Phytoremediation of Heavy Metal Contaminated Soil Using Indian Mustard and Marigold Plant

A Thesis

by

Zaki Uddin Ahmad

MASTER OF SCIENCE IN ENVIRONMENTAL ENGINEERING

Department of Civil Engineering

BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY

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Submitted to the Department of Civil Engineering, Bangladesh University of Engineering and Technology (BUET), Dhaka in partial fulfillment of the requirements for the degree

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The thesis titled "Phytoremediation of Heavy Metal Contaminated Soil Using Indian Mustard and Marigold Plant" submitted by Zaki Uddin Ahmad, Roll No: 0413042502P, Session: April 2013 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Master of Science in Environmental Engineering on 21st June, 2015.

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This is to certify that the work presented in this thesis entitled "Phytoremediation of Heavy Metal Contaminated Soil Using Indian Mustard and Marigold Plant" is the outcome of the investigation carried out by the author Zaki Uddin Ahmad (Student ID: 0413042502P) under the supervision of Dr. Mahbuboor Rahman Choudhury, Assistant Professor, Department of Civil Engineering, Bangladesh University of Engineering and Technology (BUET), Dhaka. It is also declared that neither this thesis nor any part of this thesis has been submitted or is being currently submitted anywhere else for the award of any degree or diploma except for research publication by the author.

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ABSTRACT

Rapidly increasing urban population is influencing the land use pattern causing enormous degradation to the surrounding environment. Land filling operations are being conducted in Dhaka city and many other urban areas by using dredged riverbed sediments for developing newly built urban zones. Being placed on the bank of Buriganga River, numerous development projects have been advanced using the Buriganga riverbed sediments. Huge volume of toxic waste is being discharged into Buriganga River from riverside industries without any treatment. Of all these chemical pollutants, heavy metals reaching soil maintain their presence in the pedosphere for many years, even after the removal of pollution sources. The newly developed areas containing heavy metal contaminated sediments may cause severe health hazards resulting from wind-blown dusts entering the respiratory system.

In this study heavy metal uptakes from contaminated Buriganga riverbed sediments by Indian mustard and Marigold plants, two locally available hyperaccumulators, were assessed. Initial characterization showed concentrations of chromium, lead, copper and zinc in the Buriganga sediments higher when compared to the toxicity reference values given for these heavy metals in soil for terrestrial plants, and soil invertebrate. The average background concentration of chromium, lead, copper, and zinc in the Buriganga riverbed sediments were found to be 141.5 mg/kg, 34.9 mg/kg, 38.7 mg/kg, and 287.5 mg/kg, respectively. It was observed that both Indian mustard and Marigold plants accumulated these heavy metals in different parts of the plant from the contaminated sediments and were able to maintain a growth rate of more than 90% compared to that in non-contaminated soil. The results indicated rapid phytoextraction of the heavy metals by the Indian mustard during its final growth phase, whereas rapid phytoextraction of the heavy metals was observed in case of Marigold in its initial growth phase. Total chromium, lead, copper, and zinc uptakes (in mg/kg of plant dry weight) by Indian mustard plant in 12 weeks were 102.6, 28.9, 53, and 1861.5, respectively. The uptakes (in mg/kg of plant dry weight) of the same heavy metals by Marigold plant in 12 weeks were found to be 112.3, 104.25, 82.5, and 716.75, respectively. Marigold showed higher uptake efficiency for chromium, lead, and copper; while Indian mustard was found to be more efficient in zinc uptake. Hence both of these plants can be used in an environment-friendly approach for treating heavy metal contaminated landfills developed using heavy metal contaminated riverbed sediments.

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Chapter 1
Introduction

1.1 Background

Rapidly increasing global population is exerting pressure on land use resulting insubstantial cohesion among environmental variables (Green, et. al., 1994). The rapid changes of land use and land cover in urban areas, particularly in developing nations, are characterized by: (a) rampant urban sprawling (Jat, et. al., 2008; Mundia & Aniya, 2006), (b) land degradation by agricultural development and tourism industry (Shalaby & Tateishi, 2007), (c) transformation of agricultural land into shrimp farming (Ali, 2006) ensuing enormous cost to the surrounding environment (Abdullah & Nakagoshi, 2006). The population of Dhaka, the capital city of Bangladesh, is expanding apace at an average rate of 4.24% per year and it is projected to be third largest mega city in the world by the year 2020 (The World Bank, 2007). The growth of Dhaka city is phenomenal after independence (Hossain, 2008) and this growth is mainly attributed to large influx of rural to urban migration (Islam, 1996). Land filling operations, conducted primarily by dredging the riverbed sediments (Islam, et. al., 2010), in surrounding low-lying areas has developed the newly built urban zones in Dhaka city. Being placed on the bank of the river Buriganga, numerous urban expansion/development projects have been advanced using the Buriganga riverbed sediments for land filling purposes.

Buriganga riverbank is the center of many economic activities, which includes numerous industries (e.g. textile, tannery, machine shops etc.), a busy river port and other commercial enterprises. Lack of legislative action and awareness has resulted in the discharge of heavy pollution loads from city's industrial units and dumping from combined sewer lines containing huge volume of toxic wastes directly into the river from these riverside establishments without any prior treatment (Ahmad, et. al., 2010). Of all the chemical pollutants, heavy metal, being non-biodegradable, can be concentrated in the food chain and they can impart toxic effects at distant points far away from the point of generation (Tilzer & Khondker, 1993). Heavy metals reaching the soil maintain their presence in the pedosphere for many years, even after removal of pollution sources, and many previous studies have reported increased amount of heavy metals in the upper soil layer in urban areas ((Klein, 1972; Imperato, et. al., 2003; Chen, et. al., 1997; Pichtel, et. al., 1997). Previous studies have reported high heavy metal concentration in the Buriganga riverbed sediments (Ahmad, et. al.,

2010; Saha & Hossain, 2011). To reduce heavy metal concentration from the land fill soil, a viable soil treatment method needs to be developed. Recent research works and interventions have mostly focused on the improvement of Buriganga river water quality (Ahmad, et. al., 2010; Saha & Hossain, 2011). However with increasing use of Buriganga riverbed sediments for land filling purposes, focus should be made to assess suitable methods for removing heavy metals from these sediments.

Most of the current practices used for remediating heavy metal contaminated sediments are based on encapsulation or scraping up the contaminated sediments (Pulford & Watson, 2002). Extraction or immobilization by physical and chemical processes is not economically feasible for remediating heavy metal contamination of large land areas and it is often recommended for only small areas where complete and rapid decontamination is required (Martin & Bardos, 1996; BIO-WISE, 2000). Other methods, like soil washing, have an adverse effect on biological activity, soil structure and fertility, and may require significant budget for implementation as well (Baker, et. al., 1994).

Phytoremediation technique has been identified as a cost-effective approach for remediating heavy metal contaminated sediments (Pilon-Smiths, 2005; Salt, et. al., 1998; Rugh, et. al., 2000; Meagher, et. al., 2000). Phytoremediation approaches to utilize a particular group of plants, known as hyper-accumulators, to extract and concentrate particular heavy metal elements from the environment (Salt, et. al., 1998). Hyper-accumulator plant species are capable of accumulating metals at levels 100 fold greater than those typically found in common plants (Salt, et. al., 1998; Chaney, et. al., 1997; Raskin & Ensley, 2000). These hyper-accumulator species have strongly expressed mechanism of metal sequestration and, sometimes, greater internal requirement for specific metals (Shen, et. al., 1997). It offers removal of heavy metal in a particular site by maintaining the biological activity and structure of the soils and with the possibility of bio-recovery of metals (Baker, et. al., 1994). The field of phytoremediation is harnessing greater acceptance because phytoremediation technique can offer the only effective means of restoring hundreds and thousands of square kilometers of land area and water that have been polluted by irresponsible activities of humans (Meagher, 2000). Five main subgroups of phytoremediation have been identified:

- Phytoextraction: Plants removes heavy metals and radionuclides from the soil and concentrate them in their foliage (Kumar, et. al., 1995; Brooks, et. al., 1979; Baker & Brooks, 1989).
- Phytodegradation: plants and associated microbes degrade organic pollutants (Burken & Schnoor, 1997).
- Rhizophiltration: plant roots absorb metals from waste streams (Dushenkov, et. al., 1995).
- Phytostabilisation: plants reduce the mobility and bioavailability of pollutants in the environment either by immobilization or by prevention of migration (Vangronsveld, et. al., 1995).
- Phytovolatilisation: volatilisation of pollutants into the atmosphere via plants (Burken & Schnoor, 1999; Bañuelos, et. al., 1997).

Among different types of hyper-accumulators, Indian mustard (*Brassica juncea*) and Marigold (*Tagetes patula*) plants have been known to remove heavy metals from soil (McCutcheon & Schnoor, 2003; Huq, et. al., 2005). Although other hyper-accumulator species are available for the treatment of heavy metal contaminated soil, both Indian mustard and Marigold plant species are widely available and are easily grown in different parts of the Dhaka city. In spite of abundant presence of these plants, their application in phytoremediation of soil has not been realized in the local context. Hence Indian mustard and Marigold plants have been selected as the hyper-accumulators in the present study. Use of hyper-accumulators in the treatment of land fills in Dhaka city has not been studied before.

1.2 Objective of the Study

The present research aims to study the potential use of Indian mustard and Marigold in remediating heavy metal contaminated soils. The specific objectives of the present study are:

1. To characterize the Buriganga riverbed sediments in terms of soil property and heavy metal concentration.
2. To compare the growth of Indian Mustard and Marigold in heavy metal contaminated soil samples to that in normal garden soil.
3. To assess heavy metal uptake by Indian Mustard and Marigold from the contaminated soil samples collected from Buriganga riverbed.

1.3 Organization of the Thesis

This thesis consists of five chapters. Apart from this chapter, the remainder of the thesis has been divided into four chapters.

Chapter 2 provides an overview of different techniques of soil remediation and background of phytoremediation technique. This chapter also discusses different aspects of hyperaccumulator species.

Chapter 3 provides an overall description of methodology used in this study including collection of sediment from Buriganga riverbed, physiochemical condition during plant growth, plant harvesting and elemental analysis for determining heavy metal contents.

Chapter 4 entails results and relevant discussion of the study which comprises characterization of Buriganga riverbed sediments, comparison of Growth Tolerance of Indian mustard and Marigold to Buriganga riverbed sediments, comparison of accumulation of heavy metals in Indian mustard and Marigold plants.

Chapter 5 presents the major conclusion from the study and the recommendations for future research works.

Chapter 2

Literature Review

2.1 Background of Phytoremediation

Since the dawn of civilization environmental threats from different sources have been a part of human life. Intensity of toxic metal pollution in the biosphere has been increasing since the starting of industrial revolution, posing major environmental threats and human health problems. Controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting metalliferous ores, application of sewage sludge to agricultural soil are responsible for the migration of contaminants into non-contaminated sites as dust or leachate and contribute towards contamination of our ecosystem. These contaminants include heavy metals, combustible and putrescible substances, hazardous waste, explosive and petroleum products which cover a wide range of organic and inorganic compounds. Out of all these contaminants heavy metals pose threats than organic contaminants to our ecosystem (Logan, 1987; Alloway, 1990). Soil microorganisms can degrade organic contaminants, while metal needs immobilization or physical removal from site. One of the important reason behind the toxicity characteristics of heavy metals is that they can replace essential metals in pigments or enzymes disrupting their normal function (Henry, 2000). Toxicity derived from heavy metal is also reported to be associated with loss of livestock, which sometimes hampers the economy of a country. Heavy metals are toxic, as they tend to accumulate in plants and animals. They bioconcentrate in the food chain and attack specific organs in human body (Bondada & Ma , 2003). From dawn to dusk of the period of industrialization intoxication of heavy metals will not be sequestered from human life.

The concept of using plants for cleaning up contaminated environment is an old concept. Plants were recommended for the treatment of wastewater about 300 years ago (Hartman Jr., 1975). At the end of the 19th century, ***Thlaspi caerulescens*** and ***Viola calaminaria*** were the first plant species reported to accumulate high levels of metals in leaves (Baumann, 1885). Later on it was reported that plants of the genus *Astragalus* were capable of accumulating up to 0.6% selenium in dry shoot biomass (Byers, 1935). Despite subsequent reports claiming identification of Co, Cu, and Mn hyperaccumulators, the existence of plants hyperaccumulating metals other than Cd, Ni, Se and Zn has been questioned and requires

additional confirmation (Salt, et al., 1995). The idea of using plants to extract metals from contaminated soil was reintroduced and developed by Chaney (Chaney, 1989), and the first field trial on Zn and Cd phytoextraction was conducted in 1991 (Baker, et al., 1991). In the last decade, extensive research has been conducted to investigate the biology of metal phytoextraction. Despite significant success, our understanding of the plant mechanisms that allow metal extraction is still emerging. In addition, relevant applied aspects, such as the effect of agronomic practices on metal removal by plants are largely unknown. It is conceivable that maturation of phytoextraction into a commercial technology will ultimately depend on the elucidation of plant mechanisms and application of adequate agronomic practices. Natural occurrence of plant species capable of accumulating extraordinarily high metal levels makes the investigation of this process particularly interesting.

2.2 Techniques of Soil Remediation

Heavy metal contaminated soil can be remediated by chemical, physical and biological techniques. These can be grouped into two broad categories:

2.2.1 Ex-situ Soil Remediation Methods

It requires removal of contaminated soil for treatment on or off site, and returning the treated soil to the reported site. The conventional ex-situ methods applied for remediating the polluted soils includes excavation, detoxification and/ or destruction of contaminant physically or chemically, as a result the contaminant undergo stabilization, solidification, immobilization, incineration or destruction.

Ex-situ thermal processes involve the transfer of pollutants from the soil to a gaseous phase. The pollutants are released by vaporization and the burned at high temperatures. *Ex-situ* thermal remediation is completed in three steps: soil conditioning, thermal treatment, and exhaust gas purification (Deuren, et al., 2002). Soil condition is a process in which soil is broken into small grains and sieved in preparation for thermal treatment. Thermal treatment heats the soil in order to transfer volatile pollutants to a gas phase. Heating is done by using a sintering strand, fluid bed, or rotary kiln plants. The soil is usually heated to a low temperature range of 350-550ºC. Combustion of the gases occurs over the top of the soil, but

the volatile gases are not destroyed. The gases are then burned in an after-burner chamber at approximately 1200ºC and dioxins are destroyed (Koning, et al., 2000).

Ex-situ thermal remediation processes are ideal for use when removing petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), benzene, toluene, ethylbenzene, xylenes (BTEX), phenolic compounds, cyanides, and chlorinated compounds like polychlorinated biphenyls (PCB), pentchlorphenol (PCP), chlorinated hydrocarbons, chlorinated pesticides, polychlorinated dibenzodioxins (PCDD), and polychlorinated dibenzofurans (PCDF) (Koning, et al., 2000).

The ex-situ chemical/physical remediation process known as soil scrubbing uses mechanical energy to separate the pollutants from the soil. The soil is crushed and then separated via sieving. This ensures that the soil sample is homogeneous. The soil is then dispersed in liquid. Water, which is sometimes enhanced with an additive, is used to dissolve the pollutant. The additives are used to overcome the bonding forces between the pollutants and the soil particles. The soil is then separated into two categories: low density and high-density solids. Highly polluted fine particles are then separated out and dewatered. The particles are then rinsed with uncontaminated water. The wastewater and exhaust air are then purified. Soil scrubbing is most effective when removing BTEX, TPH, PAH, PCB, heavy metals, and dioxins (Koning, et al., 2000).

Ex-situ biological processes include: composting, landfarming, biopiling and the use of bioreactors. Composting consists of excavating the soil and then mixing organics such as wood, hay, manure, and vegetative waste with the contaminated soil (Deuren, et al., 2002). The organics are chosen based on their ability to provide the proper porosity and carbon and nitrogen balances to aid in the breakdown of contaminants. Maintaining thermophilic temperatures 54 to 65ºC is an important part of composting. In most cases, the indigenous microorganisms maintain this temperature while degrading the contaminant. Composting is most effective when removing PAH, TNT, and RDX (Deuren, et al., 2002).

Landfarming is a process in which the soil is excavated and mechanically separated via sieving. The polluted soil is then place in layers no more than 0.4 meters thick. A synthetic, concrete, or clay membrane is then used to cover the contaminated soil layer. Oxygen is added and mixing occurs via plowing, harrowing, or milling. Nutrients and moisture may also

be added to aid the remediation process. The pH of the soil is also regulated (keeping it near 7.0) using crushed limestone or agricultural lime (Deuren, et al., 2002). Landfarming is most successful in removing PAH and PCP. Figure 2.1 illustrates the landfarming technique.

