ABSTRACT

Title of Thesis: DYNAMIC STUDY OF HEAVY METAL FATES IN BIORETENTION SYSTEMS

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Bioretention is a best management practice (BMP). In this research, pot prototypes filled with bioretention media were built to simulate the conditions of natural growth of three grasses: *Panicum virgatum, Kentucky-31* and *Bromus ciliatus.* Synthetic runoff was applied. The results show average removals of Zn, Cu, Pb and Cd exceed 90% by the bioretention media and the fates of input metals are 87.5-96.9% captured in soil media, 0.5-3.3% accumulated in plants and 2.0-11.6% not captured by bioretention media. Based on field biomass yields and laboratory metal concentrations in plants, it appears possible and practical to achieve removals of Zn, Cu, Pb and Cd of 20% by *Panicum virgatum,* 15% by *Kentucky-31* and 10% by *Bromus ciliatus*, respectively. If 20% of input metals are accumulated by plants, the lifetime of a bioretention cell will be extended by 1.25 times.

DYNAMIC STUDY OF HEAVY METAL FATES IN BIORETENTION SYSTEMS

by

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of the requirements for the degree of
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DEDICATION

To my loving wife Hongxia Feng, my lovely daughter Sophia Sun,

my dear mother, Zhaohua Chang and Father, Lanping Sun

For their endless encouragement

and everlasting love

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First, I would like to express my sincere and deep gratitude to my academic advisor, Dr. Davis, for his patience, encouragement and valuable advice. I appreciate the opportunity he gave me to earn my Master's degree.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

Urban non-point pollution has been identified for several years as an important cause of surface water quality degradation in the United States. Sources of this pollution include precipitation, soil erosion, accumulation and wash-off of atmospheric dust, wash-off of street dirt, fertilizers and pesticides, and direct discharge of pollutants into storm sewers (Novotny et al., 1994). Urban growth has several detrimental impacts on receiving waters. It increases the impervious land area in a region, which decreases infiltration, increases runoff, and decreases the time during which runoff occurs. In addition, detrimental water quality changes in storm water runoff accompany land-use changes coinciding with urbanization (Patrick et al., 2002). Recent studies of urban and roadway runoff have shown high levels of many pollutants, including suspended solids, and heavy metals (Barrett et al., 1998). The impact of solids, nutrients, and chloride in urban surface runoff are most severe in small streams, urban lakes, or bays and harbors, where rates of water movement are relatively small and opportunities for dilution and dispersion are limited (Waller and Hart, 1986). Many man-made engineering systems have been used to control runoff hydrology and storm water quality, such as retention ponds, wetlands, sand filters and biofilters.

Bioretention is a best management practice (BMP) and utilizes soils and both woody and herbaceous plants to remove pollutants from storm water runoff (Figure 1.1). Bioretention facilities typically treat storm water that has run over impervious surfaces at commercial, residential, and industrial areas (U. S. EPA, 1999) and provides storm water

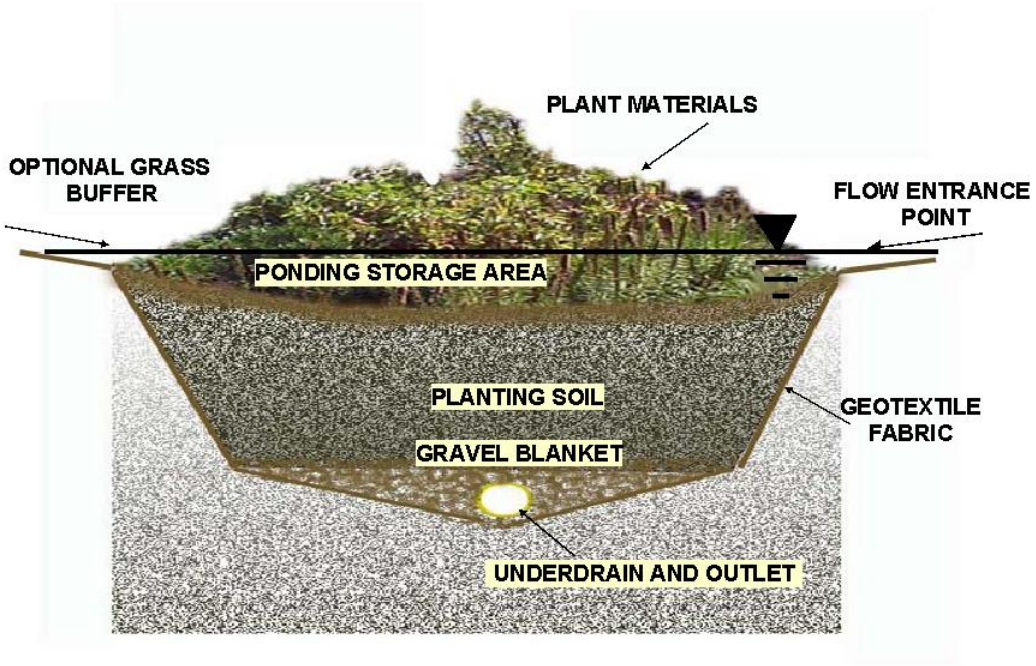


Figure 1.1. The structure of bioretention (UMD)

treatment that enhances the quality of downstream water bodies through physical, chemical and biological processes, including adsorption, filtration, plant uptake, microbial activity, decomposition, sedimentation and volatilization (U.S. EPA, 1999). Some laboratory and pilot-scale bioretention box studies have been conducted and the results showed that the removal efficiencies of Zn, Cu and Pb were typically greater than 90%, that of total phosphorus was 80% and total Kjeldahl nitrogen was 50~70% (Davis et al., 2001a). Results for both laboratory and field experiments were similar for each of the pollutants analyzed (Davis et al., 2003) and doubling or halving the influent pollutant levels had little effect on the effluent pollutants concentrations (Davis et al., 1998). All

of these studies indicated that bioretention is an effective practice to clean urban storm water runoff.

Nonetheless, the sorption capacities of bioretention media are limited. The metals may accumulate to a level where ecosystem risks may became important after 15~20 years (Davis et al., 2003). Therefore some process is necessary to remove the metals from the media in order to maintain the sorption capacities of bioretention facilities.

A simple and economical technology-phytoremediation, which uses plants to uptake pollutants from the soil media, is a growing field in cleaning storm water and soil. Two types of plants are used for phytoremediation: one is hyperaccumulator plants capable of accumulating potentially phytotoxic elements to concentrations more than 100 times than those found in nonaccumulators (Salt et al., 1998); the other method is to use nonaccumulator, but high biomass plants, possibly coupled with manipulation of soil conditions either to increase the bioavailability and, hence, increase plant uptake, or the stabilization, and so as to decrease plant uptake, of metals (Huang et al, 1997). Suitable plants can uptake pollutants from the media so as to extend the life and removal efficiency of bioretention facilities**.**

Therefore, this study tries to focus on identifying crop and crop-related species that can accumulate heavy metals while producing high biomass in response to established bioretention practices. *Panicum virgatum* (switch grass), *Kentucky-31* and *Bromus ciliatus* are erect, coarse, perennial grasses with high biomass and are easily grown in wide range habitats. They are potential candidates for accumulating metals from soil. Therefore, these three types of grasses were investigated. This study addressed the flowing aims:

1. Study the removal efficiencies of metals by bioretention systems with low and high contaminant loadings.
2. Determine if *Panicum virgatum, Kentucky-31* and *Bromus ciliatus* under lab bioretention conditions accumulate the metals Zinc (Zn), Copper (Cu), Lead (Pb) and Cadimum (Cd) and investigate the extent of accumulation and distribution in the shoots and roots.
3. Investigate the temporal and spatial variation of metal accumulation in the tissues of different grasses and compare the bioaccumulation and transport of different metals in the tissues.
4. Evaluate the potentials of metal uptake by *Panicum Virgatum, Kentucky-31* and *Bromus ciliatus* from the bioretention media in order to extend life of the bioretention facilities.
5. Investigate the metal distributions of metals in the bioretention media.

In order to achieve these goals, fourteen pot prototypes with plant lights were set up and synthetic storm water runoff with two different metals loadings were applied three times a month. Resulting effluent samples are collected. The three plants were seeded and samples of the plants and bioretention soil were collected once a month. Extraction methods were used for samples of effluent water, bioretention media, and plants. Concentrations of Zn, Cu, Pb and Cd in the samples were measured and the data were analyzed and evaluated. The role of these grasses in metal fates and bioretention performance were evaluated.

Chapter 2

BACKGROUND

2.1 Bioretention

Bioretention is a best management practice (BMP) developed in the early 1990's by the Prince George's County, MD, Department of Environmental Resources (PGDER). Bioretention utilizes soils and both woody and herbaceous plants to remove pollutants from storm water runoff. As shown in Figure 2.1, runoff is conveyed as sheet flow to the treatment area, which consists of a grass buffer strip, sand bed, ponding area, organic layer or mulch layer, planting soil, and plants (Prince George's County, 1993). The ponding area is graded and its center depressed. Water is ponded to a depth of 15 cm (6 inches) and gradually infiltrates the bioretention area or is evapotranspired. The bioretention area is graded to divert excess runoff away from itself. Stored water in the bioretention area planting soil exfiltrates over a period of days into the underlying soils. Bioretention facilities typically treat storm water that has run over impervious surfaces at commercial, residential, and industrial areas (US. EPA, 1999). For example, bioretention is an ideal storm water management BMP for median strips, parking lot islands, and swales. These areas can be designed or modified so that runoff is either diverted directly into the bioretention area or conveyed into the bioretention area by a curb and gutter collection system.

Each of the components of the bioretention area is designed to perform a specific function (U.S. EPA, 1999). The grass buffer strip reduces incoming runoff velocity and filters particulates from the runoff. The ponding area provides a temporary storage location for runoff prior to its evaporation or infiltration. Some particulates not filtered

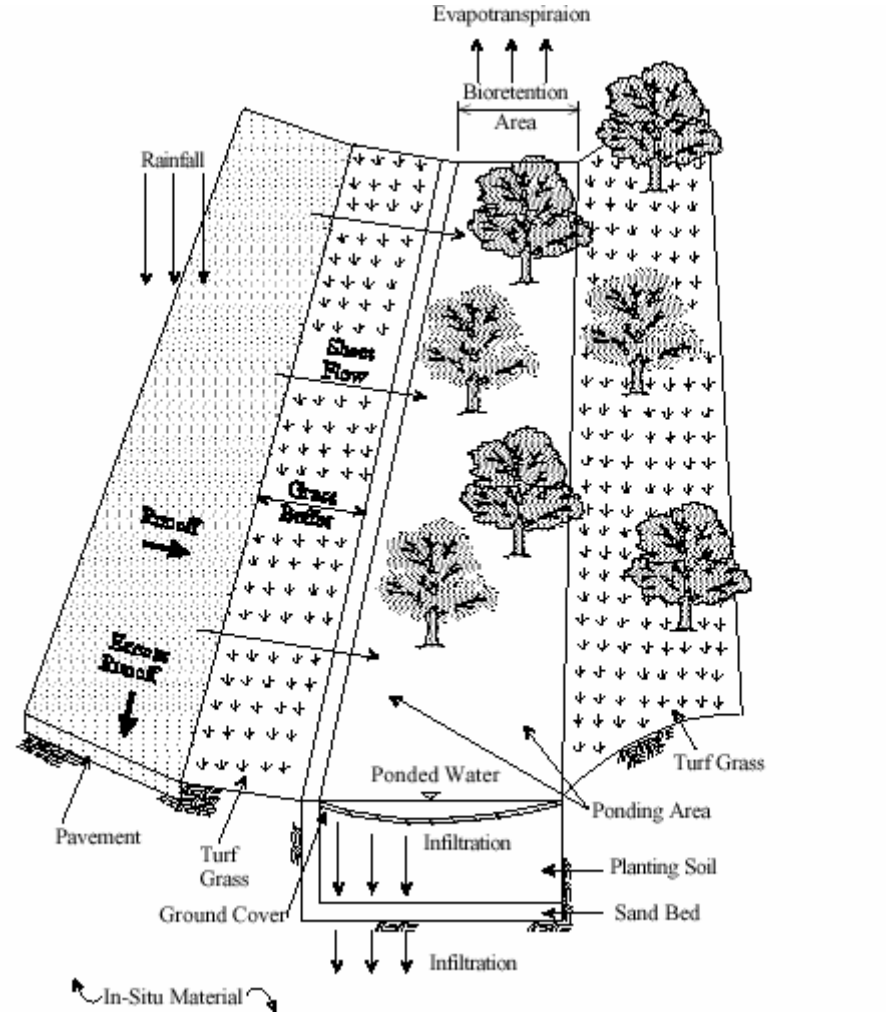


Figure 2.1 Schematic of a typical bioretention area (DER, 1993; Davis, 2001)

out by the grass filter strip or the sand bed settles within the ponding area. The organic or mulch layer also filters pollutants and provides an environment conducive to the growth of microorganisms, which degrade petroleum-based products and other organic materials. This layer acts in a similar way to the leaf litter in a forest and prevents the erosion and drying of underlying soils. Planted ground cover reduces the potential for erosion as well. The clay in the planting soil provides adsorption sites for heavy metals, nutrients and other pollutants. Storm water storage is also provided by the voids in the planting

soil. The stored water and nutrients in the water and soil are then available to the plants for uptake.

Bioretention removes storm water pollutants through physical, chemical and biological processes, including adsorption, filtration, plant uptake, microbial activity, decomposition, sedimentation and volatilization (U.S. EPA, 1999). Adsorption is the process whereby particulate pollutants attach to soil (e.g., clay) or vegetation surfaces. Adequate contact time between the surface and pollutant must be provided for in the design of the system for this removal process to occur. Therefore, the infiltration rate of the soils must not exceed those specified in the design criteria or pollutant removal may decrease. Pollutants removed by adsorption include metals, phosphorus, and some hydrocarbons. Filtration occurs as runoff passes through the bioretention area media, such as the sand bed, ground cover and planting soil.

In recent years, some research has been successfully conducted on bioretention. Batch and column sorption studies along with pilot-scale experiments have been completed successfully and the results show that removal efficiencies of metals (Zn, Cu and Pb) are more than 90% and the reductions of TKN, ammonium and phosphorus are from 60% to 80% (Davis et al., 2001a). Some field experiments were also performed and the results of the pollutant removals strongly support the laboratory observations; most of the metals are captured by the top 20 cm of bioretention depth (Davis et al., 2003). Kim et al. (2003b) reported that the nitrate plus nitrite mass can be removed by up to 80% with engineered bioretention using newspaper as an electron donor and carbon source. Hong et al. (2003) reported that the mulch layer of bioretention facilities can capture dissolved naphthalene, dissolved toluene, dissolved motor oil and particle-associated

naphthalene in storm water runoff, which demonstrated that bioretention could efficiently remove the hydrocarbon contaminants from storm water runoff.

2.2 Heavy Metals in Runoff

Runoff from construction sites, roofs, and roadways is known to contain heavy metals as trace contaminants, and can affect the bioecosystems near these runoff sites. Urban storm water runoff has been recognized as a substantial source of pollutants to receiving waters (Davis et al., 2001). Heavy metals are one type of important pollutants in runoff due to their potential toxicity. The presence of Cd, Pb Zn and Cu above trace levels in the environment is an indicator of contamination of runoff (Cardwell et al. 2002). The metals in runoff come from different sources. Wear of tires and brake pads is a source of all four metals, Cd, Cu, Pb and Zn (Makepeace et al., 1995), and building siding is also an important sources of these four metals (Davis et al., 2001). Moreover, combustion, combustion of lubricating oils, metal finishing industrial emissions, agriculture use of sludge, fertilizers, pesticides, and corrosion of galvanized metals are sources of Cd. Corrosion of building parts, wear of bearings, bushings and other moving parts in engines, metallurgical and industrial emissions and pesticides are the main source of Cu (Makepeace et al., 1995). The concentration of metals in the runoff varied greatly. Makepeace et al. (1995) reported the concentration range of Zn, Cu, Pb and Cd are 0.05 ~13730 μg/L, 0.06 ~1410 μg/L, 0.57 ~26000 μg/L, and 0.7 ~ 22, 000 μg/L respectively. Barrett et al. (1998) observed that the mean concentrations of Zn from U.S. 183 and the MoPac expressway in Texas were 0.347 and 0.129 mg/l, respectively, and those of Pb were 0.138 and 0.093 mg/l, respectively. Wu et al. (1998) reported the concentrations of Cu, Pb and Cd were 5-25, 5-25 and <5 ppb, respectively.

2.3 Metal Uptake by Plants

Heavy metals are natural elements that found at various background levels at different place throughout the world, due to various concentrations in the bedrock (Table 2.1). Soil and sediment are considered as sinks and metals are therefore accumulated in these media, resulting in high concentrations. In soils, the metals exist as different species (Table 2.2). The phytoavailability of metals depends on the form of the metal and on the plant species tested. However, even if using the same species, the uptake by plants does not necessarily correlate with the bioavailable metal concentration in the soil or the total metal concentration due to many different genotypes within the same population of the species with different metal uptake. Normal metal concentrations in pants are shown in Table 2.3.

Table 2.1. Background heavy metal levels in sediment (Forstner, 1979) and the upper limit of non-polluted soils (Temmerman, 1984)

Metal (μg/g)	Soil		Sediment	
	Sandy Loam	Loam	Lake	Sea
Cd	1	1	0.14-2.5	0.02-0.43
Cr	15	30	7-77	11-90
Cu	15	25	16-44	4-250
Hg	0.15	0.15	0.004-0.2	0.001-0.4
Ni	1	1	34-55	2-225
Pb	50	50	14-40	7-80
Zn	100	150	7-124	16-165

Recently, the mode of accumulation of heavy metal by a variety of plant species has been studied by a number of investigators. These studies have focused on the uptake and phytotoxic effects of heavy metals, the correlation between the content of heavy metals in the soil and the amount absorbed by the plant and the effect of different extractants on the bioavailability. Because metal uptake by roots depends on both soil and plants, the concentration of metals in plants varied due to the plant species, metal stress and soil conditions (Madyiwa, 2002; Kim, 2003**).**

Table 2.2. Chemical speciation of metals in the soil solution (Sposito, 1989)

Metal	Soil	Additional in	
		Acid Soil	Alkaline Soil
Cd (II)	Cd^{2+}	$CdHCO_3^+$	---
	$CdSO_4$		
	$CdCl^+$		
Cu(II)	Cu-org	Cu^+	$CuCO_3$
			$CuB(OH)_4^+$
			$Cu[B(OH)_4]_4$
Pb (II)	Pb-org	Pb^{2+}	$PbCO_3$
	$PbHCO_3^+$	$PbSO_4$	$Pb(CO_3)_2^{2-}$
		$PbOH^+$	
Zn (II)	Zn^{2+}	---	$ZnHCO_3^+$
	$ZnSO_4$		$ZnCO_3$
	Zn-org		$ZnB(OH)_4^+$
Ni (II)	Ni^{2+}	$NiSO_4$	$NiCO_3$
	$NiHCO_3^+$	Ni-org	$NiB(OH)_4^+$

Soil metal concentrations are correlated strongly with plant metals content and can be best described by linear, exponent, quadratic or cubic models (Dudka, 1996; Nan, 2002; Wang 2003b). With the increase of metals added to the soil, the concentration in the plant tissues appropriately increased (Madyyiwa, 2003). Some chemical reagents played important roles in plant metal extraction efficiency. The concentrations of elements in the plant tissues are affected by the concentrations of the heavy metals in the soil and original pH (Peralta et al., 2002). Some studies showed that EDTA at a rate of 0.5 g/kg significantly increased the shoot concentrations of Cd and Ni (Chen et al., 2001); other investigations showed that increases of metal concentrations in soil solution induced by EDTA did not increase plant total Cd uptake but appeared to stimulate the translocation of the metals from roots to shoots (Jiang et al., 2003). Both investigations drew the same conclusion that chelator toxicity reduced the plant's biomass and, therefore decreased the amount of metal accumulation.

Table 2.3. Normal composition of trace elements in plants

Metal	Conc. (μg/g dry weight)[a]	Conc. (μg/g fresh weight)[b]
Cadmium	0.05	0.2-0.8
Copper	10	4-15
Iron	150	--
Lead	1.0	0.1-10
Nickel	1.5	0.02-5
Silver	0.2	--
Zinc	50	8-400

Sources: [a] Markert (1994); [b] Allaway (1968).

2.4 Influencing Factors for Bioavailability

Plants grown in metal-enriched substrates take up metal ions to varying degrees. This uptake is largely influenced by the bioavailability of the metals, which is determined by both external and internal factors. The latter are aplant-associated factors which have been discussed above. The former are soil-associated which including chemical (pH, Eh, CEC, metal speciation), physical (size, texture, clay content, organic matter) and biological (bacteria, fungi) process and their interactions

2.4.1 pH

The chemical forms of heavy metals in soil are affected by modification to the soil pH. However, it is difficult to discuss pH influence independently from other phytochemical characteristics although the pH is a dominant factor. An increase in pH results in higher adsorption of Cd, Zn, Cu to soil particles and reduces the uptake of them by plants (Kuo, 1985). Protons (H^+) may be important competitors for metal uptake by roots. On the other hand, acidification increase the metal absorption by plants through reduction of metal adsorption to soil particles (Brown, 1994). Furthermore, solution cation concentrations are dependent on pH and pH will affect conformational changes of dissolved organic matter and may provoke its coagulation (Romkens 1998).

2.4.2 Redox Potential (Eh)

The redox potential of soil is a measure of tendency of the soil solution to accept or donate electrons. As the redox potential decreases, heavy metal ions are converted from insoluble to soluble forms. For example, under reducing conditions, Mn and Fe-oxides are reduced to Mn^{2+} and Fe^{2+}, thus increasing bioavailability (Kabata-pendias, 1984). It is therefore likely that a lower pH and Eh of the soil would enhance the mobility of most metals.

2.4.3 Cation Exchange Capacity (CEC)

The cation exchange capacity of soil is a measure of the ability of the soil to retain metal ions. The CEC increases with increasing clay content in the soil while the availability of metal ions decreases (Kabata-pendias, 1984). Modulating the CEC would therefore result in increased or decreased availability of metals to plants.

2.4.4 Soil type

The bioavailability of heavy metals in the soil also depends on the texture of the soil. A gradient of metal ion availability exists in varying soil types with the availability being lowest in clay soils, followed by clay loam and finally loams and sand. This is in part due to the low bioavailability of these metal ions, or reduced leaching as metals are bound to the soil matrix in fine textured soil (Webber, 1995). The complexation of heavy metals with organic matter, humic acid in particular, has been well documented (Friedland, 1990). High organic matter content enhances the retention of metals, drastically reducing the metal available.

2.4.5 Chelates

An essential component of the bioavailability process is the exudation of metal chelating compounds by plant roots. These chelators are synthesized by plants and can mobilize heavy metals such as copper, lead and cadmium by formation of stable complexes (Mench, 1988). Chelators are usually low molecular weight compounds such as sugars, organic acids, amino acids and phenolics that can change the metal speciation, and thus metal bioavailability.

Apart from the chelating agents produced by plants, the addition of synthetic chelating agents to contaminated soils was shown to substantially increase the metal

solubility in soil (Salt, 1995). In contrast, addition of chelates to mineral nutrient solutions has also resulted in decreased metal accumulation (Srivastava, 1995). It is likely that in contaminated soils, chelator application enhances the formation of metal-chelate complexes, reducing the sorption of metals to soil particles. Numerous studies have focused on evaluating the effects of adding synthetic chelates such as ethylene diaminetetracetic acid (EDTA), ehyleneglycoltetracetic acid (EGTA) and citrate on the uptake of metals by plants (Salt et al., 1998). All of these chelates increased the available metal content in the soil solution.

2.5 Phytoremediation

In the early 1990s, an innovative technology, phytoremediation, a process that utilizes the natural properties of plants in engineered systems to remediate hazardous waste sites-- emerged for bioremediation (Salt et al., 1998). It was also proposed that toxic organic compounds might be degraded by the action of microorganisms peculiar to the rhizosphere of plants. Plant root systems permeate soil and sediment environments with an extensive and active membrane system. The soil near the roots has microbial populations orders of magnitude greater than non-root soil (Salt et al., 1998). These benefits are provided with little or no maintenance requirements. Furthermore, plant-based systems are welcomed by the public due to their superior aesthetics and the societal and environmental benefits that their presence provides. Five main subgroups of phytoremediation have been identified:

- Phytoextraction: plants remove metals from the soil and concentrate them in the harvestable parts of plants (Kumar et al., 1995).

- Phytodegradation: plants and associated microbes degrade organic pollutants (Burken and Schnoor, 1997).
- Rhizofiltration: plant roots absorb metals from waste streams (Dushenkov et al., 1995).
- Phytostabilization: plants reduce the mobility and bioavailability of pollutants in the environment either by immobilization or by prevention of migration (Vangronsveld et al., 1995).
- Phytovolatilization: volatilization of pollutants into the atmosphere via plants (Bañuelos et al., 1997; Burken and Schnoor, 1999).

The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a `green', sustainable process. Initially, much interest focused on hyperaccumulator plants capable of accumulating potentially phytotoxic elements to concentrations more than 100 times than those found in nonaccumulators (Chaney et al., 1997; Salt et al., 1998). These plants have strongly expressed metal sequestration mechanisms and, sometimes, greater internal requirements for specific metals (Shen et al., 1997). Some species may be capable of mobilizing metals from less-soluble soil fractions in comparison to nonhyperaccumulating species (McGrath et al., 1997). Metal concentrations in the shoots of hyperaccumulators normally exceed those in the roots, and it has been suggested that metal hyperaccumulation has the ecological role of providing protection against fungal and insect attack (Chaney et al., 1997). Such plants are endemic to areas of natural mineralization and mine spoils (Brooks, 1998).

However, methods using hyperaccumulators to uptake metals are limited by the biomass and concentration of metals in some plants. Ebbs and Kochian (1995) found

Thlaspi caerulescens is a Zn hyperaccumulator, but its use in the field is limited because individual plants are very small and slow growing. The ideal plant species to remediate a heavy metal-contaminated soil would be a high biomass-producing crop that can both tolerate and accumulate the contaminants. Furthermore, the cropping of contaminated land with hyperaccumulating plants may result in a potentially hazardous biomass (Ajwa et al., 1999).

Another method is to use nonaccumulator but high biomass plants, possibly coupled with manipulation of soil conditions either to increase the bioavailability and, hence, increase plant uptake, or the stabilization, and so decrease plant uptake, of metals (Huang et al., 1997). For example, there are two major limitations to Pb phytoextraction: the low Pb bioavailability in soil and the poor translocation of Pb from roots to shoots. Huang et al. (1997) investigated the potential of adding chelates to Pb-contaminated soils to increase Pb accumulation in plants and showed that concentrations of lead in corn and pea shoots were greatly increased. Ethylenediaminetetraacetic acid (EDTA) was the most effective chelate in increasing Pb desorption from soil into the soil solution and also greatly increased the translocation of Pb from roots to shoots through prevention of cell wall retention (Cooper et al., 1999).

Salt et al. (1998) noted the potential of manipulating metal resistance mechanisms in nonhyperaccumulating plants to improve phytoextraction. This could be done by conventional plant breeding programs or by genetic manipulation. However, improved metal resistance alone may not be sufficient for successful phytoextraction, which also depends on metal bioavailability, root uptake and shoot accumulation.

Using phytoremediation, the advantages of natural plants processes can be exploited. It requires less equipment and labor than other methods, since plants do the most of the work. A site can be cleaned up without removing the polluted soil and this allows workers contact with less harmful chemicals and is safer.

2.5.1 Process of Phytoremediation

Phytoremediation is an in-situ bioremediation strategy that has been gaining increasing recognition. Phytoremediation employs a natural system or an enhanced variation thereof, to eliminate the need for removing contaminated soil to locations where remediation cannot be assured. Phytoremediation works best at sites with low to medium amounts of pollution. Plants remove harmful chemicals from the ground when their roots take in water and nutrients from polluted soils, steams and groundwater. Plants can clean up chemicals as deep as their roots can grow. Once inside the plant, chemicals can be (Figure 2.2):

- Stored in stems, roots and leaves
- Change into less harmful chemicals in the plants
- Change into gases that can be released into air when the plants breathe

Phytoremediation can occur even when the chemicals are not taken into plants by roots. For example, chemicals can stick or be adsorbed to plant roots and then be changed into less harmful chemicals by microbes near roots. Afterward, plants are harvested or destroyed.

2.5.2 Mechanism of Phytoremediation of Metals

Accumulation of a given metal in a multicellular organism is complicated. The processes affecting the accumulation rates of metals in plants are: mobilization and

uptake from the soil, compartmentation and sequestration within the root, efficiency of transport, distribution between metal sinks in the aerial parts, sequestration and storage in leaf cells. At every level, concentration and affinities of chelating molecules, as well as the presence and selectivity of transport activities, affect metal accumulation rates.

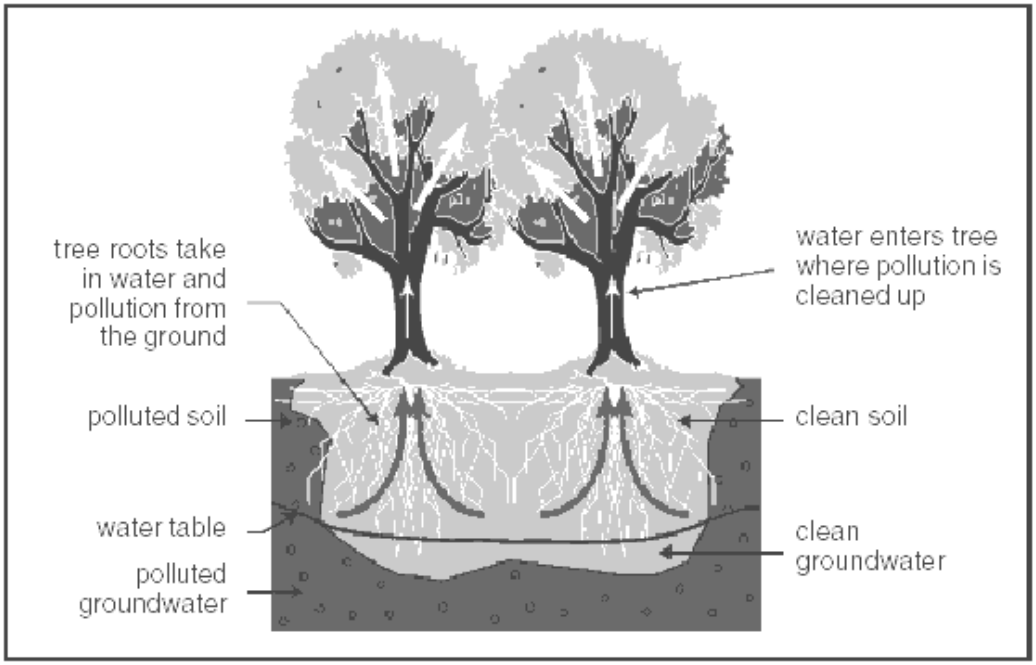


Figure 2.2. The pollutant-absorbing process by plants (U.S. EPA, 2001)

2.5.2.1 Mobilization

The elements essential for life are also among the most abundant on Earth (Frausto, 2001). However, the actual bioavailability of some metals is limited because of low solubility in water and strong binding to soil particles and sediments.

