, 2010). Soil pH measurement in deionized water or 0.01 M $CaCl_2$ in 1:1 and 1:2 soil:solution ratios are official methods adopted by the Association of Official Analytical Chemists (Kalra, 1995). Automated instruments have begun to be popular for measuring soil pH. These instruments save labor costs and improve accuracy of measurements. This chapter presents the common measurement of soil pH in a 1:1 mixture of soil to deionized water along with modifications with electrolyte solutions, a lower soil:solution ratio, and automated instruments.

Equipment and Apparatus

1. Soil scoop and leveling rod
2. pH cups
3. Holding rack for pH cups
4. Dispenser for deionized water or electrolyte solution added to soil
5. Manual pH meter or automated pH analyzer

6. Glass pH electrode with an internal reference element or a separate reference electrode

7. Analytical balance and glassware for making electrolyte solutions if they are added to soil

Reagents

1. Deionized water

2. Standardization buffers of pH 7.00 and 4.00

3. Below are quantities of $CaCl_2 \cdot 2H_2O$ and KCl to dissolve in water for preparing 0.01 M $CaCl_2$ or 1 M KCl if they are used for measuring salt pH.

    a. 0.01 M $CaCl_2$: Dissolve 1.47 g of $CaCl_2 \cdot 2H_2O$ in DI-H_2O and bring volume to
    1 L.
    b. 1 M KCl: Dissolve 74.6 g of KCl in DI-H_2O and bring volume to 1 L.

Procedure

1. Measure a volume of soil from 10 to 20 mL, or mass of soil from 10 to 20 g, and add it to a sample cup. Volume is measured with a soil sampling scoop. Mass can be measured with a scale or estimated from a volume measurement accounting for the density of soil.

2. The next step considers different variations on the type of solution added to soil.

    a. 1:1 soil:water pH: Dispense a particular volume of water to soil that is equal to the volume or mass of soil.
    b. 1:1 soil: 0.01 M $CaCl_2$ pH: Dispense a particular volume of 0.01 M $CaCl_2$ to soil that is equal to the volume or mass of soil.
    c. 1:1 soil:1 M KCl pH: Dispense a particular volume of 1 M KCl to soil that is equal to the volume or mass of soil.
    d. 1:2 soil:water pH: Dispense a particular volume of water that is twice the volume or mass of soil.

3. Stir the soil and solution vigorously and allow slurry to set from 15 minutes to 1 hour.

4. Ensure room temperature is between 20 and 25°C before proceeding with pH measurement.

5. Calibrate pH meter and electrode using pH 4 and 7 buffers.

6. Place electrode in the soil slurry to measure pH. Measurement may be taken with or without continuous stirring. If measurement is made without continuous stirring, stir the sample with a stir bar before placing electrode in the sample. Allow adequate time for pH to reach a stable reading. Stability can be ascertained by pH meter settings for manual measurements or software settings for automated instruments. Software settings used by 4 Southeastern USA laboratories for automated Lab Fit instruments range from 5 to 30 second delay time before pH measurements begin, 4 to 10 similar pH measurements obtained (one pH measurement per second) before stability has been reached, similar pH measurements ascertained with pH differences equal to or less than 0.01 to 0.03 pH units. This range in software settings results in a maximum time for pH measurement for a single sample ranging from 20 to 90 seconds.

Calculations

1. If measuring soil pH in 0.01 M $CaCl_2$ or 1 M KCl, it is convenient to report an equivalent soil-water pH since this is a more familiar pH value in relation to plant growth. The pH in 0.01 M $CaCl_2$ is approximately 0.6 units less than soil-water pH. The pH in 1 M KCl is approximately 0.9 unit less than soil-water pH. A comparison of 1186 soils in Georgia provided an equation to calculate an equivalent soil-water pH from 0.01 M $CaCl_2$ soil pH as shown below.