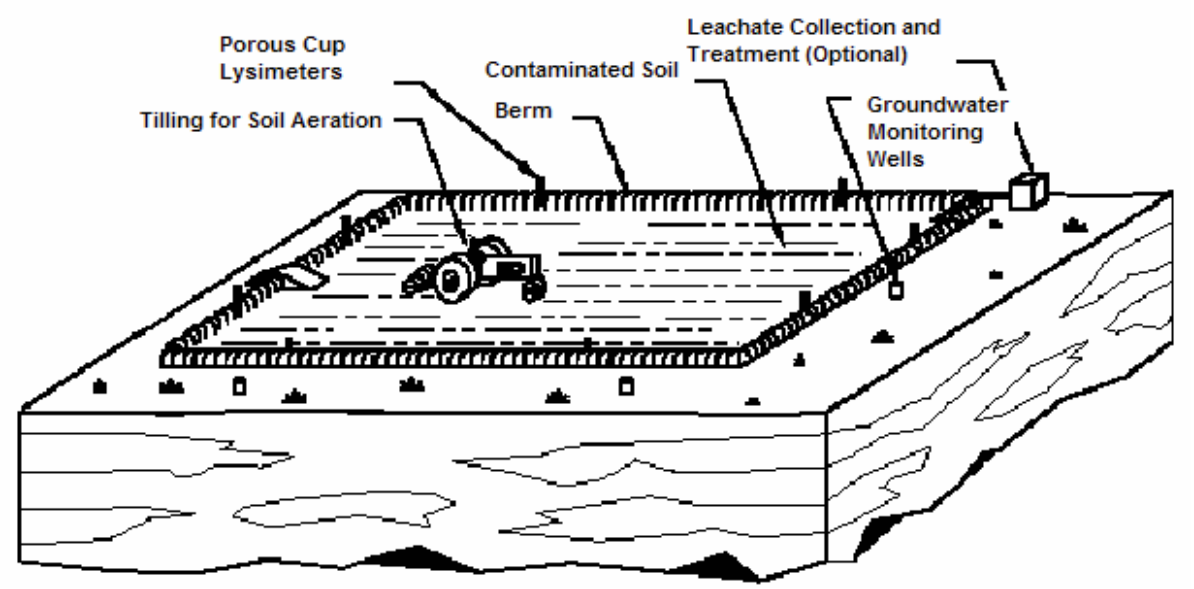

Figure 2.1. Landfarming Technique (Source: United States Environmental Protection Agency, 2004)

Biopiling is an *in-situ* process that is also known as the heap technique. The first step in the biopiling process is to perform laboratory tests that will determine the biological degradation capabilities of the soil sample. The next step involves the mechanical separation of the soil, which will homogenize the sample and remove any disruptive material such as plastics, metals, and stones. The stones will then be crushed into smaller pieces and then depending on the degree of contamination will either be added to a pile or sent out for reuse. The soil is then homogenized, meaning that the pollution concentration is averaged out across the entire soil sample. Homogenization allows for biopiling to be more effective (Schulz-Berendt, 2000).

Once the soil is piled, nutrients, microbes, oxygen, and substrate are added to start the biological degradation of the contaminants. The results of the initial laboratory tests indicate to the operators which substrates such as bark, lime, or composts needs to be added to the soil. Nutrients such as mineral fertilizers may also be added. Additionally, microorganisms such as fungi, bacteria, or enzymes could be added (Schulz-Berendt, 2000).

Static piles are usually in the form of pyramids or trapezoids. Their heights vary between 0.8 and 2.0 m depending on the type of aeration used (either passive or active). Dynamic biopiles are consistently plowed and turned to maximize their exposure to increase the bioavailability of the contaminants by constantly exposing them to oxygen, water, nutrients, and microbes (Koning, et al., 2000). No matter which types of heaps are used, the area below each heap must be covered in asphalt or concrete to prevent the seepage of contaminants and the area above the heaps must be covered in order to control temperature and moisture content conditions (Schulz-Berendt, 2000). A diagram for the heap techniques is shown in Figure 2.2.

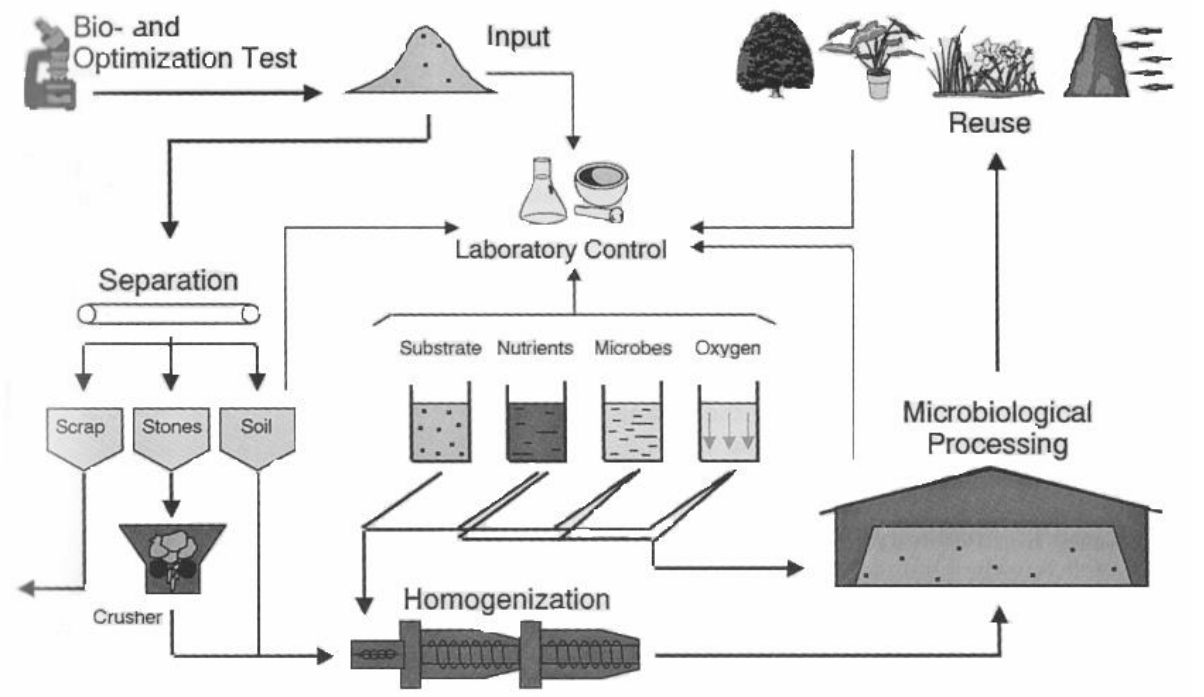

Figure 2.2. Heap Technique Diagram (Schulz-Berendt, 2000)

Biopiling is most effective in treating pollutants such as BTEX, phenols, PAHs with up to 4 aromatic rings, and explosives such as TNT and RDX (Deuren, et al., 2002; Schulz-Berendt, 2000). Each pollutant requires slight modifications to the basic technique. A specific modification is applied to volatile hydrocarbons. These volatile gases must be removed with a soil vapor extraction system and treatment biofilters and activated carbon filters. The heap technique is very economically efficient due to its low installation cost. The cost of operation is also low due to the low cost technology used in the treatment. More and more treatment plants are being built, which reduces the transportation costs, but government regulation are becoming stricter making it more expensive to transport and eventually dispose of the soil (Schulz-Berendt, 2000).

Bioreactors treat contaminated soils in both solid and liquid (slurry) phases. The solid phase treatment process mechanically decomposes the soil by attrition and mixing in a closed container. The objective of the mixing is to guarantee that the pollutants, water, air, nutrients, and microorganisms are in permanent contact. An acid or alkalinity may also be added to control the pH (Deuren, et al., 2002). In fixed bed reactors, composts is added and significantly increases the degradation rate. In rotating drum reactors, the drum has a screw like mechanism in the middle of it that rotates to mix and transport the soil. The liquid phase treatment process uses suspension bioreactors and treats soils as slurry. The slurry feed enters the system and is rinsed through a vibrating screen to remove debris. Sand is then removed using a sieve or hydrocyclone. If a hydrocyclone is used to remove the sand, the sand falls to the bottom of the cyclone and the fines remain on top. The fines are then treated in a bioreactor. After the treatment, the slurry must be dewatered and the water is then treated with standard wastewater techniques (Kleijntjens & Luyben, 2000). A typical slurry bioreactor setup is illustrated in Figure 2.3.

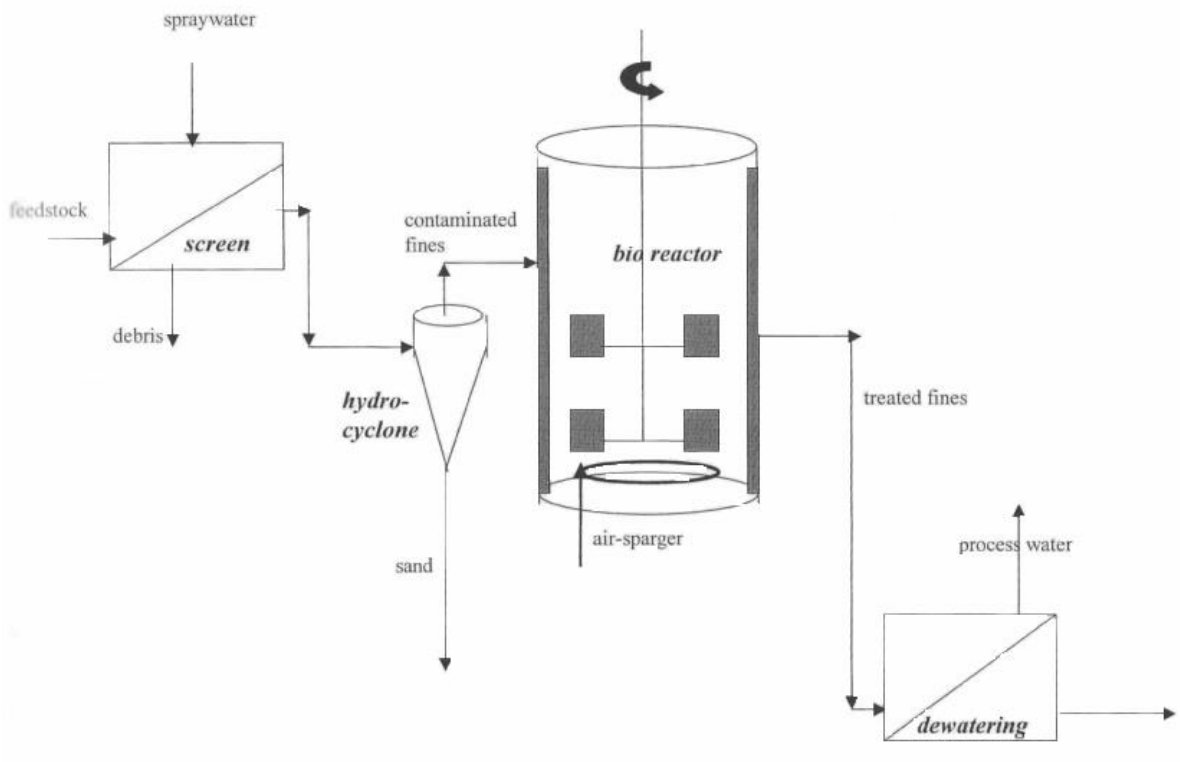

Figure 2.3. Typical Slurry Bioreactor (Kleijntjens & Luyben, 2000)

A major advantage of ex-situ bioremediation processes is that most of the decontaminated soil can be reused. Due to the ex-situ techniques used to decontaminate the soil, much of the soil cannot be used as filling or agricultural material. The soil can, however, be used for landscaping purposes. If soils are treated with thermal processes or a wet scrubber they may be reused as filling material. A key factor in determining the applicability of soil reuse is the toxicological assessment. Bioassays must be conducted in order to determine the impacts the soil will have on the surrounding area (Koning, et al., 2000).

2.2.2 In-situ Soil Remediation Methods

In-situ method of soil remediation is the remediation technique without excavation of contaminated soils. Reed et al. defined in-situ method of soil remediation as reduction of bioavailability and separation of the contaminant from the bulk soil by means of destruction and/ or transformation and immobilization of the contaminant (Reed, et al., 1992). In-situ techniques have the advantages over ex-situ techniques due to their low cost and reduced impact on the ecosystem. Conventionally, the ex-situ technique is the excavation of heavy metal contaminated soil and their burial in landfill site (McNeil & Waring, 1992).

In-situ remediation includes techniques such as bioventing, biosparging, bioslurping and phytoremediation along with physical, chemical, and thermal processes. In situ remediation is less costly due to the lack of excavation and transportation costs, but these remediation techniques are less controllable and less effective (Koning, et al., 2000). Figure 2.4 illustrates the localization of selected in-situ bioremediation processes.

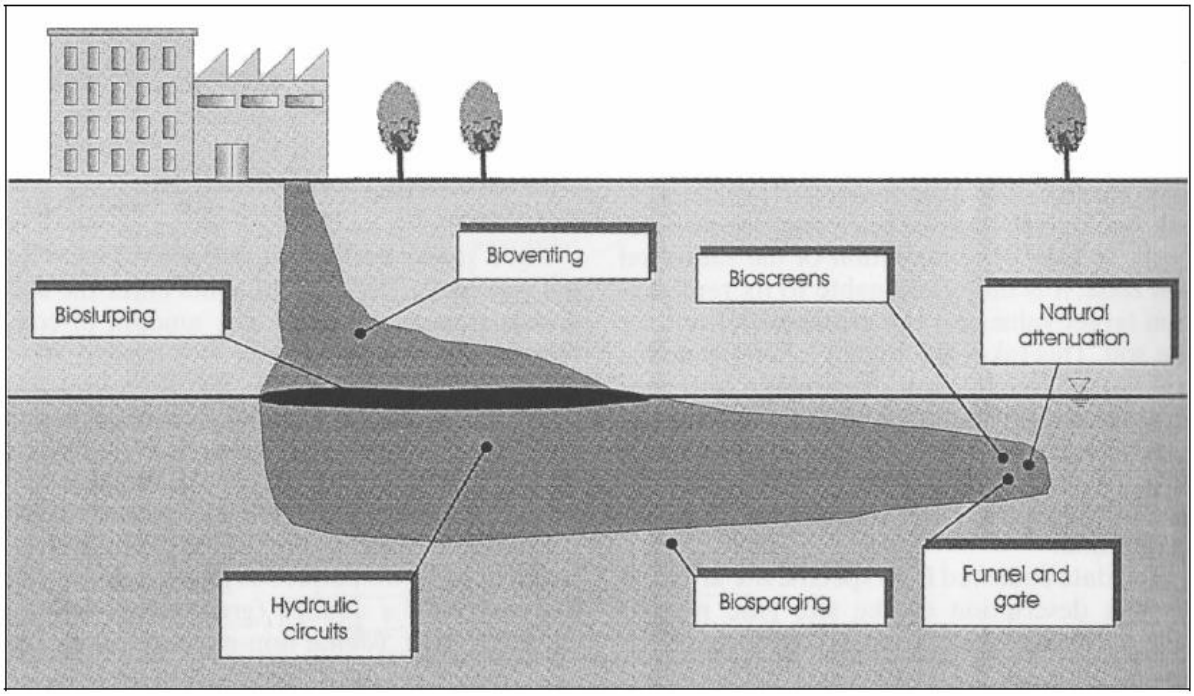

Figure 2.4. Localization of different microbial *in situ* technologies (Held & Dörr, 2000)

In-situ thermal processes are still in the developmental phase. The process involves injecting a steam-air mixture at 60-100ºC into the soil. In order to avoid the transport of pollutants to the groundwater, the steam-air mixture must stay in that temperature range. After the injection, volatile and semi-volatile compounds transport from the soil to the gas phase. The gases are then removed from the subsurface using a soil vapor extraction system and then treated at the surface. In situ thermal remediation is limited for use in only certain soil types, namely homogeneous soils with high permeability and low organic content. In-situ thermal processes are only appropriate for removing pollutants, which can be stripped in the lower temperature range (e.g. BTEX) (Koning, et al., 2000).

In-situ chemical/physical processes are sometimes referred to as pump and treat processes. The pump and treat process pumps water into the subsurface in order to draw out the contaminants. Surfactants are sometimes added to the water to increase the solubility of the pollutants. The water is then treated with standard wastewater treatment techniques. The pump and treat process is extremely limited by the permeability of the soil. Chemical oxidation is also employed to destroy contaminants such as PAHs and trychloroethylene (TCE) (Koning, et al., 2000). Chemicals such as ozone, permanganate, and peroxide have all been injected into the soil and used to accelerate the destruction of toxic organic compounds (Deuren, et al., 2002).

Another in-situ chemical/physical process used is soil vapor extraction. Vacuum blowers are used to extract volatile pollutants for the soil through perforated pipes. The volatile pollutants are then treated at the site using activated carbon filters or compost filters. The effectiveness of this technique is dependent on soil characteristics such as moisture content, temperature, and permeability. A high percentage of fine soil or a high degree of saturation can also hinder the effectiveness of soil vapor extraction (Deuren, et al., 2002). Complete decontamination of the soil is rarely achieved with this technique.

Bioventing is the only *in situ* bioremediation technique that allows for the treatment of unsaturated soil. Bioventing is not effective if the water table is within several feet of the surface (Deuren, et al., 2002). This system uses a vacuum enhanced soil vapor extraction system. Due to the pressure gradient in the soil, atmospheric oxygen flows into the subsurface. This oxygen starts an aerobic contaminant decomposition process. In many cases it is necessary to add nitrogen salts as an additive by sprinkling a nutrient solution on top of the soil or by injecting them into the soil above the contaminated soil zone (Held & Dörr, 2000).

Sufficient airflow is very important in the design of a bioventing system. The geometry of the exfiltration wells and the need for active or passive air injections are two particular design concerns. If a high concentration of pollutants exists, clogging of the soil pores may occur. In this case, pulsed soil vapor extraction is needed. Low permeability will also hinder Bioventing. If the soil vapors are volatile, they be treated at the surface with an activated carbon filter or a biofilter. Bioventing is effective in removing petroleum hydrocarbons, aromatic hydrocarbons, and non-volatile hydraulic oils (Held & Dörr, 2000). Low temperatures hinder the effectiveness of bioventing. Bioventing is normally only effect in areas with high temperatures (Deuren, et al., 2002). Figure 2.5 illustrates a typical bioventing system.