Hyperaccumulator species are able to accumulate higher metal concentrations in their shoots even from soil containing nonphytotoxic background levels of metals **(**Baker et al., 1991**).** One possible mechanism to explain this enhanced metal accumulation could be an enhanced ability to solubilize metals within the rhizosphere of the hyperaccumulator. This is supported by evidence on the ability to extract zinc from the immobile fraction of the soil, although further studies are needed to confirm this (McGrath, 1997). For example, EDTA can increase the metal availability and enhance the uptake of metals by plants, but the risk of these techniques may cause the metal-chelate complexes to leach into the groundwater (Chen et al., 2001).

Root-colonizing bacteria have a large impact on the availability of heavy metals for plant uptake. For instance, soil microorganisms significantly enhance Zn accumulation in the shoots of the hyperaccumulator *Thlaspi caerulescens* (Whiting et al*.,* 2001). Consequently, specific modifications of the rhizosphere could greatly enhance metal accumulation. Modification of the rhizosphere pH or redox potential by plant roots have also been reported to contribute to the mobilization of plant nutrients in some species (Marscher, 1995).

2.5.2.2 Root Uptake and Sequestration

Metals are first bound by the cell wall, an ion exchanger of comparatively low affinity and low selectivity. Uptake of metal ions is likely to take place through

secondary transporters such as channel proteins and/or H^+-coupled carrier proteins (Hirsch et al., 1998). The membrane potential, which is negative on the inside of the plasma membrane and might exceed -200 mV in root epidermal cells (Hirsch et al., 1998), provides a strong driving force for the uptake of cations.

Several cation transporters have been identified in recent years with the use of molecular techniques. Most of the transporters thought to be involved in the uptake of micronutrients are in the ZIP (ZRT, IRT-like protein) and the Nramp (natural resistance-associated macrophage protein) family (Guerinot, 2000). For some of them, expression in roots and up-regulation under deficiency conditions indicate a role in uptake from the soil. Direct evidence demonstrating the contribution of a specific transporter to transition metal acquisition is scarce.

Sequestration drives the passage of transition metal ions across the plasma membrane. Several processes known to contribute to metal tolerance are associated with metal accumulation at the same time. *Saccharomyces cerevisiae* cells synthesizing phytochelatins, glutathione-derived metal-binding peptides, show significantly higher Cd^{2+} tolerance and increased Cd accumulation even at subtoxic concentrations (Clemens et al., 1999).

2.5.2.3 Root to Shoot Translocation

Three processes govern the movement of metals from the root into the xylem: sequestration of metals inside root cells, simplistic transport into the stele and release into the xylem (Tester et al., 2001). This process is mediated by root pressure and transpiration. The transport of ions into the xylem is generally a tightly controlled

process mediated by membrane transport proteins (Gaymard, 1998). Most likely, some degree of cycling of metal cations occurs from the shoots back to the root.

Inside the root, chelation with certain ligands appears to route metals primarily to the xylem (Senden et al., 1995). Inside the xylem, a pH-dependent equilibrium exists between low-molecular weight chelators, free hydrated metal cations and metal chelates in the mobile transpiration stream, and stationary metal-binding sites in the cell wall material surrounding the xylem vessels (Evans et al., 1992) (Figure 2.3).

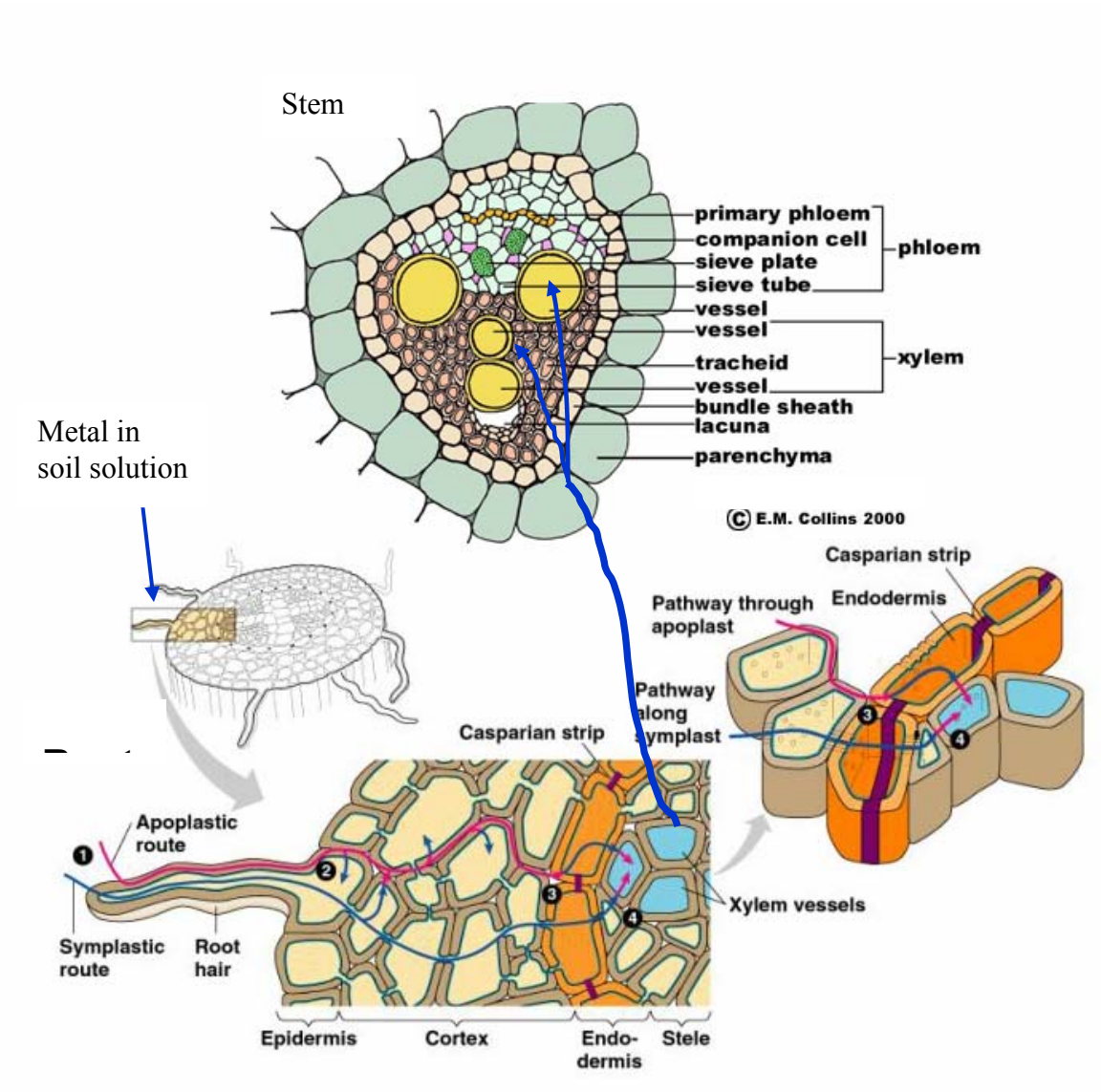


Figure 2.3. Transport process of metals from roots to shoots (UALR, 2004)

2.5.2.4 Unloading and Storage

Transition metals reach the apoplast of leaves in the xylem sap, from where they have to be scavenged by leaf cells (Marschner, 1995). Transporters mediate uptake into the symplast, and distribution within the leaf occurs via the apoplast (Karley et al., 2000). Trafficking of metals occurs inside every plant cell, maintaining the concentrations within the specific physiological ranges in each organelle and ensuring delivery of metals to metal-requiring proteins (Himelblau et al., 1998). Excess essential metals, as well as non-essential metals, are sequestered in leaf cell vacuoles (Vögeli-Lange and Wagner, 1990). Different leaf cell types show pronounced differential accumulation. The distribution pattern varies with plant species and element. Zn accumulation in *Thlaspi caerulescens* leaves is 5.0–6.5-fold higher in epidermis cells than in mesophyll cells (Küpper et al., 1999), whereas in metal-treated *A. halleri*, the mesophyll cells are thought to contain more Zn and Cd than the epidermal cells (Küpper et al., 2000).

2.6 Plants Investigated

Three plants are investigated in this study and their characters are discussed below.

2.6.1 *Panicum virgatum* (Switch grass)

Switch grass is a native, erect, coarse, warm-season perennial grass. The foliage height of mature plants is mostly between 0.9 and 1.5 m; the inflorescence, a 15 to 46 cm long open panicle, often extends to a height of 1.5 to 2.1 m (Weaver, 1960).

Switch grass reproduces both sexually and vegetatively. Rhizomes are responsible for vegetative expansion, but spreading ability depends upon growth form. Some rhizomes of sod-forming ecotypes may extend to lengths of 0.3 to 0.6 m, while those of bunch-forming ecotypes may extend only a few inches (Beaty, 1978). The primary site

of nonstructural carbohydrate storage is in the stem bases, roots, and rhizomes. Germination begins when soil temperatures reach 20 ^{o}C (Vogel et al., 1985).

Switch grass tillering and rhizome production generally begins 5 to 7 weeks after germination, unless competition is severe. Three months after germination, plants may be 30-50 cm tall, and roots may be 30 to 76 cm deep (Weaver, 1968). Switch grass is a mesic grass that grows on a wide variety of soil textures if soil moisture is adequate (Wasser et al., 1982). Studying its distribution along a water gradient in Kansas, Knapp et al. (1984) found that switch grass favored mesic sites, and concomitant physiological studies showed it was less able to adjust osmotically to drought than big or little bluestem. Deep-rooted switch grass grows well on the sand dunes because even small amounts of precipitation penetrate the coarse sand and thus subsurface moisture is available throughout the growing season (Barnes et al., 1984). Besides mesic prairies, switch grass also commonly grows in fresh and brackish marshes, on dunes and along lakeshores, and in oak and pine savannas. Switch grass is tolerant of spring flooding but not of high water tables. It is tolerant of moderate soil salinity and acidity. It grows in soils ranging in pH from about 4.5 to 7.6 (Vogel et al., 1985).

2.6.2 *Kentucky-31* (Tall fescue)

Tall fescue is a coarser-bladed, dense, clumping grass that grows well in shady areas and is often mixed with other grasses for just this quality. It was brought to the U.S. in the early 1800's for pasturage purposes and now grows in about 4/5 of our country (Agriculture publication G4669, 2000). Tall fescues are used extensively on lawns, athletic fields, baseball fields, play fields, polo fields, hospitals, and everywhere that a good, dense utility-grade lawn is desired. Under ideal growing conditions, tall fescue

may reach 48 inches or more in height with a loosely branching panicle for a seed head. Leaf blades are 1/8 to 1/2 inch wide and from 4 to 24 inches long. Leaves may be a yellowish to dark green color and have a dull upper leaf surface with distinct veins running the length of the leaf. Lower leaf surfaces are smooth and glossy and slightly keeled. Leaf margins are usually rough (Agriculture MU Guide, 2000).

Kentucky 31 tall fescue was established from original plants found in Kentucky. Originally introduced from Europe, it adapted itself to soil conditions of the Kentucky region, and has since gained importance because of its ability to adapt to a wide variety of other types of soils, including poorly drained areas. It is one of the more popular varieties planted throughout the U.S.. Kentucky-31 Tall fescue is favored for its adaptation to grow well in the shadier northern areas and throughout the transition areas where cool season grasses will not withstand warmer climate. Kentucky 31 also performs well in the upper areas where the warm season grasses will not tolerate the cooler weather. Kentucky 31 tall fescue has a medium light green color, coarse leaf blades, and a somewhat open growth habit (Grass Varieties in the United States, 1994). Kentucky 31 requires a moist, weed-free, firm seedbed. These characteristics make it an acceptable grass for utility areas, but not desirable for a home lawn or other high viability area. Kentucky 31 remains popular still because of its lower price and good overall usage qualities.

2.6.3 *Bromus ciliatus* (Fringed brome)

Fringed brome is a native perennial grass (Fulbright et al., 1982). Culms are slender, usually 0.5 to 1.2 m tall, but up to 1.6 m tall in the Great Plains (Fulbright et al., 1982). The blades are flat, 3 to 15 mm wide and 15 to 25 cm long (Harper et al., 1992). The

panicle is narrowly elongate 7 to 18 cm long with branches ascending to drooping (Munz et al., 1973). Fringed brome has a well-developed root system (Harper et al., 1992).

Fringed brome reproduces exclusively from seed. Seeds are non-dormant and can show high germination rates. Tests were conducted with light and dark regimes, with or without stratification, and with a variety of thermal periods. Fringed brome is wind pollinated (Harper et al., 1992).

Fringed brome occurs in a variety of habitats including woodlands, forest openings, thickets, grasslands, shrublands, prairies, meadows, marshes, bogs, fens, and stream and lake margins. It is commonly found in moist places such as wet meadows, benches, and along streams (Munz et al., 1973). Fringed brome also occurs on moist to seasonally dry, open or densely shaded habitats in valleys and montane zones (Fitzhugh et al., 1987).

Fringed brome grows best on moist to semi-wet soils, but is tolerant of poorly drained and subirrigated conditions (Butterwick et al., 1992). It grows best on loam, silty loam, and sand, but occurs on stony or bouldery substrates as well (Butterwick et al., 1992). In heavily shaded habitat types, fringed brome may become the dominant understory species (Fitzhugh, 1987).

Chapter 3

METHODOLOGY

3.1 Bioretention Media

The bioretention media was a mixture of planting soil, mulch, and sand and its composition is shown in Table 3.1.

Table 3.1 The composition of bioretention media *

Items	Composition by Volume		Sources
	Top layer	Bottom layer	
Planting soil	50%	30%	Department of transportation Forestville, MD
Shredded 2x Hardwood Mulch	50%	20%	Department of Public Works College Park, MD
Sand	--	50%	Mystic White® US Silica Company

* Special provisions, 300-bioretention facilities-draft, Department of water resource, Prince George County.

3.2 Plant Seeds

The seeds of *Panicum virgatum* and *Bromus ciliatus* were obtained from the Ion Exchange- Native Prairie Seed Company (Harpers Ferry, IA) and those of Kentucky-31 came from the Plant Nursery Company (College Park, MD). The concentrations of heavy metals in the seeds were measured using the HNO_3-$HClO_4$ (V/V: 3:1) method (Miller 1998) which will be discussed below. The results are shown in Table 3.2.

3.3 Experimental Design

Pot prototypes (31 cm diameter and 31 cm height) were employed to simulate the conditions for natural growth of plants (Fig. 3.1). Fourteen plastic pots with soft PVC

pipe (10 cm) at the bottom of each were used for experiment (Fig. 3.2). The pots were filled with 25 cm of soil, leaving about 6 cm above the surface of soil to prevent water overflow when the synthetic runoff was pumped in. The top 0~5 cm media was a mixture of 50% soil and 50% mulch to promote fertility. The lower 5~25 cm was a mixture of 50% sand, 30% soil and 20% mulch. The background concentrations of Zn, Cu, Pb and Cd in the media layers are shown in Table 3.3.

Table 3.2. Background metal concentrations of the seeds

Concentration (μg/g)	**Panicum virgatum** (Switch grass)	**Kentucky-31** (Tall fescue)	**Bromus ciliatus** (Fringed brome)
Zinc	115±5	8±1	8±1
Copper	16±1	44±1	60±3
Lead	1±0	0.8±0	1±0
Cadmium	0.2±0	2±0	0.1±0

Table 3.3. Background concentrations of metals in soil

	Concentration (μg/g)	
	Top Layer (0~5cm)	Bottom layer (5~25 cm)
Zinc	223±15	102±14
Copper	23±4	7±3
Lead	44±6	29±4
Cadmium	0.2±0.2	0.1±0.1

Twelve pots were seeded and two pots were used for control experiments without any plants. The soil surface area in each pot is about 0.07 m^2. About 1.5 g seeds were sown for each pot. The surface soil was watered with deionized water and covered by

plastic wrap to maintain the temperature and keep in the moisture of the soil. After 7days, the plants germinated. After 47 days, the plants grew very well (Fig. 3.3) and the first synthetic runoff was applied.

The study was conducted under controlled light and ambient temperature. Agro-lite indoor plant lights with 100 and 40 W power ratings were used (Home Depot, College Park, MD). To maintain the healthy growth of plants and to simulate the natural light, the light was controlled by timers, which keep the lights on and off about 12 hrs each day.

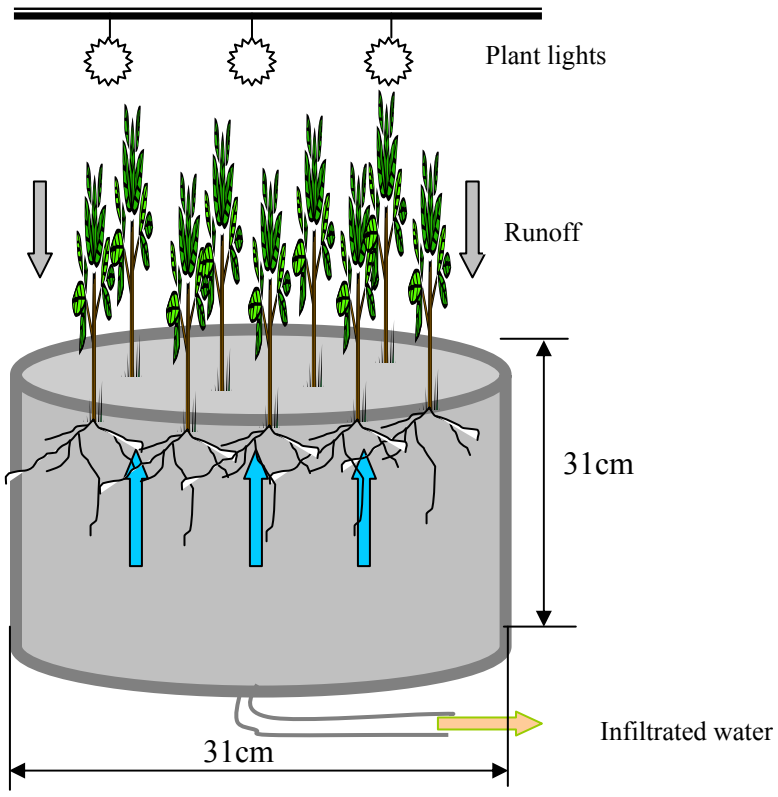


Figure 3.1. The pot experiment setup to plant grasses

Synthetic storm water runoff was prepared using tap water that was left to stand at room temperature for 24 hours to dechlorinate and to thermally equilibrate. The pH of synthetic runoff was adjusted to 7 using Na(OH) solution. The required chemicals and concentration for the runoff are shown in Table 3.4. The synthetic runoff was prepared in 200 L plastic drums and was applied to each pot at 4.1 cm/hr for 6 hours three times each month using a calibrated Masterflex pump. The flow rate was based on the assumption that the drainage area being served by the bioretention basin received 1.6 cm of rainfall over 6 hours and the area of bioretention basin was 5% of the drainage area with a runoff coefficient of 0.8.

The volume of runoff applied to each pot was about 15 L during one event. No water head built up above the surface of soil during the events due to the high permeability coefficient of the bioretention media. The overall experiment lasted 6 months and runoff was applied 20 times. Two different pollutant loadings and four metals were investigated. $CaCl_2$ at 40 mg/l was applied as a fixed background electrolyte. Moreover, N, P, K nutrients at concentrations of 2.55×10^{-4} M KNO_3, 9.68×10^{-4} M KH_2PO_4 (Peralta et al. 2002) and 1×10^{-4} M NH_4NO_3 (Jarvis et al., 2001) were included with the runoff once per month.

Table 3.4. Composition of the synthetic urban runoff used in the study

Element	Chemical Used	Source	Concentration (low) (mg/l)	Concentration (high) (mg/l)
Copper	Cupric Sulfate ($CuSO_4$)	Fisher Scientific	0.08	0.2
Lead	Lead Chloride ($PbCl_2$)	Fisher Scientific	0.08	0.2
Zinc	Zinc Chloride ($ZnCl_2$)	Fisher Scientific	0.6	1.5
Cadmium	Cadmium Chloride ($CdCl_2$)	Fisher Scientific	0.02	0.05
Calcium	Calcium Chloride ($CaCl_2$)	Fisher Scientific	40	40

Figure 3.2. The experimental setup

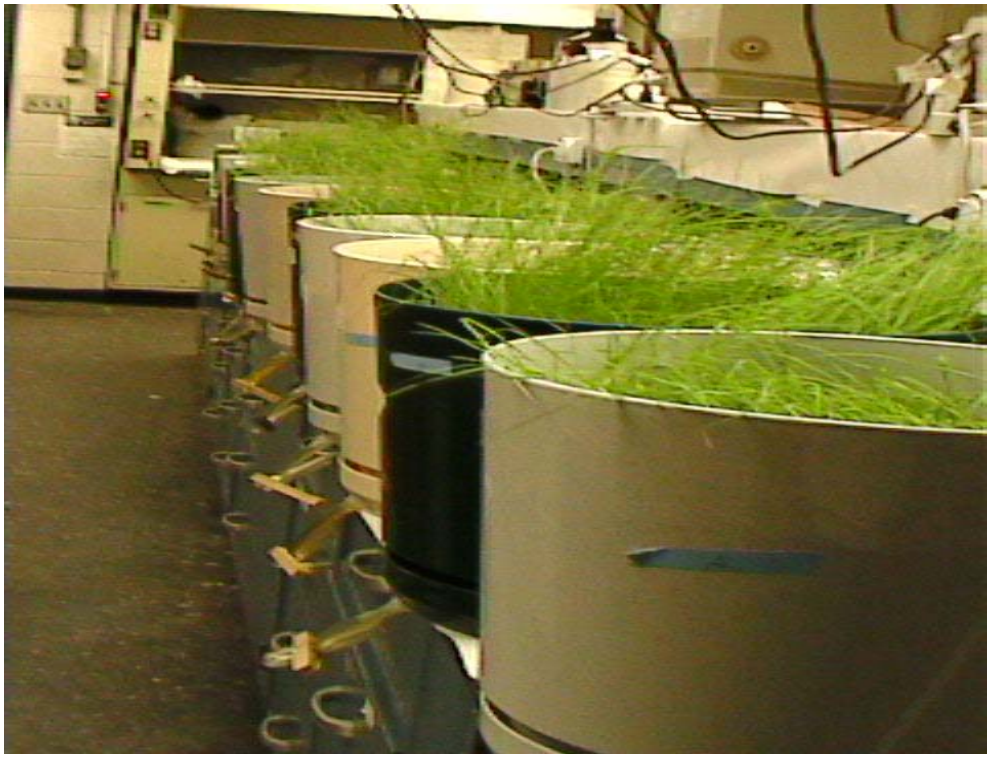


Figure 3.3a. The plants used for experiments

Figure 3.3b. The plants used for experiments (Switch Grass)

Fig 3.3c. The plants used for experiments (Kentucky -31)

3.4 Sampling and Pretreatment

3.4.1 Soil

Soil samples were collected every 30 days with a stainless steel sampler. Samples from two soil depths (0~5 cm and 20~25 cm) were taken and stored in transparent plastic bags during sampling to avoid excessive desiccation and prevent airborne contamination. Each sample has two replicates. The sample soil was air dried, ground and sieved (<2 mm) for future use. Soil pH was tested with 2.5:1 (volume of 0.01 M $CaCl_2$: weight of soil) after shaking 2 hrs (Wenzel 1999).

3.4.2 Vegetation

Plant samples were randomly collected once a month starting after fifty days of growth. Fresh plant material was separated from soil by washing with tap water then DI water to remove adhering soil particles and dust (Dahmani, 2000). Special attention was given to the roots, which were scrubbed free of soil and rinsed thoroughly. Roots and shoots were separated and air dried at room temperature covered with aluminum foil to avoid airborne contamination.

Air-dried plant samples were cut into pieces, placed in aluminum trays, and dried to a constant mass at 80°C for 12 hr. This temperature was used because below this temperature all moisture may not be removed from the sample, and above this temperature thermal decomposition may occur, resulting in a reduction in dry weight (Campbell and Flank 1998; Cardwell 2002). Subsamples were chopped finely with stainless scissors and ground with mortar and pestle to <1.0 mm to ensure homogeneity and to facilitate organic matter digestion.

3.4.3 Input and Infiltrated Water

Three input samples (0~1, 3~4 and 5~6 hrs) were collected and analyzed for all four metals to determine the input concentration. Infiltrated water samples were collected at the bottom of pots. Infiltrated water was collected as a composite for 0~1, 1~ 2, 2~ 4 and 4~6 hrs, for a total of 4 samples for the first two months, and for 0~1, 1~3 and 3~6 hrs, for a total of 3 samples for the remainder, by which enough effluent samples were taken to calculate the output metals. After collection, samples were placed in 125 ml plastic bottles, one drop Trace Metal Grade HNO_3 (EMD Chemicals Omni Trace Grade or Fisher Scientific Metal Grade for Atomic Absorption) was added. Samples were refrigerated until they were analyzed.

3.4.4 Metal Extraction

Soil: Subsamples were digested using aqua regia (Berrow and Stein, 1983; Blum et al. 1989; Wenzel, 1999; Madyiwa, 2002). 1.000 g of soil was mixed with 20 mL aqua regia (HCl: HNO3 (3:1)) in a 125 mL Pyrex beaker and the mixture was heated for 3 hrs without boiling dry on a steam bath. After cooling to room temperature (23 ± 1^{o}C), the residue was extracted with 0.01 M HCl, quantitatively transferred to a 50 mL volumetric flask and diluted with DI water. The volumetric flasks were sealed with laboratory film (Parafilm, Chicago, IL) and shaken a few minutes manually. Subsamples were filtered through a 0.45 μm filter (Gelman Sciences sterile aerodisc). The filtered samples were analyzed for metals.

Additional metal extraction was conducted with strontium nitrate. For strontium nitrate extractable metals, $Sr(NO_3)_2$ (0.01 M) was added to give a 1:2 (W/V) soil: solution

ratio. Typical extractions were conducted in 20 ml glass bottles. All bottles were sealed with caps and taped to reduce the gas exchange with the atmosphere during experiment. All extractions were conducted at the room temperature, 23 ± 1^{o}C. The suspension was shaken for 2 hrs using a large reciprocal shaker. Then the suspension was centrifuged at 3000 rpm for 10 min and then the supernatant liquid was removed with a pipette. After filtration with a 0.45 μm filter, the liquid was stored in clean polyethylene bottles (VWR scientific) at 4^{o}C prior to analysis.

Plant samples: Subsamples were digested by a mixture of concentrated nitric acid and 70% perchloric acid. 0.500 g (shoot) or 0.200 g (root) subsamples were transferred to 125 ml bottles for digestion. 6.0 ml HNO_3 and a boiling chip were added and swirled to wet the sample. After standing about 12 hrs, the digestion bottles were heated for 1 hr at 150°C on the digestion plate, then removed and cooled to room temperature and 2.0 mL $HClO_4$ were slowly added into the mixture. After the HNO_3 fumes evolved, the digestion bottles were heated continuously for 2 hrs at 215°C and 10 mL deionized water was added after 20 minutes cooling (Miller 1998). The digested samples were transferred to 25 mL volumetric flasks and diluted with DI water. Subsamples were filtered using 0.45 μm filters, stored in 50 ml clean polyethylene bottles, and refrigerated.

Water samples: 100 ml of well-mixed, acid-preserved sample was transferred to a flask, and 5 ml concentrated HNO_3 and a few glass bead boiling chips were added. During slow boil on a hot plate, 15~20 ml of mixture was obtained before precipitation occurred. Five ml concentrated HNO_3 was then added to the mixture and the mixture was covered with a watch glass and heated to obtain a gentle reflux. Addition of concentrated

HNO_3 was continued until digestion was completed, signified by a clear solution with light color. The final digested solution was filtered with a 0.45 μm filter and transferred to a 50 ml volumetric flask. This solution was then cooled and diluted for metal analysis (APHA Standard Methods, 1995).

3.5 Metal Analysis

All the aqueous samples were tested using a Perkin Elmer Model 5100 ZL Atomic Absorption Spectrophotometry (AAS). Different standards were used due to the different concentration ranges of the metals in the samples. Analysis for zinc concentration was carried out via the flame module of the AAS against three standards ranging from 0.025 to 2 mg/l. The concentrations of Cu and Pb were measured with the furnace module of the AAS against two sets of standards ranging between 2 and 50 □g/l, while those of Cd were tested against the standards ranging from 1 to 20 □g/l. All standards were prepared from 1000 mg/L stock solution (Pb, VWR Scientific; Cd, Cu, Zn, Fisher Scientific). The detection limit for the flame module under the operating conditions used for zinc analysis was 0.025 mg/l, while those of furnace module used for copper, lead and cadmium were 2, 2 and 1 □g/L, respectively. Varion Techtron Hollow cathode lamps were used for determination of Zn, Cu, Pb and Cd at a wavelengths of 213.9, 324.8, 283.3 and 228.8 nm, respectively. For samples having concentration higher than the highest standards, initial dilutions were carried out manually by an amount appropriate to lower the concentration to within the ranges specified; further dilutions for Cu, Pb and Cd, if necessary, were carried out using the autodilution feature of the module, which can dilute concentration up to 20 times the concentration of the highest

standard used. The measured concentrations were multiplied by dilution factor to obtain final concentrations.

3.6 Data Analysis and Calculation

3.6.1 Cumulative Volume and Mass

The cumulative volume of inflow and outflow during 20 events are calculated by:

$$V_{\text{in}} = \int_0^t Q(in)dt = \sum_i^{20} V_i \tag{3.1}$$

$$V_{out} = \int_0^t Q(out)dt = \sum_0^{66} V_j \tag{3.2}$$

Where V_{in} and V_{out} are the total cumulative volume of inflow and outflow, respectively. V_i is in cumulative volume for one event; i is the number of events. V_j is the interval volume, j is number of sample intervals; t is the experimental time.