   1:1 soil:water pH = 0.92 x 0.01 M $CaCl_2$ soil pH + 1.10 $r^2 = 0.91$

   A comparison of 240 soils in Kentucky provided an equation to calculate soil-water pH from 1 M KCl soil pH as shown below.

   1:1 soil:water pH = 0.91 x 1 M KCl soil pH + 1.34 $r^2 = 0.98$

2. The pH in a 1:2 soil:water mixture is only about 0.1 pH units greater than pH in a 1:1 soil:water mixture. A comparison of median values for 1:1 soil:water versus 1:2 soil:water from 134 samples in the North American Proficiency Testing program resulted in the following relationship.

   1:1 soil-water pH = 0.99 x 1:2 soil:water pH – 0.04 $r^2 = 0.996$

3. To determine if lime is needed, the measured pH is evaluated to determine if it is below some threshold pH for the specific crop to be grown. Lime is then recommended to reach a target pH most often between 5.7 to 6.5 for 1:1 soil:water pH or 1:2 soil:water pH. If using pH in 0.01 M $CaCl_2$ or 1 M KCl, correct the threshold and target pH for measurements in the electrolyte solution to reflect the lower pH compared to pH measured in water (see Calculations, 1)

4. To determine how much lime is needed to reach a target pH, refer to Chapter X with presentation of various methods to quantify the lime requirement (LR) using buffers or $Ca(OH)_2$.

Analytical Performance

Range and Sensitivity

1. Soil-water pH is most often within a range from 4 to 8.

Precision and Accuracy

1. pH measurements can be made to the nearest 0.1 or 0.01 pH unit. There is no need to measure pH with more than 2 decimal places since this level of accuracy is not achievable or required. If measurements are made to the nearest 0.01 pH unit, pH can be rounded to 0.1 pH units before reporting to clients.

2. Typical measurements of interlaboratory precision for pH in 1:1 soil:water, 1:1 soil:0.01 M $CaCl_2$, and 1:1 soil:1 M KCl are shown below. Each measurement was taken on different days.

Method	Number of measurements	Mean	Standard deviation
1:1 soil:water	10	5.73	0.09
1:1 soil:0.01 M $CaCl_2$	yet to be added	yet to be added	yet to be added
1:1 soil:1 M KCl	yet to be added	yet to be added	yet to be added

Interferences

1. Differences in pH will occur with electrode placed in a soil-slurry or in the supernatant after the soil has settled. The differences are more pronounced with soil pH in water compared to electrolyte solutions. To avoid this variability in pH, it is important to stir the soil slurry right before

measurement. With sandy soils, the settling time of soil particles is rapid and continuous stirring during measurement is recommended.

2. Glass electrodes have a short life span when measuring pH of sandy soils. The sand particles are abrasive to the glass resulting in electrode breakage or malfunction. When electrodes fail to measure pH of calibration buffers or quality control samples show more error than expected, replace electrodes.

Interpretation

1. There is a wide variation of pH values for optimum plant growth. Most agronomic crops require soil-water pH values between 5.7 and 7. Some plants, such as blueberries and azaleas, require acidic soil conditions with soil-water pH below 5. A soil pH measurement just provides a measure of whether lime is needed or not. If soil pH is below a threshold pH value, some method of quantifying residual acidity is required to determine how much lime is needed to reach the target pH.

Effects of Storage

1. Air-dried soils may be stored several months without affecting the soil pH measurement provided they are stored in an ammonia free environment or in a tightly sealed container.

2. The electrodes used for pH measurement should be maintained and stored according to the manufacturer's instructions. Any automated instrument used for pH measurement should be maintained according to manufacturer's directions.

Safety and disposal

1. The chemicals used in this procedure pose no safety risk and therefore can be stored and disposed of according to routine laboratory procedures.