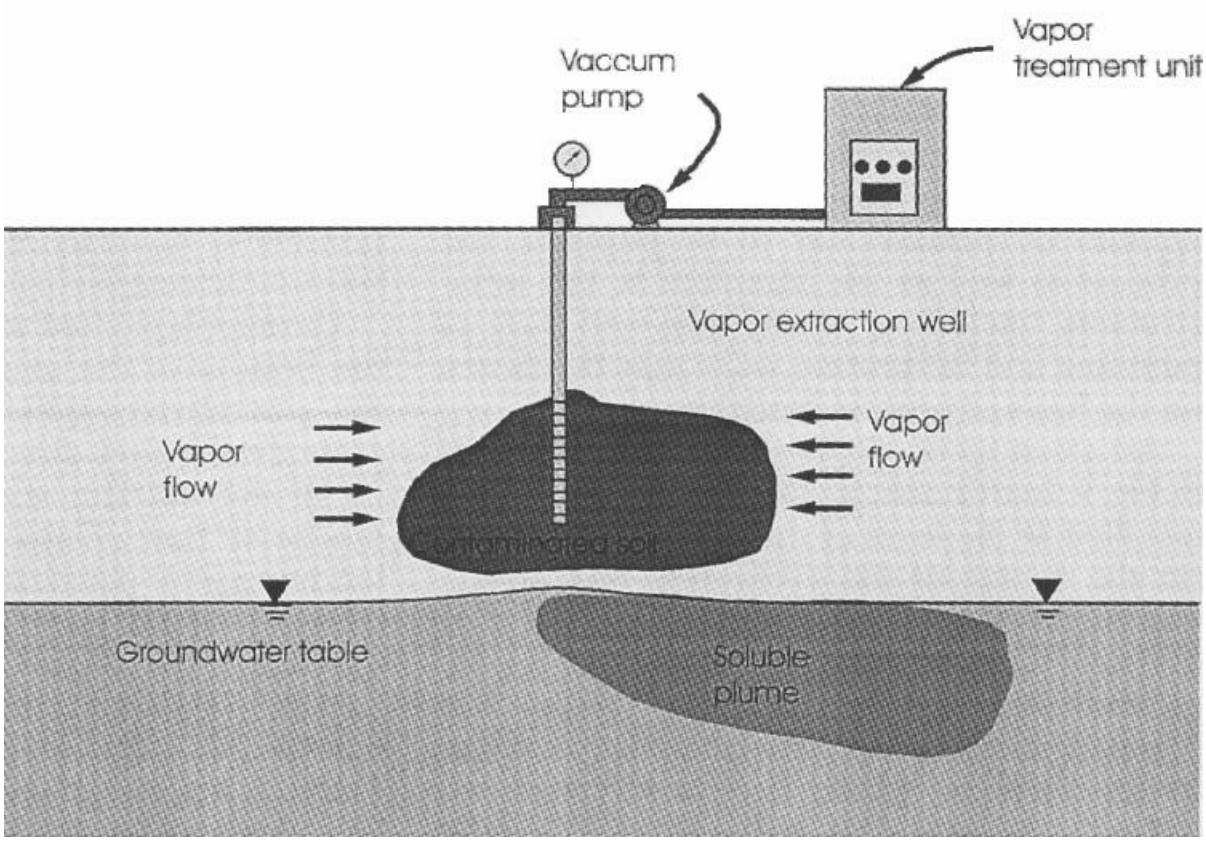

Figure 2.5. Illustration of Bioventing System (Held & Dörr, 2000)

Phytoremediation is an *in situ* technique that uses plants to remediate contaminated soils. Phytoremediation is most suited for sites where other remediation options are not costs effective, low-level contaminated sites, or in conjunction with other remediation techniques. Deep rooted trees, grasses, legumes, and aquatic plants all have application in the phytoremediation field. Phytoremediation has been used to remove TPH, BTEX, PAH, 2, 4, 6-trinitrotoluene (TNT), and hexahyro-1, 3, 5-trinitro-1, 3, 5 triazine (RDX) (Schnoor, 2000).

Plants are able to remove pollutants from the groundwater and store, metabolize, or volatilize them. Also, roots also help support a wide variety of microorganisms in the subsurface. These microorganisms can then degrade the contaminants. The roots also provide organic carbon sources to promote cometabolism in the rizosphere. The rizosphere is the soil in the area of the vegetative roots. Figure 2.6 illustrates different phytoremediation techniques.

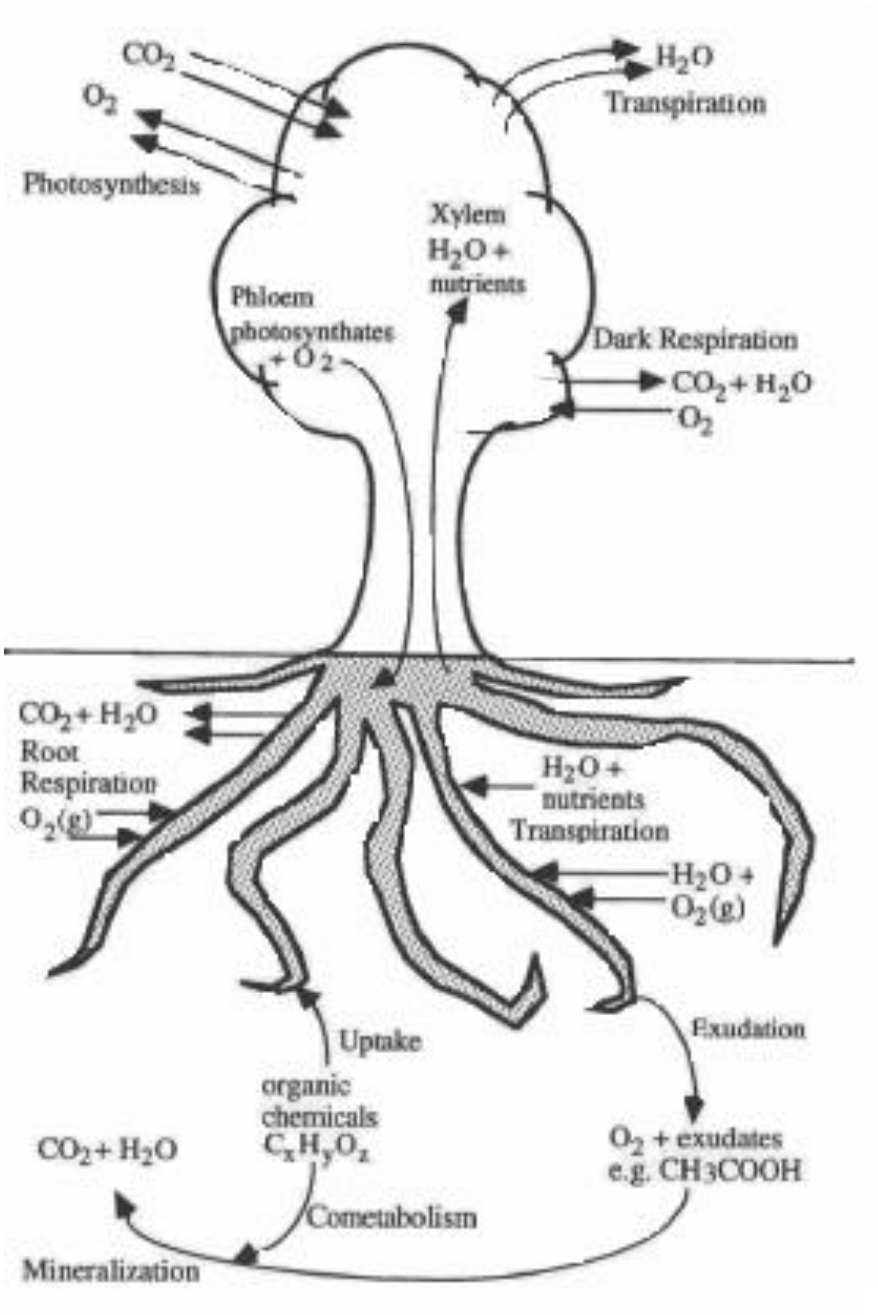

Figure 2.6. Illustration of Phytoremediation (Schnoor, 2000)

2.3 Heavy Metals in Soils

2.3.1 Sources of contamination

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand specificity, etc.) and atomic number >20. The most common heavy metal contaminants are: Cd, Cr, Cu, Hg, Pb, and Zn. Metals are natural components in soil. Contamination, however, has resulted from industrial activities, such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application, and generation of municipal waste (Pendias, 1989). Soil concentration range and regulatory limits for several major metal contaminants are shown in Table 2.1 (Riley & Zachara, 1992; NJDEP, 1996).

Table 2.1. Soil concentration ranges and regulatory guidelines for some toxic metals

Metals	Soil Concentration Range[a] (mg/kg)	Regulatory limits[b] (mg/kg)
Pb	1.00-6900	600
Cd	0.10-345	100
Cr	0.05-3950	100
Hg	<0.01-1800	270
Zn	150-5000	1500

Source: [a] Riley et al., 1992

[b] Nonresidential direct contact soil cleanup criteria (NJDEP, 1996)

High levels of metals in soil can be phytotoxic. Poor plant growth and soil cover caused by metal toxicity can lead to metal mobilization in runoff water and subsequent deposition into nearby bodies of water. Furthermore, bare soil is more susceptible to wind erosion and spreading of contamination by airborne dust. In such situations, the immediate goal of remediation is to reclaim the site by establishing a vegetative cover to minimize soil erosion and pollution spread.

2.3.2 Risk assessment

Soil remediation is needed to eliminate risk to humans or the environment from toxic metals. Human disease has resulted from Cd (Nogawa, et al., 1987; Kobayashi, 1978; Shiwen, et al., 1990), Se (Yang, et al., 1983), and Pb in soil (Chaney, et al., 1999). Livestock and wildlife have suffered from Se poisoning (Kopsell & Randle, 1999; Ohlendorf, et al., 1986; Berti & Jacobs, 1996). In addition, soil contamination with Zn, Ni and Cu caused by mine wastes and smelters is known to be phytotoxic to sensitive plants (Chaney, et al., 1999). One of the greatest concerns for human health is caused by Pb contamination. Exposure to Pb can occur through multiple pathways, including inhalation of air and ingestion of Pb in food, water, soil or dust. Excessive Pb exposure can cause seizures, mental retardation and behavioral disorders. The danger of Pb is aggravated by low environmental mobility even under high precipitations.

2.3.3 Bioavailability of metals in soil

In soil, metals are associated with several fractions: (1) in soil solution, as free metal ions and soluble metal complexes, (2) adsorbed to inorganic soil constituents at ion exchange sites, (3) bound to soil organic matter, (4) precipitated such as oxides, hydroxides, carbonates, and (5) embedded in structure of the silicate minerals. Soil sequential extractions are employed to isolate and quantify metals associated with different fractions (Tessier, et al., 1979). For phytoextraction to occur, contaminants must be bioavailable (ready to be absorbed by roots). Bioavailability depends on metal solubility in soil solution. Some metals, such as Zn and Cd, occur primarily in exchangeable, readily bioavailable form. Others, such as Pb, occur as soil precipitate, a significantly less bioavailable form.

The chemistry of metal interaction with soil matrix is central to the phytoremediation concept. In general, sorption to soil particles reduces the activity of metals in the system. The higher the cation exchange capacity (CEC) of the soil, the greater the sorption and immobilization of the metals. In acidic soils, metal desorption from soil binding sites into solution is stimulated due to H+ competition for binding sites. Soil pH affects not only metal bioavailability, but also the very process of metal uptake into roots. This effect appears to be metal specific. For example, in *T. caerulescens,* Zn uptake in roots showed small pH

dependence, whereas uptake of Mn and Cd was more dependent on soil acidity (Brown, et al., 1995).

2.4 Phytoremediating Plants

2.4.1 Uptake of toxic metals by plants

To grow and complete the life cycle, plants must acquire not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients such as Fe, Zn, Mn, Ni, Cu, and Mo. Highly specific mechanisms have been evolved by plants to take up, transport, and store these nutrients. For example, metal movement across biological membranes is mediated by proteins with transport functions. In addition, sensitive mechanisms maintain intracellular concentration of metal ions within the physiological range. In general, the uptake mechanism is selective, plants preferentially acquiring some ions over others. Ion uptake selectivity depends upon the structure and properties of membrane transporters. These characteristics allow transporters to recognize, bind and mediate the trans-membrane transport of specific ions. For example, some transporters mediate the transport of divalent cations, but do not recognize mono- or trivalent ions.

2.4.2 Hyperaccumulator species

Interest in phytoremediation has grown significantly following the identification of metal hyperaccumulator plant species. Hyperaccumulators are conventionally defined as species capable of accumulating metals at levels 100-fold greater than those typically measured in common nonaccumulator plants. Thus, a hyperaccumulator will concentrate more than: 10 ppm Hg; 100 ppm Cd; 1,000 ppm Co, Cr, Cu, and Pb; 10,000 ppm Ni and Zn. To date, approximately 400 plant species from at least 45 plant families have been reported to hyperaccumulate metals. Most hyperaccumulators bioconcentrate Ni, about 30 absorb either Co, Cu, and/or Zn, even 11 fewer species accumulate Mn and Cd, and there are no known natural Pb-hyperaccumulators (Reeves & Baker, 2000).

2.4.3 Plant tolerance of high metal concentration

Ecological studies have revealed the existence of specific plant communities, endemic floras, which have adapted on soils contaminated with elevated levels of Zn Cu, and Ni. Different ecotypes of the same species may occur in areas uncontaminated by metals. Metal tolerance is an indispensable property to plants exposed to metal-contaminated soils. In comparison, in related populations inhabiting uncontaminated areas, a continuous gradation between ecotypes with high and low tolerance usually occurs. Plants evolved several effective mechanisms for tolerating high concentrations of metals in soil. In some species, tolerance is achieved by preventing toxic metals uptake into root cells. These plants, called excluders, have little potential for metal extraction. Such an excluder is "Merlin," a commercial variety of red fescue (*Festuca rubra*), used to stabilize erosion-susceptible metal contaminated soils. A second group of plants, accumulators, does not prevent metals from entering the root. Accumulator species have evolved specific mechanisms for detoxifying high metal levels accumulated in the cells. These mechanisms allow bioaccumulation of extremely high concentration of metals. In addition, a third group of plants, termed indicators, shows poor control over metal uptake and transport processes. In these plants, the extent of metal accumulation reflects metal concentration in the rhizospheric soil. Indicator species have been used for mine prospecting to find new ore bodies (Raskin, et al., 1994).

2.4.4 Mechanisms of metals uptake

It is important to note that of the total amount of ions associated with the root, only a part is absorbed into cells. A significant ion fraction is physically adsorbed at the extracellular negatively charged sites (COO^-) of the root cell walls. The cell wall-bound fraction cannot be translocated to the shoots and, therefore, cannot be removed by harvesting shoot biomass (phytoextraction). Thus, it is possible that a plant exhibiting significant metal accumulation into the root, to express a limited capacity for phytoextraction. For example, many plants accumulate Pb in roots, but Pb translocation to shoot is very low. In support of this, Blaylock and Huang (1999) concluded that the limiting step for Pb phytoextraction is the long distance translocation from roots to shoots (Blaylock & Huang, 1999). Binding to the cell wall is not the only plant mechanism responsible for metal immobilization into roots and subsequent inhibition of ion translocation to the shoot. Metals can also be transformed into metal complex and sequestered in cellular structures (e.g., vacuole), which become unavailable for

translocation to the shoot (Lasat, et al., 1998). In addition, some plants, coined excluders, possess specialized mechanisms to restrict metal uptake into roots. However, the concept of metal exclusion is not well understood (Peterson, 1983).

Uptake of metals into root cells, the point of entry into living tissues, is a step of major importance for the process of phytoextraction. However, for phytoextraction to occur metals must also be transported from the root to the shoot. Movement of metal-containing sap from the root to the shoot, termed translocation, is primarily controlled by two processes: (a) root pressure and (b) leaf transpiration. Following translocation to leaves; metals can be reabsorbed from the sap into leaf cells. A schematic representation of metal transport processes that take place in roots and shoots are shown in Figure 2.7.

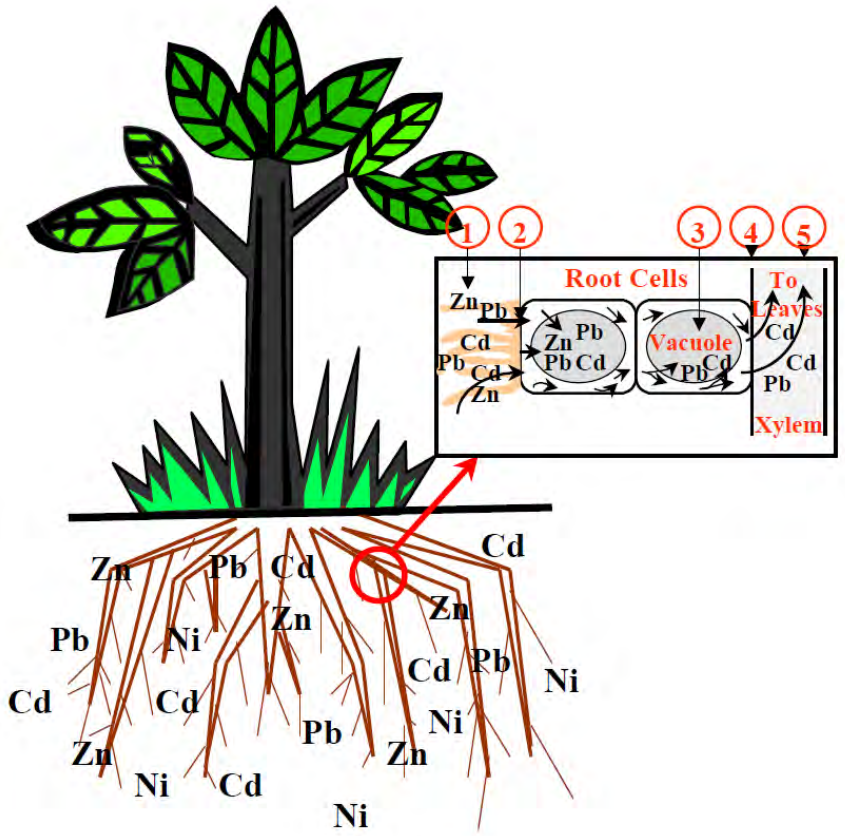

Figure 2.7. Schematic Representation of Metal uptake and accumulation in plants (1. A metal fraction is absorbed at root surface, 2. Bioavailable metal moves across cellular membrane into root cells, 3. A fraction of the metal absorbed into roots is immobilized in the vacuole, 4. Intracellular mobile metal crosses cellular membranes into root vascular tissue (xylem), 5. Metal is translocated from the root to aerial tissues (stems and leaves) (Lasat, et al., 1998)

2.4.5 Plant-metal interaction in the rhizosphere

A major factor limiting metal uptake into roots is slow transport from soil particles to root surfaces (Nye & Tinker, 1977; Barber, 1995). With the possible exception of volatile mercury, for all other metals, this transport takes place in soil solution. In soil, metal solubility is restricted due to adsorption to soil particles. Some of the soils binding sites are not particularly selective. For example, they bind Cd as strong as Ca. Nonspecific binding occurs at clay cation exchange sites and carboxylic groups associated with soil organic matter. Other sites are more selective and bind Cd stronger than Ca. For example, most clay particles are covered with a thin layer of hydrous Fe, Mn, and Al oxides. These selective sites maintain Cd activity in the soil solution at low levels (Chaney, 1988). Lead, a major contaminant, is notorious for the lack of soil mobility, primarily due to metal precipitation as insoluble phosphates, carbonates and hydroxides (Blaylock & Huang, 1999). Therefore, increasing solubility of metals in the soil is an important prerequisite to enhance the potential for Pb phytoextraction.