Total retention of metals mass in the pots was calculated as:

$$\text{M} = \text{M}_{\text{in}} - \text{M}_{\text{out}} = \sum_{i=1}^{20} C_i V_i - \sum_{j=1}^{66} C_j V_j \tag{3.3}$$

Where M is the total retention mass in the pots, and C_i and C_j are the measured average metal concentrations of influent and effluent samples respectively.

3.6.2 Concentration of Metals in Plants and Soils

The concentration of the metals in the plants and soils, L, are calculated as:

$$\mathrm{L} = \frac{(\mathrm{C_d} - \mathrm{C_b}) \times V}{m} \tag{3.4}$$

Where C_d is the concentration of diluted solutions; Cb is the concentration of method blanks; V is the volume of diluted solutions and *m* is the dry mass of the sample material.

3.6.3 Mass Balance Analysis

The distribution of the metals in the bioretention media and plants are calculated and checked shown as below:

$$\mathrm{M_T} = \mathrm{M_s} + \mathrm{M_p} + \mathrm{M_e}$$
$$\sum_{\mathrm{i=1}}^{20} \mathrm{C_i V_i} = \mathrm{L_s} m_s + \mathrm{L}_p m_p + \sum_{\mathrm{j=1}}^{66} \mathrm{C_j V_j} \tag{3.5}$$

Where M_T is the total metal mass input; $M_{s,}$ M_p and M_e are the total metal mass in the bioretention media, plants and effluents respectively. L_s and L_p are the concentrations of metals in the bioretention media and plants respectively. m_s and m_p are the total masses of bioretention media and plants respectively.

3.6.4 Statistical Analysis

Metal concentrations of soil, plants and influent and effluents of pots are the means of two replicate samples. For each sample set, standard deviations are calculated using the statistical function available in Microsoft EXCEL 2000. Regression analysis was undertaken for evaluating the relationship between the concentrations of metals in plants and bioretention media using the statistical package SAS and the one-way ANOVA was performed to compare the difference between the concentration of metals of the effluents from control pots and plant-growing pots using the SAS.

Chapter 4

RESULTS AND DESSCUSSION

4.1 Effluent Metal Concentrations

The total volumes of influent and effluent for the pots with *Panicum virgatum* (PV), *Kentucky-31* (K-31), *Bromus ciliatus* (BC) and control pots (C) obtained by Equations 3.1 and 3.2 are shown in Table 4.1-1.

Table 4.1-1. Influent and effluent volumes for pots with different plants

Pots	PV	K-31	BC	C
Influent volume (L)	300	300	300	300
Effluent volume (L)	279±2	276±4	277±2	282±1
Collection ratio	0.93	0.92	0.92	0.94

From Table 4.1-1, it can be seen that 92 -93 % of the total influent water volume was collected from the bottom outlet over the duration of the run. With the same influent volume, a slightly higher effluent volume was obtained for the control pots than for the others. These results may be due to the plants growing in the pots, which can enhance water evaporation. Murphy et al. (2001) established pot experiments (2×2 m) to investigate evaporation with the different plant densities and reported that the presence of pasture plants increased total evaporation, with a maximum of 4.0 mm/day recorded in both experimental days. Our observations agree with these results.

Table 4.1-2. Concentration of metals in influent and effluent with low and high loading

Pollutants			Zn (mg/l)	Cu (µg/l)	Pb (µg/l)	Cd (µg/l)
Low loading	Input		0.655±0.11	71±5	67±6.1	21±2.4
	PV	Range	< 0.025 - 0.153	< 2 -15	< 2 -7.5	< 1-2.8
		Average ± S.D	0.04±0.02	8 ±3	3.3±1.5	<1
		Removal Efficiency (%)	93±4	88±4.5	95±2	>95
	K-31	Range	< 0.025 - 0.11	< 2-19.3	< 2-9.14	<1-2.98
		Average ± S.D	0.039±0.022	8.6± 4.1	3.4±1.6	<1
		Removal Efficiency (%)	94±3	87±5.5	95 ±2	>95
	BC	Range	< 0.025 - 0.085	< 2 -17	< 2 -6.4	<1-2.5
		Average ± S.D	0.037±0.014	7.8±3.2	3.4±1.0	<1
		Removal Efficiency (%)	94±2	88±5	95±2	>95
	C	Range	< 0.025 - 0.12	< 2 -22	< 2 -12	<1 - 3.9
		Average ± S.D	0.037±0.02	8.9± 5.3	3.2±1.7	<1
		Removal Efficiency (%)	94±3	86±7	95 ±2	>95
High loading	Input		1.435±0.12	167±19	162±18	48±7.2
	PV	Range	< 0.025 - 0.42	2.5 - 22	< 2 - 21	<1 - 3
		Average ± S.D	0.049±0.025	8.6±4.1	4.7±2.9	<1
		Removal Efficiency (%)	97±2	94±2	97±2	>98
	K-31	Range	< 0.025-0.15	< 2 - 20	< 2 -13	<1 - 4.5
		Average ± S.D	0.049 ± 0.028	8.9±3.5	4.1±2.1	<1
		Removal Efficiency (%)	97±2	93±2	97±1	>98
	BC	Range	< 0.025 - 0.14	< 2 - 25	< 2 - 9.2	<1 - 2.9
		Average ± S.D	0.047 ± 0.02	9.8±4.5	4.7±1.5	<1
		Removal Efficiency (%)	97±1	93±2	97±1	>98
	C	Range	< 0.025 - 0.109	< 2 - 22	< 2 -8.9	<1 - 3.1
		Average ± S.D	0.047 ± 0.023	8.3±3.9	4.5±1.7	<1
		Removal Efficiency (%)	97±2	93±3	97±2	>98

Two influent metal loadings were applied to the pots. In the low loading influents, the concentrations of Zn, Cu, Pb and Cd were 0.66±0.11 mg/l, and 71±5.6, 67±6 and 21±2.4 µg/l, respectively. In the high loading influents the concentrations of Zn, Cu, Pb and Cd were 1.44±0.12 mg/l, and 167±19, 165±18 and 48±7 µg/l, respectively.

The metal concentrations in the effluents show some variation. Some of them are below the detection limit, while others are unexplainable high (Table 4.1-2). The variations of the concentrations in the effluent samples are due to many factors, such as different input etc. The data below the detection limit were determined by fitting a normal distribution to the above-reporting-limit data and extrapolating the values below the limit (Helsel, 1990). In order to evaluate the concentration distribution of different metal in the effluents, probability distribution plots of metal concentrations were created to statistically determine the most possible concentration range. Probability plots were created by ranking the observed concentrations in ascending order. Each value is assigned a rank from 1 to the total number of observed values. The plotting position for each value on the probability scale is determined as follows:

$$p = \frac{i}{(n+1)} \qquad (4.1)$$

Where p is the probability, i is the rank number and n is the total sample number. Based on this method, the probability distributions of the concentrations in effluent are shown in Figures. 4.1-1 to 4.1-4. The distribution plots indicate the concentrations of Zn, Cu Pb and Cd in most effluent samples fall into ranges of 0.025 to 0.05 mg/l, 4 to 12 µg/l, 2 to 5 µg/l and 0 to 1 µg/l, respectively, with low loading and the ranges are 0.025 to 0.075 mg/l, 4 to 12 µg/l, 3 to 6 µg/l and 0 to 1.5 µg/l, respectively, with the high loading. The tick marks on the cumulative percentage are not uniform, but arranged to match the

distance between the quantiles of a normal distribution. As a result, a linear distribution curve on the probability plot corresponds to a normally distributed data set. The distribution curve of the data is linear

One-way analysis of variance was carried out to compare mean concentrations of metals in the effluents from the different pots. The results show that there are no significant differences between the mean concentrations of metals in the effluents from plant-growing pots and control ones with 95% confidence levels (Table 4.1-3).

Table 4.1-3. The F and P values using the one-way ANOVA method (α=0.05)*

Metals		Zn		Cu		Pb		Cd	
		F	P	F	P	F	P	F	P
Low loading	PV/C	0.18	0.67	2.6	0.11	0.001	0.93	1.1	0.3
	K-31/C	0.004	0.94	0.87	0.35	0.51	0.47	0.44	0.51
	BC/C	0.49	0.49	2.16	0.14	0.4	0.52	5.47	0.02
	PV/K-31	0.22	0.64	0.57	0.45	0.42	0.52	0.17	0.67
	PV/BC	1.1	0.29	0.33	0.57	0.3	0.58	2.1	0.15
	K-31/BC	0.31	0.58	0.34	0.57	0.06	0.81	3.5	0.06
High loading	PV/C	0.17	0.68	0.12	0.72	0.27	0.6	0.44	0.51
	K-31/C	0.16	0.69	0.7	0.4	0.94	0.33	1.37	0.24
	BC/C	0.001	0.97	4.1	0.04	0.65	0.42	0.1	0.75
	PV/K-31	0	1	0.2	0.65	1.47	0.22	2.77	0.1
	PV/BC	0.21	0.64	2.8	0.1	0	1	1.1	0.3
	K-31/BC	0.2	0.65	1.8	0.18	3	0.08	1.1	0.3

* One-Way ANVOA is a statistical method for making a single test to find out whether two or more sets of data have the same mean. If p-value <α (significance level), means of two set of data are significantly different. If the p-value >α, the means are not significantly different.

Therefore, the average concentrations in effluents for the same metal were essentially the same for different pots when the input was the same. For example, the concentration of Zn was about 0.039 mg/l for all experiment systems with the low loading. For Zn, Cu and Pb, the average effluent concentrations with low and high loading were nearly constant. The double loading did not increase the effluent concentrations significantly, This is very important for bioretention facilities, to capture the metals in the first flush in which the majority of the event pollutant load is contained. For Cd, the concentrations in the most effluent samples were below the detection limit with both low and high loading. Therefore the mean concentrations in the effluent are less than 1 μg/l, resulting in the >95% and >98% of removed efficiencies.

Based on the average effluent concentrations, 93% removal for zinc was observed for PV, and 94% for K-31, BC and C, respectively, with the low loading. While with the high loading, the removal efficiencies were 97% for PV, K-31, BC and C, respectively. For copper, a removal of 88% for PP, 87% for PK, 88% for PB and 86% for PC was observed with the low loading, but with the high loading, the removal efficiencies were higher, 94% for PV, 93% for K-31, 93% for BC and 93 % for C, respectively. For lead, the removals showed the same values, 95% and 97%, with low and high loading, respectively, for all four types of pots. The Cd concentrations in most effluent samples were below the detection limit, resulting in high removal efficiencies. All the removal efficiencies indicated high affinity of metals to the bioretention media. Higher removal efficiencies were achieved with higher input for the same metal in both plant-growing and control pots. The removal values are based on the average concentrations of effluent and influent. Davis et al. (2001) carried out two box prototype bioretention system

experiments and the results showed that when the input of Zn, Cu and Pb were 600±8 140±32 and 61± 3 µg/l, respectively, 91%, 95% and 93% reduction of Zn, Cu and Pb were achieved, respectively, by18 cm of bioretention depth in the small system, and more than 97% of these three metals were sorbed when the depth of the media was increased to 61 cm. In the large bioretention system, 89%, 92% and 88% of metals were removed, respectively, by the 25 cm depth and 93% of Cu and more than 98% of Zn and Pb were captured by the 59 cm depth media. Observations from the Greenbelt bioretention facility showed reductions of Zn, Cu and Pb all more than 90% by the depth of 20cm (Davis et al. 2003).

Comparing the results of the large system to those of this study, better reductions of metals are found with 25 cm of bioretention media in this investigation, although the effluent concentrations in this study are higher than those of the large system, which is caused by the higher input in the present study. This may be due to the difference of experiment conditions, such as components of the bioretention media, the depth of the mulch layer, which is an important component to capture metals in the runoff, and the grasses growing in the top surface. Regardless, all of those results broadly agree with this study and are consistent.

In order to evaluate mass removals, total retention mass and percentage of metals in the pots are calculated based on equation 3.3. The same reduction trends are observed as noted for concentration (Table 4.1-4). With the same influent loading, no significant difference in metal retention was observed between plant-growing pots and control ones. The plots of concentration versus time for different metals indicated that the plants did not affect the effluent concentrations and removal efficiencies of metals within a short

time. Based on both the concentration and total mass removals, cadmium showed higher removal efficiency than zinc, copper and lead. The percent removal trend by the media was Cd > Pb > Zn > Cu.

The metal ions in the influents were immobilized by different physico-chemical processes like precipitation and adsorption, and the metal ions are expected to be bonded to the organic matter. In this study, the 0-5 cm layer of media with 50% soil and 50% mulch, in which the organic matter concentration is high, results in large capacities for metal adsorption and immobilization of the metals. Udom et al. (2004) reported that there were highly positive and significant ($P < 0.01$) correlation coefficients between organic matter (OM) and Zn and between OM and Cd in sewage soil (mixture of sewage and soil). Robertson et al. (1982) found that the CEC and OM contents can be used as good predictors of heavy metal mobility in soils. Hence, as soil OM increases, there is the tendency for more Zn, Cd, Cu and Pb to be adsorbed on the soil complex, thus reducing their mobility and ability to concentrate to phytotoxic levels in the soil. But with an increasing amount of applied metals, the capacities of soil and mulch will decrease as a result of declining metal reduction efficiencies. Using the plants to uptake those metals may provide an approach to clean the bioretention media and maintain high adsorption capacities. The roles of the plants for metal uptake and immobilization will be discussed in the following section.

Table 4.1-4. Retained metal mass and percentage in different pots

			Zn (mg)	Cu (mg)	Pb (mg)	Cd (mg)
Low loading	Input		203	21.6	20.8	6.6
	PV	Retention	191±0.6	19.5±0.1	19.9±0	6.4±0.1
		Retention percentage (%)	94.5	90.5	95.7	96.9
	K-31	Retention	191±0.2	19.3±0	19.8±0.1	6.4±0.1
		Retention percentage (%)	94.6	89.4	95.5	96.3
	BC	Retention	202±1.3	19.4±0.2	20.1±0.2	6.3±0
		Retention percentage (%)	99.5	90.0	96.7	94.6
	C	Retention	192±0.5	19.2±0	19.9±0	6.4±0
		Retention percentage (%)	94.9	89.0	95.8	96.9
High loading	Input		434	51.4	49.3	14.8
	PV	Retention	420±3.4	49.2±0.5	48±0	14.5±0.1
		Retention percentage (%)	96.7	95.6	97.5	97.8
	K-31	Retention	423±1.9	49.1±0	48.2±0.1	14.5±0.1
		Retention percentage (%)	97.4	95.4	97.8	98.0
	BC	Retention	425±1.7	49.3±0.1	48.9±0.1	14.5±0
		Retention percentage (%)	98.0	95.9	99.1	97.9
	C	Retention	421±1.2	48.9±0	48±0	14.5±0
		Retention percentage (%)	97.0	95.1	97.3	98.0

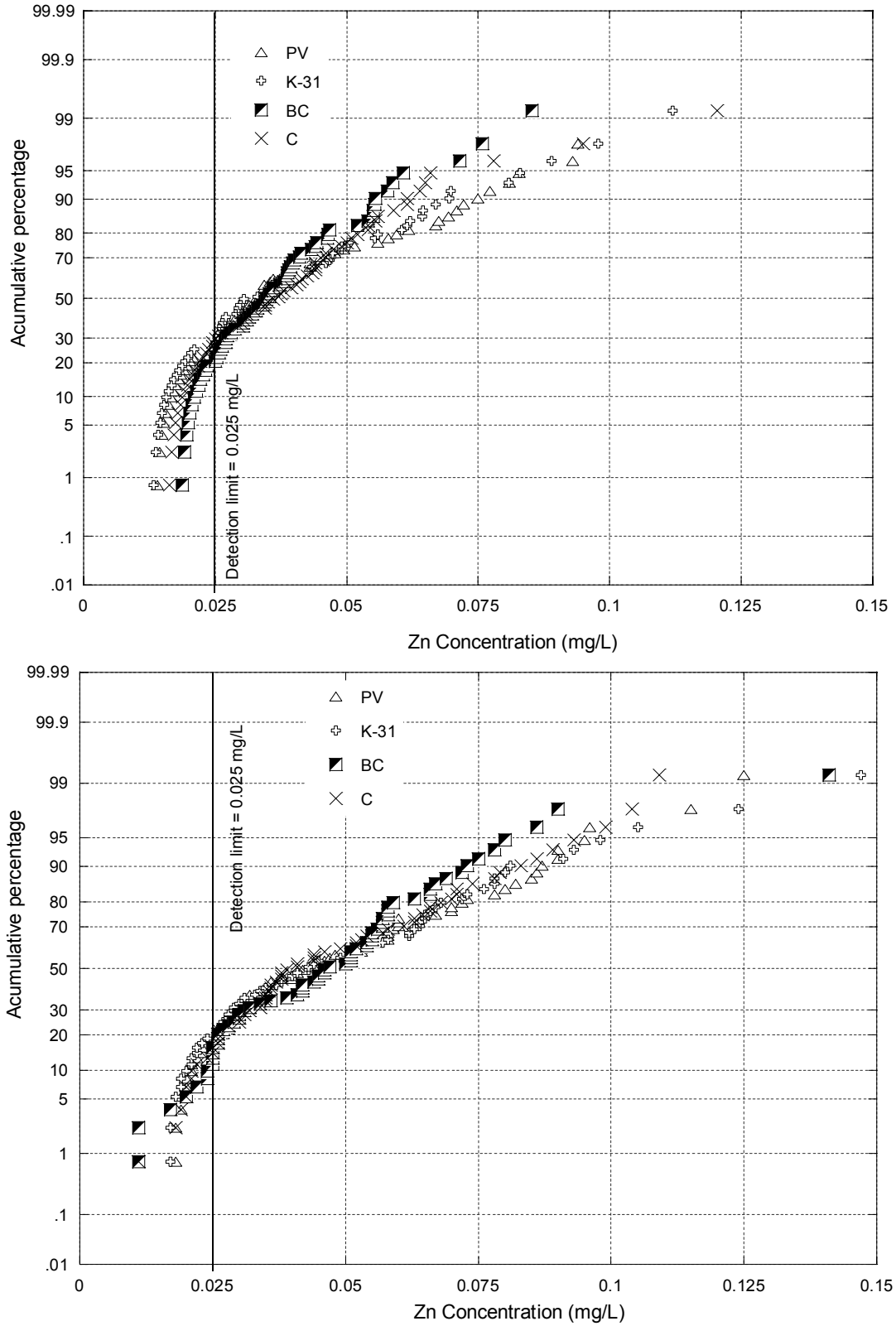


Figure 4.1-1. The distribution of different Zn concentrations in the effluent with low (top) and high (bottom) loading

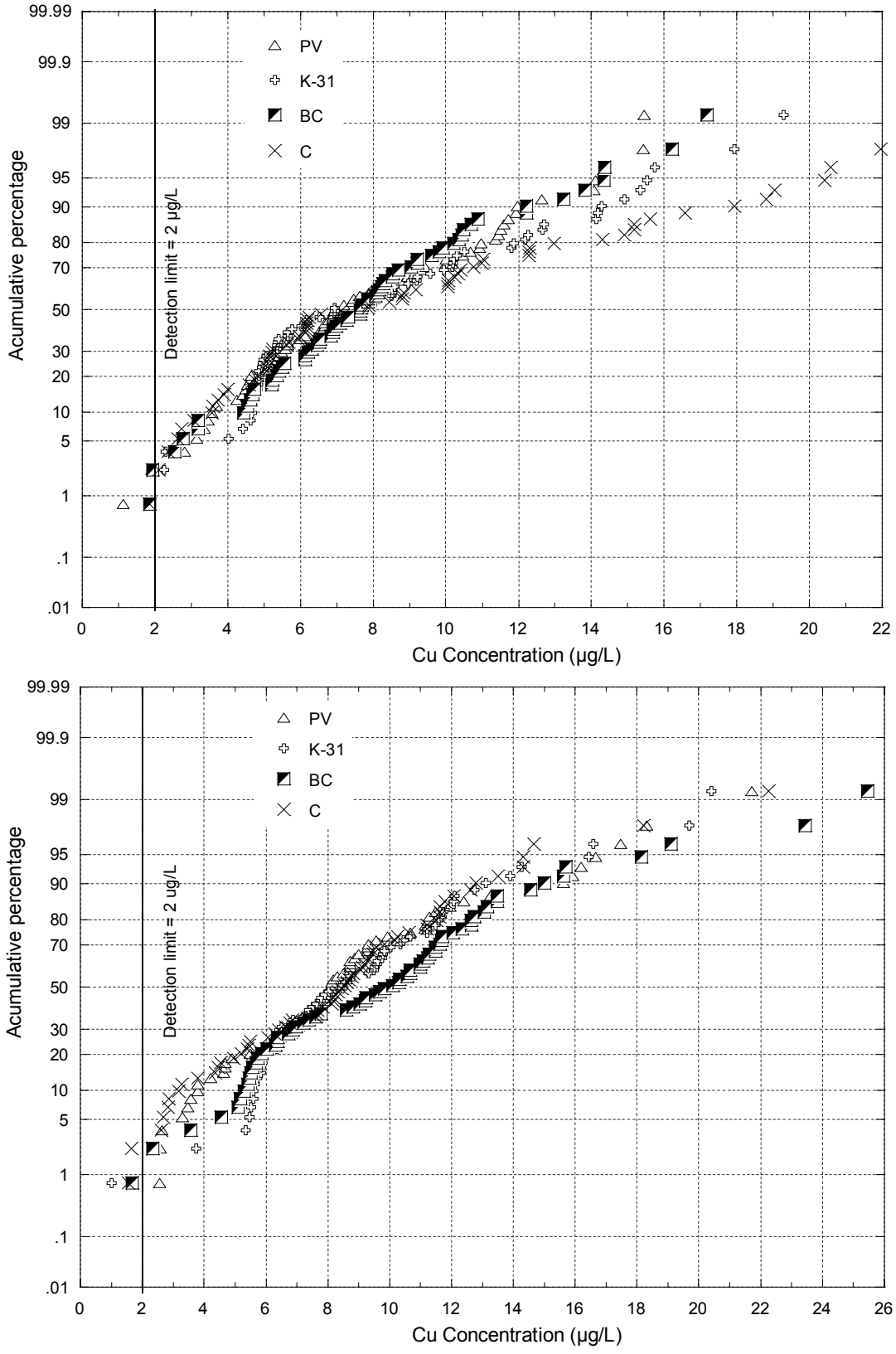


Figure 4.1-2. The distribution of different Cu concentrations in the effluent with low (top) and high (bottom) loading

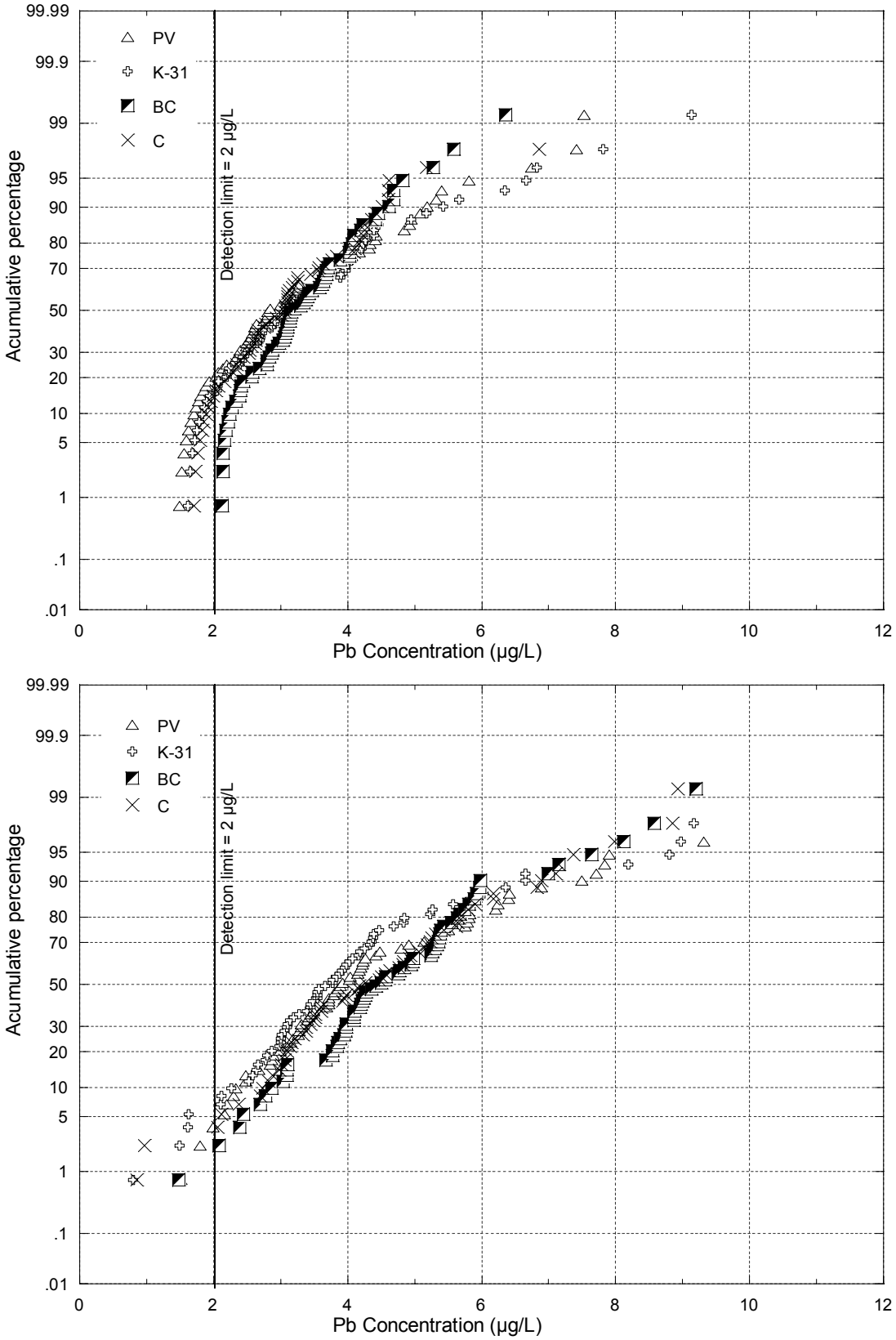


Figure 4.1-3. The distribution of different Pb concentrations in the effluent with low (top) and high (bottom) loading

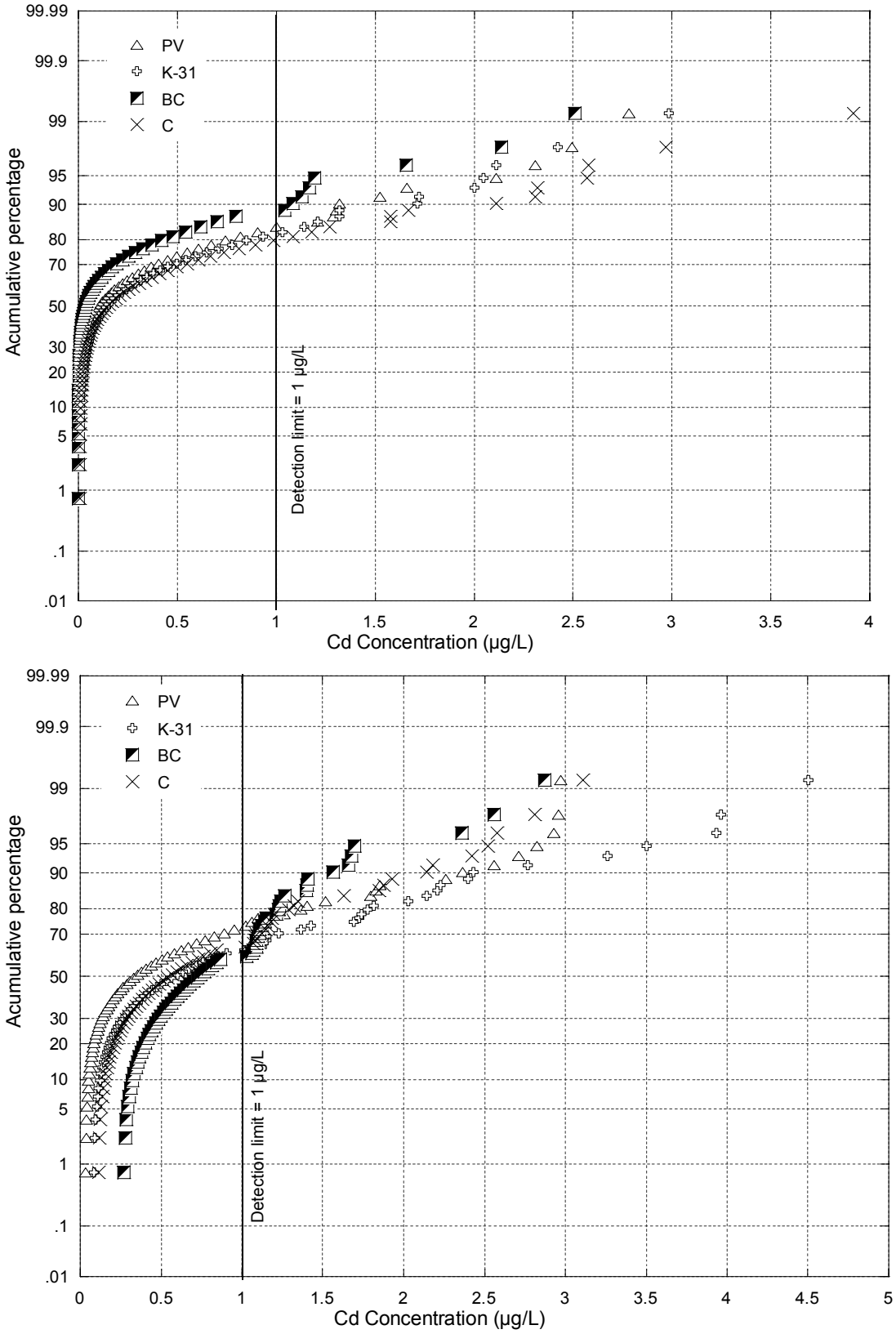


Figure 4.1-4. The distribution of different Cd concentrations in the effluent with low (top) and high (bottom) loading

4.2 Metal Accumulation in Plants

4.2.1 Temporal Variation of Concentration

The changes of Zn, Cu, Pb and Cd concentrations in *Panicum Virgatum* (PV), *Kentucky-31* (K-31) and *Bromus ciliatus* (BC) with time are shown in Tables 4.2-1 to 4.2-3.