Appendix B: Data of soil and plant analysis

Appendix B-1

Total Cd uptake and percentage Cd removal by marigold at different soil pH

Soil pH	Desired Cd (mg/kg)	Initial Cd applied in pot (mg/pot)	Final Cd in pot (mg/pot)	Total Cd uptake (mg/pot)	% Cd removal by marigold
5.0	50	343.13	329.55	13.58	3.96
5.0	50	349.29	336.94	12.34	3.54
5.0	50	330.50	309.44	21.05	6.38
6.3	50	339.10	318.87	20.23	5.97
6.3	50	362.66	353.54	9.12	2.52
6.3	50	390.73	382.52	8.20	2.10
7.0	50	408.71	396.60	12.11	2.97
7.0	50	410.93	394.36	16.57	4.04
7.0	50	428.09	416.69	11.40	2.67
7.5	50	433.19	425.69	7.50	1.73
7.5	50	411.76	399.32	12.44	3.02
7.5	50	405.78	395.13	10.65	2.63
5.0	100	747.36	725.29	22.07	2.96
5.0	100	774.15	757.51	16.64	2.15
5.0	100	755.61	733.19	22.42	2.97
6.3	100	803.43	777.62	25.80	3.21
6.3	100	742.43	727.08	15.35	2.07
6.3	100	806.38	789.53	16.86	2.09
7.0	100	787.06	759.65	27.40	3.49
7.0	100	822.76	800.88	21.87	2.66
7.0	100	809.52	793.63	15.89	1.96
7.5	100	768.38	751.03	17.36	2.26
7.5	100	800.00	788.02	11.98	1.50
7.5	100	796.66	783.38	13.28	1.67

Note: 7 kg soil/pot was used in this experiment

Appendix B-2

Total Cd uptake and percentage Cd removal by marigold at different Zn applied to soil under various Cd treatments

Cd: Zn applied to soil (mg/kg)	Desired Cd (mg/kg)	Initial Cd applied in pot (mg/pot)	Final Cd in pot (mg/pot)	Total Cd uptake (mg/pot)	% Cd removal by marigold
50:0	50	487.69	472.46	15.23	3.13
50:0	50	439.04	430.69	8.35	1.90
50:0	50	440.79	430.55	10.24	2.32
50:500	50	444.99	423.04	21.95	4.94
50:500	50	444.22	419.87	24.35	5.49
50:500	50	451.29	430.41	20.88	4.63
50:1500	50	423.43	415.85	7.58	1.79
50:1500	50	446.39	441.45	4.94	1.11
50:1500	50	443.73	438.07	5.66	1.28
50:2500	50	457.38	453.82	3.56	0.78
50:2500	50	443.38	439.10	4.28	0.97
50:2500	50	456.40	452.33	4.07	0.89
100:0	100	733.95	709.67	24.28	3.31
100:0	100	745.99	712.98	33.01	4.43
100:0	100	714.42	684.24	30.18	4.23
100:1000	100	725.90	716.84	9.06	1.25
100:1000	100	741.44	731.58	9.86	1.33
100:1000	100	717.22	710.04	7.18	1.00
100:3000	100	738.15	735.04	3.11	0.42
100:3000	100	722.12	717.29	4.83	0.67
100:3000	100	728.00	723.07	4.93	0.68

Note: 7 kg soil/pot was used in this experiment

Appendix B-3

Total Cd uptake and percentage Cd removal by marigold at different EDTA applied to soil under various Cd treatments