Mainly two mechanisms are responsible for transporting metal ions from the bulk soil to the plant root zone: 1) convection or mass flow, and 2) diffusion (Barber, 1995; Corey, et al., 1987). Soluble metal ions move from soil solids to root surface due to convection or mass flow. From the rhizosphere, roots to replace water, which has left the plants by transpiration process, absorb water. Water uptake from rhizosphere creates a hydraulic gradient directed from the bulk soil to the root surface. Roots absorb some ions faster than the rate of supply via mass flow. Thus, a depleted zone is created in soil immediately adjacent to the root. This generates a concentration gradient directed from the bulk soil solution and soil particles holding the adsorbed elements, to the solution in contact with the root surface. This concentration gradient drives the diffusion of ions toward the depleted layer surrounding the roots.

2.4.6 Plant limitations and improving phytoremediating plants

When the concept of phytoextraction was reintroduced (approximately two decades ago), engineering calculations suggested that a successful plant-based decontamination of even moderately contaminated soils would require crops able to concentrate metals in excess of 1-2%. Accumulation of such high levels of heavy metals is highly toxic and would certainly kill

the common nonaccumulator plant. However, in hyperaccumulator species, such concentrations are attainable. Nevertheless, the extent of metal removal is ultimately limited by plant ability to extract and tolerate only a finite amount of metals. On a dry weight basis, this threshold is around 3% for Zn and Ni, and considerably less for more toxic metals, such as Cd and Pb. The other biological parameter, which limits the potential for metal phytoextraction, is biomass production. With highly productive species, the potential for biomass production is about 100 tons fresh weight/hectare. The values of these parameters limit the annual removal potential to a maximum of 400 kg metal/ha/yr. It should be mentioned, however, that most metal hyperaccumulators are slow growing and produce little biomass. These characteristics severely limit the use of hyperaccumulator plants for environment cleanup.

It has been suggested that phytoremediation would rapidly become commercially available if metal removal properties of hyperaccumulator plants, such as *T. caerulescens,* could be transferred to high-biomass producing species, such as Indian mustard (*Brassica juncea*) or maize (*Zea mays*) (Brown, et al., 1995). Biotechnology has already been successfully employed to manipulate metal uptake and tolerance properties in several species. For example, in tobacco (*Nicotiana tabacum*) increased metal tolerance has been obtained by expressing the mammalian metallothionein, metal binding proteins, genes (Lefebvre & Laliberte, 1987; Maiti, et al., 1991). Possibly, the most spectacular application of biotechnology for environmental restoration has been the bioengineering of plants capable of volatilizing mercury from soil contaminated with methyl-mercury. Methyl-mercury, strong neurotoxic agents, is biosynthesized in Hg contaminated soils. To detoxify this toxin, transgenic plants (*Arabidopsis* and tobacco) were engineered to express bacterial genes *merB* and *merA*. In these modified plants, *merB* catalyzes the protonolysis of the carbon-mercury bond with the generation of Hg^{2+}, a less mobile mercury species. Subsequently, *MerA* converts Hg (II) to Hg (0) a less toxic, volatile element which is released into the atmosphere (Rugh, et al., 1996; Heaton, et al., 1998). Although regulatory concerns restrict the use of plants modified with *merA* and *merB,* this research illustrates the tremendous potential of biotechnology for environment restoration. In an effort to address regulatory concerns related to phytovolatilization of mercury, Bizily et. al. (1999) demonstrated that plants engineered to express *MerBpe* (an organomercurial lyase under the control of a plant promoter) may be used to degrade methyl-mercury and subsequently remove ionic mercury via extraction (Bizily, et al., 1999). Despite recent advances in biotechnology, little is known about the

genetics of metal hyperaccumulation in plants. Particularly, the heredity of relevant plant mechanisms, such as metal transport and storage (Lasat, et al., 2000) and metal tolerance (Ortiz, et al., 1992; Ortiz, et al., 1995) must be better understood. Recently, Chaney et al. (1999) proposed the use of traditional breeding approaches for improving metal hyperaccumulator species and possibly incorporating significant traits such as metal tolerance and uptake characteristics into high biomass producing plants (Chaney, et al., 1999). Partial success has been reported in the literature. For example, in an effort to correct for small size of hyperaccumulator plants, Brewer et al. (1999) generated somatic hybrids between *T. caerulescens* (Zn hyperaccumulator) and *Brassica napus* (canola) followed by hybrid selection for Zn tolerance (Brewer, et al., 1999). High biomass hybrids with superior Zn tolerance were recovered. These authors have also advocated a coordinated effort to collect and preserve germplasm of accumulator species. A list plant species used for phytoremediation is provided in Appendix A.

2.5 Summary

Due to prevalence of contaminated soil in urban environment, phytoremediation has been used as a technique for in-situ remediation of contaminated soil. It is important to assess the effectiveness of remediation of heavy metal contaminated soil using locally available plants such as Indian Mustard (*Brassica juncea*) and Marigold (*Tagetes patula*). No previous study has been reported for studying effectiveness of these hyperaccumulator species for treatment of heavy metal contaminated soil in Bangladesh before.

Chapter 3

Methodology

3.1 Introduction

In this study an assessment of remediation of contaminated soil has been done using hyperaccumulator plant species, namely Indian Mustard and Marigold. In this chapter, collection scheme of Buriganga riverbed sediments, hyperaccumulator plant types, and experimental methods have been described. Buriganga riverbed sediments have been collected three times for assessment of heavy metal contamination. To demonstrate that both plant species (*Brassica juncea and Tagetes patula*) show better growth potential when planted in contaminated soil to that growth in non-contaminated soil (garden soil), a comparison of total growth of both plant types were assessed in terms of dry mass yield per unit surface area. After consultation with gardener of a local nursery, the final growth period of both plant types were fixed at 12 weeks. The 8-week time period was considered to study an intermediate accumulation scenario. After these time periods, plants were harvested from germination basket and divided into plants parts (roots, shoots and leaves). The elemental analysis of soil samples and plant samples were conducted according to Standard Methods.

3.2 Collection of the Buriganga Riverbed Sediments and Plants

Buriganga riverbed sediments were collected during the winter season of 2013. Riverbed sediments were collected on three different occasions from the location indicated in Figure 3.1 (having GPS coordinates: 23.72º N, 90.36º E) to assess the quality and heavy metal content of the sediments. Bulk amount of sediments were collected from channel bed of Buriganga at third time and the collected samples were air dried before using for plantation. Sediments from this sampling were used in laboratory experiments to assess uptake of heavy metal by Indian mustard and Marigold plants. Garden soils for control condition were collected from the garden adjacent to the premises of Civil Engineering Building, BUET. Indian mustard *(Brassica juncea)* seeds were obtained from Narayangonj district, Bangladesh. Marigold plants (*Tagetes patula*) seedlings were obtained from a local nursery situated in Farmgate, Dhaka. Both Indian mustard and Marigold plants are available in abundance in Dhaka city.

Figure 3.1 Sediment sampling location in the Buriganga River. Inset pictures show pipelines that are used to convey riverbed sediments for land filling purpose.

3.3 Experimental setup

Both Indian Mustard and Marigold plants were studied for heavy metal uptake from Buriganga riverbed sediments. The plantation scheme for Indian Mustard and Marigold plants for the present study are given below:

(1) Seeds of Indian Mustard were scattered in the germination basket to achieve a uniform density all over the plantation area of the germination basket.

(2) Seedlings of Marigold plants were planted in two different densities.

- Density 1: Six seedlings were planted in a germination basket (plan area is 52 × 32 cm). The depth of sediments in the germination basket was maintained at 28 cm.
- Density 2: Twelve seedlings were planted in a germination basket (plan area is 52 × 32 cm). This higher density of plantation was done to evaluate the effect of plant density on heavy metal uptake. The depth of sediments in the germination basket was kept as maintained in density 1.

The collected seeds of Indian mustard and seedlings of Marigold were planted in germination basket during winter season (November, 2013). The seeds were scattered and seedlings were individually planted in germination basket. The spacing between the seedlings was determined through consultation with experienced gardeners of a local nursery for optimum growth of the plants. The average high and low temperatures experienced by the Indian Mustard and Marigold plants (density 1) during its growth period were 26ºC and 13ºC, respectively. An average relative humidity of 50% existed in the surrounding environment during the growth of the plants. There was no rainfall after plantation. Germination baskets were kept at a protected place to prevent entry of birds and other animals. Soil moisture content was maintained at near the field capacity level by adding water periodically as required during the crop period. To study the effect of plant density on heavy metal uptake, another trial was carried out using twelve seedlings of Marigold plants (density 2) during the next winter season (December, 2014). The average high and low temperatures experienced by the Marigold plants (density 2) during its growth were 23ºC and 12ºC, respectively.

3.4 Plant Harvesting

Each germination basket, having a plan area of 52 cm × 32 cm, was filled with collected riverbed sediments from Buriganga River up to a depth of 28 cm. Both Indian mustard and Marigold plants were harvested after 8 and 12 weeks for measurement of heavy metal accumulation in different parts of the plants. Figure 3.2 and Figure 3.3 show Indian mustard and Marigold plants (density 1) immediately after plantation and before the 8-week and 12-week harvesting period. Plan area of the germination basket was divided into two parts and half of the plants were harvested after 8 weeks, while the remaining half were harvested after 12 weeks for Indian mustard plants. For Marigold plants, plan area of the germination basket was divided into three parts and one-third of the plants were harvested after 8 weeks, 10 weeks and 12 weeks. The 12-week time period was designed considering the usual growth and life cycle of the Indian mustard and Marigold plants in the climatic condition of Dhaka city, after discussion with gardeners and nursery owners. The 8-week time period was selected to study an intermediate growth rate and accumulation scenario in the plants. Similar germination baskets were prepared for both Indian mustard and Marigold plants using garden soil (control condition) mixed with cow dung (5% by weight) to evaluate growth tolerance of the plants in contaminated sediments. Figure 3.4 shows Marigold plants immediately after plantation and before the 8-week, 10-week and 12-week harvesting period. For the second

trial run of Marigold plant with an increased density (density 2), plan area of the germination basket was divided into four parts and one-fourth of the total sample was harvested after 6 weeks, 8 weeks, 10 weeks and 12 weeks.

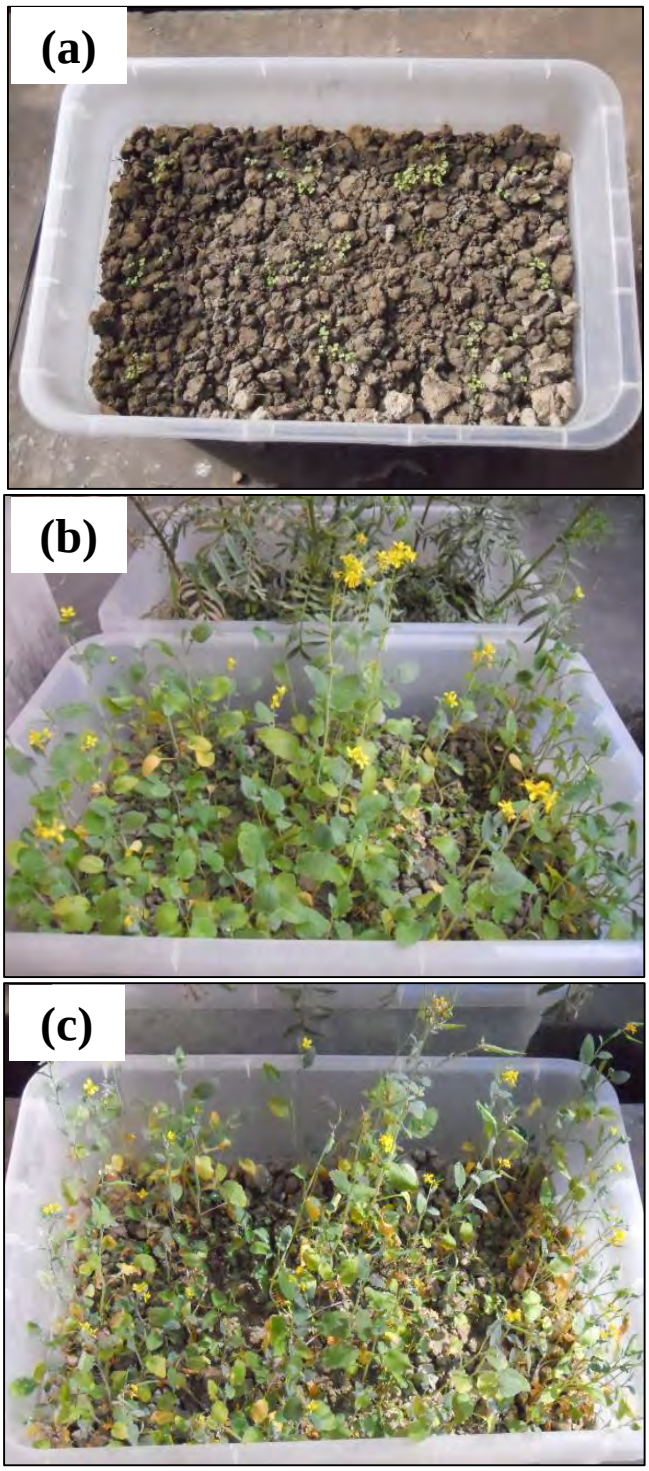

Figure 3.2. Indian mustard plants (a) after plantation, (b) before 8-week harvesting, (c) before 12-week harvesting in the germination baskets filled with heavy metal contaminated Buriganga riverbed sediments.

Figure 3.3. Marigold plants (density 1) (a) after plantation, (b) before 8-week harvesting, (c) before 12-week harvesting in the germination baskets filled with heavy metal contaminated Buriganga riverbed sediments.

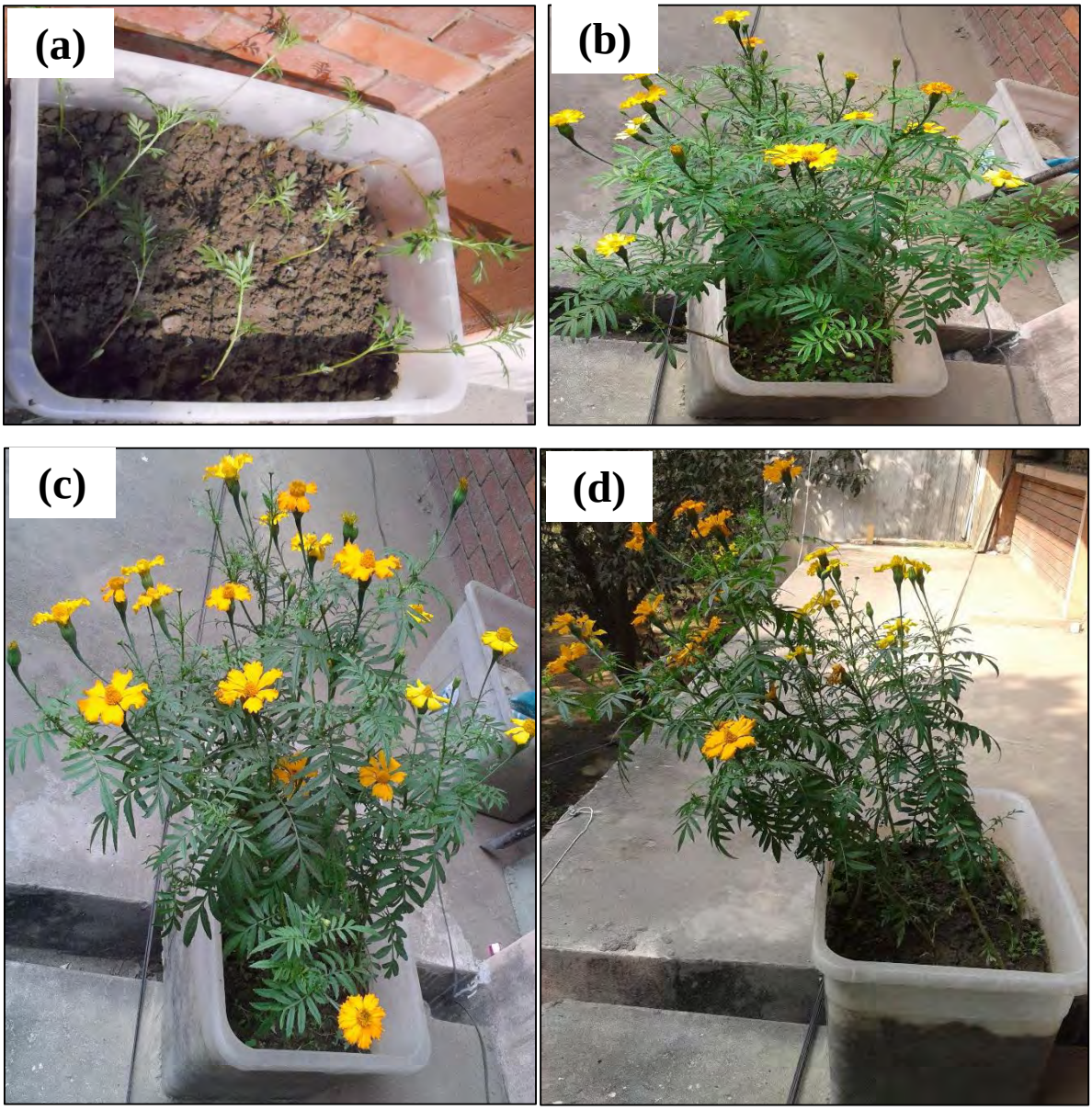

Figure 3.4. Marigold plants (density 2) (a) after plantation, (b) before 8-week harvesting, (c) before 10-week harvesting (d) before 12-week harvesting in the germination baskets filled with heavy metal contaminated Buriganga riverbed sediments.