In *Panicum Virgatum*, the levels of Zn, Cu, Pb and Cd contents in shoots ranged from 96 to 255, 9.2 to 24, 0.9 to 21 and 0.8 to 8.9 μg/g, respectively, and those in roots ranged from 217 to 658, 18 to 60, 2.2 to 37 and 1.9 to 9.6 μg/g, respectively, with the low loading. With the high loading, the content levels in the shoots were observed from 117 to 543, 12.5 to 31, 1.9 to 25 and 0.6 to 14 μg/g, respectively, and those in the roots ranged from 309 to 136, 30 to 148, 8.4 to 60 and 3.3 to 21 μg/g, respectively.

Similar accumulation patterns were found for all metals and a wide range of concentration in roots and shoots of *Kentucky-31* and *Bromus ciliatus* are observed. The concentrations of Zn, Cu, Pb and Cd in different grass species are different. Based on the concentrations of 230-day growing plants, the ability to bioconcentrate Zn in shoots increased in the order of K-31 >PV>BC, and in the roots in the order of K-31>BC>PV with low loading and those are K-31 >PV>BC and BC>K-31 >PV in shoots and roots , respectively, under high loadings. The accumulation of Cu in the shoots ranked as K-31>PV>BC, while in the roots in the order of K-31 >PV >BC. The accumulation patterns of Pb and Cd are PV>K-31>BC and PV>K-31>BC, respectively, in the shoots and PV>K-31>BC and K-31> BC >PV, respectively, in the roots, with low loading. With the high loading, the rank of Pb and Cd in shoots are BC>PV>K-31 and PV> K-31 >BC, respectively, and in roots are K-31 >BC>PV and K-31 >PV>BC, respectively.

Table 4.2-1. Concentrations of Zn, Cu, Pb and Cd in the tissues of *panicum virgatum* (average±S.D. μg/g)

Time (d)			80	110	140	170	200	230
Zn	low	Shoot	96±3.2	106±3.3	132±5.3	162±6.6	255±14	220±3.6
		Root	243±4.5	217±3.1	383±6.3	658±10	611±10	571±11
	high	Shoot	132±3.5	117±2.9	265±8.9	323±12.6	542±24	345±13
		Root	309±8.8	405±10	694±6.5	1362±15	921±32	828±16
Cu	low	Shoot	9.2±0.2	11±2.2	22±2.1	24±3.5	15±2.8	16±1.3
		Root	18±1.4	23±1.2	51±1.5	60±2.6	49±3.3	41±2.1
	high	Shoot	13±0.7	13±1.1	29±2.8	31±1.3	23±2.4	14±3.1
		Root	30±1	38±1.3	70±5.9	148±13	70±5.8	80±5.2
Pb	low	Shoot	1±0.4	0.9±0.1	6.4±0.6	15±1.4	21±1.8	21±3.0
		Root	4±0.3	2.2±0.3	18±1.6	37±5.7	28±3.8	24±3.2
	high	Shoot	3±0.3	2±0.2	18±2.3	17±2.9	19±4.5	25±1.3
		Root	10±0.2	8.4±1.2	28±2.9	50±3	60±5.0	47±2.4
Cd	low	Shoot	1±0	0.8±0.1	1.2±0.1	5.1±0.6	8.2±1.2	8.9±1.4
		Root	2±0	2.4±0.1	4.2±1.0	9.6±1.1	8.2±1.4	6.2±0.5
	high	Shoot	0.8±0	0.6±0.1	1.6±0.2	8±0.4	14±3.1	14±2.9
		Root	3.3±0.1	3±0.1	6±0.9	17±2.4	21±3.6	18±4.3

Table 4.2-2. Concentrations of Zn, Cu, Pb and Cd in the tissues of *kentucky-31*(average±S.D. μg/g)

Time (d)			80	110	140	170	200	230
Zn	low	Shoot	95±3.0	169±10	139±5.7	295±10	303±18	308±4.2
		Root	321±10	513±15	615±7.9	808±7.3	703±15	1176±21
	high	Shoot	82±7.1	111±5.6	158±10	263±19	325±13	373±10
		Root	290±12	577±19	789±20	1071±32	1564±14	1431±21
Cu	low	Shoot	8.3±0.2	9.2±0.5	20±2.5	21±1.6	22±3.7	19±2.6
		Root	21±0.3	31±1.1	50±5.1	78±6.3	45±4.7	54±6.6
	high	Shoot	8.4±0.2	9.6±0.6	24±.6	28±3.7	23±5.8	21±2.3
		Root	27±0.1	35±1.3	60±2.5	87±9.8	77±10	79±11
Pb	low	Shoot	2.2±0.8	1.3±0.2	7.8±1.5	9.7±2.3	15±0.1	20±2.8
		Root	6.0±0.2	5.2±0.8	30±4.2	32±2.7	29±3.2	25±2.4
	high	Shoot	1.8±0.2	1.8±0.5	7.0±0.6	12±1.5	20±2.5	25±2.3
		Root	12±0.3	8.3±0.7	61±5.2	62±3.5	83±6.5	83±2.2
Cd	low	Shoot	1.0±0.1	0.9±0.0	1.5±0.2	4.1±1.4	3.2±1.5	5.6±1.1
		Root	2.9±0.0	2.2±0.0	5.2±0.1	11±1.7	15±3.7	16±0.4
	high	Shoot	0.9±0.0	1.0±0.1	1.9±0.1	4.6±1.3	9.8±2.1	7.3±2.1
		Root	2.9±0.1	3.2±0.2	5.6±0.4	20±0.9	42±5.8	36±3.9

Table 4.2-3. Concentrations of Zn, Cu, Pb and Cd in the tissues of *Bromus ciliatus* (average±S.D. µg/g)

Time (d)			80	110	140	170	200	230
Zn	low	Shoot	105±5.9	108±11	170±14	174±12	181±21	193±11
		Root	472±16	822±24	876±18	922±20	1043±22	1014±31
	high	Shoot	127±5.3	130±19	242±12	256±22	267±34	343±21
		Root	761±11	983±20	1129±26	2233±12	2510±31	2758±31
Cu	low	Shoot	10.6±1.3	13.2±3.9	6.2±2.1	8.1±1.5	9.9±3.5	6.5±4.2
		Root	18±2.1	26±2.6	16±4.2	19±3.4	31±2.2	28±2.8
	high	Shoot	11±2.7	14±1.2	12±2.4	18±3.4	23±3.3	11±4.2
		Root	30.2±5.2	44±3.4	36±5.7	50±6.4	73±9.2	51±11
Pb	low	Shoot	6.1±0.8	5±1.1	7.5±1.2	7.7±1.1	6.3±2.1	8.7±2.3
		Root	11±2.3	14±2.5	9.1±1.4	12±2.7	22±3.2	15±2.3
	high	Shoot	7.3±0.8	6.2±1	14.1±1.9	21±1.5	35±2.2	28±3.5
		Root	21±2.3	24±2.7	26±3.2	28±3.6	31±4.3	29±6.5
Cd	low	Shoot	1.1±0.2	2.5±0.6	2.9±1.1	1.1±0.2	1.3±0.2	2.1±0.3
		Root	4.4±0.7	6.8±2.3	4.2±2.5	5.4±0.1	7.3±1.5	7.2±2.1
	high	Shoot	1.3±0.3	2.9±0.7	7.4±3	5.2±2.1	5.1±1.1	5.0±0.6
		Root	7.6±0.4	19±2.3	15±3.7	11±1.1	9.3±2.4	9.9±1.4

Therefore the highest Zn and Pb bioaccumulation abilities were observed in K-31 and PV respectively, whereas the lowest Cu and Cd accumulation capacities occurred in BC. The accumulation patterns in whole plants of PV, K-31 and BC increased in the order of Zn>Cu>Pb>Cd with both low and high loading following the trend of the input metal concentrations.

The results also show the contents of Zn, Cu, Pb and Cd in the shoots and roots of PV, K-31 and BC vary greatly in different growing phases (Fig. 4.2-1 to 4.2-12). After the first two months of runoff application, the contents of four metals in both roots and shoots are very low comparing to later values, except that of Cd in the root of BC. These phenomena may be due to the low cumulative metal input into the pots. After 120 days, the contents of metals in the shoots and roots reach the highest values gradually, although the profiles are not smooth during this time. The highest and lowest metal contents in the different parts of the plants are presented in Tables 4.2-4 to 4.2-5.

For these three plants, all four metal concentrations are typically higher in the roots than in shoots with both high and low loadings. Pilon et al. (2002) investigated the accumulation of Zn, Cu, Pb and Cd in four wetland plant species and reported that the concentrations of these four metals in the roots were higher than those in the shoots. MacFarlane et al. (2003) observed that Zn, Cu and Pb were accumulated in the largest proportions in root tissues of *Avicennia marina,* which indicated most absorbed metals were confined to the root in the outer cortex. Similar results have been obtained in laboratory studies with *Avicennia marina* (MacFarlane et al., 2002). Concentrations of

Table 4.2-4. Maximum and minimum metal contents in the roots and shoots of different plants with low loading

Metals			Zn		Cu		Pb		Cd	
			Max	Min	Max	Min	Max	Min	Max	Min
PV	Shoot	Content ±S.D (µg/g)	255±14	96±3.2	24±3.5	9.2±0.2	21±3	0.9±0.1	8.9±1.4	0.8±0.1
		Time (d)*	150	30	120	30	180	60	180	60
	Root	Content ±S.D (µg/g)	658±10	217±3.1	60±2.6	18±1.4	37±5.7	2.2±0.3	9.6±1.1	1.9±0
		Time (d)*	120	60	120	30	120	60	120	30
K-31	Shoot	Content ±S.D (µg/g)	308±4.2	95±3.0	22±3.7	8.3±0.2	20±2.5	1.3±0.2	5.6±1.1	0.9±0
		Time (d)*	180	30	150	30	180	60	180	60
	Root	Content ±S.D (µg/g)	1176±21	321±10	78±6.3	21±0.3	32±4.2	5.7±0.8	16±0.3	2.2±0
		Time (d)*	180	30	120	30	120	60	230	60
BC	Shoot	Content ±S.D (µg/g)	193±11	105±5.9	13±3.9	6.2±2.1	8.7±2.3	5.0±1	2.9±1.1	1.1±0.2
		Time (d)*	180	30	60	90	180	60	90	120
	Root	Content ±S.D (µg/g)	1014±31	472±16	31±2.2	16±3.6	22±3.2	9.1±1.4	7.3±1.5	4.2±2.5
		Time (d)*	180	30	150	60	150	90	150	90

* The time at which the corresponding content in the plant parts occurred.

Table 4.2-5. Maximum and minimum metal contents in the roots and shoots of different plants with high loading

Metals			Zn		Cu		Pb		Cd	
			Max	Min	Max	Min	Max	Min	Max	Min
PV	Shoot	Content ±S.D (μg/g)	542±24	117±2.9	30.7±1.3	12.5±0.7	25±1.1	1.9±0.2	14±3	0.6±0.1
		Time (d)*	150	60	120	30	180	60	180	60
	Root	Content ±S.D (μg/g)	1362±15	309±8.1	148±13	30±1	60±5.2	8.4±1.2	21±4	3.3±0.1
		Time (d)*	120	30	120	30	150	60	150	30
K-31	Shoot	Content ±S.D (μg/g)	373±10	82 ±7.1	28±3.7	8.4±0.2	25±2.3	1.8±0.2	9.8±2.1	0.9±0
		Time (d)*	180	30	120	30	180	30	150	30
	Root	Content ±S.D (μg/g)	1564±14	290±12	86.6±9.8	27±0.1	83±6.5	8.3±0.7	42±5.8	2.9±0.1
		Time (d)*	150	30	120	30	180	60	150	30
BC	Shoot	Content ±S.D (μg/g)	342±21	127±5.3	23±3.3	11±2.7	35±2.2	6.2±1.1	7.4±3	1.3±0.3
		Time (d)*	180	30	150	30	150	60	90	30
	Root	Content ±S.D (μg/g)	2758±31	761±11	73±9.3	30±5.2	31±4.2	21±2.3	19±2.3	7.6±0.4
		Time (d)*	180	30	150	30	150	30	60	30

* The time at which the corresponding content in the plant parts occurred.

Pb and Cd in roots of *Vetiver, Bahia, St. Augustine* and *Bana* growing on oil shale mined land were higher than those in the shoots (Xia, 2004). Dahmani et al. (2000) found that Zn, Pb, Cd and Cu concentrations were significantly higher in the roots than in the stems and green leaves of *Armeria maritime ssp. Halleri.*

Moreover, the concentrations of metals in shoots and roots exposed to the high loading were higher than those with low loading. This indicates that the high contaminant input enhanced the metal uptake of the tissues. Dudka et al. (1996) concluded from a field study that the concentrations of Zn, Pb and Cd in tissues would increase with increase of metals in soils. With the increase of metals added to the soil, the concentration in the plant tissues appropriately increased (Madyiwa et al. 2003). Jiang et al. (2003) explained relationships between Cd uptake by plants and Cd loading, and observed that the concentrations of Cd in the shoots and roots were enhanced by the addition of Cd. The current findings were broadly agreement with those reports. The concentrations of the four metals in the tissues increased with increasing input metal loading.

The concentrations of metals in the plants (80 days after seeding and 30 days after input applied) with low and high loadings are compared to those of plants no metal input in Figures 4.2-1-4.2-12. The results show the concentrations of metals in the tissues were in the order of $C_{high}>C_{low}>C_{blank}$, except Zn in root of the K-31, in which the Zn content showed the reverse order of $C_{blank} >C_{low}> C_{high}$, and Zn, Pb and Cd in shoots of K-31 in which the concentration ranks were $C_{low}> C_{high}> C_{blank}$. The reasons are unclear. The trends of metals accumulation in the tissues of plants under the different metal input

conditions indicated that the metal input enhanced the concentrations of metals in plant tissues, as discussed above.

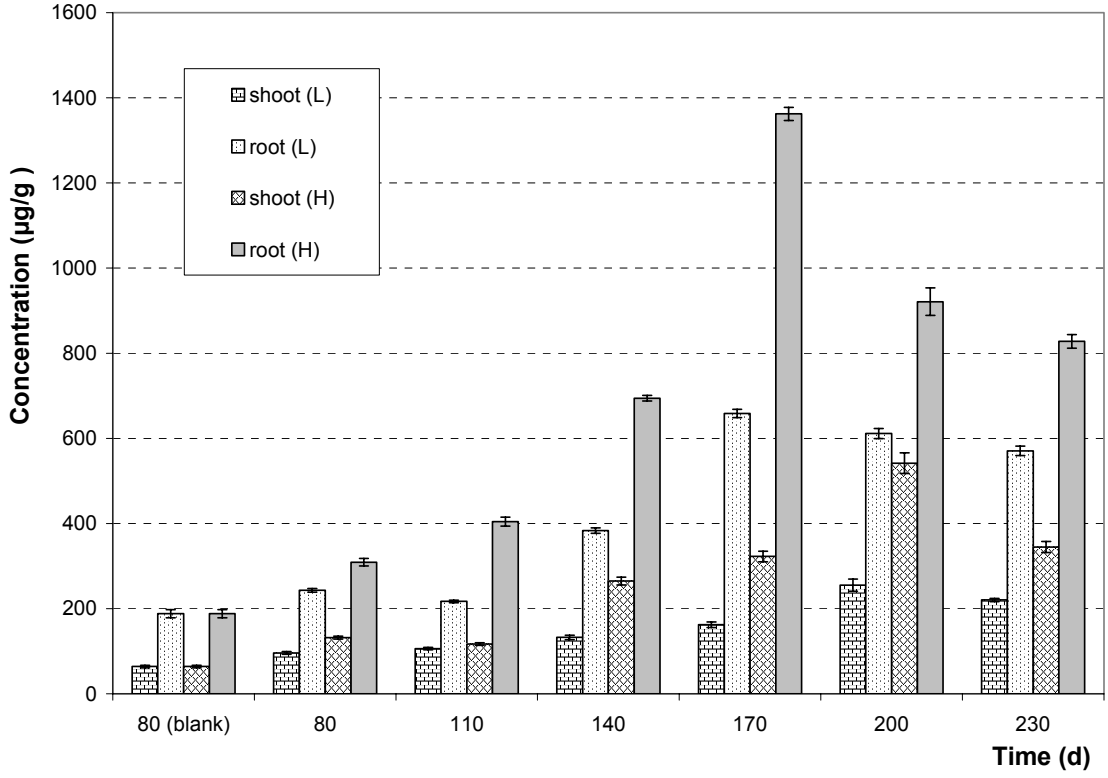


Figure 4.2-1. The concentrations of Zn in *Panicum Virgatum* at various time and both low and high loadings

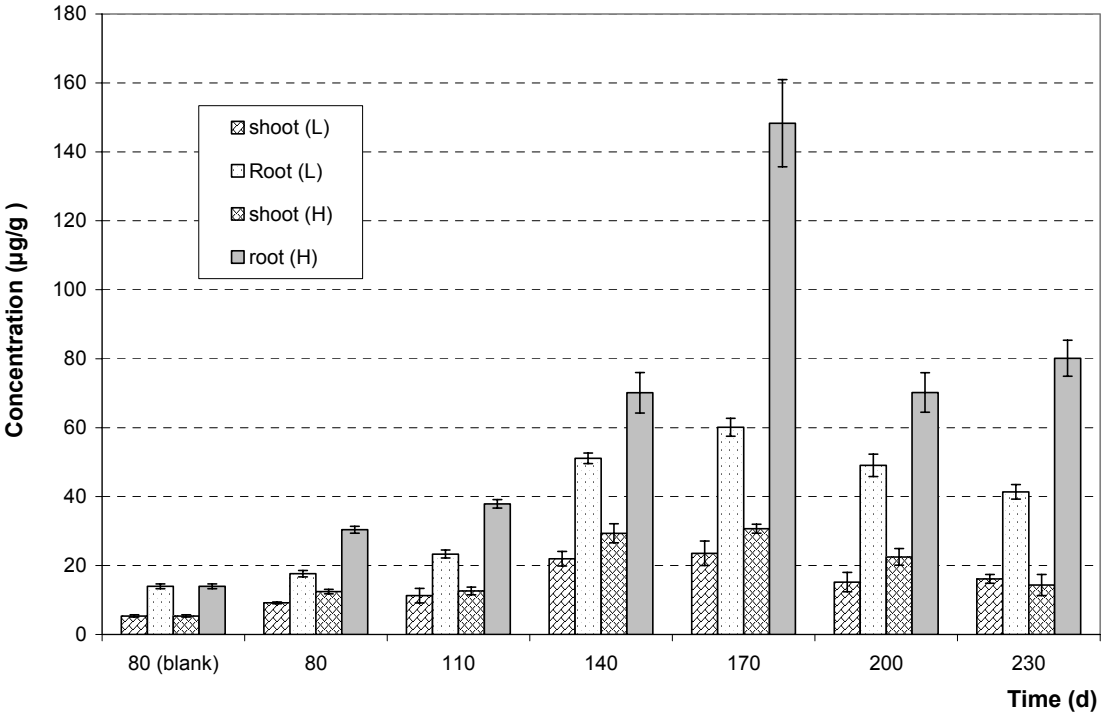


Figure 4.2-2. The concentrations of Cu in *Panicum Virgatum* at various time and both low and high loadings

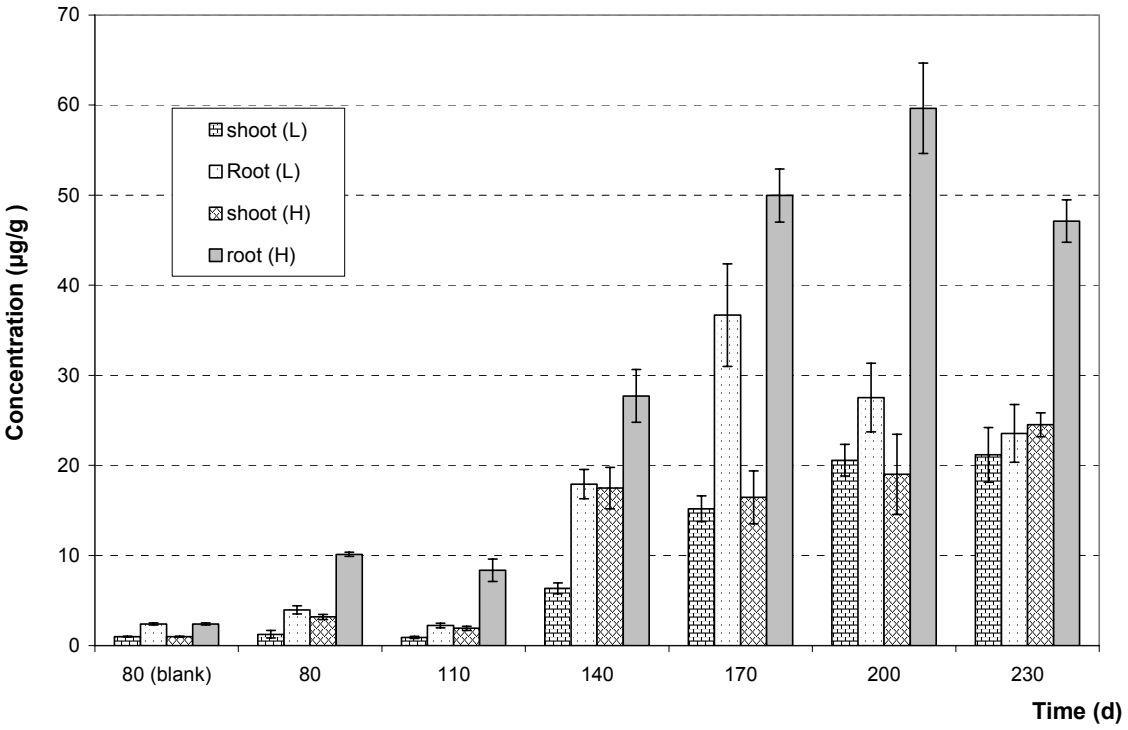


Figure 4.2-3. The concentrations of Pb in *Panicum Virgatum* at various time and both low and high loadings

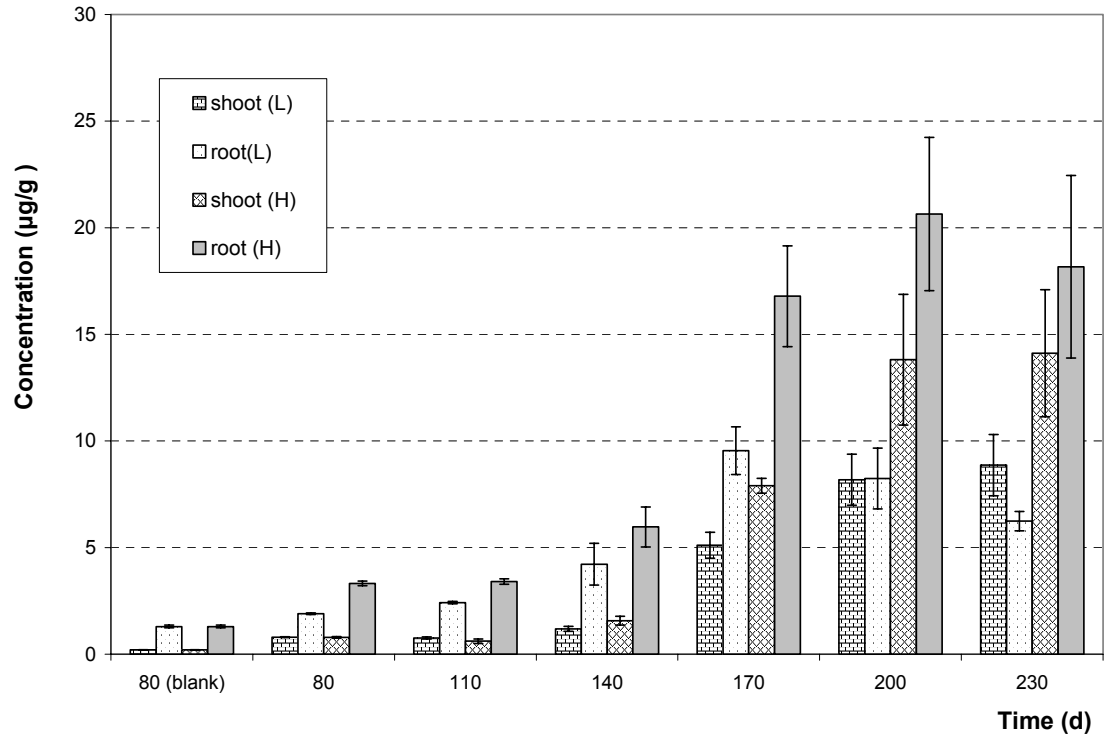


Figure 4.2-4. The concentrations of Cd in *Panicum Virgatum* at various time and both low and high loadings

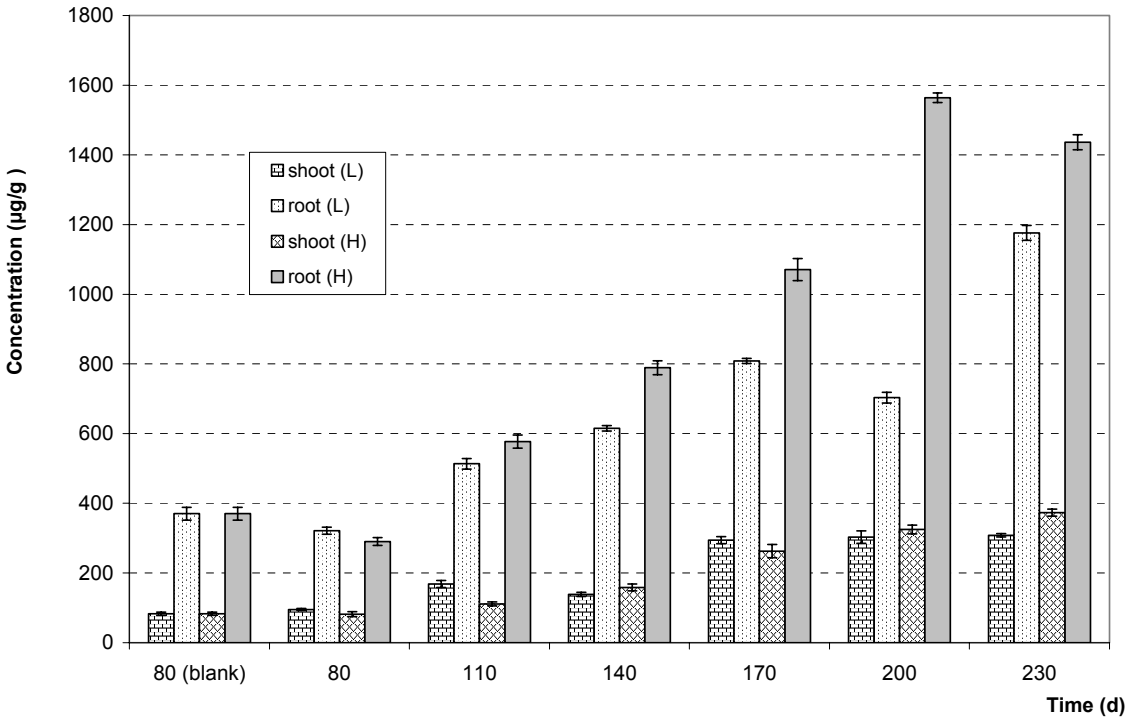


Figure 4.2-5. The concentrations of Zn in *Kentucky-31* at various time and both low and high loadings

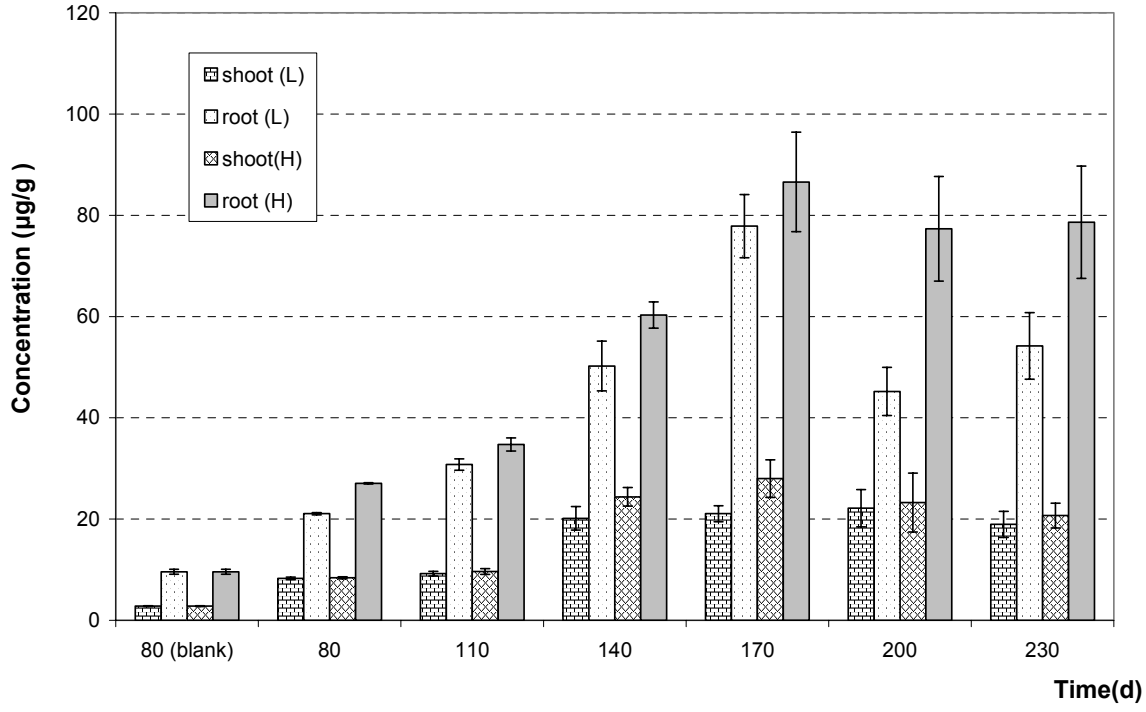


Figure 4.2-6. The concentrations of Cu *in Kentucky-31* at various time and both low and high loadings