Cd:EDTA treatment (mg/kg)	Desired Cd (mg/kg)	Initial Cd applied in pot (mg/pot)	Final Cd in pot (mg/pot)	Total Cd uptake (mg/pot)	% Cd removal By marigold
50:0	50	260.87	240.91	19.96	7.67
50:0	50	276.53	258.77	17.77	6.44
50:0	50	251.21	234.58	16.63	6.63
SD		12.78	12.54	1.69	0.66
50:25	50	269.40	256.35	13.06	4.86
50:25	50	290.51	277.76	12.75	4.40
50:25	50	267.31	250.87	16.44	6.16
SD		12.83	14.26	2.05	0.92
50:50	50	267.35	254.16	13.19	4.95
50:50	50	261.80	247.06	14.74	5.64
50:50	50	263.94	249.42	14.52	5.51
SD		2.80	3.62	0.84	0.37
100:0	100	513.03	496.33	16.70	3.26
100:0	100	498.84	479.85	18.99	3.82
100:0	100	529.64	502.68	26.96	5.10
SD		15.42	11.79	5.39	0.94
100:50	100	550.22	529.89	20.33	3.70
100:50	100	487.36	472.16	15.21	3.13
100:50	100	505.53	470.18	35.35	7.01
SD		32.35	33.92	10.47	0.95
100:100	100	522.11	508.75	13.37	2.57
100:100	100	489.65	474.27	15.38	3.15
100:100	100	505.86	487.45	18.41	3.65
SD	100	16.23	17.40	2.54	0.54

Note: 6 kg soil/pot was used in this experiment

Appendix B-4

Total Cd uptake and percentage Cd removal by Guinea grass at different soil pH

Soil pH	Desired Cd (mg/kg)	Initial Cd applied in pot (mg/pot)	Final Cd in pot (mg/pot)	Total Cd uptake (mg/pot)	% Cd removal by Guinea grass
5.0	50	386.11	367.73	18.38	4.76
5.0	50	393.86	371.10	22.76	5.79
5.0	50	383.60	366.90	16.69	4.36
6.3	50	405.84	396.34	9.51	2.34
6.3	50	375.72	370.49	5.23	1.39
6.3	50	378.47	373.18	5.29	1.40
7.0	50	371.13	366.78	4.35	1.17
7.0	50	406.82	402.28	4.54	1.12
7.0	50	388.26	383.53	4.74	1.22
7.5	50	370.91	367.25	3.67	0.99
7.5	50	387.65	383.21	4.44	1.15
7.5	50	376.70	371.23	5.46	1.45
5.0	100	696.94	664.12	32.82	4.71
5.0	100	636.55	602.11	34.45	5.42
5.0	100	642.28	601.38	40.91	6.38
6.3	100	640.72	624.01	16.71	2.61
6.3	100	630.52	616.93	13.59	2.16
6.3	100	629.00	616.95	12.05	1.92
7.0	100	637.08	628.98	8.10	1.27
7.0	100	626.04	618.49	7.55	1.21
7.0	100	651.87	640.65	11.22	1.72
7.5	100	677.64	668.88	8.77	1.30
7.5	100	674.69	669.52	5.17	0.77
7.5	100	653.21	640.27	12.95	1.98
5.0	200	1160.72	1136.11	24.61	2.12
5.0	200	1188.05	1169.75	18.29	1.54
5.0	200	1218.32	1201.66	16.66	1.37
6.3	200	1162.54	1144.27	18.27	1.57
6.3	200	1152.61	1131.21	21.40	1.86
6.3	200	1207.89	1194.53	13.36	1.11
7.0	200	1211.84	1191.13	20.72	1.71
7.0	200	1195.85	1182.96	12.89	1.08
7.0	200	1179.53	1162.46	17.07	1.45
7.5	200	1083.77	1067.08	16.69	1.54
7.5	200	1148.93	1140.00	8.93	0.78
7.5	200	1195.18	1180.61	14.57	1.22

Note: 7 kg soil/pot was used in this experiment

Appendix B-5

Total Cd uptake and percentage Cd removal by Guinea grass at different Zn applied to soil under various Cd treatments