3.5 Elemental Analysis

3.5.1 Digestion of Soil Sample

For determination of aqua-regia extractable metal, the soil samples were taken in aluminum bowl and kept in an oven at 110ºc for 24 hours. After drying for 24 hours, the sample was ground in a grinder. The grinded soil sample was digested with aqua-regia for extraction of metal ions. For digestion, 2.5 ml concentrated nitric acid and 7.5 ml concentrated hydrochloric acid were added to 5 gm grinded oven dried sample taken in a 500 ml volumetric flask. The sample was kept overnight in the flask and it was heated to boiling for two hours. Afterwards distilled water was added up to 500 ml graduation mark. The contents of the flask were stirred for 5 minutes, then cooled and finally filtered using a filter paper (0.45 micron). The filtrate was stored in a plastic bottle for analysis using an AAS or atomic absorption spectrophotometer (Shimadzu AA6800).

3.5.2 Digestion of Plant Sample

Before analysis, Indian mustard and Marigold plants were divided into three parts: (i) Leaf (ii) Root (iii) Shoot. For analysis of heavy metal, the different parts/ segments of the plant samples were digested separately.

A number of similar but different digestion procedures are available in the literature (Bennett, et al., 2000; Chen & Folt, 2000). At first three different digestion procedures were tested and compared (in term of extraction efficiency and reproducibility). The procedure reported in Shimadzu AAS Cookbook (Shimadzu Corporation, 2002) was found to be more satisfactory than the others and was selected for the study. First of all, the plant samples were washed with distilled water and plant sample was divided into parts (as described above). Weight of each part of the sample was determined and the sample was oven dried for 48 hours in aluminum bowl and the weight of oven dried sample was taken. Approximately 2 grams of oven-dried sample was taken in a volumetric flask and a few ml of distilled water was added, then 25 ml of nitric acid was added to the sample and kept overnight. The flask was heated to boiling for two hours, then after cooling the sample 10 ml of perchloric acid was added to the flask and heated again for one hour to boiling. If the color of the sample turns yellow, the digestion process is assumed to be completed; if color of the sample turns dark, 2 to 3 ml of nitric acid is added to the flask and heat is applied; the process is repeated until the sample color turns yellow. Finally distilled water was added up to the 200ml graduation mark of the

volumetric flask. The content of the flask was stirred for 5 minutes, then cooled and finally filtered using a filter paper (0.45 micron). The filtrate was used to find heavy metal concentration in the root, shoot, or leaf part of the plant sample by atomic absorption spectrophotometry using an AAS (Shimadzu, AA6800).

3.6 Limitations

One of the major limitations of the present study is the short period of winter season. The growth of Indian Mustard and Marigold plants is attributed to winter period which lasts from December month to February month. For this short duration, only a little uptake is possible by the selected plant species. However variations of some marigold plants can be planted throughout the year, however for this study the plants were planted only during the winter season.

Chapter 4

Results and Discussion

4.1 Characteristics of Buriganga Riverbed Sediments

Physical properties like specific gravity, liquid limit, plastic limit, plasticity index, organic content, silt content, and clay content in the Buriganga riverbed sediments and the garden soil (control condition) were measured and presented in Table 4.1. From the physical properties, it can be established that the sediments are clayey silt. The Unified Soil Classification System (USCS) classification of both the Buriganga riverbed sediment and the garden soil is CL (ASTM Standard D2487-11, 2011). The Indian mustard plant grows well in loamy-acidic soil, whereas the Marigold plant can be grown in a wide range of soils with well aeration, drainage, and moist condition (Gilman & Howe, 1999).

Table 4.1. Selected physical properties of Buriganga riverbed sediments and garden soils.

Soil Properties	Sample	
	Buriganga Riverbed Sediment	**Garden Soil**
LL (%)	46	49
PL (%)	23	18
PI (%)	23	31
Specific Gravity	2.67	2.70
OC (%)	4 – 6	8.8 – 9.4
Silt (%)	91.5	58
Clay (%)	8.5	17

*Cow dung was mixed at 5% by weight of garden soil in the germination basket at the beginning of the experiments.

Sediment pH ranges from 4.5 to 6.5, which indicates that the soil sample was circum-neutral to acidic. Electrical conductivity of contaminated sediments have a value of 684.5 ± 96.9 μS/cm. Table 4.2 shows the heavy metal concentrations in the garden soil and Buriganga riverbed sediments along with the Toxicity Reference Values (TRVs) of the heavy metals in freshwater bed sediments and in soil for terrestrial plants, and soil invertebrate (USEPA, 1999). From the values given in Table 4.2, it is evident that the concentrations of heavy metals (Pb, Cr, Cu, and Zn) in Buriganga riverbed sediments are very high for freshwater riverbed sediments. The TRV of these metal in soil (Table 4.2) shows that use of the Buriganga riverbed sediments for land filling purpose would pose great risk to both terrestrial plants and soil invertebrates.

Table 4.2. Concentrations (in mg/kg dry weights) of selected heavy metals in the Buriganga riverbed sediments[a].

Metal	Garden Soil (Control Condition)	Buriganga Riverbed Sediment (Present Study)	TRV in Freshwater Bed Sediment	TRV in Soil for Terrestrial Plant	TRV in Soil for Soil Invertebrate
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Lead (Pb)	1.3-4.2	22.8 – 47	31	4.6	100
Chromium (Cr)	0.8-1.3	116 – 167	26	0.018*	0.2*
Copper (Cu)	1.5-2.6	33.1 – 44.3	16	1	32
Zinc (Zn)	95-128	120 – 455	110	0.9	199

[a]: Screening Level Ecological Risk Assessment Protocol, Appendix E: Toxicity Reference Values, U.S. EPA, August 1999.

* Concentration value corresponds to hexavalent chromium only.

4.2 Growth Tolerance of Indian mustard and Marigold to Buriganga Riverbed Sediments

On harvesting Indian mustard plants from the contaminated sediments after 8 weeks, root and shoot lengths of the plants varied between 6-15 and 9-27 cm, respectively. After 12 weeks, root and shoot lengths of the plants, collected from the contaminated sediments, varied between 7-16.5 cm and 12-38 cm, respectively. On harvesting of Marigold plants (density 1) from the contaminated sediments after 8 weeks, root and shoot lengths of the plants varied between 9-15 cm and 43-45 cm, respectively. After 10 weeks, the same lengths varied between 10-12.5 cm and 44-48 cm, respectively for plants collected from the contaminated sediments. After 12 weeks, the same lengths varied between 12-18 cm and 48-58 cm, respectively for plants collected from the contaminated sediments. Leaves, shoots and roots were separated from harvested plants (from both contaminated sediment baskets and control condition baskets), washed with deionized water, oven dried at 105-110ºc for 2 days, grounded in a porcelain grinder and weighed to measure the dry mass yield after 8 weeks, 10 weeks and 12 weeks, respectively.

On harvesting of Marigold plants (density 2) from contaminated sediments after 8 weeks, root and shoot length of the plants varied between 8-15 cm and 38-44 cm, respectively. After 10 weeks, the same lengths varied between 10-12 cm and 40-48.5 cm, respectively for plants collected from the contaminated sediments. After 12 weeks, the same lengths varied between 14-19 cm and 52-62 cm, respectively for plants collected from the contaminated sediments. Similarly, leaves, shoots and roots were separated from harvested plants, washed with deionized water, oven dried at 105-110ºc for 2 days, grounded in a porcelain grinder and weighed to measure the dry mass yield after 8 weeks, 10 weeks and 12 weeks, respectively.

Dry mass yields per unit surface area (g/m^2) of both Indian mustard and Marigold plants in garden soil (control condition) were higher than that in contaminated soil after 12 weeks (Figure 4.1). However, both Indian mustard and Marigold attained about 92% and 95% overall growth, respectively, in the contaminated sediments when compared to their growth in control condition. Indian mustard attained a total above ground (shoots and leaves) dry weight per unit area in contaminated soil that is about 88% of that on garden soil. Similarly, Marigold attained a total above ground (shoots and leaves) dry weight per unit area in contaminated soil that is about 95% of that of Marigold grown on clean soil. From figure 4.1, it can be perceived that both Indian mustard and Marigold can demonstrate better growth potential when planted on highly contaminated sediments, compared to that on non-contaminated garden soil (control condition).

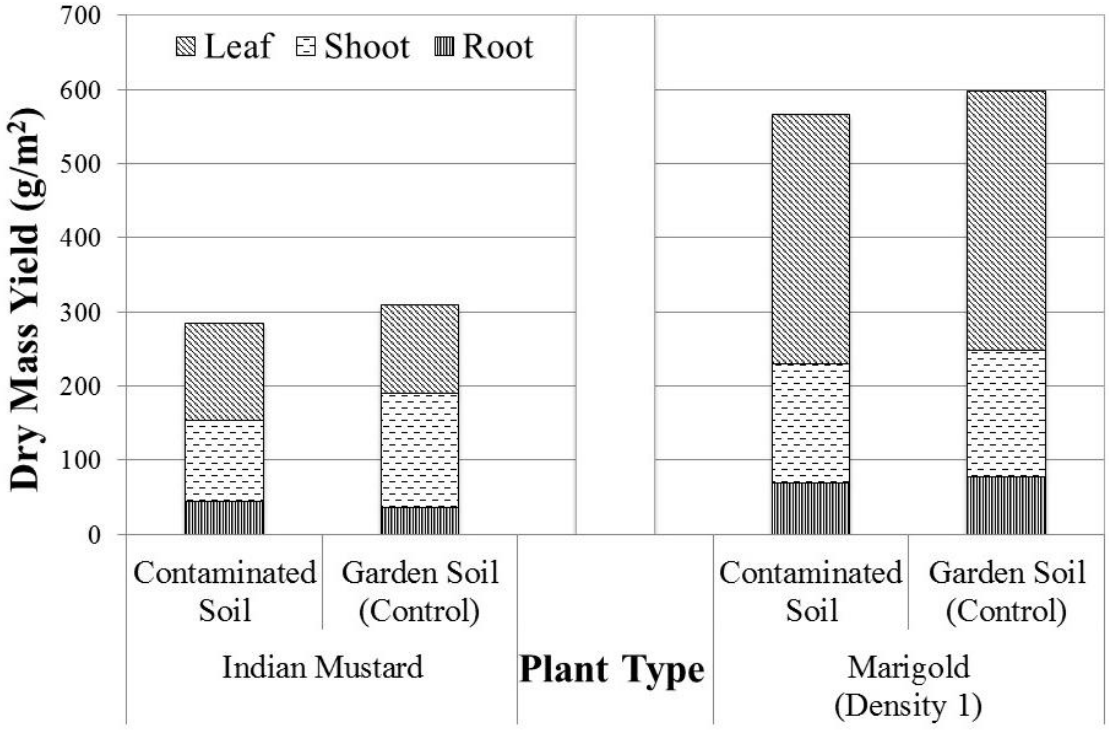

Figure 4.1. Dry Mass Yield of Indian Mustard and Marigold after 12 weeks of plantation. Comparison of dry weights of leaves, shoots and roots of plants grown on contaminated Buriganga riverbed sediments and non-contaminated garden soil (control condition).

On the other hand, comparison of dry mass yield per unit surface area (g/m^2) of both Indian mustard and Marigold plants in contaminated Buriganga riverbed sediments is shown in figure 4.2. Indian mustard and Marigold plants attained about 30% and 57% overall growth in the first eight weeks of growth, respectively, in the contaminated sediments when compared to their total growth in contaminated sediments. In the first eight weeks, Indian Mustard attained a total above ground (shoot and leaves) yield of 71.88 gm/m^2 which is about 30% of the above ground (shoot and leaves) yield attained in the twelve weeks (239.75 gm/m^2). Similarly, in the first eight weeks, Marigold plants attained a total above ground (shoot and leaves) yield of 263.91 gm/m^2 which is about 53% of the total above ground (shoot and leaves) yield attained in the twelve weeks which is 497.54 gm/m^2. From figure 4.2, it is well demonstrated that Marigold plant shows better growth potential when planted on highly contaminated sediments compared to Indian mustard plants.

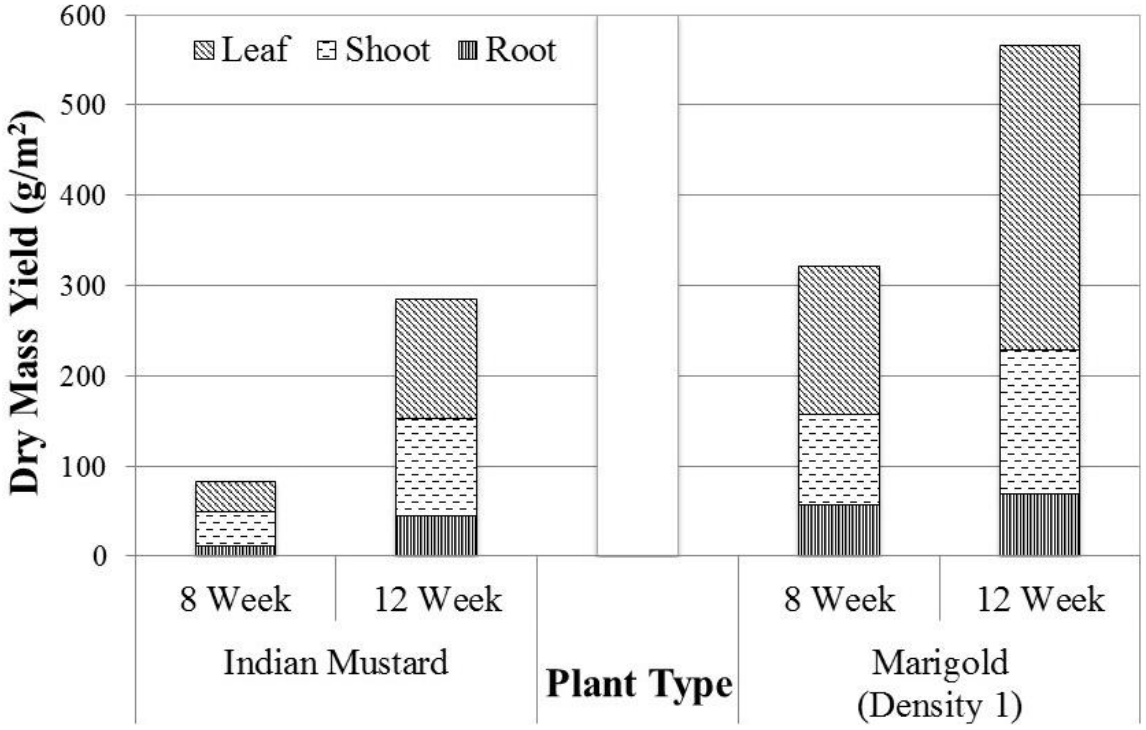

Figure 4.2. Dry Mass Yield of Indian Mustard and Marigold (Density 1) after 8 weeks and 12 weeks of plantation. Comparison of dry weights of leaves, shoots and roots of plants grown on contaminated Buriganga riverbed sediments between 8 weeks and 12 weeks.

Comparison of dry mass yield per unit surface area (g/m^2) of Marigold plants in contaminated Buriganga riverbed sediments for different density is shown in figure 4.3. Marigold plant (density 1) attained about 57% growth in the first eight weeks when compared to its total growth on contaminated sediments, whereas Marigold plant (density 2) attained about 26% growth in the first eight weeks when compared to its total growth on contaminated sediments. Marigold plant (density 1) attained about 93% growth in the first ten weeks when compared to its total growth on contaminated sediments, whereas Marigold plant (density 2) achieved about 49% growth in the first ten weeks when compared to its total growth on contaminated sediments based on dry mass yield of plant samples. From figure 4.3, it is well demonstrated that in density 1, rapid early growth was observed in Marigold plants, whereas in density 2, majority of plants growth took place in the last four weeks.