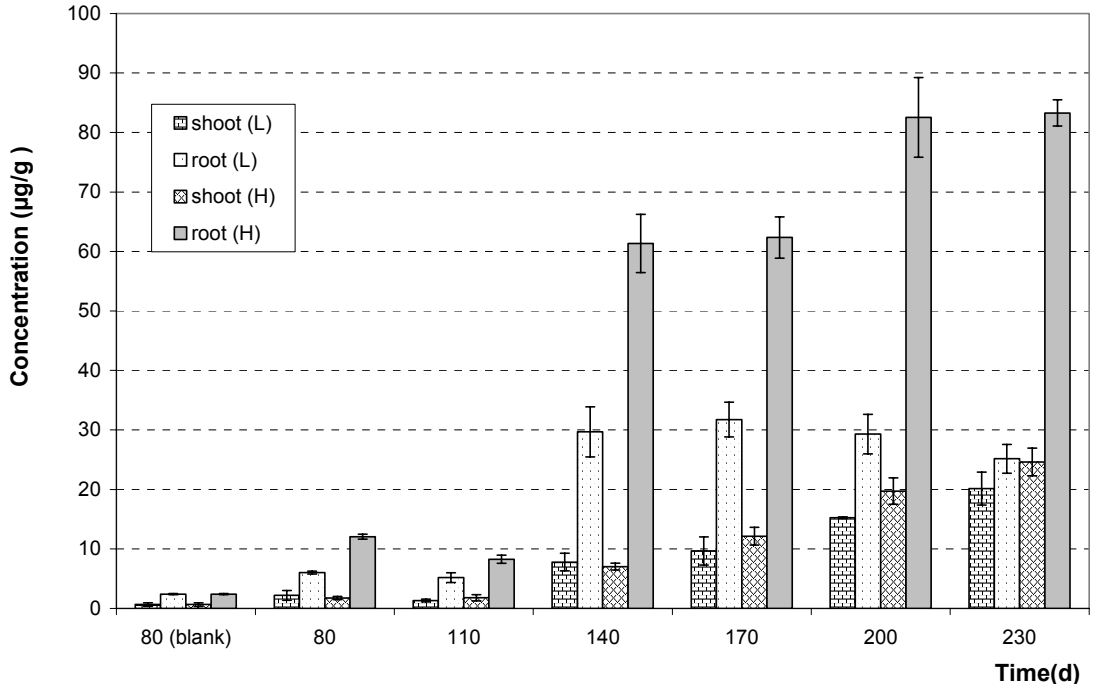


Figure 4.2-7. The concentrations of Pb *in Kentucky-31* at various time and both low and high loadings

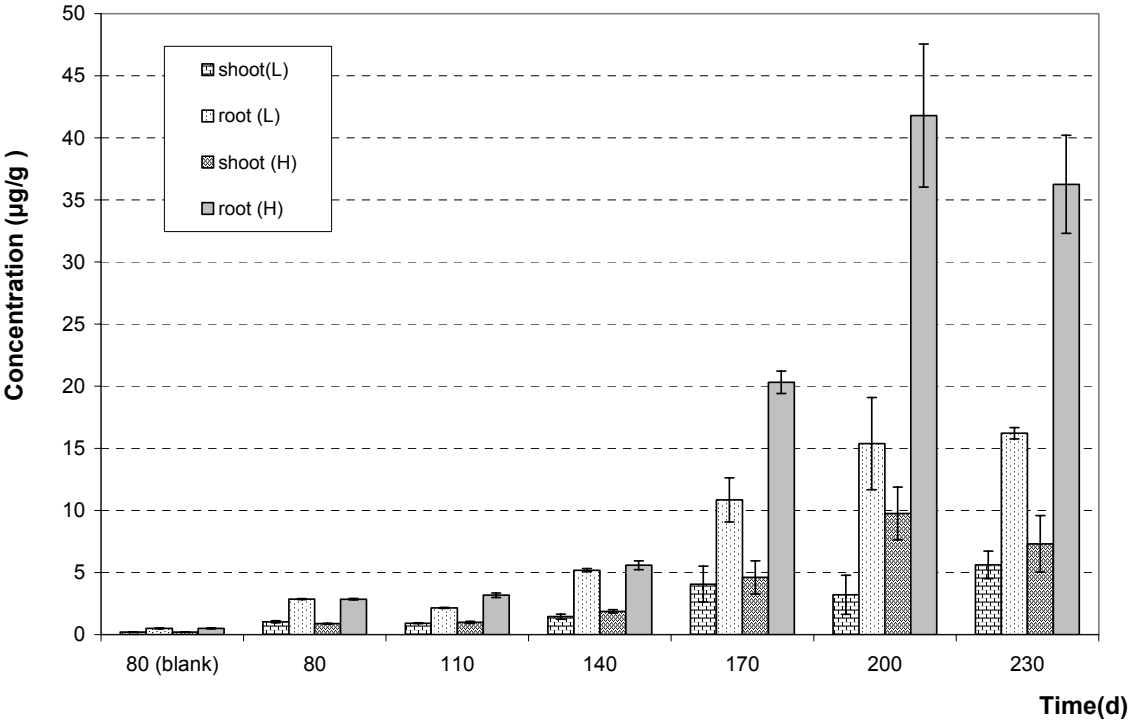


Figure 4.2-8. The concentrations of Cd *in Kentucky-31* at various time and both low and high loadings

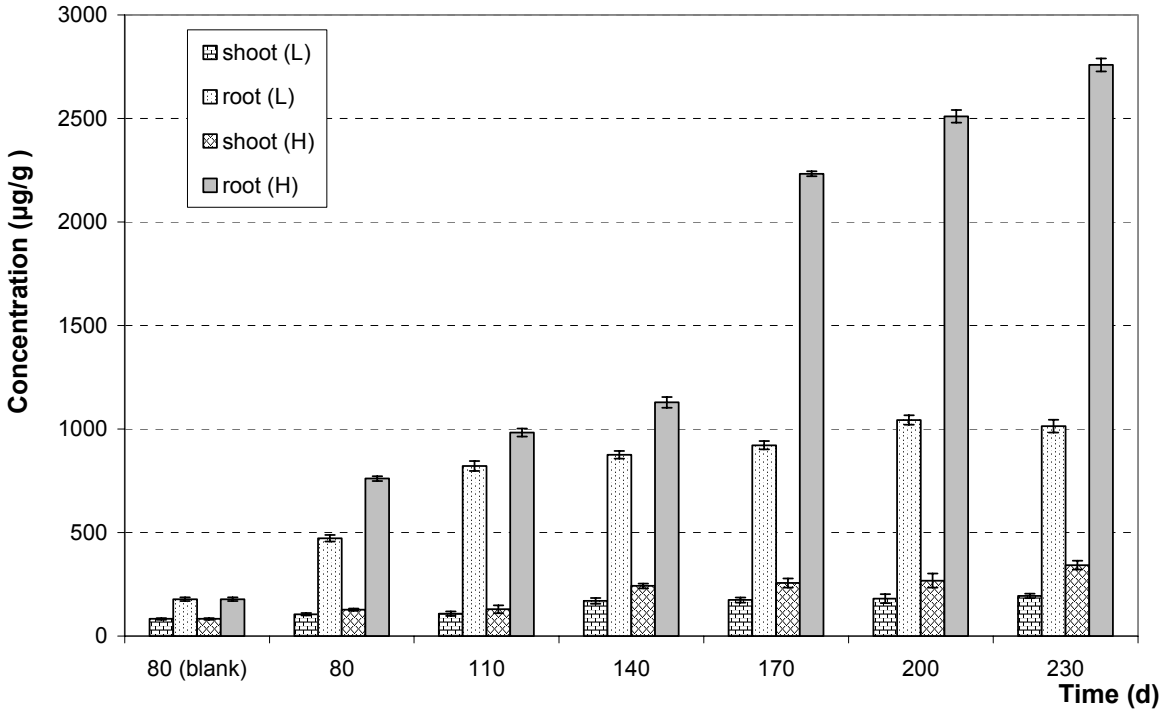


Figure 4.2-9. The concentrations of Zn *in Bromus ciliatus* at various time and both low and high loadings

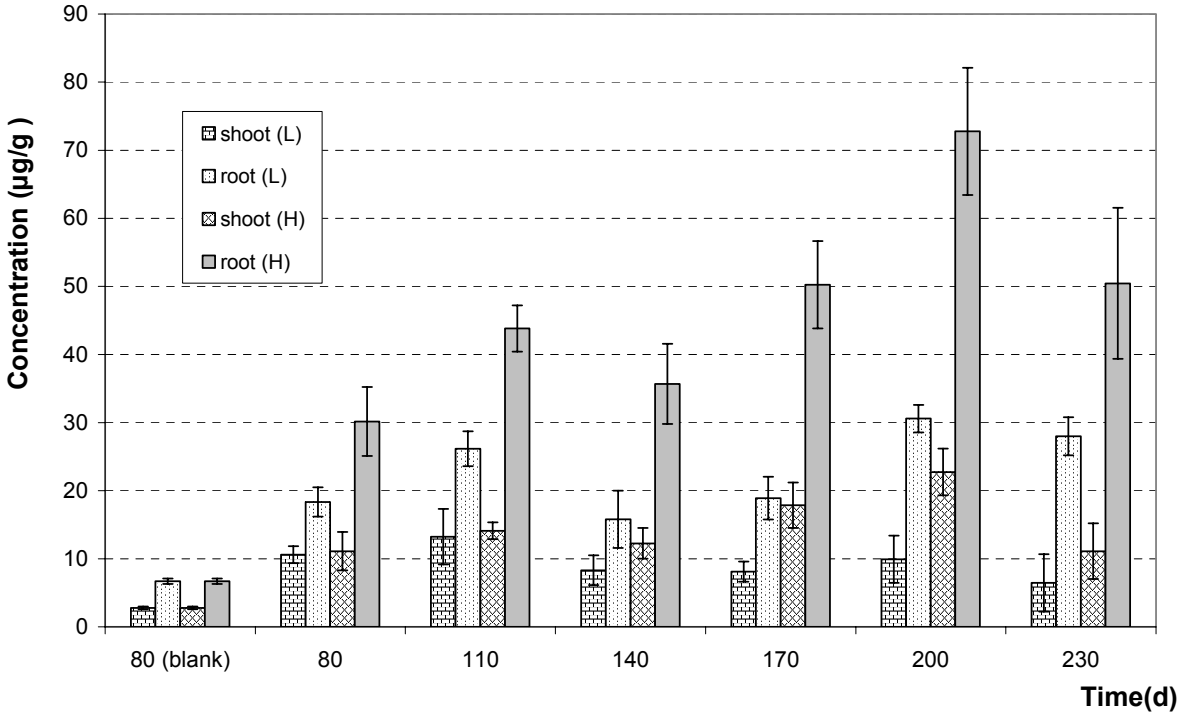


Figure 4.2-10. The concentrations of Pb *in Bromus ciliatus* at various time and both low and high loadings

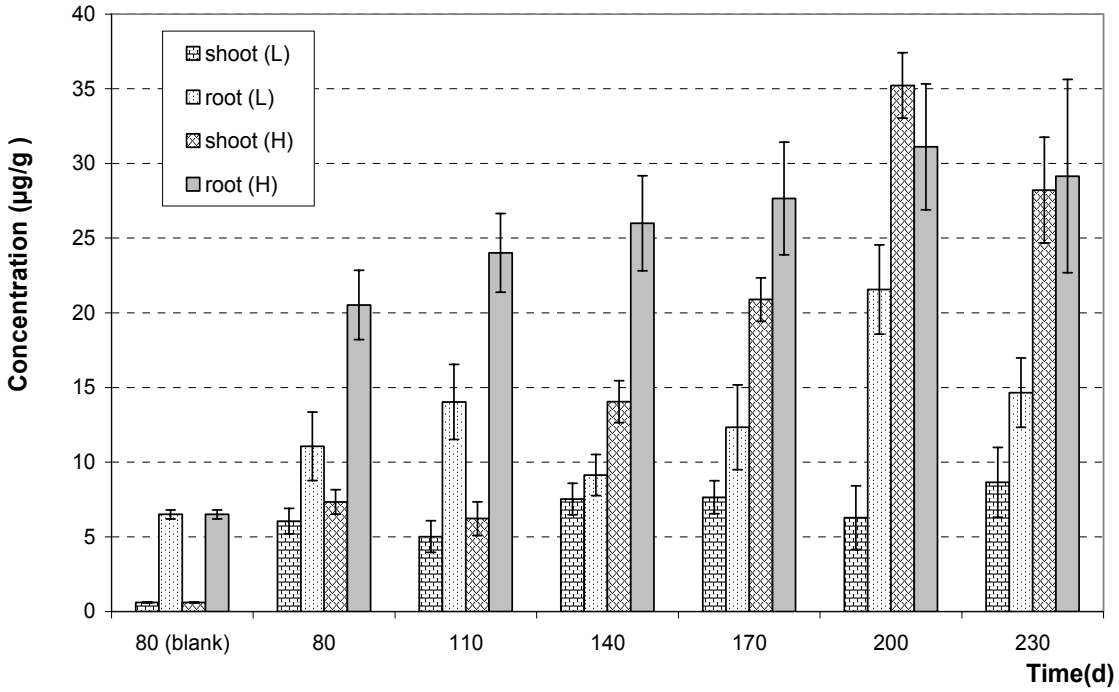


Figure 4.2-11. The concentrations of Pb *in Bromus ciliatus* at various time and both low and high loadings

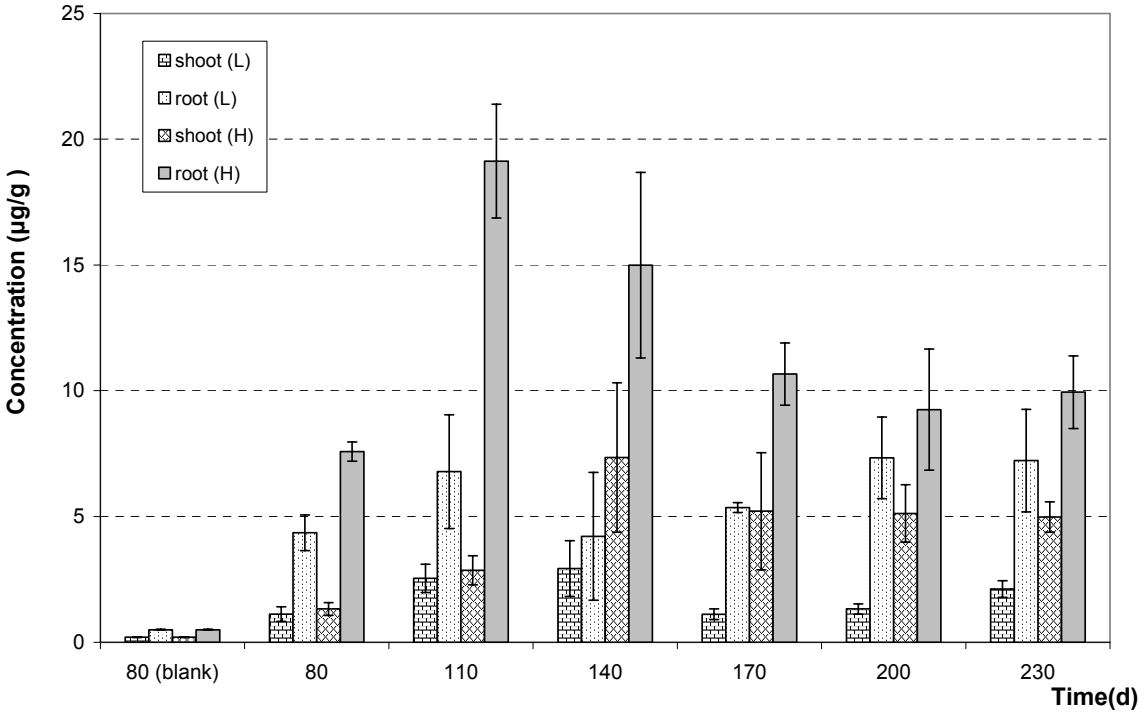


Figure 4.2-12. The concentrations of Cd *in Bromus ciliatus* at various time and both low and high loadings

Complicated relations were observed between the metal concentrations in the roots and shoots with low and high loading. First, the interaction relationships of the four different metals in the same tissue are complex (in shoots or roots). Regression analysis showed that there were significant ($R>0.4$) and positive correlation between the Zn, Cu, Pb and Cd concentrations in both roots and shoots for PV, with exceptions of (Cu and Zn), and (Cu and Cd) in the shoots under the low loadings, and Cu and Cd in shoots and roots under high loading (Table 4.2-6 and 4.2-7). For K-31, the concentrations of Zn, Cu, Pb and Cd showed significant and positive correlations in the shoots and roots with low and high loading except that the correction between concentration of Zn in shoot and that of Cd in root was negative with high loading.

The relationships of the concentration of theses metals for BC were complicated. At the low loading, there were no significant correlations between the concentration of Cu, Pb and Cd in the shoots and those in the roots, while there were significant corrections between Pb and Zn, and Pb and Cu in the shoots. With the high loading, Cd concentration in the root showed no significant relations with those of Zn, Cu and Pb, both in the shoots and roots.

MacFarlane et al. (2002) found that increasing concentrations of Pb and Zn in sediments resulted in a greater accumulation of Pb to both roots and leaves. Liu et al. (2003) observed that there were significant and positive corrections between Cd and Zn, and Cd and Cu for their concentrations in both roots and leaves of rice plants. Luo and Rimmer (1995) investigated the interactions of Zn and Cu in spring barleys which grew in pots with quantities of metal added; results showed Zn uptake in the shoot was increased by Cu additions and the interaction of Zn-Cu was synergic. These results agreed with

Table 4.2-6. Relationship coefficients between the concentrations in shoots and roots with low metal loading *

Plants			Shoot				Root			
Elements			Zn	Cu	Pb	Cd	Zn	Cu	Pb	Cd
PV	shoot	Zn	1							
		Cu	+	1						
		Pb	0.953	0.42	1					
		Cd	0.954	+	0.979	1				
	root	Zn	0.842	0.652	0.931	0.855	1			
		Cu	0.564	0.932	0.672	0.512	0.847	1		
		Pb	0.701	0.787	0.833	0.72	0.975	0.926	1	
		Cd	0.749	0.767	0.849	0.762	0.974	0.867	0.975	1
K-31	shoot	Zn	1							
		Cu	0.724	1						
		Pb	0.832	0.759	1					
		Cd	0.888	0.589	0.901	1				
	root	Zn	0.827	0.649	0.893	0.941	1			
		Cu	0.703	0.802	0.535	0.692	0.672	1		
		Pb	0.659	0.988	0.706	0.626	0.62	0.988	1	
		Cd	0.921	0.768	0.964	0.908	0.833	0.654	0.706	1
BC	shoot	Zn	1							
		Cu	-0.79	1						
		Pb	0.812	0.944	1					
		Cd	+	--	+	1				
	root	Zn	0.831	-0.396	0.62	0.302	1			
		Cu	+	+	--	+	0.556	1		
		Pb	+	+	--	-0.336	0.551	0.585	1	
		Cd	+	+	+	--	0.661	0.9472	0.83	1

* + The coefficients are positive and less than 0.3, -- The coefficients are negative and less than 0.3
The $P<0.05$.

Table 4.2-7. Relationship coefficients between the concentrations in shoots and roots with high metal loading *

Plants			Shoot				Root			
Element			Zn	Cu	Pb	Cd	Zn	Cu	Pb	Cd
PV	Shoot	Zn	1							
		Cu	0.447	1						
		Pb	0.786	0.479	1					
		Cd	0.866	+	0.807	1				
	Root	Zn	0.663	0.744	0.744	0.617	1			
		Cu	0.457	0.747	0.747	0.449	0.967	1		
		Pb	0.945	0.518	0.519	0.905	0.845	0.588	1	
		Cd	0.905	+	0.333	0.965	0.791	0.639	0.975	1
K-31	Shoot	Zn	1							
		Cu	0.669	1						
		Pb	0.988	0.402	1					
		Cd	0.926	+	0.924	1				
	Root	Zn	0.968	+	0.949	0.959	1			
		Cu	0.886	0.638	0.826	0.779	0.878	1		
		Pb	0.908	0.512	0.906	0.845	0.92	0.918	1	
		Cd	-	+	0.954	0.993	0.959	0.781	0.846	1
BC	Shoot	Zn	1							
		Cu	0.338	1						
		Pb	0.84	0.616	1					
		Cd	0.806	+	0.49	1				
	Root	Zn	0.859	0.467	0.929	0.43	1			
		Cu	0.587	0.858	0.866	0.3	0.785	1		
		Pb	0.893	0.634	0.933	0.669	0.905	0.873	1	
		Cd	-	-	-0.48	-	-	+	-	1

* + The coefficients are positive and less than 0.3, -- The coefficients are negative and less than 0.3
The $P<0.05$.

the reports of Hinsely et al. (1984), who reported that repeated sludge applications resulted in additional increase of both Cd and Zn contents in corn leaves and grain in calcareous soil. He et al. (2004) reported that pollution of Pb and Cd in soil restrained the absorption of Zn and Cu, while Smilde et al. (1992) found mainly antagonistic effects, in which applied Zn reduced the plants uptake of Cd in a range of crop plants grown in soil. McKenna et al. (1993) found similar effects for lettuce and spinach grown in nutrient solutions. The different results may be due to the different metal contents in soil and different soil and plant species. The synergic uptake mechanism of those four metals will be helpful for using one typical plant to remove the different metals in bioretention media.

Second, the interactions of the four metals in different tissues (between the roots and shoots) are complicated. From the Tables 4.2-6 and 4.2-7, statistically positive strong relationships were observed between concentrations of Zn, Cu, Pb and Cd in the shoots and those in the roots, respectively, in PV and K-31. But in BC, with low loading, low and non-significant correlations were found between the concentrations of Cu, Pb and Cd in the shoots and those in the roots respectively. With the high loading, high and significant relationships were observed between the concentrations of Zn, Cu and Pb in the shoots and those in the roots, respectively, while there is a low and negative correlation between the concentration of Cd in the shoots and that in the roots. The high positive relationship coefficient indicate the less antagonism between metals in roots and shoots, which means one metal presence in shoots does not decrease the transport both for the same metal and different metals from shoots to roots. For example, under the low loading, the relationship coefficient of Pb in shoot and Zn in root of PV is 0.931. Therefore, the Pb-Zn interaction is not antagonistic and the presence of Pb in shoots do

not decrease the relocation of Zn from roots to shoots. Campbell et al. (1985) reported that the relationship between Cu content in shoots and that in roots was significant in the hydrovascular plant, *Nuphar Variegatum*, whereas there was no apparent relationship between the content of Zn in shoots and roots. These contrasting observations are likely due to complex interactions between different kinds of metal ions and plant species, which lead to variability of heavy metal absorption and assimilation by growing plants.

4.2.2 Spatial Distribution of Metals in Plants

The concentration changes of Zn, Cu, Pb and Cd with different grass heights are shown in Figures 4.2-13 to 4.2-16. Nearly all of the concentrations of four metals decreased with height in the order of *Root >(0-15cm) >(15-25) cm > (>25 cm).* The exception was Cd, in which concentration in the root of PV was less than that in the 0-15 cm shoot. For the same height, generally, the concentrations of Zn, Cu, Pb and Cd in all three grasses with low loading were less than those with high loading. It is obvious that the distribution of these heavy metals in the grass is far from homogeneous. The variability of within-plant distribution of the four metals in the plants may be caused by compartmentalization and translocation in the vascular (Kim et al., 2003a**).** It also shows that the translocation of heavy metals to above-ground parts of plants was minimized in order to minimize the toxic effects caused by the presence of metals in the soil (Peralta-Videa et al., 2002). This observation is consistent with the common behavior of plants in their response towards environmental stress. MacFarlane et al. (2003) found the Cu, Zn and Pb were accumulated in the tissues of *A. marina* and Cu and Zn showed some mobility in the plants, being accumulated in leaf tissues in the levels of approximately 10% of the root level. Pb showed little mobility with the levels in leaf tissue only 3% that of root levels.

Some significant correlations between the concentrations of Zn, Cu, Pb and Cd at different height of grasses were observed and the various relationships between the four metals are shown in Figures 4.2-17 to 4.2.19. The relationships between plant concentrations of different metals are linear (Table 4.2-8).

Table 4.2-8. Relationship between the concentrations of Zn, Cu, Pb and Cu in different grass heights

Plants	Equation	R value	P value
PV	[Cu]=-14.238+0.104[Zn]	0.953	0.001
	[Pb]=6.985+0.044 [Zn]	0.925	0.001
	[Cd]=1.8534+0.019[Zn]	0.737	0.05
	[Cu]=13.364+0.3963[Pb]	0.701	0.002
	[Cu]=5.3471+0.1632[Cd]	0.636	0.1
	[Pb]=-1.1147+0.4331[Cd]	0.791	0.05
K-31	[Zn]=1.291+0.05[Cu]	0.979	0.001
	[Zn]=7.047+0.0392[Pb]	0.775	0.05
	[Zn]=-1.0237+0.02135[Cd]	0.914	0.001
	[Cu]=3.838+0.8564[Pb]	0.865	0.01
	[Cu]=-1.951+0.4396[Cd]	0.961	0.001
	[Pb]=-1.444+0.4303[Cd]	0.931	0.001
PB	[Zn]=3.975+0.0164[Cu]	0.992	0.001
	[Zn]=6.652+0.0084[Pb]	0.919	0.001
	[Zn]=1.675+0.0033[Cd]	0.914	0.001
	[Cu]=4.612+0.5124[Pb]	0.926	0.001
	[Cu]=0.9837+0.1944[Cd]	0.886	0.01
	[Pb]=-0.521+0.359[Cd]	0.904	0.01

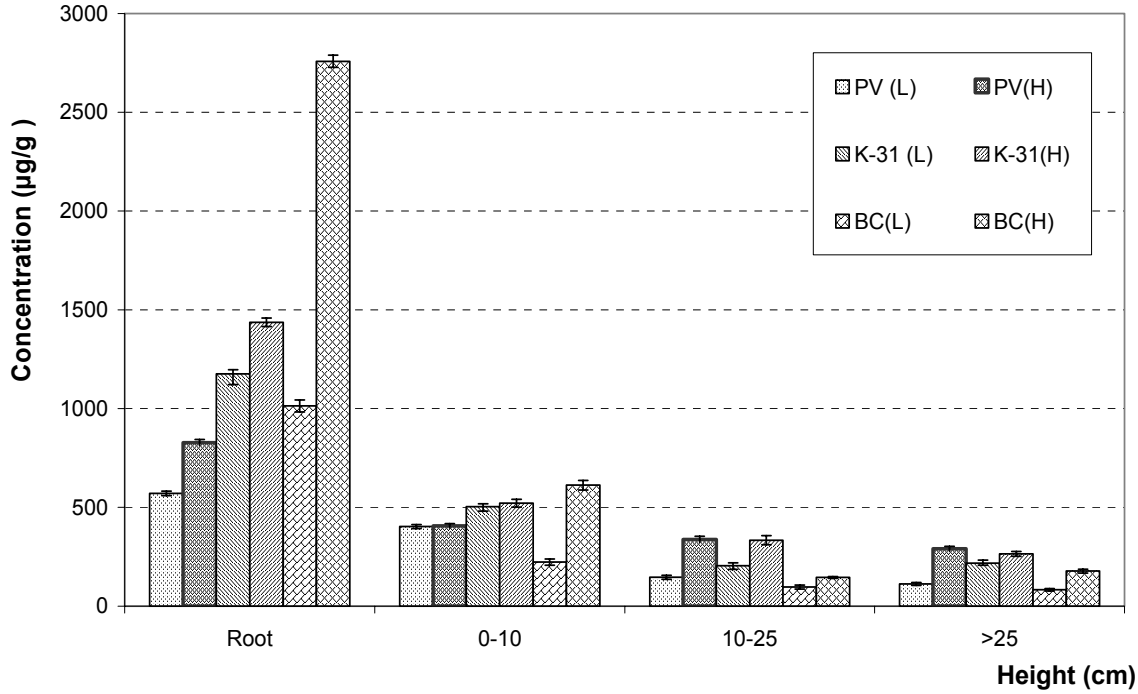


Figure 4.2-13. The concentrations of Zn in different heights with high and low loading

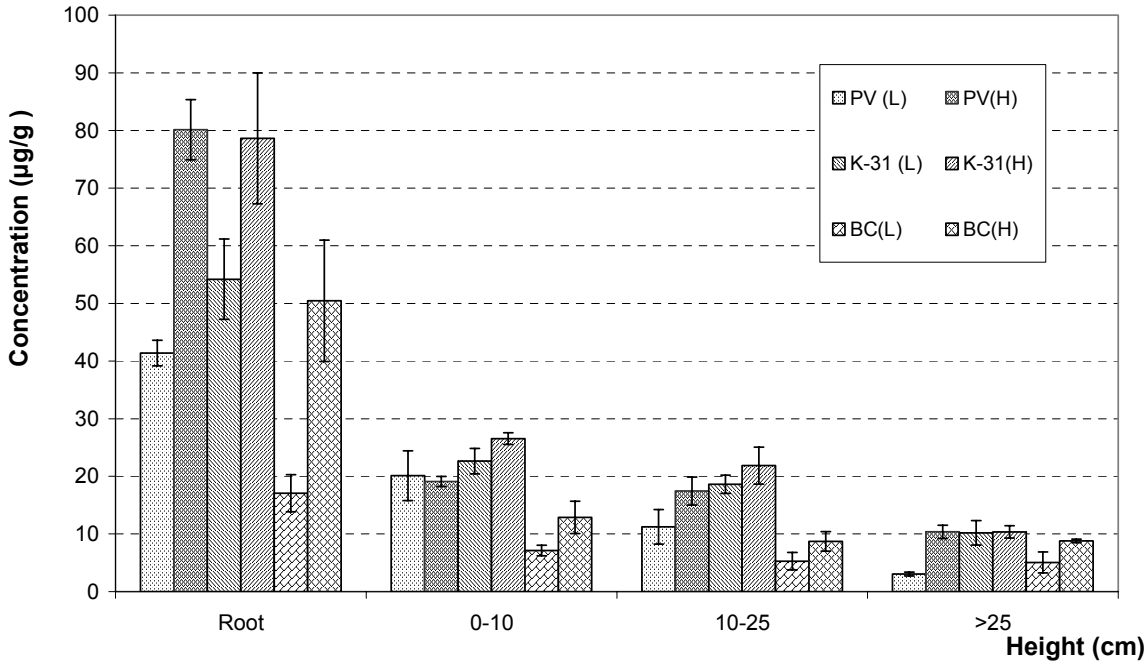


Figure 4.2-14. The concentration of Cu in different heights with high and low loading