Cd: Zn applied to soil (mg/kg)	Desired Cd (mg/kg)	Initial Cd applied in pot (mg/pot)	Final Cd in pot (mg/pot)	Total Cd uptake (mg/pot)	% Cd removal by Guinea grass
50:0	50	368.60	365.60	3.00	0.82
50:0	50	361.39	358.24	3.15	0.87
50:0	50	376.94	373.61	3.33	0.88
50:500	50	399.37	395.48	3.89	0.98
50:500	50	389.65	385.55	4.10	1.05
50:500	50	395.89	390.66	5.23	1.32
50:1500	50	384.58	381.42	3.16	0.82
50:1500	50	368.03	361.44	6.60	1.79
50:1500	50	331.10	324.74	6.36	1.92
50:2500	50	376.18	374.52	1.66	0.44
50:2500	50	369.72	368.15	1.57	0.43
50:2500	50	402.01	400.78	1.23	0.31
100:0	100	710.40	704.15	6.25	0.88
100:0	100	664.29	658.94	5.36	0.81
100:0	100	695.11	693.99	1.12	0.16
100:1000	100	706.06	697.50	8.56	1.21
100:1000	100	685.52	678.82	6.70	0.98
100:1000	100	710.09	698.25	11.84	1.67
100:3000	100	691.09	687.56	3.53	0.51
100:3000	100	701.83	699.41	2.42	0.35
100:3000	100	698.97	694.25	4.72	0.68
200:0	200	1265.91	1259.77	6.15	0.49
200:0	200	1243.35	1235.65	7.70	0.62
200:0	200	1187.63	1183.26	4.37	0.37
200:2000	200	1251.72	1247.93	3.78	0.30
200:2000	200	1208.36	1205.68	2.68	0.22
200:2000	200	1254.30	1251.20	3.11	0.25

Note: 7 kg soil/pot was used in this experiment

Appendix B-6

Total Cd uptake and percentage Cd removal by Guinea grass at different EDTA applied to soil under various Cd treatments

Cd:EDTA treatment (mg/kg)	Desired Cd (mg/kg)	Initial Cd applied in pot (mg/pot)	Final Cd in pot (mg/pot)	Total Cd uptake (mg/pot)	% Cd removal by Guinea grass
50:0	50	274.14	252.46	21.68	7.92
50:0	50	290.34	272.09	18.25	6.30
50:0	50	278.94	261.53	17.41	6.25
SD		8.32	9.82	2.26	0.95
50:25	50	298.62	278.93	19.69	6.61
50:25	50	271.02	246.71	24.31	8.99
50:25	50	281.28	258.71	23.11	8.23
SD		13.96	16.33	2.40	1.22
50:50	50	299.04	277.94	21.10	7.07
50:50	50	284.52	264.80	19.72	6.94
50:50	50	252.6	234.87	17.73	7.03
SD		23.76	22.07	1.69	0.06
100:0	100	582.66	565.89	16.77	2.88
100:0	100	536.58	516.19	20.39	3.81
100:0	100	546.54	527.99	18.55	3.40
SD		24.25	25.96	1.81	0.46
100:50	100	572.04	546.10	25.94	4.54
100:50	100	553.56	536.65	16.91	3.06
100:50	100	535.68	512.05	23.63	4.42
SD		18.18	17.58	4.69	0.82
100100	100	587.88	566.01	21.87	3.73
100100	200	544.08	523.08	21.00	3.87
100100	200	538.08	515.75	22.33	4.16
SD		27.19	27.15	0.68	0.22
200:0	200	1037.64	1023.69	13.95	1.35
200:0	200	1074.78	1056.26	18.52	1.73
200:0	200	1033.2	1016.66	16.54	1.60
SD		22.83	21.13	2.29	0.19
200:100	200	1121.28	1108.11	13.17	1.18
200:100	200	928.50	915.12	13.38	1.44
200:100	200	947.10	932.52	14.58	1.54
SD		106.34	106.75	0.76	0.19
200:200	200	1126.86	111.18	15.68	1.39
200:200	200	1164.06	1151.83	12.23	1.05
200:200	200	1003.68	992.49	11.19	1.12
SD		83.94	82.79	2.35	0.18

Note: 6 kg soil/pot was used in this experiment