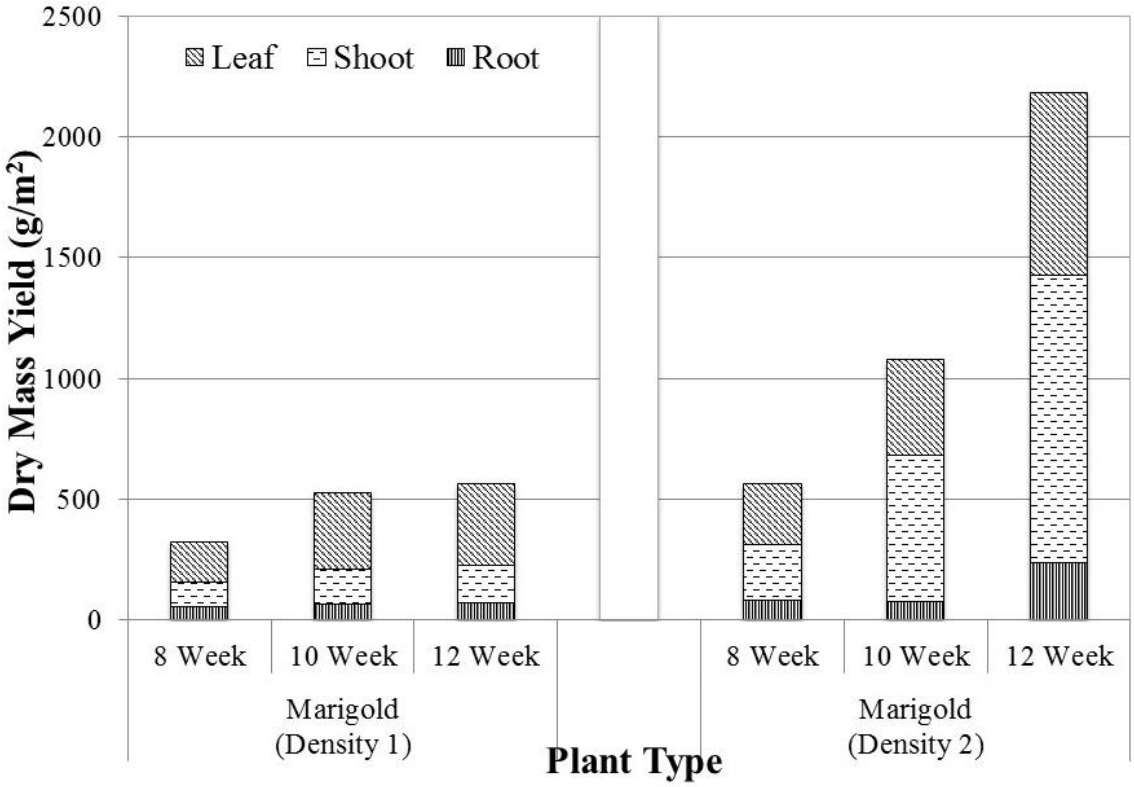

Figure 4.3. Dry Mass Yield of Marigold plants after 8 weeks, 10 weeks and 12 weeks of plantation. Comparison of dry weights of leaves, shoots and roots of plants grown on contaminated Buriganga riverbed sediments among 8 weeks, 10 weeks and 12 weeks.

4.3 Accumulation of Heavy Metals in Indian mustard and Marigold

4.3.1 Comparison of Heavy Metal Uptake by Indian mustard and Marigold plants

Figure 4.4 to figure 4.7 indicates the heavy metal uptake in different parts (leaf, shoot, and root) of Indian mustard and Marigold plants harvested from the contaminated Buriganga riverbed sediments and from the non-contaminated garden soil. The figure also indicates the heavy metal uptake data of two different times of harvesting, i.e. 8 weeks (corresponding to approximately 30% of full growth for Indian mustard and 57% of full growth for Marigold plant by dry mass yield) and 12 weeks (corresponding to full growth of the plants), from the contaminated Buriganga riverbed sediments. Duplicate leaf, shoot, and root samples were prepared and analyzed from each harvest for determination of heavy metal uptake in these parts. The average value of the duplicate analysis was used for preparing figure 4.4 to figure 4.7. It can be observed that for the four different types of heavy metals (i.e. chromium, lead, copper, and zinc) analyzed in this present study, very insignificant uptake of the heavy metals were observed for the plants harvested from the control condition experiments. This is due to the fact that the garden soil used in the control condition had very little heavy metal content.

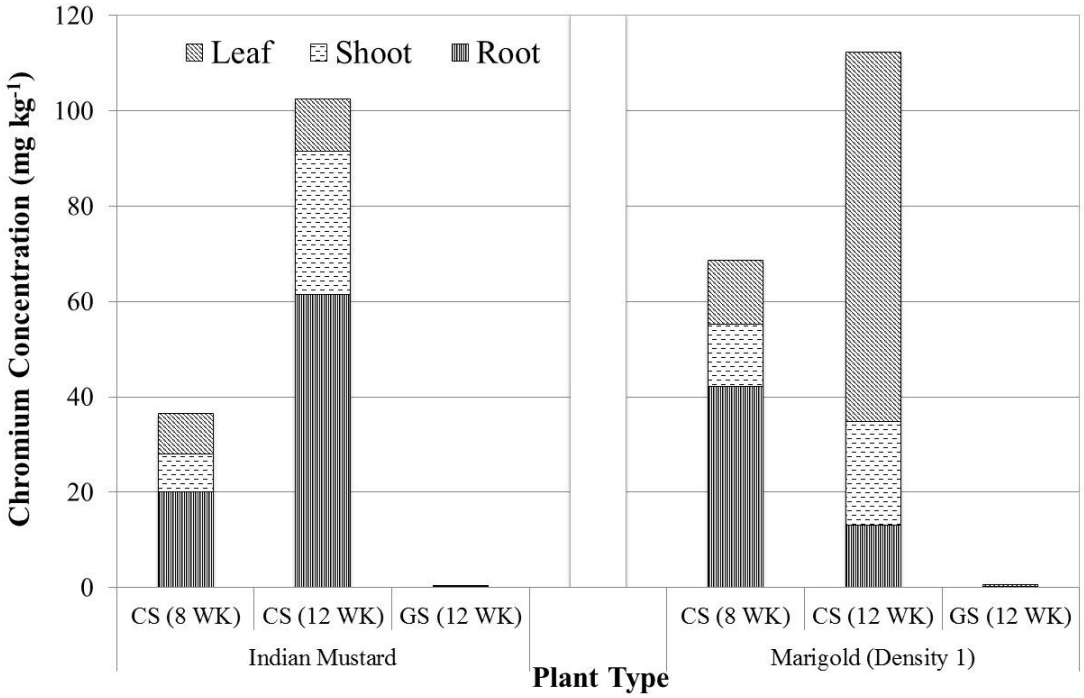

Figure 4.4. Chromium uptake in different parts (leaf, shoot, and root) of Indian mustard and Marigold plants harvested from the contaminated Buriganga riverbed sediment (CS) and from the non-contaminated (control condition) garden soil (GS).

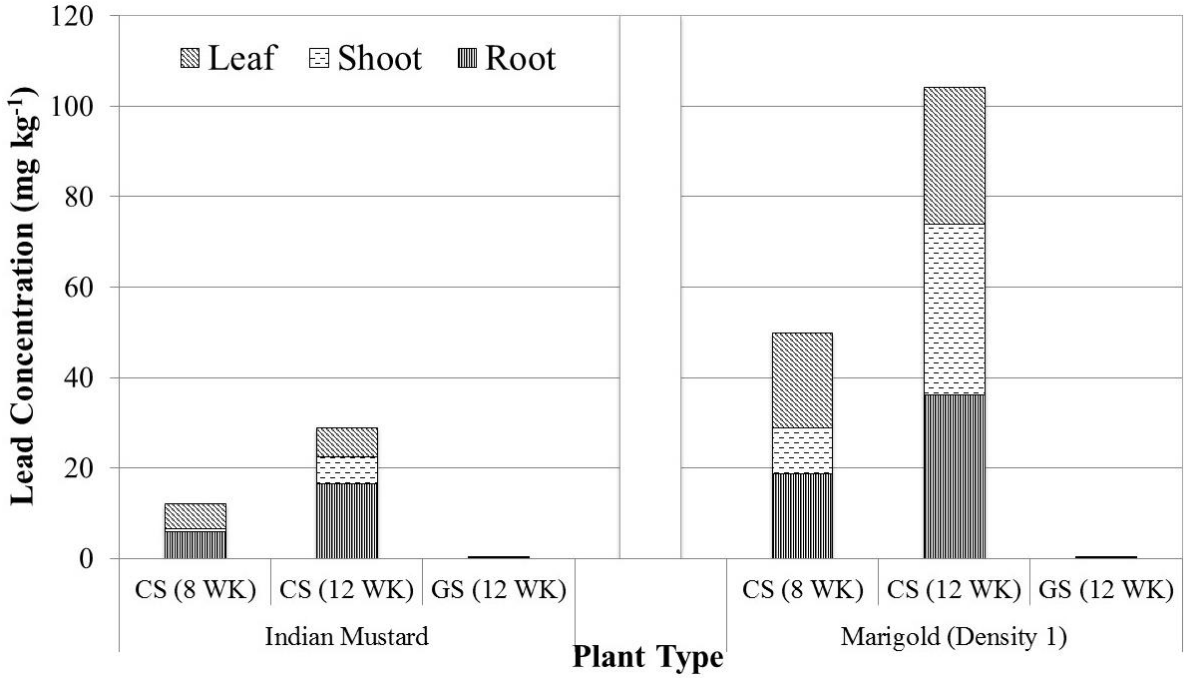

Figure 4.5. Lead uptake in different parts (leaf, shoot, and root) of Indian mustard and Marigold plants harvested from the contaminated Buriganga riverbed sediment (CS) and from the non-contaminated (control condition) garden soil (GS).

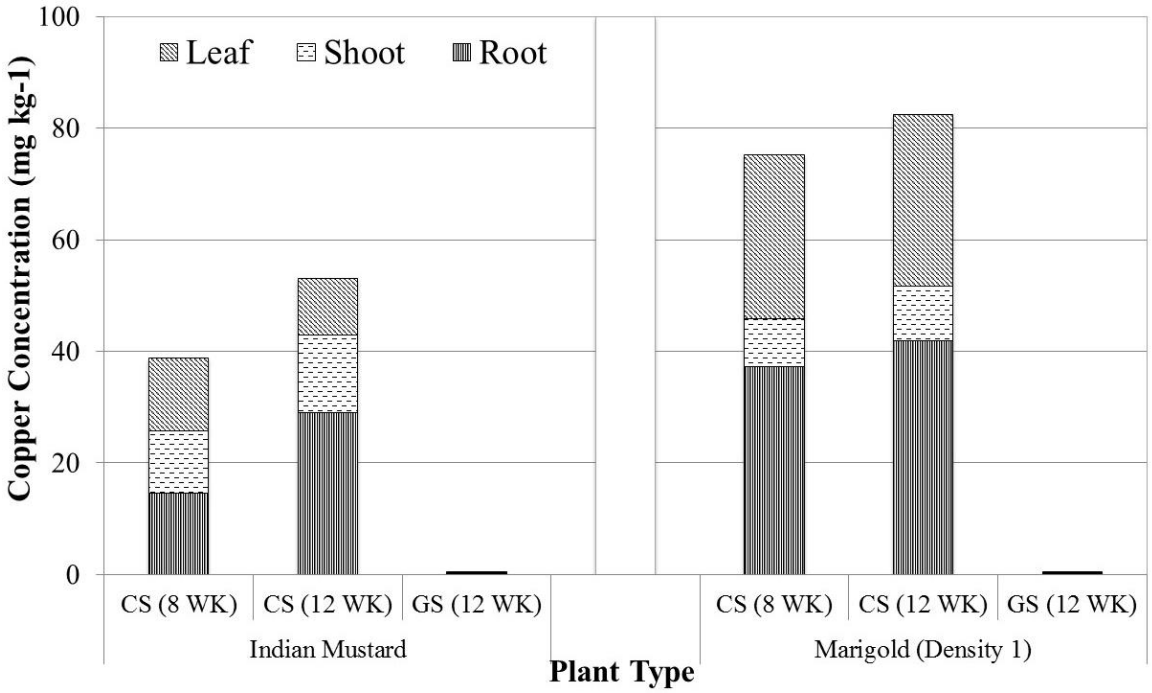

Figure 4.6. Copper uptake in different parts (leaf, shoot, and root) of Indian mustard and Marigold plants harvested from the contaminated Buriganga riverbed sediment (CS) and from the non-contaminated (control condition) garden soil (GS).

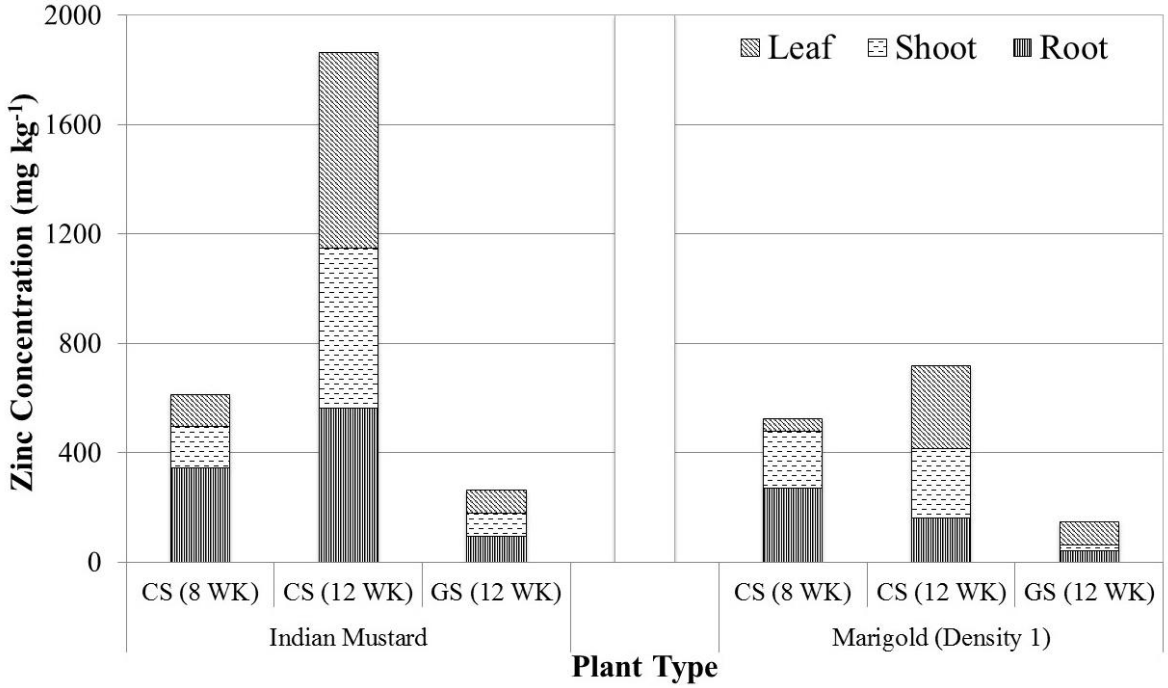

Figure 4.7. Zinc uptake in different parts (leaf, shoot, and root) of Indian mustard and Marigold plants harvested from the contaminated Buriganga riverbed sediment (CS) and from the non-contaminated (control condition) garden soil (GS).

Chromium, lead, and zinc uptake by Indian mustard plant in the first 8 weeks (corresponding to approximately 30% of total growth by dry weight yield) accounted for approximately 36%, 42%, and 33% of the total uptake observed at harvesting after 12 weeks. Only in case of copper an uptake of 73% was observed in the first 8 weeks by Indian mustard plant. This indicated that more heavy metal was phytoextracted by the Indian mustard during the last 4 weeks of its growth. For Marigold plants, uptake of chromium, lead, copper, and zinc in the first 8 weeks (corresponding to approximately 57% of total growth by dry weight yield) accounted for approximately 61%, 48%, 91%, and 73% of the total uptake, respectively, observed at harvesting after 12 weeks. This indicated a rapid phytoextraction by Marigold during the initial growth phase (first 8 weeks) of the plant. This may be due to rapid initial growth of the Marigold plant in the first 8 weeks after plantation. The distribution of total metal uptake from the sediments in the initial 8 weeks and final 4 weeks of the plant growth period for both Indian mustard and Marigold plants are given in Table 4.3. In a view of the fact that the Indian mustard and Marigold plants were in the germination basket for such a short period (i.e. 12 weeks) and the distribution of the roots of the plants were random within the sediment sample, detection of significant decrease in heavy metal in the sediment samples

seemed unlikely (Bañuelos, et. al., 2005). Hence no attempt was made to measure post-harvesting changes of heavy metals in soil.

Table 4.3. Distribution of total metal uptake (in mg/kg of plant dry weight) from the sediment by Indian mustard and Marigold plants.

Heavy Metal	Heavy metal uptake by Indian mustard		Heavy metal uptake by Marigold	
	Week 1-8[a]	**Week 9-12[b]**	**Week 1-8[a]**	**Week 9-12[b]**
Chromium	36.5 *(36%)*	66.1 *(64%)*	68.7 *(61%)*	43.6 *(39%)*
Lead	12.1 *(42%)*	16.8 *(58%)*	49.9 *(48%)*	54.4 *(52%)*
Copper	38.8 *(73%)*	14.2 *(27%)*	75.2 *(91%)*	7.3 *(9%)*
Zinc	610.5 *(33%)*	1251.1 *(67%)*	525.2 *(73%)*	191.6 *(27%)*

Note: [a]Week 1-8 corresponds to approximately 30% of full growth for Indian mustard and 57% of full growth for Marigold plant by dry mass yield. [b]Week 9-12 corresponds to approximately 70% of full growth for Indian mustard and 43% of full growth for Marigold plant by dry mass yield. The value in the parenthesis indicates percentage of total metal uptake in the Week 1-8 and Week 9-12 of plant growth period.

Chromium, lead, and copper accumulation per unit dry mass of Marigold plant were higher than that of Indian mustard. Only zinc accumulation per unit dry mass of Indian mustard was found to be higher than that of Marigold plant. The total uptake of chromium, lead, copper, and zinc by Marigold was found to be 2.7, 5.6, 2.4, 0.6 times of that by Indian mustard, respectively, considering the dry mass yield of the plants. Hence, it is evident that Indian mustard showed higher affinity in extracting zinc from the sediments, while Marigold showed higher affinity in extracting chromium, lead, and copper from a given surface area of contaminated sediments. Some other observations were found from different studies. For example, Pb is extremely insoluble and not generally available for plant uptake in the normal range of soil pH. Thus, vegetation growing in heavily contaminated areas often has less than 50 mg/kg Pb in shoots. Even plants that have a genetic capacity to accumulate Pb (e.g. *B.*

juncea) will not contain much Pb in roots or shoots if cultivated in Pb-contaminated soil. The solution to the metal availability problem came with the discovery that certain soil-applied chelating agents greatly increase the translocation of heavy metals, including Pb, from soil into the shoots. EDTA (Ethylenediaminetetraacetic acid) was particularly effective in facilitating the phytoextraction of Cd, Cu, Ni, Pb and Zn. The application of 10 mmol kg^{-1} of EDTA to soil containing 1200 mg/kg Pb resulted in a 1.6% Pb accumulation in the shoots of *B. juncea*. These values are much higher than the root, shoot and leaf concentration obtained from the present study (Blaylock, et. al., 1997).