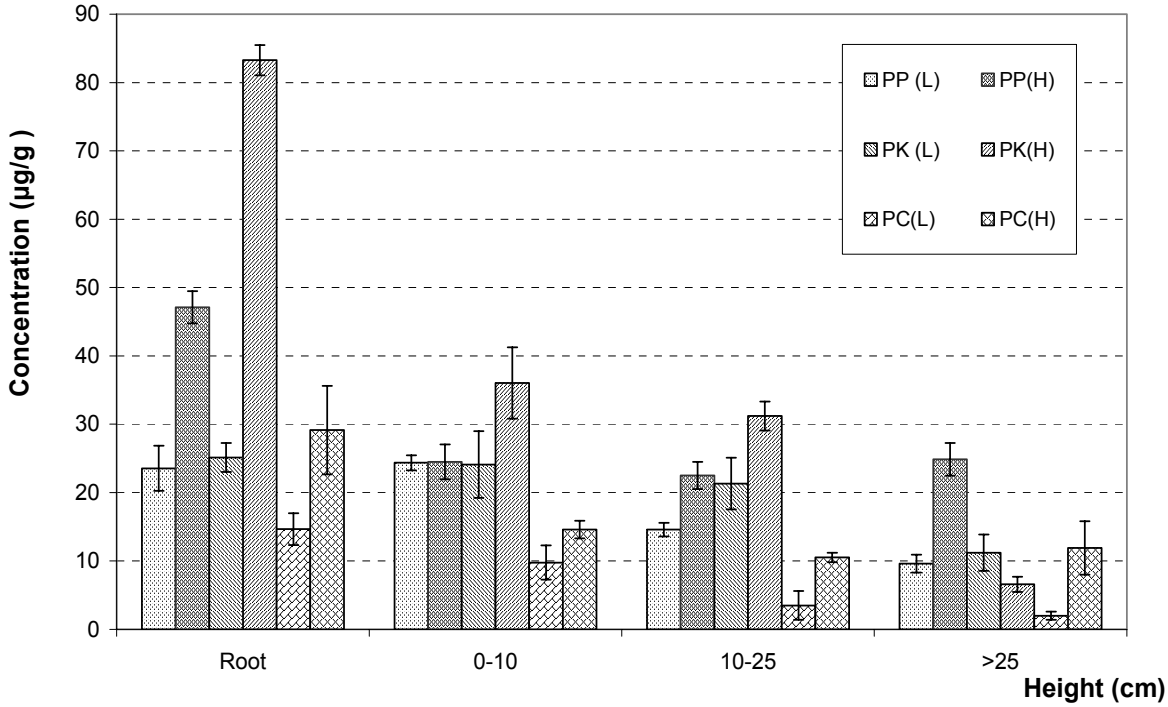


Figure 4.2-15. The concentration of Pb in different heights with high and low loading

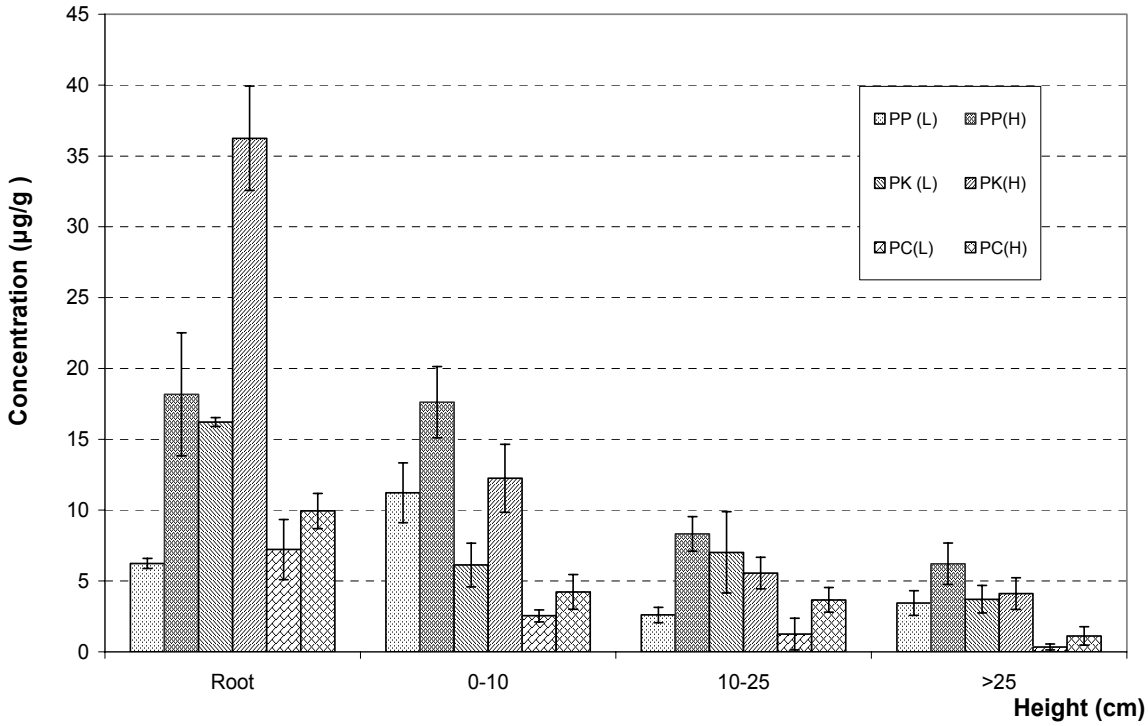


Figure 4.2-16. The concentration of Cd in different heights with high and low loading

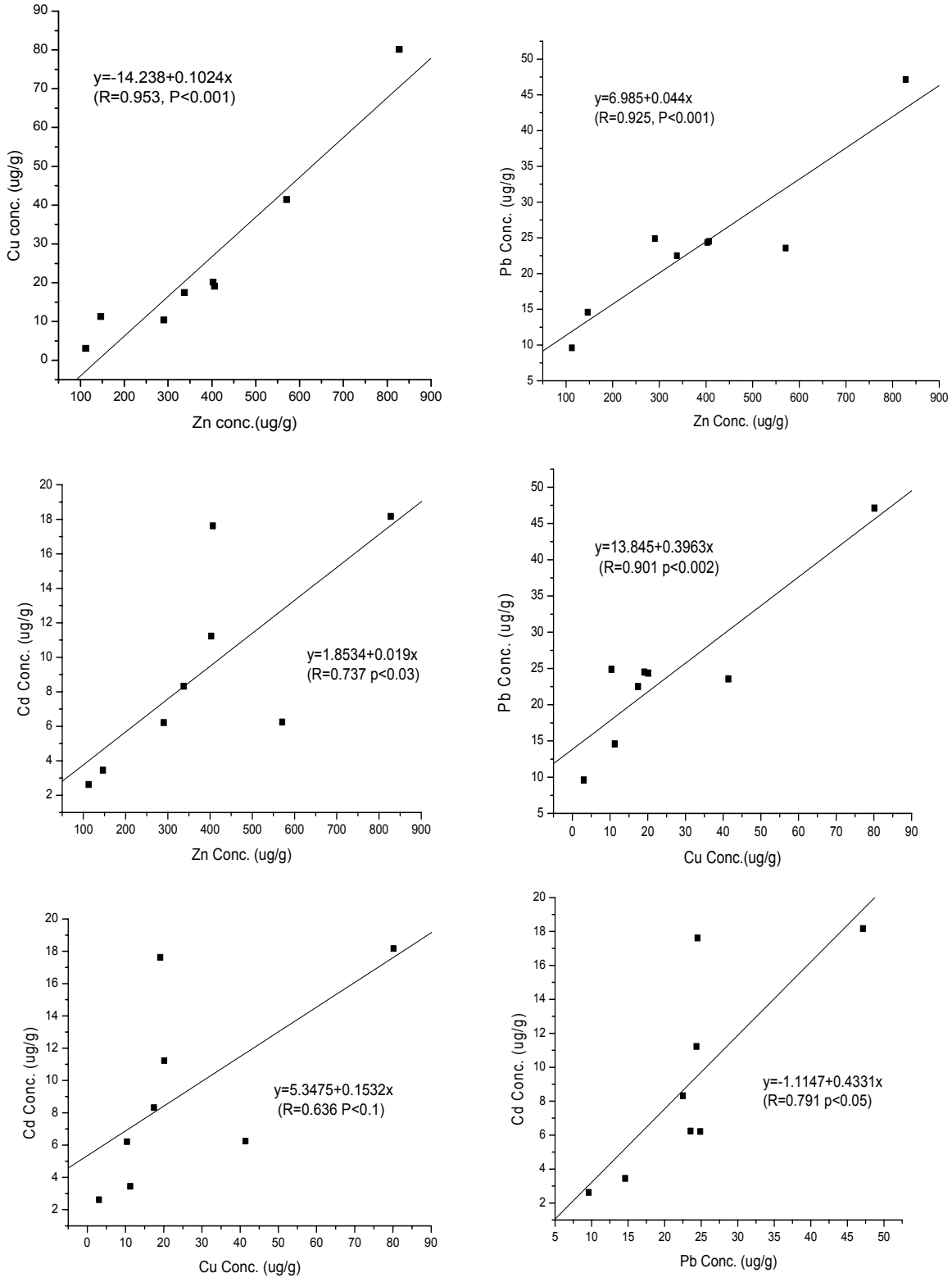


Figure 4.2-17. The concentration relationship of different metals in PV (Time: 230 days; Data: root, 0-15, 15-25 and >25 cm)

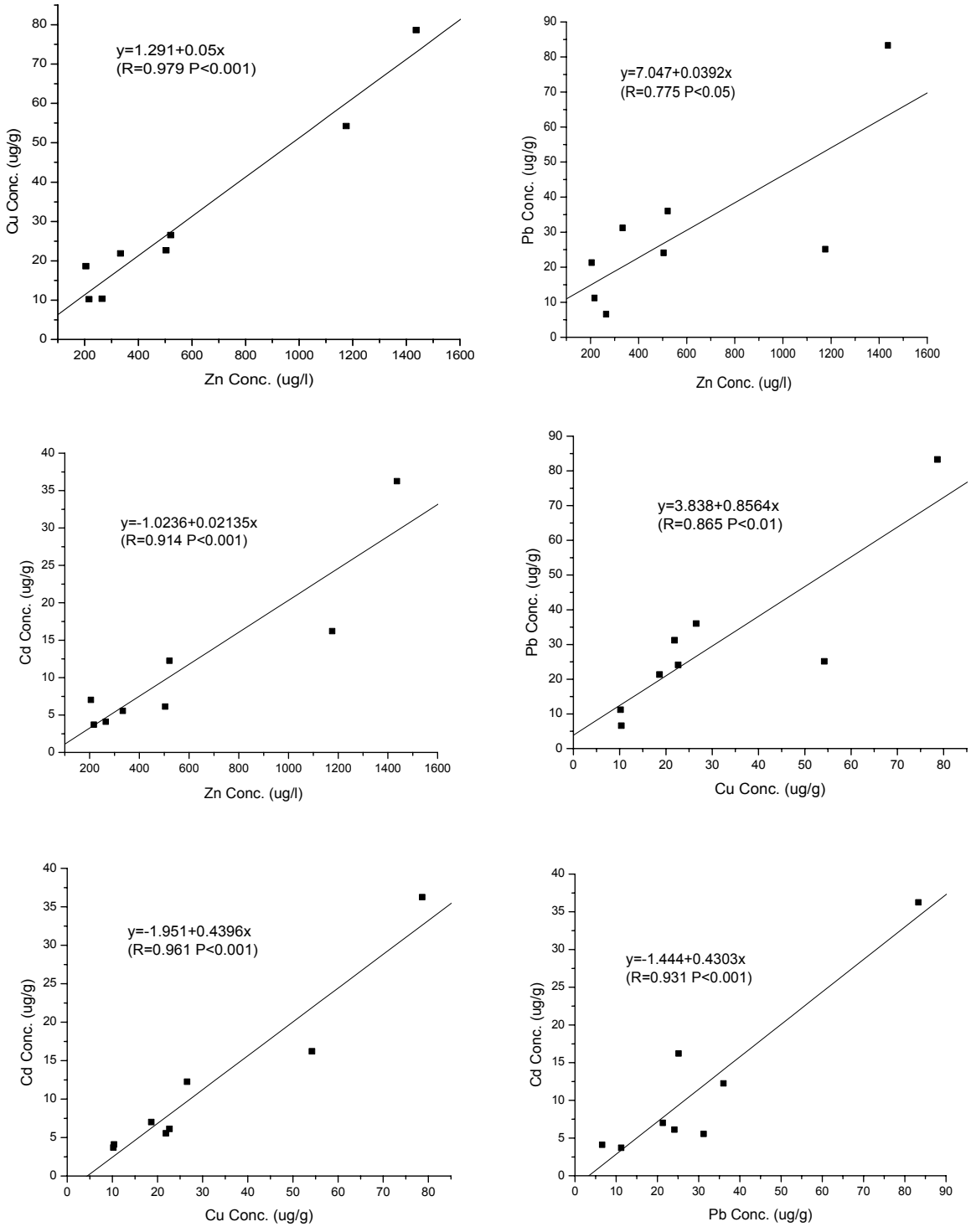


Figure 4.2-18. The concentration relationship of different metals in K-31 (Time: 230 days; Data: root, 0-15, 15-25 and >25 cm)

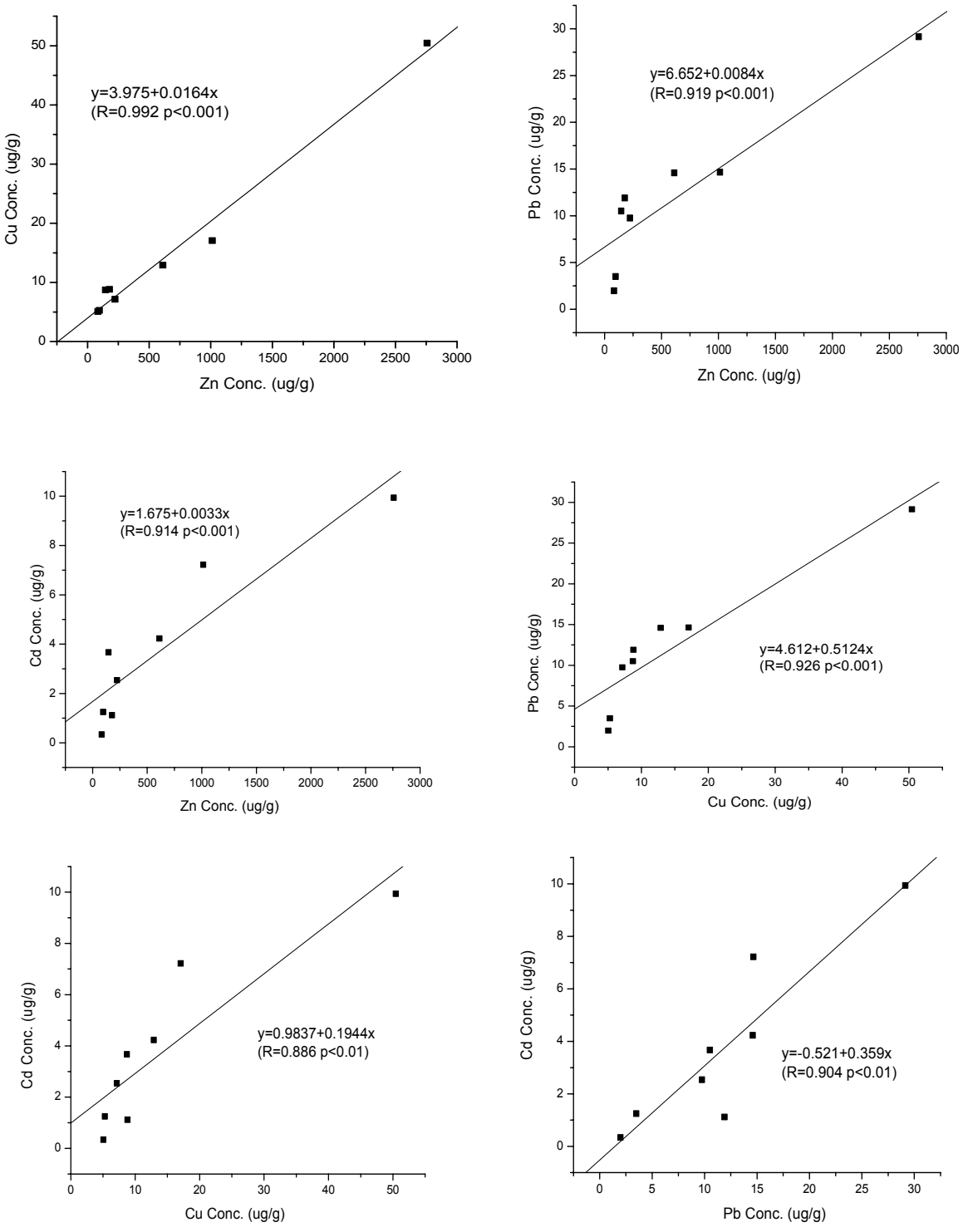


Figure 4.2-19. The concentration relationship of different metals in BC (Time: 230days; Data: root, 0-15, 15-25 and >25 cm)

4.2.3 Accumulation of metals in the plants

In order to investigate the bioaccumulation capacities of the pants, bioconcentration factor, BCF and transfer ratio, TR were determined as:

$$\text{BCF} = \frac{\text{concentration in the root (dry wt)}}{\text{concentration in the soil (dry wt)}} = \frac{C_{root}}{C_{soil}} \qquad (4.2)$$

$$\text{TR} = \frac{\text{concentration in the shoot (dry wt)}}{\text{concentration in the root (dry wt)}} = \frac{C_{shoot}}{C_{root}} \qquad (4.3)$$

The BCF has also been called uptake efficiency, and can be used to express the transport potentials of heavy metals in plants (Wu and Yu 1998). The BCF and TR values of the Zn, Cu, Pb and Cu for the three plants are shown in Tables 4.2-9 and 4.2-10. The bioconcentration factor and transfer ratio showed markedly temporal variation due to the different growth phases and response towards environmental stress of the plants. For the four metals, the high values of BCF occur during the days 140-200 in which the plants grow fast and have high uptake capacities. For example, the highest BCF values for PV occurred at 140 days with high and low loading for all four metals. The average BCF values (Figures 4.2-20 and 4.2-21) in these three plants follow Cd>Zn>Cu>Pb with both high and low loading. Similar observation has been reported by Wu and Yu (1998) who studied the effects of heavy metals on the growth of paddy plants and found that the concentrations of Zn, Cu, Pb and Cd in the roots were higher than those in the soil and the BCF values flowed the order of Cd>Zn>Cu>Pb. It is not surprising that the two trace metals, Zn and Cu, have high BCF values because these two elements are essential for plant growth, although they will be toxic to plants when the concentrations are high in the soil. Due to the toxic effect of Pb, the BCF of Pb of these three plants is very low and all of the values are less than 1, which means uptake efficiencies of these plants for Pb

are very low. The BCF of Cd is high although Cd is also toxic for plants. One of the possible reasons is due to the relative low concentrations of Cd in the soil, which causes the relative high BCF, even under low accumulation in the plants.

For Zn, Cu, Pb and Cd, the sequences of BCF are BC>K-31>PV, K-31>PV>BC, K-31>PV>BC and K-31>BC>PV, respectively, under the low loading, while with the high loading, the orders of the four metals are PB>K-31>PV, PV>K-31>BC, K-31>PV>BC and BC>K-31>PV, respectively. It is obvious that PV has lowest uptake capacities for Zn and Cd under both low and high loading conditions, while BC has lowest uptake capacities for Cu and Pb and highest ones for Zn and Cd, respectively. Comparing the BCF for different pollutant loadings shows that the higher the pollutant loading, the higher BCF value, except for that of Cd in PV and K-31, and Cu in K-31. So it seems that to some extent, the high pollutant loading enhanced the uptake of metals by plants.

The transfer ratio (TR) of plants showed time-dependent variation as the BCF did. All of the TR values for different metals in these three plants are less than 1 and most are less than 0.5, which shows that the metals were retained by the roots and translocation of the four metals from roots to shoot is rather slow. The average values of TR increase in the order of Pb>Cu>Cd>Zn in BC and K-31, while that of Cd >Pb>Cu >Zn in PV under the low loading conditions. Under the high loading, the transport patterns of those metals are complicated and the TR increase in the rank of Cd > Pb > Zn > Cu, Cu > Cd > Zn > Pb, and Pb > Cd > Cu > Zn for the PV, K-31 and BC, respectively. TR values of the plants with low pollutant loading are higher than those of plants with high loading with the exception of Cu and Pb in BC, which are in contrast with the BCF.

The results revealed that increased pollutant loading does not enhance the TR but does BCF. In other words, heavy metals mobility within these plants cannot be enhanced in the presence of massive quantities of metals in the pollutant soil. The roots have high accumulation capacities and some restriction of internal transport from roots to shoots (Dahmani-Muller et al., 2000). But translocation of heavy metals to above-ground parts of plants was minimized in order to minimize the toxic effects caused by the metals in soil. This observation is also consistent with the common behavior of plants in their response towards environmental stress (Wang et al., 2003a). Such metal immobilization in roots is referred to as an exclusion strategy of plants towards metals (Baker and Brooks, 1989; Dahmani-Muller et al., 2000). Gigliotti et al. (1996) reported that even though Pb uptake by plants grown in a greenhouse was 100 times greater than in plants grown on an amended soil, the Pb distribution within the corn plant was unchanged, which is similar to what was observed here. Different metal tolerance strategies, such as restriction of root to shoot transport of heavy metals may exist for tolerant nonaccumulators (Khan, 2001).

The TR of Zn is lowest among the four metals, which means Zn is the most difficult element for transporting from root to shoot, while Pb is relatively easier to transport. Most heavy metals are transported from roots to shoots in terrestrial plants, but the extents are different (Kim et al., 2003a). Within a certain concentration range, Cu and Zn extensively translocated, as they are essential to the plant enzymes (Delhaize et al., 1985). In contrast, cadmium and lead are apparently non-essential and can be toxic to photosynthetic activity and antioxidant enzymes (Somashekaraiah et al., 1992). Barazani et al. (2004) reported that the Zn was more easily translocated through the vascular system of *Nicotiana glauca graham* than Cu. But this study did not show some of

Table 4.2-9. The variation of BCF with time

Time (d)		80	110	140	170	200	230	Average	SD
Low loading									
Panicum Virgatum	Zn	1.04	0.85	1.45	2.23	1.94	1.74	1.54	0.53
	Cu	0.71	0.86	1.73	1.94	1.58	1.25	1.35	0.49
	Pb	0.09	0.05	0.35	0.75	0.54	0.44	0.37	0.27
	Cd	1.51	1.68	3.01	6.50	5.39	3.92	3.67	2.00
Kentucky-31	Zn	1.41	1.91	2.38	2.71	2.16	3.66	2.37	0.77
	Cu	0.95	1.28	2.01	2.88	1.46	1.75	1.72	0.68
	Pb	0.15	0.13	0.58	0.63	0.59	0.51	0.43	0.23
	Cd	4.77	1.79	3.39	7.75	10.26	9.01	6.16	3.35
Bromus Ciliatus	Zn	1.93	3.22	3.28	3.30	3.47	3.24	3.07	0.57
	Cu	0.80	1.00	0.55	0.59	0.85	0.78	0.76	0.17
	Pb	0.28	0.31	0.19	0.25	0.43	0.28	0.29	0.08
	Cd	10.88	6.16	2.99	3.64	4.58	4.63	5.48	2.85
High loading									
Panicum Virgatum	Zn	1.10	1.29	1.99	3.74	2.59	2.08	2.13	0.96
	Cu	1.01	1.11	2.00	3.80	1.56	1.57	1.84	1.02
	Pb	0.23	0.19	0.52	0.92	0.92	0.70	0.58	0.32
	Cd	2.46	1.79	2.39	4.94	3.97	2.98	3.09	1.16
Kentucky-31	Zn	1.22	2.18	2.83	3.59	4.84	3.94	3.10	1.30
	Cu	1.18	1.19	1.77	2.22	1.83	1.54	1.62	0.40
	Pb	0.36	0.19	1.16	1.18	1.43	1.46	0.96	0.55
	Cd	2.86	2.40	2.66	5.98	10.72	7.11	5.29	3.30
Bromus Ciliatus	Zn	2.76	3.30	3.58	6.81	6.82	6.95	5.04	2.02
	Cu	1.21	1.51	1.02	1.29	1.59	0.89	1.25	0.27
	Pb	0.50	0.50	0.50	0.58	0.53	0.49	0.52	0.03
	Cd	6.16	13.28	5.35	3.05	2.80	2.55	5.53	4.08

Table 4.2-10. The variation of TR with time

Time (d)		80	110	140	170	200	230	Average	S.D.
Low loading									
Panicum Virgatum	Zn	0.39	0.49	0.35	0.25	0.42	0.39	0.38	0.08
	Cu	0.52	0.48	0.43	0.39	0.31	0.39	0.42	0.07
	Pb	0.32	0.40	0.35	0.41	0.75	0.90	0.52	0.24
	Cd	0.42	0.31	0.28	0.53	0.99	1.42	0.66	0.45
Kentucky-31	Zn	0.29	0.33	0.23	0.36	0.43	0.26	0.32	0.07
	Cu	0.39	0.30	0.40	0.27	0.49	0.35	0.37	0.08
	Pb	0.36	0.25	0.26	0.30	0.52	0.80	0.42	0.21
	Cd	0.36	0.43	0.28	0.38	0.21	0.35	0.33	0.08
Bromus Ciliatus	Zn	0.22	0.13	0.19	0.19	0.17	0.19	0.18	0.03
	Cu	0.58	0.51	0.39	0.43	0.32	0.23	0.41	0.12
	Pb	0.55	0.36	0.82	0.62	0.29	0.59	0.54	0.19
	Cd	0.26	0.37	0.70	0.21	0.18	0.29	0.33	0.19
High loaing									
Panicum Virgatum	Zn	0.43	0.29	0.38	0.24	0.59	0.42	0.39	0.12
	Cu	0.41	0.33	0.42	0.21	0.32	0.18	0.31	0.10
	Pb	0.31	0.23	0.63	0.33	0.32	0.52	0.39	0.15
	Cd	0.24	0.18	0.26	0.47	0.67	0.78	0.43	0.25
Kentucky-31	Zn	0.28	0.19	0.20	0.25	0.21	0.26	0.23	0.04
	Cu	0.31	0.28	0.40	0.32	0.30	0.26	0.31	0.05
	Pb	0.15	0.22	0.11	0.19	0.24	0.30	0.20	0.07
	Cd	0.31	0.31	0.33	0.23	0.23	0.20	0.27	0.06
Bromus Ciliatus	Zn	0.17	0.13	0.21	0.11	0.11	0.12	0.14	0.04
	Cu	0.37	0.32	0.34	0.36	0.31	0.22	0.32	0.05
	Pb	0.36	0.26	0.54	0.76	1.13	0.97	0.67	0.34
	Cd	0.17	0.15	0.49	0.49	0.55	0.50	0.39	0.18

the same results, especially for Zn, which is in contrast to some previous results. This transport may be affected by many factors like the solubility and bioavailability of metals in the soil and the experimental conditions.

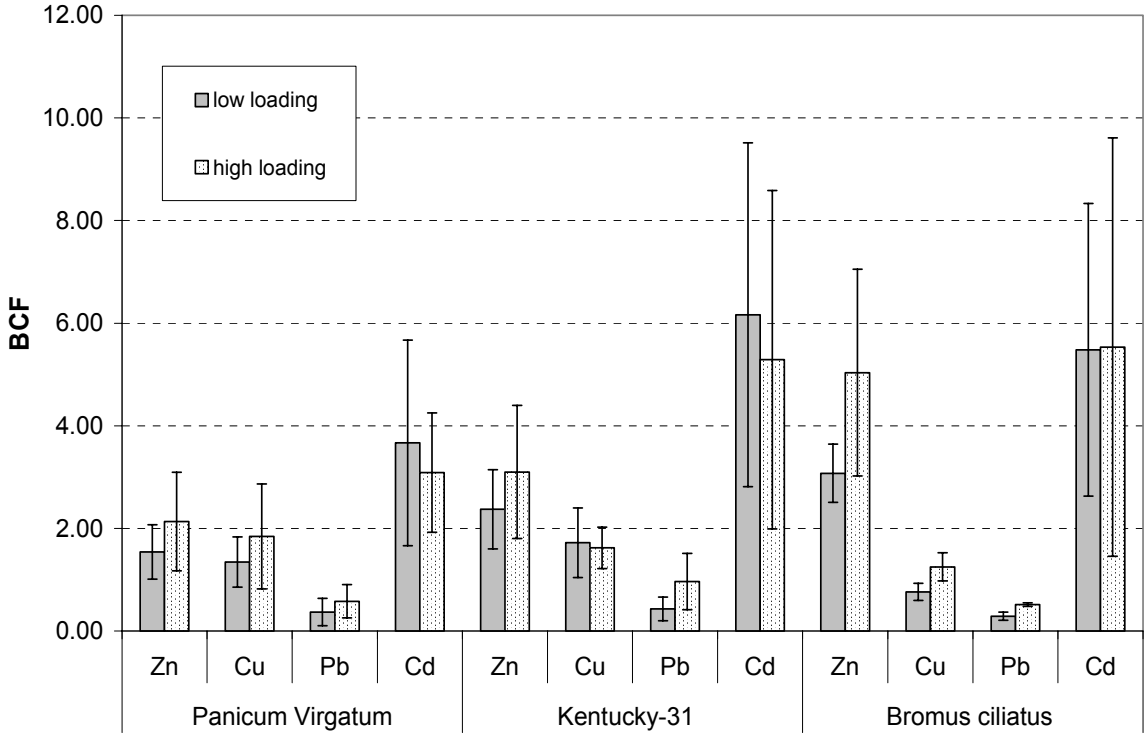


Figure 4.2-20. The BCF of the metals for the different plants

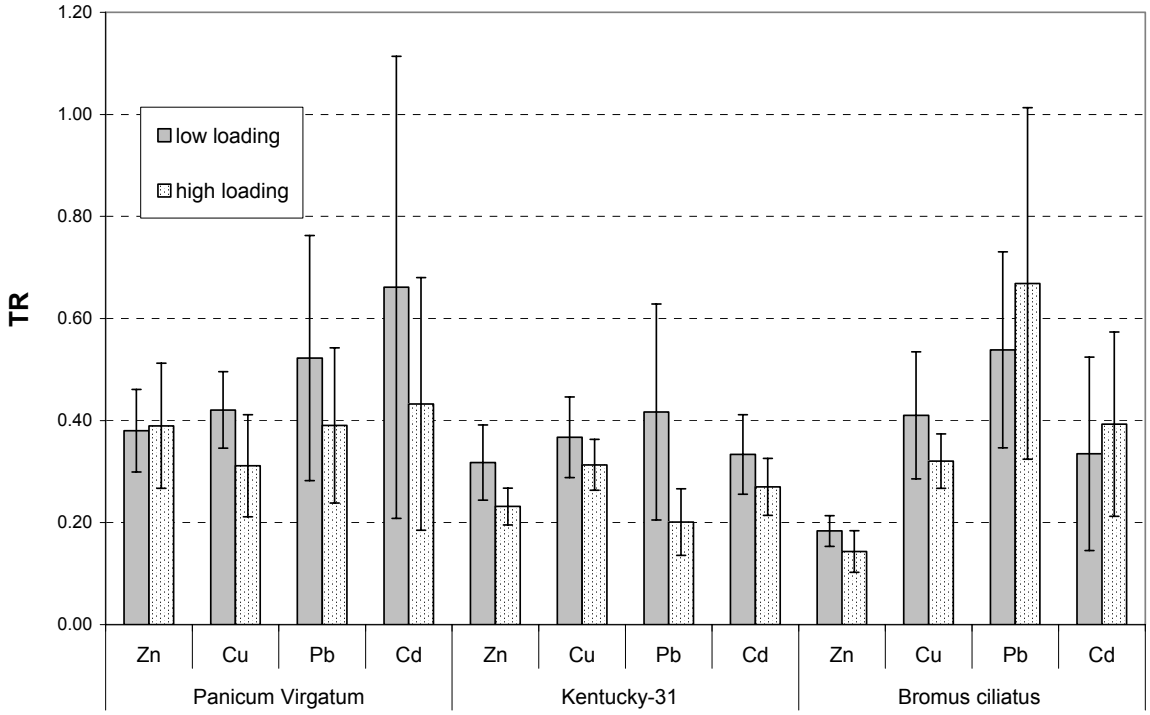


Figure 4.2-21. The TR of the metals for the different plants

4.2.4 Biomass and Heavy Metal Accumulation

The biomass of the three plants after 230-day growth is shown in Table 4.2-11. With both low and high loading, shoot biomass of these plants ranked K-31>BC>PV. The root biomass increased in the order of PV>K-31>BC with low loading while in the order of PV>BC>K-31 for high loading. The difference of total biomass between the two pollutants loading was 3.4%, 5.0% and 6.7% for PV, K-31 and BC, respectively. This indicated that the different pollutant loading did not have significant affect on the growth of plants and the heavy metals added to the pots are within the tolerance of those species.