Chromium uptake by roots of wild type Indian Mustard was found to be more than 1500 mg/kg whereas uptake of chromium by shoots of wild type Indian Mustard was reported to be only about 220 mg/kg from another study (Reisinger, et. al., 2008). Another study revealed the total accumulation of Pb (47.3-64.5 mg/kg) and Cr (16.0-41.4 mg/kg) by Marigold plants when the experiment was carried out in small pot. No chelating agents were added during the experimental procedure (Huq, et. al., 2005). Another study showed chelating agents enhanced accumulation of Zn, Cu, and Pb by roots, shoots and leaves as compared to control condition by Marigold plants. Among the heavy metals, Zn accumulated in the largest amount (527 mg/kg of plant dry weight) followed by Cu (443.14 mg/kg of plant dry weight), Pb (393 mg/kg of plant dry weight) in plants (Sinhal, et. al., 2010). Whenever chelating agents are used, enhanced phytoextraction has been observed.

Similar kind of study was carried out using vegetables such as Spinach, Red Amaranth and Amaranth. Spinach showed an accumulation of 4.78 mg/kg of Pb, 23.9 mg/kg of Cr; Red Amaranth showed an uptake of 5.0 mg/kg of Pb, 22.4 mg/kg of Cr; and Amaranth showed an uptake of 5.16 mg/kg of Pb, 21.9 mg/kg of Cr (Naser, et. al., 2011). These values are much lower than the values obtained from the present study which indicates that Indian Mustard and Marigold plants are much efficient than Spinach, Red Amaranth and Amaranth in extracting heavy metals from the contaminated soil.

4.3.2 Comparison of Heavy Metal Uptake by Different Density of Marigold Plants (Density 1 and Density 2)

Figure 4.8 to figure 4.11 indicates the heavy metal uptake in different parts (leaf, shoot, and root) of Marigold plants (both density 1 and density 2) harvested from the contaminated Buriganga riverbed sediments and from the non-contaminated garden soil. The figure also indicates the heavy metal uptake data of two different times of harvesting, i.e. 8 weeks (corresponding to 57% of full growth for Marigold plant on the basis of dry mass yield for density 1 and 26% of full growth for Marigold plant on the basis of dry mass yield for density 2), 10 weeks (corresponding to 93% of full growth for Marigold plant on the basis of dry mass yield for density 1 and 49% of full growth for Marigold plant on the basis of dry mass yield for density 2) and 12 weeks (corresponding to full growth of the plants), from the contaminated Buriganga riverbed sediments. Duplicate leaf, shoot, and root samples were prepared and analyzed from each harvest for determination of heavy metal uptake in these parts. The average value of the duplicate analysis was used for preparing figure 4.8 to figure 4.11. It can be observed that for the four different types of heavy metals (i.e. chromium, lead, copper, and zinc) analyzed in this present study, very insignificant uptake of the heavy metals were observed for the plants harvested from the control condition experiments like previous observations. This is due to the fact that the garden soil used in the control condition had very little heavy metal content.

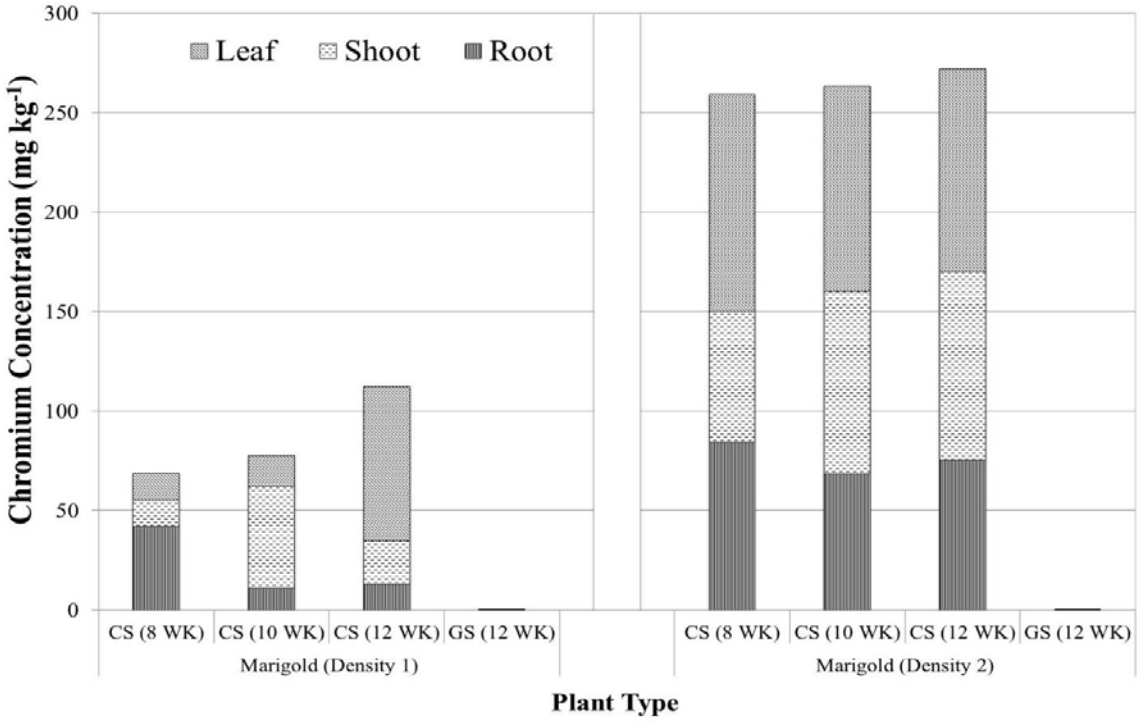

Figure 4.8. Chromium uptake in different parts (leaf, shoot, and root) of Marigold plants (Density 1 and Density 2) harvested from the contaminated Buriganga riverbed sediment (CS) and from the non-contaminated (control condition) garden soil (GS).

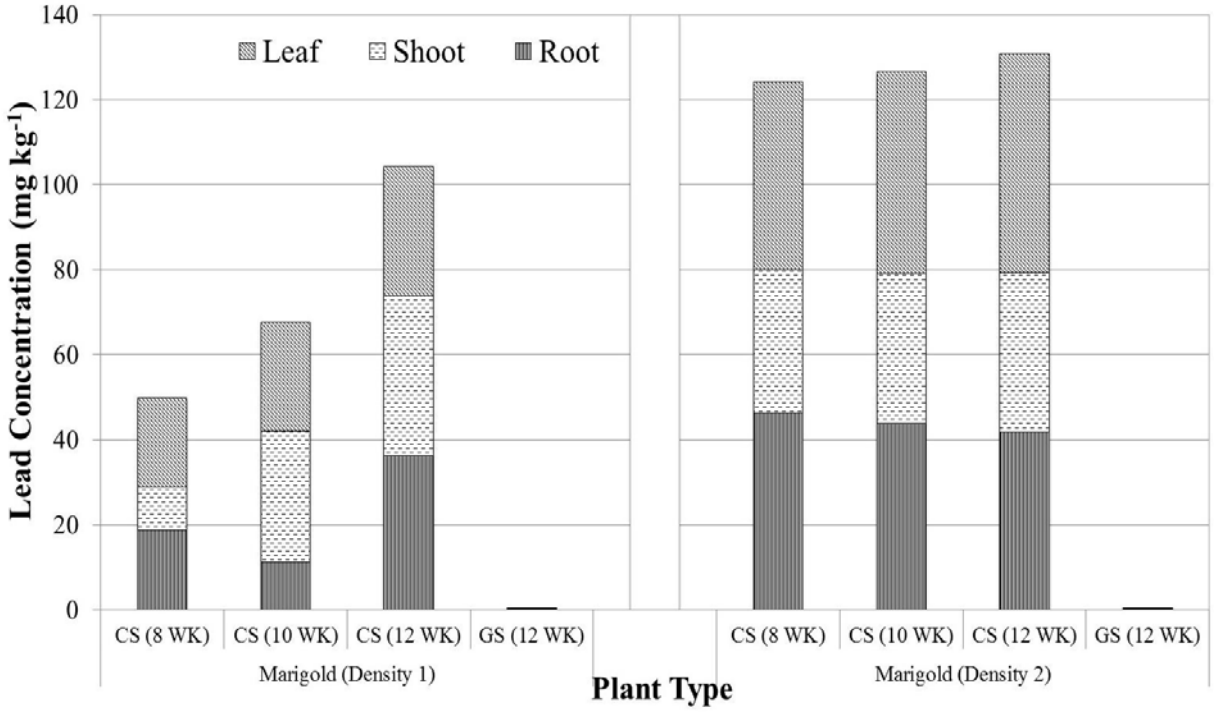

Figure 4.9. Lead uptake in different parts (leaf, shoot, and root) of Marigold plants (Density 1 and Density 2) harvested from the contaminated Buriganga riverbed sediment (CS) and from the non-contaminated (control condition) garden soil (GS).

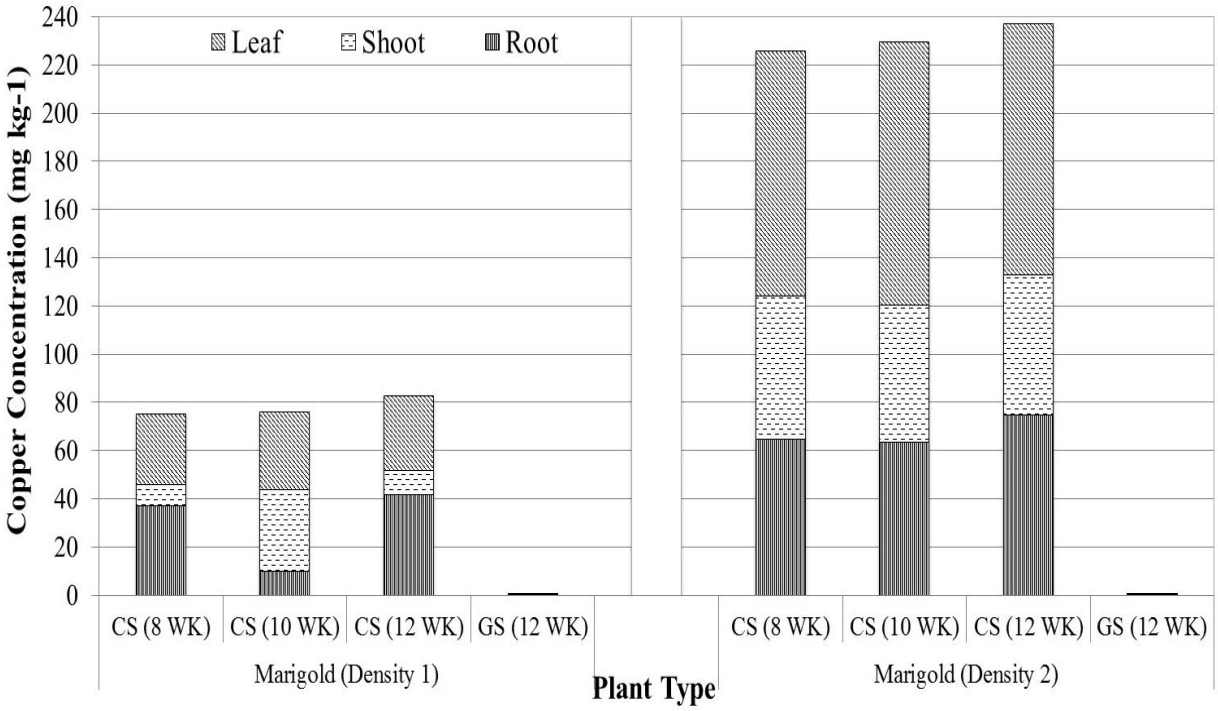

Figure 4.10. Copper uptake in different parts (leaf, shoot, and root) of Marigold plants (Density 1 and Density 2) harvested from the contaminated Buriganga riverbed sediment (CS) and from the non-contaminated (control condition) garden soil (GS).

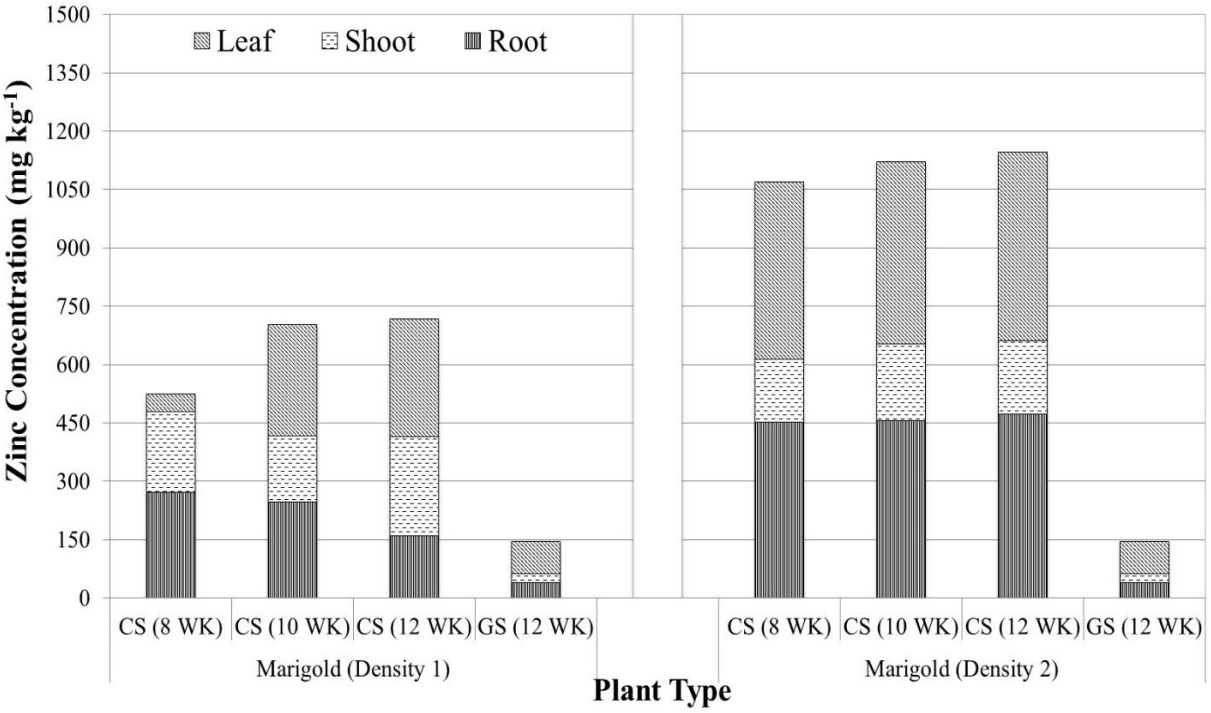

Figure 4.11. Zinc uptake in different parts (leaf, shoot, and root) of Marigold plants (Density 1 and Density 2) harvested from the contaminated Buriganga riverbed sediment (CS) and from the non-contaminated (control condition) garden soil (GS).

Heavy metal uptake of chromium, lead, copper, and zinc in the first 8 weeks (corresponding to approximately 57% of the total growth based on dry mass yield) accounted for approximately 61%, 48%, 91%, and 73% of the total uptake, respectively, by Marigold plants (density 1). On the other hand, chromium, lead, copper uptake accounted for approximately 95% of the total uptake and zinc uptake accounted for 93% of the total uptake, respectively, in the first 8 weeks (corresponding to 26% of the total growth based on dry mass yield) by Marigold plants (density 2). The distribution of total metal uptake (in mg/kg of plant dry weight) from the sediment in the initial 8 weeks and final 4 weeks of the plant growth period for Marigold plants with different density is shown in table 4.4. Form these observations it can be concluded that during overall growth of the plant, slower growth rate was observed during period of high heavy metal uptake though Marigold plant is a hyper-accumulator species. The total uptake of chromium, lead, copper and zinc by Marigold plant (density 2) was found to be 6.8, 5.0, 11.2 and 4.6 times of that Marigold plant (density 1), respectively, considering the dry mass yield of the plants. It can be concluded that density is not directly/ linearly related to total uptake of heavy metal. This may be due to the uneven distribution of root in different zones of soil with varying heavy metal concentrations.

Table 4.4. Distribution of total metal uptake (in mg/kg of plant dry weight) from the sediment by Marigold plants with different density.

Heavy Metal	Heavy metal uptake by Marigold (Density 1)		Heavy metal uptake by Marigold (Density 2)	
	Week 1-8[a]	**Week 9-12[b]**	**Week 1-8[c]**	**Week 9-12[d]**
Chromium	68.7 *(61%)*	43.6 *(39%)*	259.4 *(95%)*	12.7 *(5%)*
Lead	49.9 *(48%)*	54.4 *(52%)*	124.2 *(95%)*	6.8 *(5%)*
Copper	75.2 *(91%)*	7.3 *(9%)*	225.8 *(95%)*	11.5 *(5%)*
Zinc	525.2 *(73%)*	191.6 *(27%)*	1069.4 *(93%)*	76.6 *(7%)*

Note: [a]Week 1-8 corresponds to approximately 57% of full growth for Marigold (Density 1) plant by dry mass yield. [b]Week 9-12 corresponds to approximately 43% of full growth for Marigold plant (Density 1) by dry mass yield. [c]Week 1-8 corresponds to approximately 26% of full growth for Marigold plant (Density 2) by dry mass yield. [d]Week 9-12 corresponds to 74% of full growth for Marigold plant (Density 2) by dry mass yield. The value in the parenthesis indicates percentage of total metal uptake in the Week 1-8 and Week 9-12 of plant growth period.