The total mass accumulation of the four metals in these species is shown in Figure 4.2-21a and 4.2-21b. The amount of different metals uptaken by these three species

varied greatly due to different concentrations of metals and plant biomass. The accumulation amounts of Zn, Cu, Pb and Cd in the shoots and roots of these three species exposed to the high loading are greater than that exposed to low loading. This is mainly due to the high concentration of the tissue in the plants with high loading because the biomass yields are similar between the two loadings. Madyiwa et al. (2003) reported that increased uptake of Pb and Cd would decrease plant yield because of the toxicity of the metals resulting from substitution of vital nutrients and their metabolic functions due to the relative abundance of bioavailable Pb and Cd compared to other ions. In this study, the yields of biomass did not decrease while the concentrations in the tissues were enhanced with the increasing pollutant input. For PV, roots are the main storage for the metals, while for BC shoots are the main pool. For K-31, the roots and shoots play similar roles for the retention of metals. The K-31 has the highest phyto-extration capacity among the three species due to the high concentration of tissues and great biomass yield followed by PV and BC. Although these capacities are not comparable to those of the hyperccumulators, it is significant to extract the pollutants from relatively low-pollutant concentration media like bioretention cell soil.

Table 4.2-11 Comparison of biomass of four plants after 230 days (g/pot)

Species	Low loading			High loading		
	Total Shoot	Total Root	Root/Shoot	Total Shoot	Total Root	Root/Shoot
Panicum Virgatum	7.53	4.36	0.58	8.12	4.18	0.52
Kentucky-31	12.14	3.28	0.27	13.33	2.91	0.22
Bromus Ciliatus	8.02	2.88	0.36	8.41	3.28	0.39

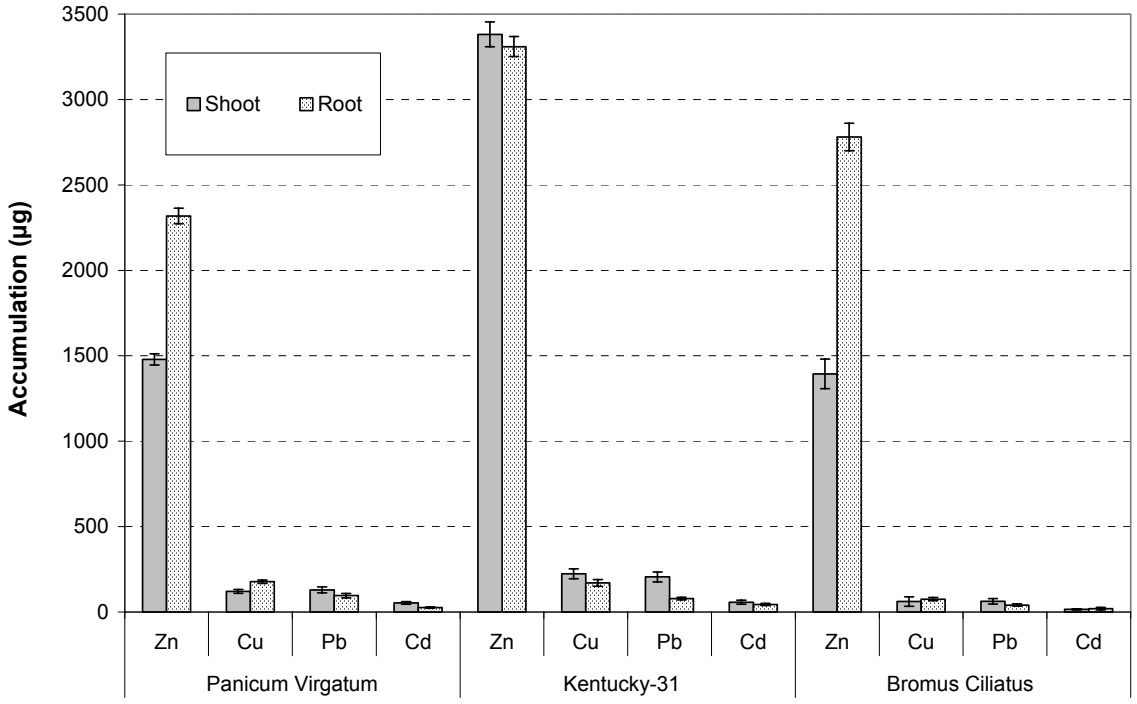


Figure 4.2-21a. The total mass accumulation of metals by different plants at the low loading

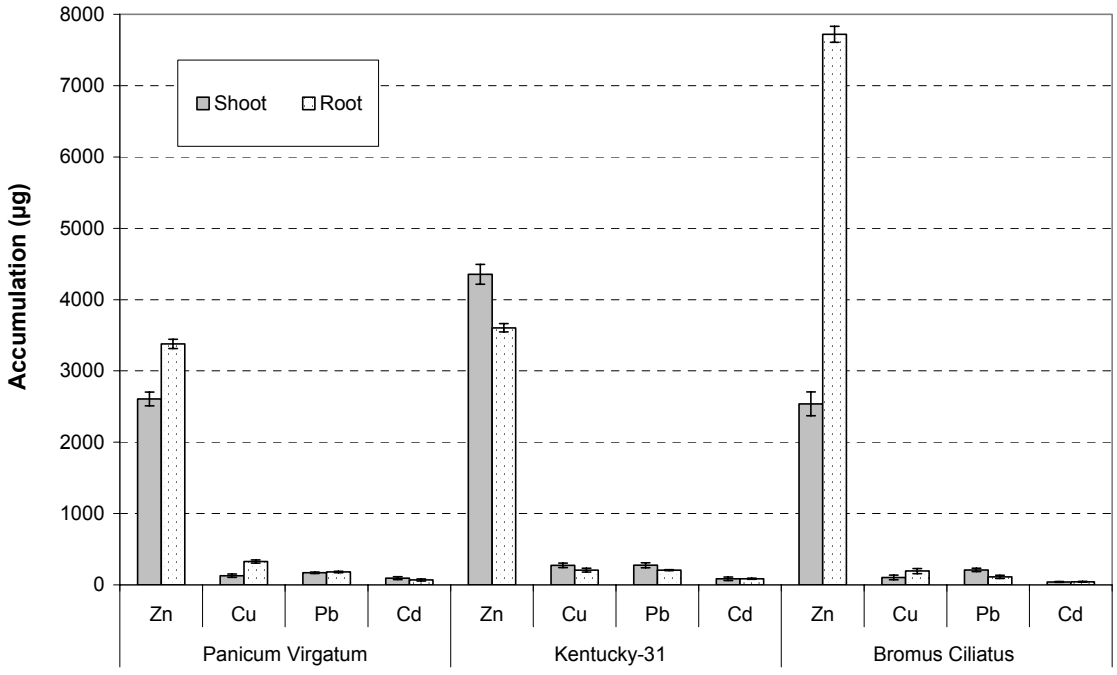


Figure 4.2-21b. The total mass accumulation of metals by different plants at the high loading

4.3 Metals in Soil

The concentrations of metals in the top and bottom soil layers are shown in Tables 4.3-1 and 4.3-2. Trends of increasing concentrations of Zn, Cu, Pb and Cd in both layers with the time are observed. There are no significant differences between the concentrations of Zn, Cu, Pb and Cd in the soil of pots with plants and that of the control pots, indicating that the plant uptake had negligible impact on the soil metals levels.

Vertical distributions of metals in the soil profile are shown in Figures 4.3-1 and 4.3-2. Relatively high metal concentrations within the top 5 cm were observed, which declined sharply with depth. Considering the different background concentrations, the concentrations of Zn, Cu, Pb and Cd in the top layer increased about 103±12, 11±2, 8±3 and 2±0 μg/g respectively, with the low loading and 161±16, 32±4, 19±6 and 5±1 μg/g respectively, with high loading. Those in bottom layer increased about 14±8, 7±1, 2±1 and 0.7±0.2 μg/g, respectively, with low input and 40±9, 11±2, 4±1 and 1.5±0.4 μg/g respectively, with high input. Compared to the top layer, the increases of Zn, Cu, Pb and Cd in second layer (5-10cm) are relatively low and are 55±16, 9±1, 3±5 and 1.1±0.3 μg/g, respectively, with low loading and 86±22, 13±4, 6±3 and 2.2±6 μg/g, respectively, with high loading. These results show that most input metals are captured by the top soil layer. Considering the high organic content in the top layer, the metals are possibly held by organic matter in top layer, making them immobile, and thereby confirming their high affinity to organic matter. McGrath et al. (1989) reported high correlation between the heavy metals and organic content of the top layer of soil (R^2=0.88-0.99). High organic content in the top media of bioretention cells may be necessary to produce high removal efficiencies of metals from runoff.

Table 4.3-1. Total metal concentrations in the soil (µg/g) (low loading)

Times (Days)			0	80	110	140	170	200	230
Species	Metal	Depth (cm)							
PV	Zn	0-5	223±15	234±13	255±3	265±11	296±9	315±4	328±9
		20-25	102±14	106±12	109±14	111±3	112±6	110±9	112±12
	Cu	0-5	23±4	25±5	27±1	30±4	31±2	31±5	33±2
		20-25	7±3	8±2	8±1	9±2	9±4	11±3	13±7
	Pb	0-5	44±6	42±5	48±4	51±7	49±10	51±7	53±11
		20-25	29±4	29±6	28±7	30±11	29±6	29±5	30±5
	Cd	0-5	0.21±0.2	1.3±0.2	1.4±0.2	1.4±0.4	1.5±0.5	1.5±0.4	1.6±0.8
		20-25	0.1±0.1	0.1±0.02	0.3±0.04	0.6±0.04	0.6±0.01	0.9±0.03	0.9±0.04
K-31	Zn	0-5	223±15	228±10	269±12	258±13	298±9	326±15	321±18
		20-25	102±14	103±10	104±8	110±6	108±12	103±18	106±9
	Cu	0-5	23±4	22±3	24±6	25±7	27±8	31±10	31±7
		20-25	7±3	10±3	12±4	13±7	14±1	16±6	14±4
	Pb	0-5	44±6	41±5	40±4	51±3	50±6	50±6	49±7
		20-25	29±4	30±4	31±6	29±1	30±3	31±3	31±7
	Cd	0-5	0.21±0.2	0.6±0.3	1.2±0.5	1.5±0.3	1.4±0.4	1.5±0.7	1.8±0.5
		20-25	0.1±0.1	0.2±0.01	0.4±0.04	0.4±0.2	0.5±0.2	0.4±0.5	0.6±0.3
BC	Zn	0-5	223±15	245±12	255±10	267±11	279±12	301±15	313±18
		20-25	102±14	104±4	106±5	110±10	106±6	111±9	124±7
	Cu	0-5	23±4	23±2	26±3	29±1	32±4	36±6	36±4
		20-25	7±3	8±2	9±5	11±4	11±8	12±12	14±10
	Pb	0-5	44±6	40±4	46±5	47±7	49±9	50±6	52±8
		20-25	29±4	32±6	30±2	30±3	30±7	30±9	32±3
	Cd	0-5	0.21±0.2	0.4±0.1	1.1±0.4	1.4±0.5	1.5±0.2	1.6±0.6	1.6±0.6
		20-25	0.1±0.1	0.3±0.1	0.4±0.2	0.3±0.2	0.5±0.4	0.9±0.3	1.0±0.3
C	Zn	0-5	223±15	233±13	256±19	269±21	263±21	279±23	341±20
		20-25	102±14	104±4	106±11	113±5	121±10	122±12	122±12
	Cu	0-5	23±4	24±3	26±5	29±10	30±3	33±9	34±5
		20-25	7±3	9±5	11±8	12±9	12±2	13±4	13±3
	Pb	0-5	44±6	39±4	40±6	46±3	52±2	52±3	55±4
		20-25	29±4	30±3	30±4	32±5	31±2	31±11	32±10
	Cd	0-5	0.21±0.2	0.8±0.2	0.9±0.1	1.1±0.5	1.4±0.3	1.8±0.6	2.1±0.1
		20-25	0.1±0.1	0.4±0.1	0.6±0.2	0.6±0.01	0.7±0.1	0.7±0.4	0.9±0.2

Table 4.3-2. Total metal concentrations in the soil (µg/g) (high loading)

Times (Days)			**0**	**80**	**110**	**140**	**170**	**200**	**230**
Species	Metal	Depth (cm)							
PV	Zn	0-5	223±15	281±12	313±10	349±5	364±14	355±15	398±7
		20-25	102±14	105±5	112±7	121±9	123±12	134±11	133±11
	Cu	0-5	23±4	30±6	34±7	35±3	39±12	45±5	51±2
		20-25	7±3	7±3	11±6	13±3	14±3	17±5	21±8
	Pb	0-5	44±6	45±3	44±5	53±7	54±8	65±7	67±12
		20-25	29±4	30±5	32±7	31±12	32±6	32±7	31±6
	Cd	0-5	0.21±0.2	1.4±2	1.9±1	2.5±0.3	3.4±0.5	5.2±0.3	6.1±0.5
		20-25	0.1±0.1	0.3±0.2	0.3±0.2	0.9±0.3	1.0±0.2	0.7±0.3	0.9±0.3
K-31	Zn	0-5	223±15	238±12	265±1	279±10	298±9	323±12	365±5
		20-25	102±14	119±8	121±5	134±12	145±12	156±10	147±11
	Cu	0-5	23±4	23±7	29±4	34±5	39±9	42±5	51±8
		20-25	7±3	12±5	15±7	17±9	15±3	20±10	17±13
	Pb	0-5	44±6	33±5	44±10	53±9	53±5	58±7	57±13
		20-25	29±4	30±9	31±3	31±7	30±12	32±11	33±6
	Cd	0-5	0.21±0.2	1.0±0.4	1.3±0.2	2.1±0.1	3.4±0.2	3.9±0.3	5.1±0.3
		20-25	0.1±0.1	0.4±0.1	0.9±0.2	1.4±0.1	1.6±0.3	2.1±0.5	1.8±0.4
BC	Zn	0-5	223±15	276±12	298±11	315±14	328±12	368±20	397±21
		20-25	102±14	107±5	112±5	121±9	130±1	134±15	136±7
	Cu	0-5	23±4	25±2	29±4	35±9	39±8	46±12	57±4
		20-25	7±3	8±2	12±4	11±6	15±5	17±12	17±10
	Pb	0-5	44±6	41±6	48±6	52±3	48±12	59±8	60±12
		20-25	29±4	30±5	31±6	30±4	32±5	31±10	32±11
	Cd	0-5	0.21±0.2	1.2±0.2	1.4±0.1	2.8±0.4	3.5±0.6	3.3±0.6	3.9±0.7
		20-25	0.1±0.1	0.5±0.1	0.9±0.3	0.9±0.4	1.4±0.1	1.5±0.2	1.8±0.4
C	Zn	0-5	223±15	279±10	302±9	312±17	338±12	352±15	377±10
		20-25	102±14	108±4	113±3	125±3	136±5	147±6	153±7
	Cu	0-5	23±4	26±3	37±5	38±6	45±6	51±5	59±10
		20-25	7±3	9±2	11±2	14±4	15±6	15±10	17±8
	Pb	0-5	44±6	46±4	52±6	55±9	65±10	69±11	69±12
		20-25	29±4	30±4	30±6	31±7	31±6	32±4	34±4
	Cd	0-5	0.21±0.2	1.4±0.3	1.6±0.3	2.4±0.5	4.1±1.1	5.9±1.2	6.5±1.2
		20-25	0.1±0.1	0.2±0.3	0.9±0.1	0.9±0.2	1.1±0.2	1.3±0.4	1.8±0.3

The ratios of the metal concentration increase in the soil layers and mass input (unit: μg/g/g input) can indicate the mobility of metals in the soil profile shown as:

$$R = \frac{\text{Increment of metals conc.}}{\text{Metal mass input}} = \frac{\Delta C}{M} \qquad (4.4)$$

Equation 4.4 indicates that the higher the R value of a metal, the easier the movement from top soil layer to the bottom layer. The R values of the bottom layer for Zn, Cu, Pb and Cd are 7.2, 33.6, 11.5 and 11.7 $\mu g/g^2$, respectively, with low loading and 9.5, 22.2, 7.0 and 9.0 $\mu g/g^2$, respectively, with high loading. Therefore, mobility follows the order Cu>Cd>Pb>Zn under the low loading and Cu>Zn>Cd>Pb with high loading, respectively. These results indicate that Cu is more mobile from top to bottom than Zn, Pb and Cd. Nyamangara et al. (1999) investigated concentrations of EDTA-extractable Zn, Cu, Ni and Pb in the 0-90 cm profiles of soil amended with sewage sludge for more than 19 years and found that, compared with the control, the concentrations of Zn and Cu increased more than those of Pb and Ni in the 50-90 cm, which indicated that Cu and Zn are more mobile than Ni and Pb. In present study, the results showed the mobility of Pb is higher than that of Zn under low loading, which is in contrast to what Nyamangara et al. observed. The movement of metals from top to lower soil layers could be due to several reasons, including differences in solubility of the metal forms and availability of exchange sites in the lower layers which were reversely occupied by specific metals.

Since plants take up most metals from the soil solution, it is often assumed that dissolved metals are readily available to organisms (Barber et al., 1984). Determination of dissolved metals may provide useful information on metal bioavailability and toxicity

(Knight et al. 1998). The bioavailability of metals in soils is very complicated and some studied have attempted to relate metals extracted by different reagents to the metals uptake by plants. Reagents include $CaCl_2$, EDTA, EDPA, NH_4NO_3, water, etc. In the present study, $Sr(NO_3)_2$ is used as the reagent to extract the metals from the soil. The results show that the exchangeable fraction (extractable metals by $Sr(NO_3)_2$/ total metals) in the top and lower layers varied greatly with time (Figures 4.3-3 to 4.3-8). The plots show that bioavailabile metals in soils changed, likely due to the uptake of plants and continued addition from the input. After the plants grew 110 days, the bioavailabile metals in the soil decreased because of absorption by plants, then increased to higher levels which may be due to continuous metal input and low requirement of plants during this time. The time-dependent variation of bioavailabile metals indicated that the amounts of metals which can be absorbed by the PV, K-31 and BC differ during different growth phases. The concentrations of bioavailabile metals in the control pots were not higher than the other pots. This indicated the bioavailability in the soil did not only depend on the uptake by plants.

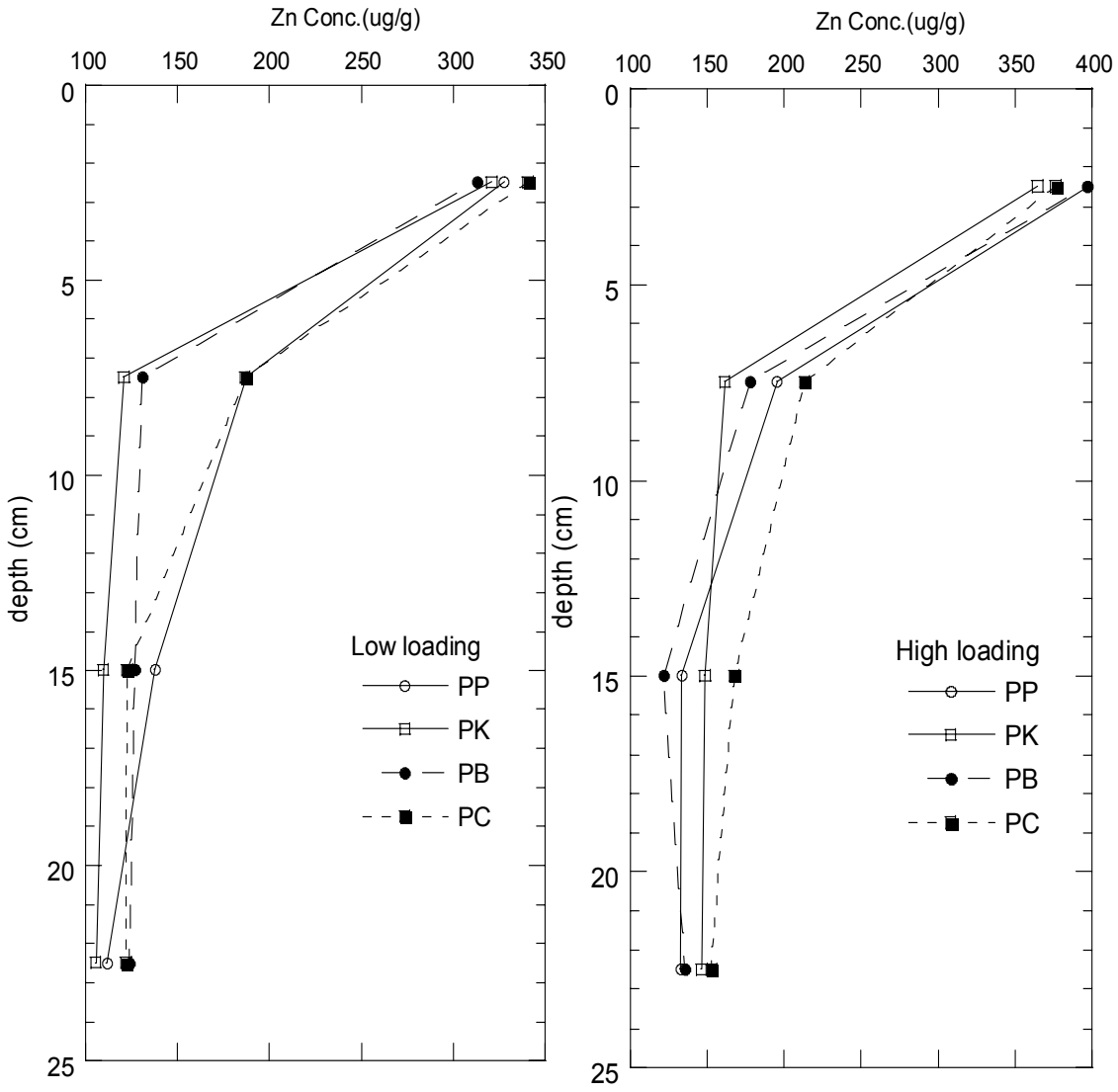


Figure 4.3-1a. The vertical distribution of total Zn in the soil of different pots after 230 days

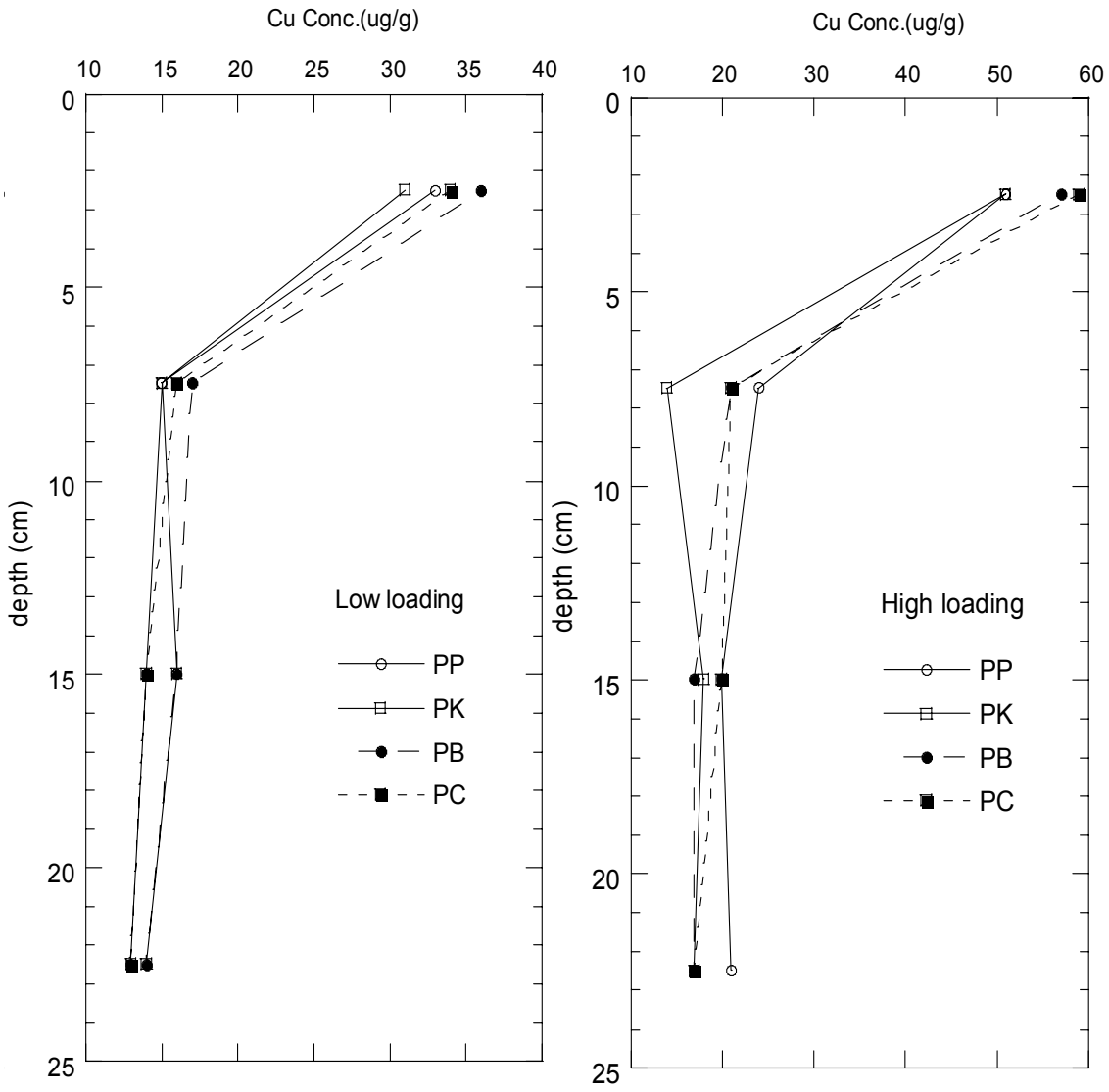


Figure 4.3-1b. The vertical distribution of total Cu in the soil of different pots after 230 days

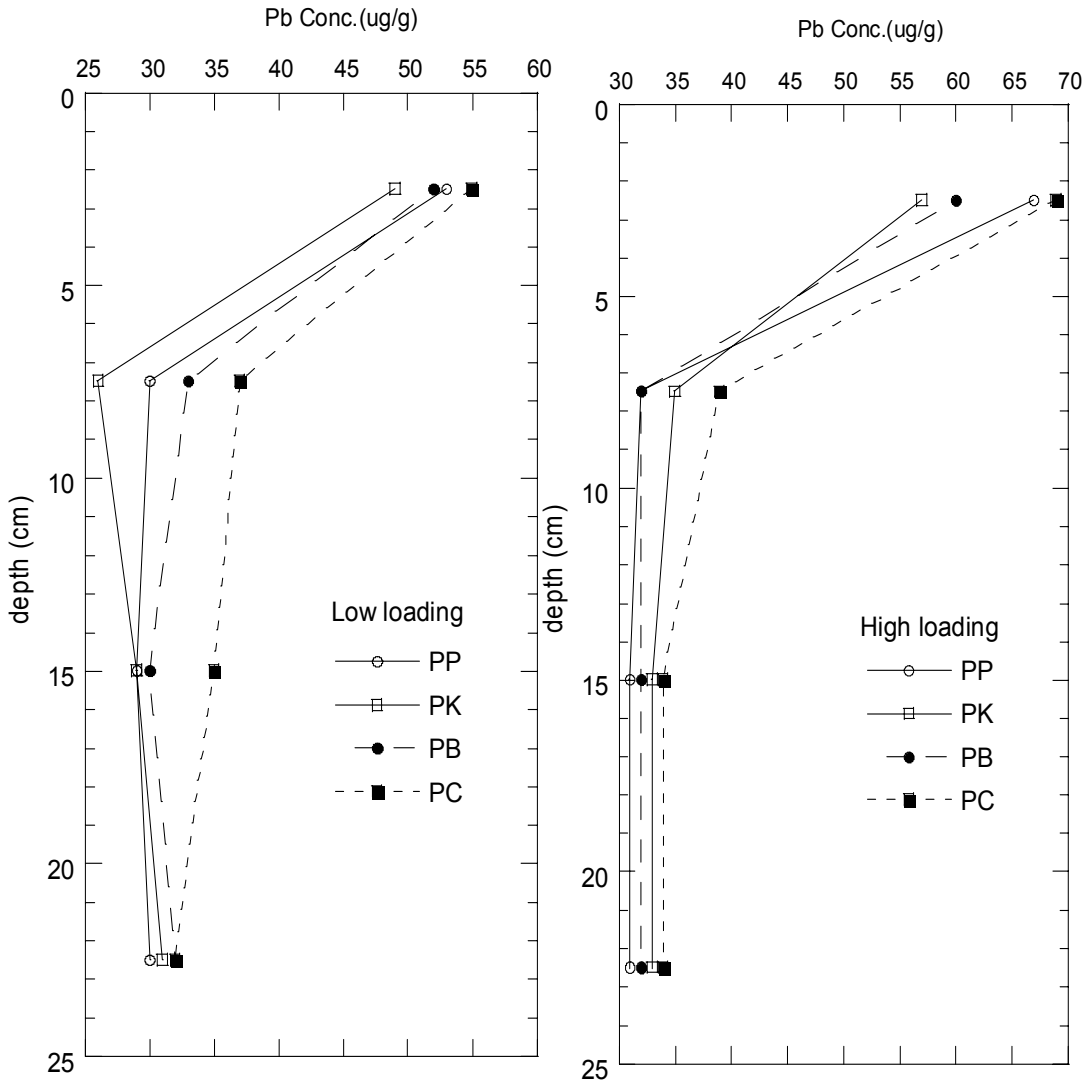


Figure 4.3-2a. The vertical distribution of total Pb in the soil of different pots after 230 days