Chapter 5
Conclusion and Recommendation

5.1 Introduction

In this study an assessment of remediation of contaminated soil was done using hyperaccumulator plant species, namely Indian Mustard and Marigold. For this purpose, Buriganga riverbed sediments have been collected three times for assessing heavy metal contamination in the soil. A comparison of total growth of both plants, Indian Mustard and Marigold, was done in heavy metal contaminated soil samples to that in normal garden soil to demonstrate that both plant species show better growth in contaminated soil and can be used for remediation purpose. After certain period of plant growth, plants were harvested and divided into different parts (roots, shoots and leaves) for assessing heavy metal uptake by both plants from contaminated sediments.

5.2 Conclusion

The results of the present research works are summarized below:

1. Indian mustard (*B.juncea*) and Marigold (*T.patula*) plants were able to accumulate heavy metals in different parts of the plant from heavy metal contaminated sediments and at the same time maintain a growth rate of more than 90% when compared to similar plants grown in non-contaminated soil.
2. The growth rate of Indian mustard was slow (about 30%) in the first 8 weeks after plantation and almost 70% of total growth took place in the last 4 weeks of plantation. This resulted in more phytoextraction of heavy metals (chromium, lead, and zinc) by the Indian mustard during the last 4 weeks of its growth. Extraction of lead was relatively higher in the initial growth phase for Indian mustard. On the other hand Marigold plant showed higher growth rate (about 57%) in the first 8 week time period and higher rate of phytoextraction of chromium, copper, and zinc in this time period. Lead extraction rate by Marigold plant followed relatively similar trends of observed plant growth rate.
3. Higher rate of phytoextraction of chromium, lead, copper and zinc was observed by Marigold plants with an increased density in the initial 8 weeks of growth considering only 26% of total growth of Marigold plants.

4. Marigold plants were found to be more efficient in extracting heavy metals (except for zinc, for which Indian mustard plant showed higher uptake efficiency) from a given surface area of the sediments.

5.3 Recommendation

More studies are needed to better understand the uptake of heavy metal by hyperaccumulator species in climatic condition of Bangladesh. It is further recommended to study enhanced phytoremediation by application of Chelating agents for bioremediation of heavy metal contaminated sediments. The potential of remediating of heavy metal contaminated soil using *Ethylenediaminetetraacetic Acid* (EDTA) for increased uptake of heavy metals such as Pb, Cr, Cu, Ni and Zn by different plant parts can be further investigated. No previous study has been reported for enhanced phytoremediation using chelating agents for remediation of heavy metal contaminated soil in Bangladesh. It is also recommended to study potential effects of phytoremediation through repeated cycle on the same contaminated soil and therefore, correlation between percentage decrease of heavy metal in soil and heavy metal concentration in different plant parts can be established. Potential application of other variations of marigold plants in other seasons throughout the year can also be observed to study its efficiency in remediating heavy metal contaminated soil throughout the year.

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Appendix A
List of Plant Species Used for Phytoremediation

Table 1A. Different Plant Species Studied for Phytoremediation

Grasses/Legumes			
Species/Common Name	**Contaminant**	**Process**	**Comments**
Agropyron smithii Western Wheat Gr ass	Hydrocarbons	Rhizodegradation	Perennial grass used in pastures/lawns; shown in studies to enhance degradation of TPH and PAH in soils.
Agrostis castellana Colonial bentgrass	Metals	Hyperaccumulation	Perennial *A. castellana* has been shown to accumulate As, Pb, Zn, Mn, and Al
Bouteloua gracilis Blue gamma grass	Hydrocarbons	Rhizodegradation	Used for low-water use lawn and pasture grass. Has shown promise in grass mixes to enhance degradation of PAHs in soils.
Buchloe dactyloides Buffalo grass	Hydrocarbons	Rhizodegradation/ Accumulation	Perennial grass; low maintenance, drought tolerant lawn requiring little/ no mowing. In studies has been shown to reduce TPH and PAHs in soil.
Cerastium arvense Field chickweed	Cadmium	Uptake/ accumulation	Tufted perennial, white flowers. A Northwest (NW) native, a recent study on Vashon Island indicated uptake of Cadmium (Institute of Environmental Research and Education, 2003). Additional chickweed varieties found in the NW include *C. beringianum* (Bering chickweed) and *C. fischerianum* (Fisher's chickweed).
Claytonia perfoliata Miner's lettuce	Cadmium	Uptake/ accumulation	A somewhat succulent annual with white or pink flowers. Also known as *Montia perfoliata*. A smaller attractive variety is *Montia spathulata*. A recent study on Vashon Island indicated uptake and accumulation of Cadmium (Institute of Environmental Research and Education, 2003).
Cynodon dactylon	Hydrocarbons	Rhizodegradation/ Accumulation	Lawn grass; minimum maintenance but needs mowing and can be invasive. In studies where mixed with other grasses, it has reduced TPH

Bermuda grass	and PAHs in soils.

Grasses/Legumes			
Species/Common Name	**Contaminant**	**Process**	**Comments**
Elymus Canadensis Canadian wild rye	Hydrocarbons	Rhizodegradation/ Accumulation	In combination with grasses, was shown to reduce PAH in soils. *E. mollis* is a NW native wild rye.
Festuca arundinacea Tall Fescue	Pyrene, PAHs	Rhizodegradation/ Accumulation	Introduced perennial grass common in the NW; studies have shown enhanced degradation of recalcitrant PAHs (McCutcheon, 2003). Also helpful in uptake of nutrients, nitrogen, phosphorous, and Potassium.
Festuca rubra Red Fescue	Hydrocarbons	Rhizodegradation	Perennial grass often used in lawn mixes; studies have shown enhanced degradation of TPH and PAHs.
Lolium perenne English ryegrass	Hydrocarbons/ Nutrients	Rhizodegradation/ uptake	Perennial grass shown to uptake nutrients and to significantly enhance degradation of TPH and PAHs in soil.
Lupinus albus White lupin	Arsenic	Rhizoaccumulation	A nitrogen fixing legume capable of growth in acidic soils with low nutrients availability. A recent study indicated an ability to take up arsenic, primarily stored in the root structure (Esteban, Vazquez & Carpena, 2003). A number lupine varieties are native to the NW, including *Lupinus arcticus* (Artic lupine), *L. littoralis* (Seashore lupin), *L. nootkatensis* (Nootka lupine)
Lotus corniculatus Birds-foot trefoil	Hydrocarbons	Rhizodegradation/ Accumulation	An introduced European annual herb; when mixed with grasses was shown to reduce TPH and PAHs in soils (McCutcheon & Schnoor, 2003). This plant is generally not recommended for introduction into constructed wetlands of Puget Sound Region.

Grasses/Legumes			
Species/Common Name	**Contaminant**	**Process**	**Comments**
Melilotus officinalis Yellow sweet clover	Hydrocarbons	Rhizodegradation	Tall, sweet smelling annual; M. alba is more common in NW region. When mixed with other grass was shown to degrade TPH in soils (McCutcheon & Schnoor, 2003). Also helpful in uptake of nutrients: nitrogen, phosphorous and potassium.
Panicum virgatum Switch grass	Hydrocarbons	Rhizodegradation	Enhance degradation of PAHs in soils. *P. occidentale* is a species found in the NW.
Stellaria calycantha Northern starwort	Cadmium	Uptake/ Accumulation	Low sprawling perennial. A number of varieties are common in the NW including *S. longifolia* (long leaved starwort) and *S. longipes* (long stalked starwort). A recent study on Vashon Island indicated uptake and accumulation of Cadmium.
Stenotaphrum secundatum St. Augustine grass	Hydrocarbons	Rhizodegradation	Perennial grass often used in lawns; coarse-textured. Decreases TPH and PAHs in soils.
Trifolium pratense Red Clover	Hydrocarbons	Rhizodegradation	Introduced perennial herb common in the NW. When mixed with other grass was shown to degrade TPH in soils.
Trifolium repens White clover	Hydrocarbons, PCBs	Rhizodegradation/ Metabolization	Introduced perennial herb, deep rooting; enhances microbial activity and degradation of PAHs, nitrogen fixer and PCB metabolizer.
Vicia spp. Vetch	Nutrients/ Metals	Uptake	Perennial herb, takes up nutrients (nitrogen, phosphorous, and potassium); *V. faba* has been shown to accumulate Al.

Other Forbes			
Species/Common Name	**Contaminant**	**Process**	**Comments**
Achillea millefolium Yarrow	Cadmium	Uptake/ Accumulation	Perennial aromatic herb native to the NW. Also known as *A. borealis*. A recent study on Vashon Island indicated uptake and accumulation of Cadmium.
Allium schoenoprasum Chives	Cadmium	Hyperaccumulation	Perennial onion relative. A recent agricultural study in Israel indicated Cd was accumulated in roots and leaves.
Atriplex hortensis Garden Orach	PCBs	Metabolism	Of the spinach family, Orache is an extremely variable species; *A. patula* (Spearscale), *A. subspicata* common in the NW. Shows promise transforming PAH and Garden Orach metabolizes PCBs.
Brassica juncea Indian Mustard	Metals	Rhizofiltration/ Hyperaccumulation	Various species applicable for removing heavy metals (Pb, Cr, Cd, Cu, Ni, Zn and Ur) from soil or water (McCutcheon & Schnoor, 2003). *B. campestris* (also known as *B. rapa*) and *B. camestris* are common annual herb species in the NW.
Brassica rapa Field Mustard	Cadmium, Zinc	Hyperaccumulation	Known to accumulate metals.
Digitalis purpurea Common Foxglove	Cadmium	Phytoextraction	A recent study on Vashon Island indicated uptake of Cadmium; *D. lanata* (Grecian foxglove) shown to transform digitoxigenin.
Helianthus annuus Sunflower	Metals, PAHs	Extraction/ Metabolism, Rhizodegradation	The common sunflower has been the subject of numerous studies and is used to extract heavy metals (Pb, Cr, Cd, Cu, Ni, Zn, Ur, Mn, Sr, and Cs). Has shown promise in degrading PAHs in soil.
Pteris vittata Brake Fern	Arsenic	Hyperaccumulation	*P. vittata* accumulates arsenic in its above ground shoots.

Other Forbes			
Species/Common Name	**Contaminant**	**Process**	**Comments**
Senecia glaucus	Crude Oil	Rhizodegradation	Observed to rhizodegrade crude oil Kuwait; *Senecio triangularis* (Arrow leaved groundsel), *S. pseudoarnica* (Beach groundsel), and *S. intergerrimus* (Western groundsel) are among the related perennial herbs in the NW.
Solidago hispida Hairy Golden Rod	Metals	Hyperaccumulation	Shown to accumulate *Al. Solidago* species shows promise for metabolizing TCE (McCutcheon & Schnoor, 2003). Related NW species include *S. Canadensis* (Canada goldenrod) and *S. multiradiata* (Northern Goldenrod).
Thlaspi caerulescens Alpine pennycress	Cadmium, Zinc, Nickel	Hyperaccumulation	The plant is well recognized for its ability to hyperaccumulate metals. *T. arvense* (Field pennycress) is common NW annual weed.

Trees, Shrubs and Vines **Species/Common Name**	**Contaminant**	**Process**	**Comments**
Acer rubrum Red maple	Leachate	Uptake	Fairly fast growing deciduous trees that have been utilized to uptake land fill leachate along with hybrid poplars (McCutcheon & Schnoor, 2003). NW species include *A. macrophyllum* (organ maple), *A. circinatum* (Vine maple) and *A. glabrum* (rocky mountain maple).
Betula pendula European White Birch	PAHs, PCBs	Phytodegradation	Attractive European native, has been shown in laboratory tests to degrade PAHs and PCBs in solution.

Trees, Shrubs and Vines **Species/Common Name**	**Contaminant**	**Process**	**Comments**
Gleditsia triacanthos Honey locust	Lead	Phytoextraction	Common honey locust (many cultivars available) has shown promise in the extraction and accumulation of lead.
Ilex spp. Holly	Cadmium	Accumulation	Evergreen shrub or tree. Recently shown to take up and accumulate Cadmium.
Liquidambar styraciflua American sweet gum	Perchlorate	Phytodegradation/ Rhizodegradation	A native of eastern U.S. grows to 60 ft. and is tolerant of damp soil. Has shown promise for phytoremediation of perchlorate.
Maclura pomifera Osage Orange	PCBs	Rhizodegradation	A deciduous tree that can withstand heat, cold, wind, drought, and poor soil condition. Roots have been shown to stimulate PCB-degrading bacteria in the soil.
Morus rubra Mulberry	PAHs PCBs	Rhizodegradation	The mulberry is one of few trees producing phenolic compounds stimulating PCB degrading bacteria, and thus enhances the degradation of this pollutant. Mulberry has also shown in the lab to degrade PAHs.
Populus spp. Poplars	Chlorinated solvents, PAHs, atrazine, DDT, Carbon tetrachloride.	Phytodegradation, Phytoextraction	Deciduous trees known for deep rooting and rapid growth. The focus of major attention in the field of phytoremediation, hybrids and clones has been developed for very fast growth and colonization. Poplars can absorb nutrients, such as nitrogen, at a high rate and are used in treatment of land applications of wastewater (McCutcheon & Schnoor, 2003). Known to take up and transform TCE from groundwater [1]. Varieties tested include *P. deltoids* (Eastern cottonwood), *P. trichocarpa* (Black cottonwood), *P. simonii* (Chinese poplar) and *P. nigra* (Lombardy poplar). *P. trichocarpa* is a NW native.

Trees, Shrubs and Vines **Species/Common Name**	**Contaminant**	**Process**	**Comments**
Populus tremula Aspen	Lead	Extraction	*P. tremula, P. treumloides* (Trembling aspen) and hybrids have shown potential to remediate contaminated water, either from the soil or water table, specially the extraction of lead.
Rosa spp. Paul's scarlet rose	Organic contaminants	Phytodegradation	Paul's scarlet rose is a red, natural climbing rose that can metabolize tetrachlorinated PCB 77. There are, of course many varieties *R. gymnocarpa* (Dwarf rose) and *R. nutkana* (Nootka rose) are two Washington natives.
Salix spp. Willow	Perchlorate	Phytodegradation/ Rhizodegradation, Phytoextraction	Deciduous trees or shrubs needing plenty of water. *S. caroliniana* (Coastal plain willow) and *S. nigra* (Black willow) shown to uptake and degrade perchlorate in soils as well as phytoextract metals (Cd, Zn and Cu). Additional *Salix spp.* and hybrids have extracted metals (Cr, Hg, Se and Zn) (McCutcheon & Schnoor, 2003). Species in the NW includes *S. commutata* (Undergreen willow), *S. lucida* (Pacific willow), and *S. sitchensis* (Sitka willow). A study on Vashon Island indicated uptake/ accumulation of Cadmium by *S. scouleriana* (Scouler's willow).
Viola spp. Violets	Metals	Phytoextraction/ Hyperaccumulation	Perennial flowering plants with many varieties. *Hybanthus floribundus* (Shrub violet) from Australia has been found to accumulate high concentrations of metals. A study on Vashon Island, WA found violets growing naturally to have accumulated Cadmium (Institute of Environmental Research and Education, 2003). There are many varieties in the NW include: *V. adunca* (Early blue violet), *V. langsdorfii* (Alaskan violet), *V. palustris* (Marsh violet) and *V. glabella* (Yellow wood violet).

Appendix B
Accumulation of Heavy Metals by Different Plant Parts of Indian mustard and Marigold

Table 1B. Accumulation of heavy metals in different parts (root, shoot, leaf) of Indian mustard after 8 weeks and 12 weeks of plantation period in mg/kg of dry weight of plant part.

Heavy Metal	Accumulation of Heavy Metal (mg/kg of dry weight of plant parts) in different plant parts					
	After 8th Week			After 12th week		
	Root	Shoot	Leaf	Root	Shoot	Leaf
Pb	5.85	0.625	5.6	16.5	6	6.4
Cr	20.1	7.95	8.4	61.55	30	11
Cu	14.65	0	13.05	29	13.9	10.1
Ni	15	3.35	14.2	15.7	13.4	13.15
Zn	342.1	151.6	116.75	562	584.5	715

Table 2B. Accumulation of heavy metals in different parts (root, shoot, leaf) of Marigold (Density 1) after 8 weeks, 10 weeks and 12 weeks of plantation period in mg/kg of dry weight of plant part.

Heavy Metal	Accumulation of Heavy Metal (mg/kg of dry weight of plant parts) in different plant parts								
	After 8th Week			After 10th week			After 12th week		
	Root	Shoot	Leaf	Root	Shoot	Leaf	Root	Shoot	Leaf
Pb	18.8	10	21.05	11.2	30.85	25.65	36.25	37.65	30.35
Cr	42.15	13.15	13.4	11	51.05	15.75	13	21.9	77.4
Cu	37.25	8.55	29.35	10.05	31.8	32.25	21.85	9.75	30.9
Ni	18.95	5	15.65	12.25	15.45	36.1	16.4	10.5	14.2
Zn	271	208.25	45.95	246.3	179.5	301	159.05	256.15	301.55

Table 3B. Accumulation of heavy metals in different parts (root, shoot, leaf) of Marigold (Density 2) after 8 weeks, 10 weeks and 12 weeks of plantation period in mg/kg of dry weight of plant part.

Heavy Metal	Accumulation of Heavy Metal (mg/kg of dry weight of plant parts) in different plant parts								
	After 8th Week			After 10th week			After 12th week		
	Root	Shoot	Leaf	Root	Shoot	Leaf	Root	Shoot	Leaf
Pb	46.3	33.65	44.2	43.7	35.3	47.6	41.95	37.25	51.7
Cr	84.3	65.65	109.45	68.4	92	103.05	75.5	94.5	102.05
Cu	64.6	59.65	101.5	63.55	56.7	109.25	74.7	58	104.55
Ni	6.35	2.5	22.85	6.85	13.65	25.85	7.35	2.85	28.3
Zn	452.05	161.6	455.75	463.95	178.7	486.15	472.7	187.55	485.75