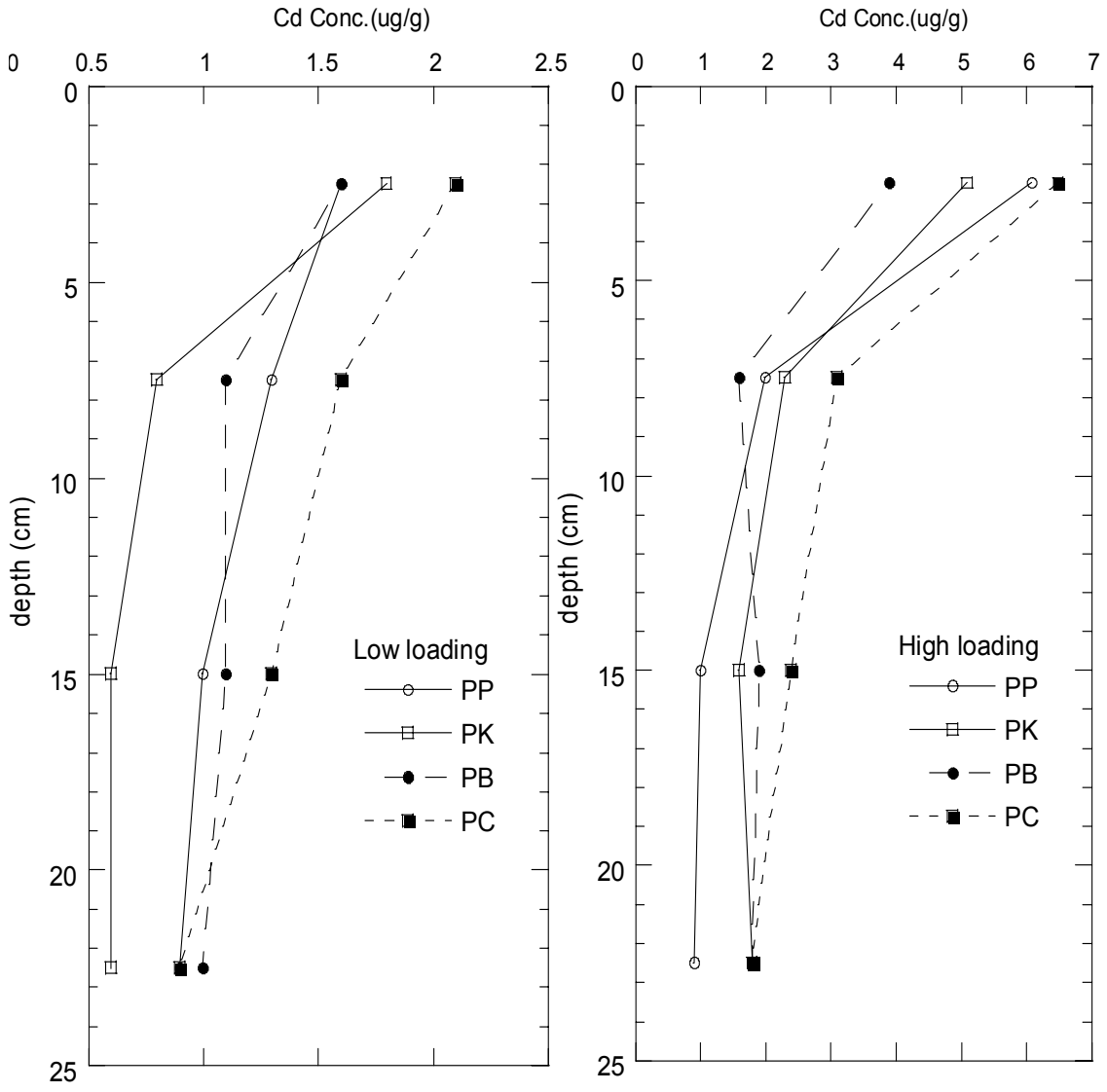


Figure 4.3-2b. The vertical distribution of total Cd in the soil of different pots after 230 days

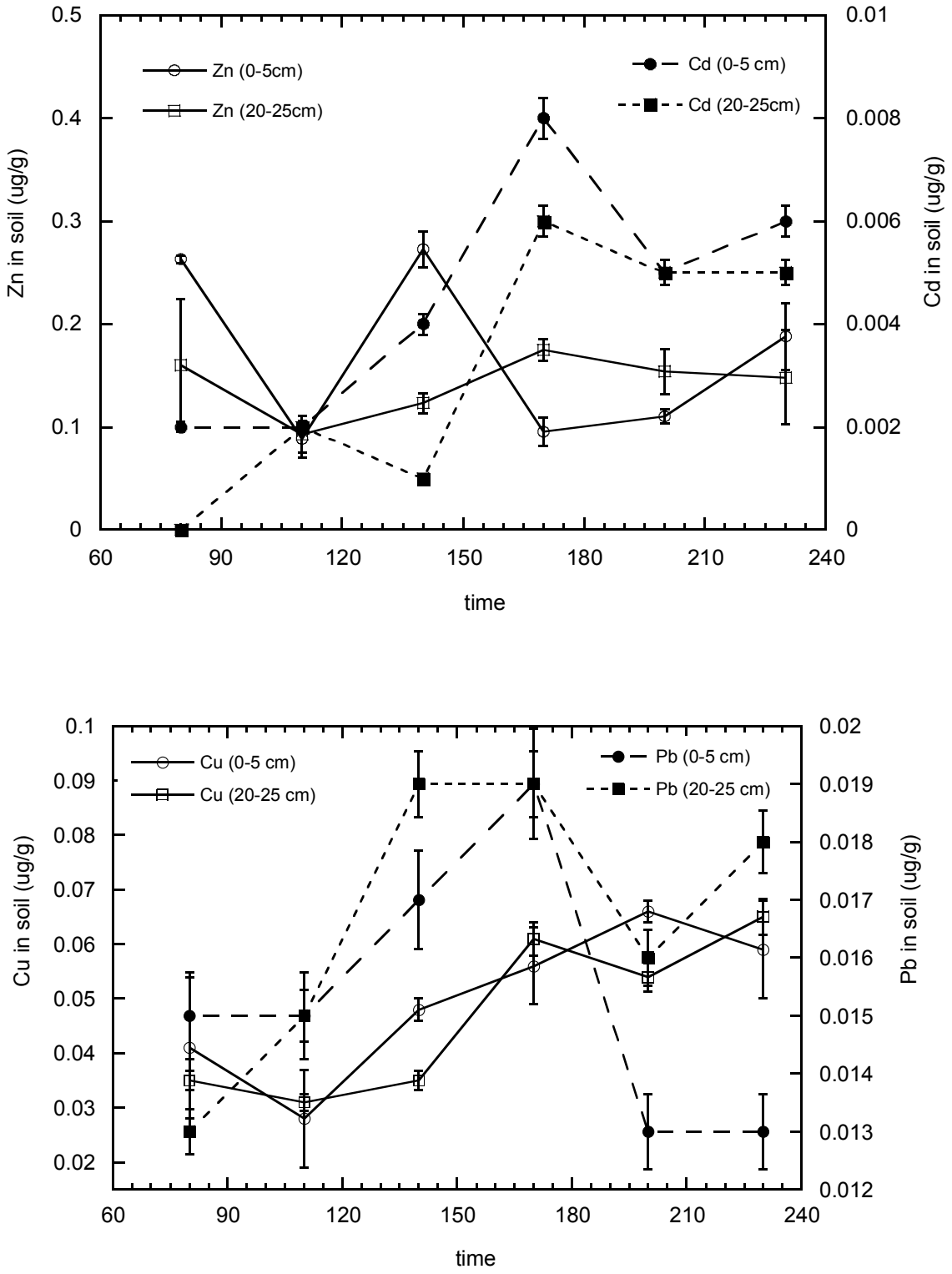


Figure 4.3-3. The concentration of $Sr(NO_3)_2$ extractable (bioavailable) metals in soil of pots with PV with low loading input

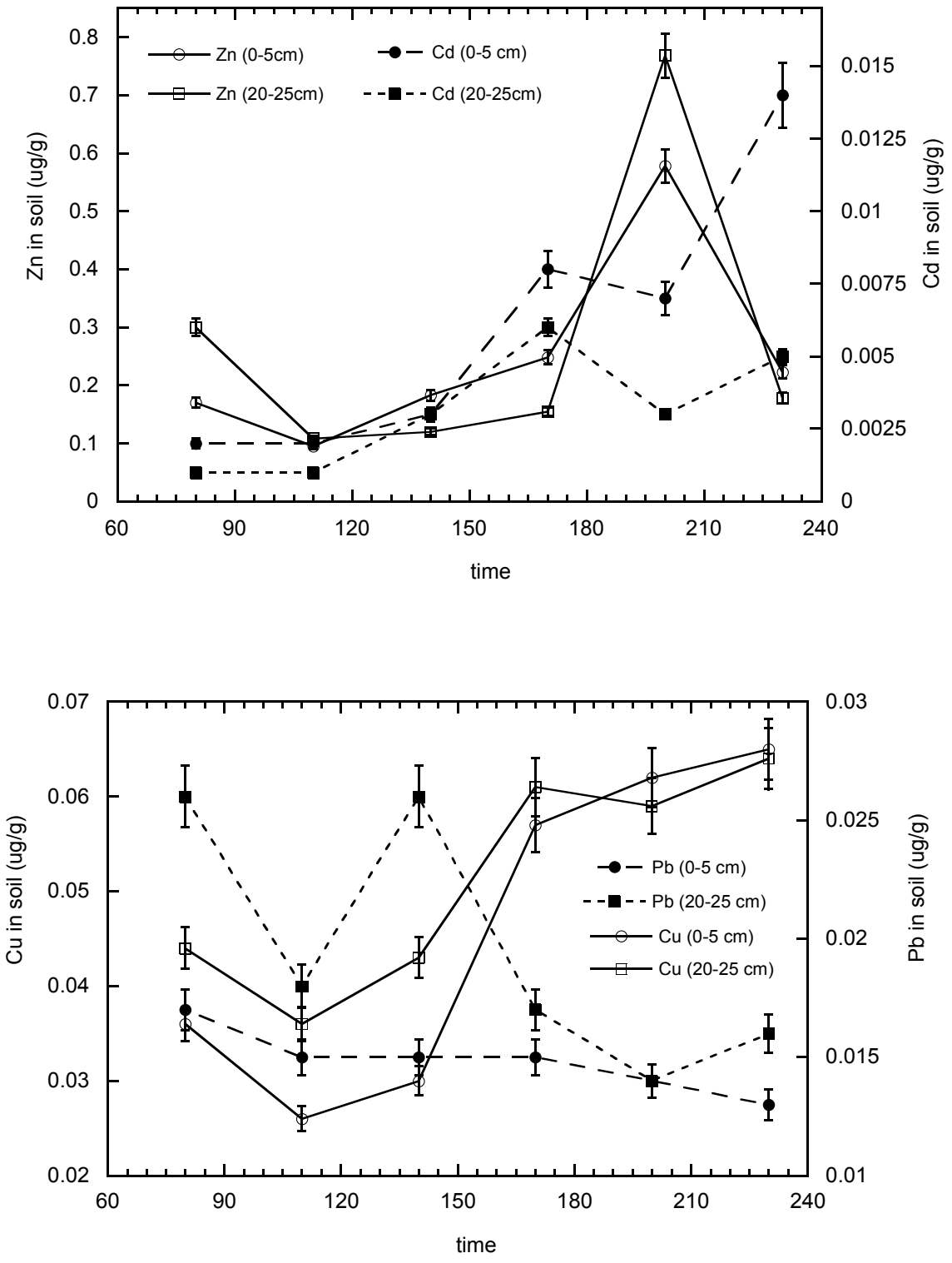


Figure 4.3-4. The concentration of $Sr(NO_3)_2$ extractable (bioavailable) metals in soil of pots with K-31 with low loading input

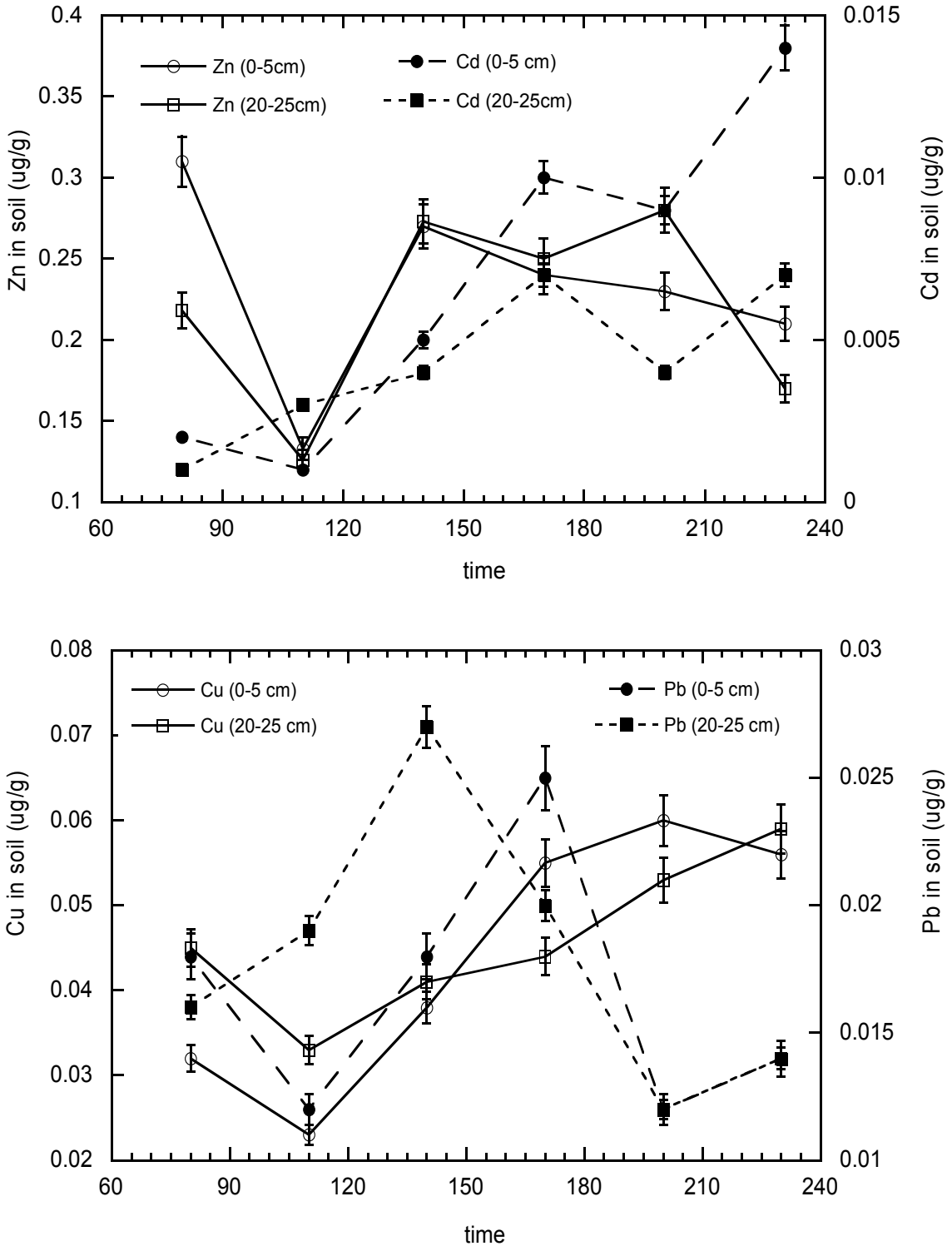


Figure 4.3-5. The concentration of $Sr(NO_3)_2$ extractable (bioavailable) metals in soil of pots with BC with low loading input

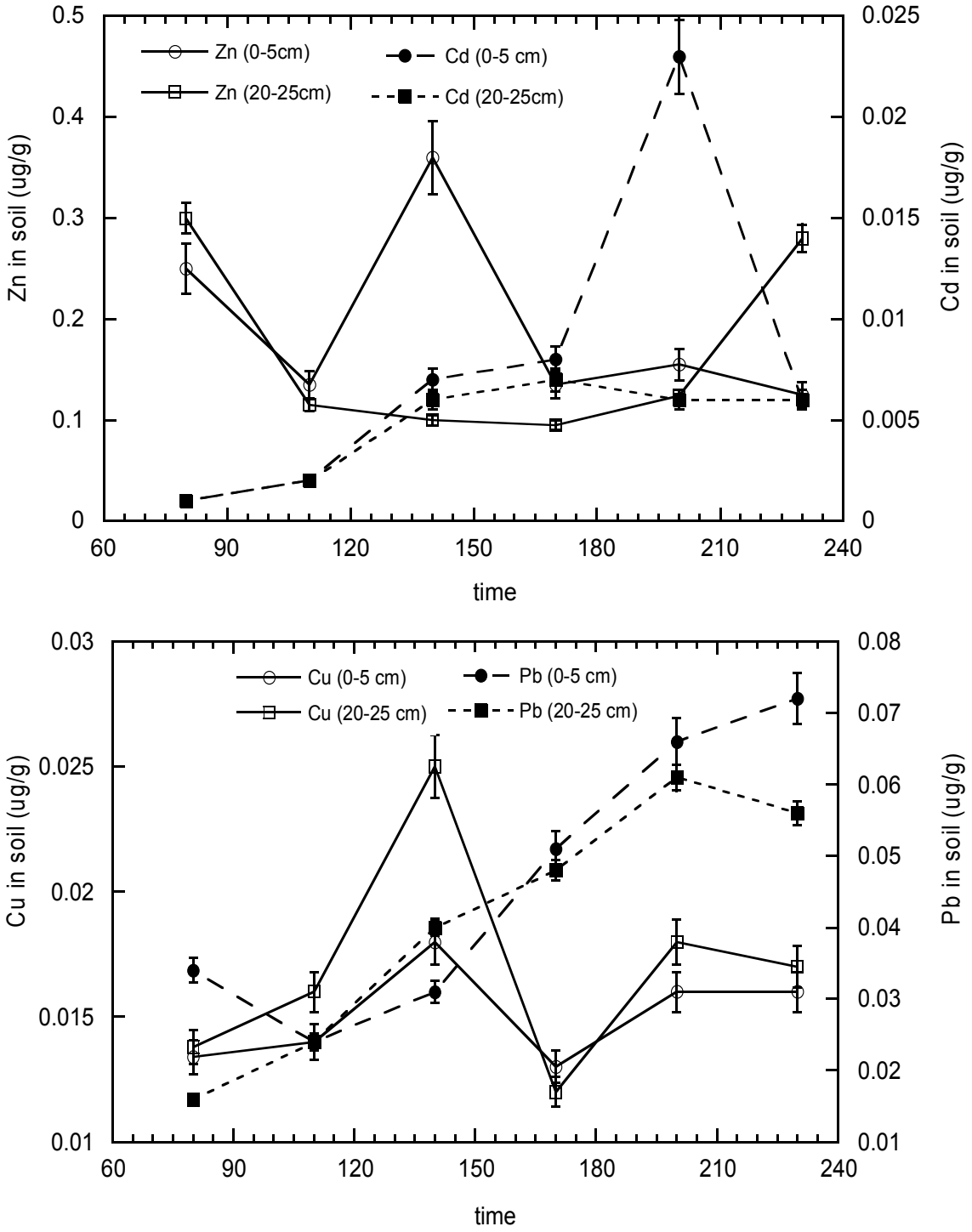


Figure 4.3-6. The concentration of $Sr(NO_3)_2$ extractable (bioavailable) metals in soil of Control pots with low loading input

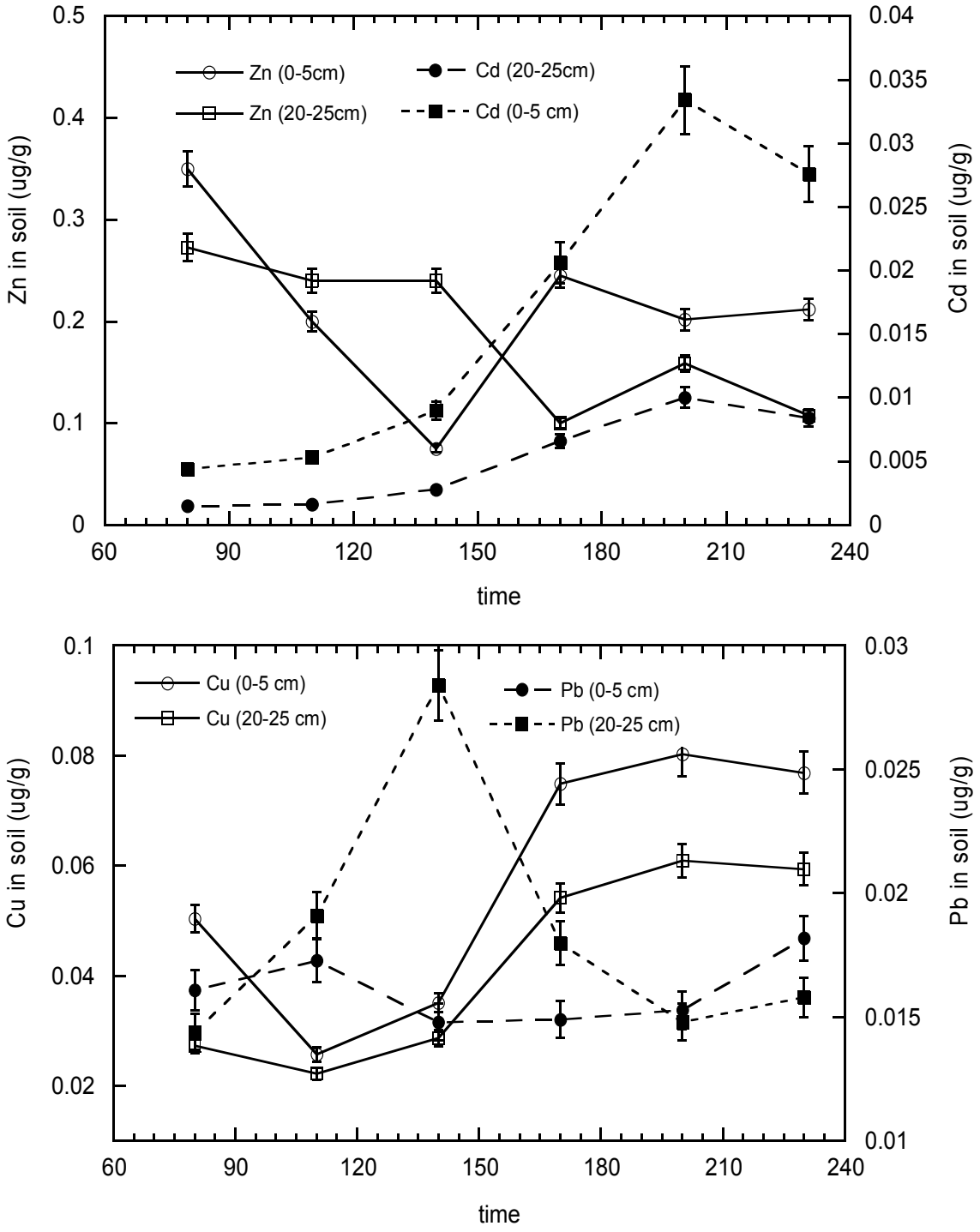
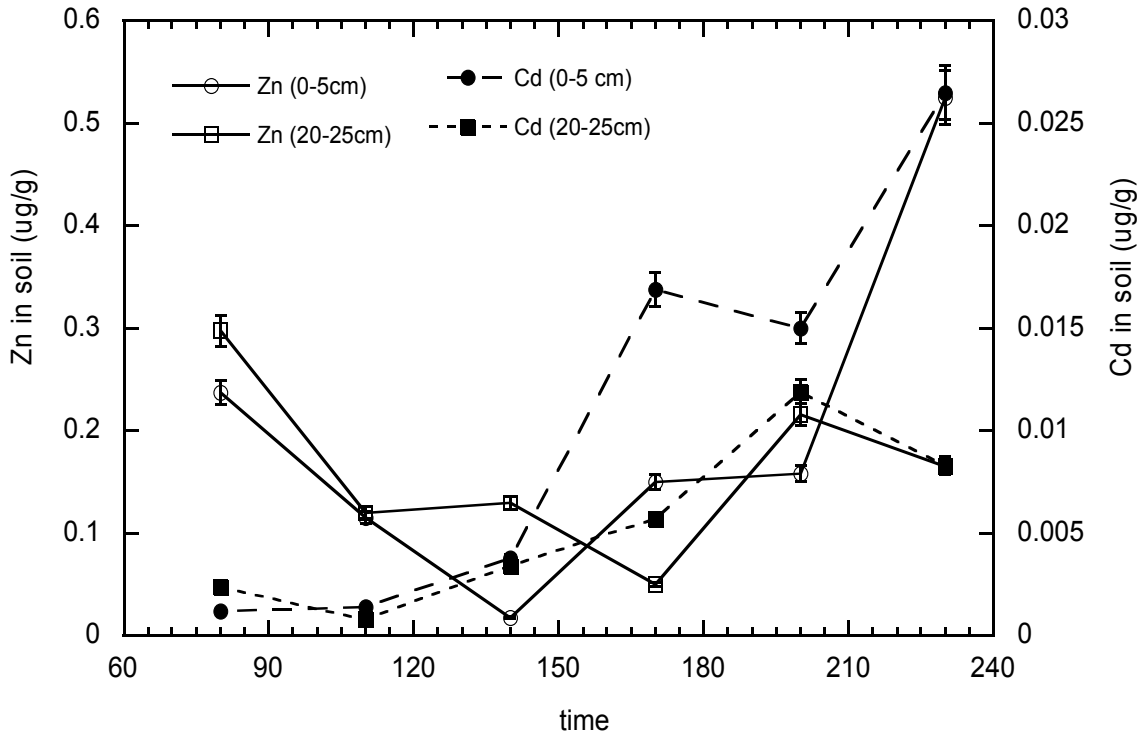


Figure 4.3-7. The concentration of $Sr(NO_3)_2$ extractable (bioavailable) metals in soil of pots with PV with high loading input



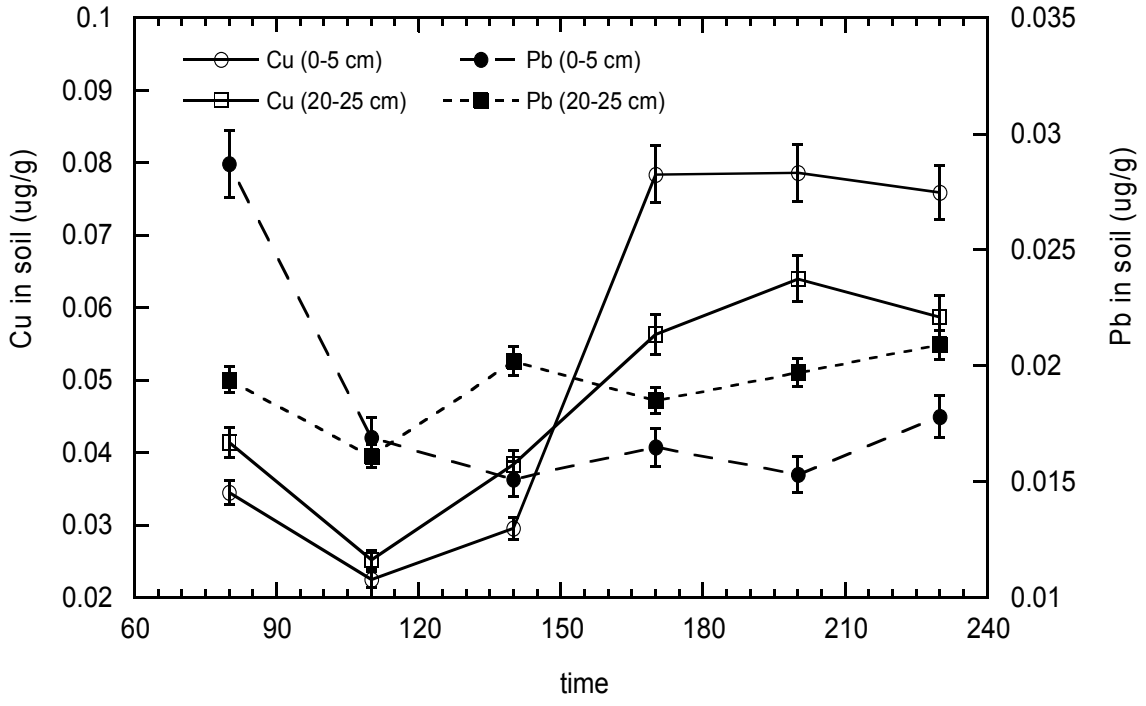


Figure 4.3-8. The concentration of $Sr(NO_3)_2$ extractable (bioavailable) metals in soil of pots with K-31 with high loading input

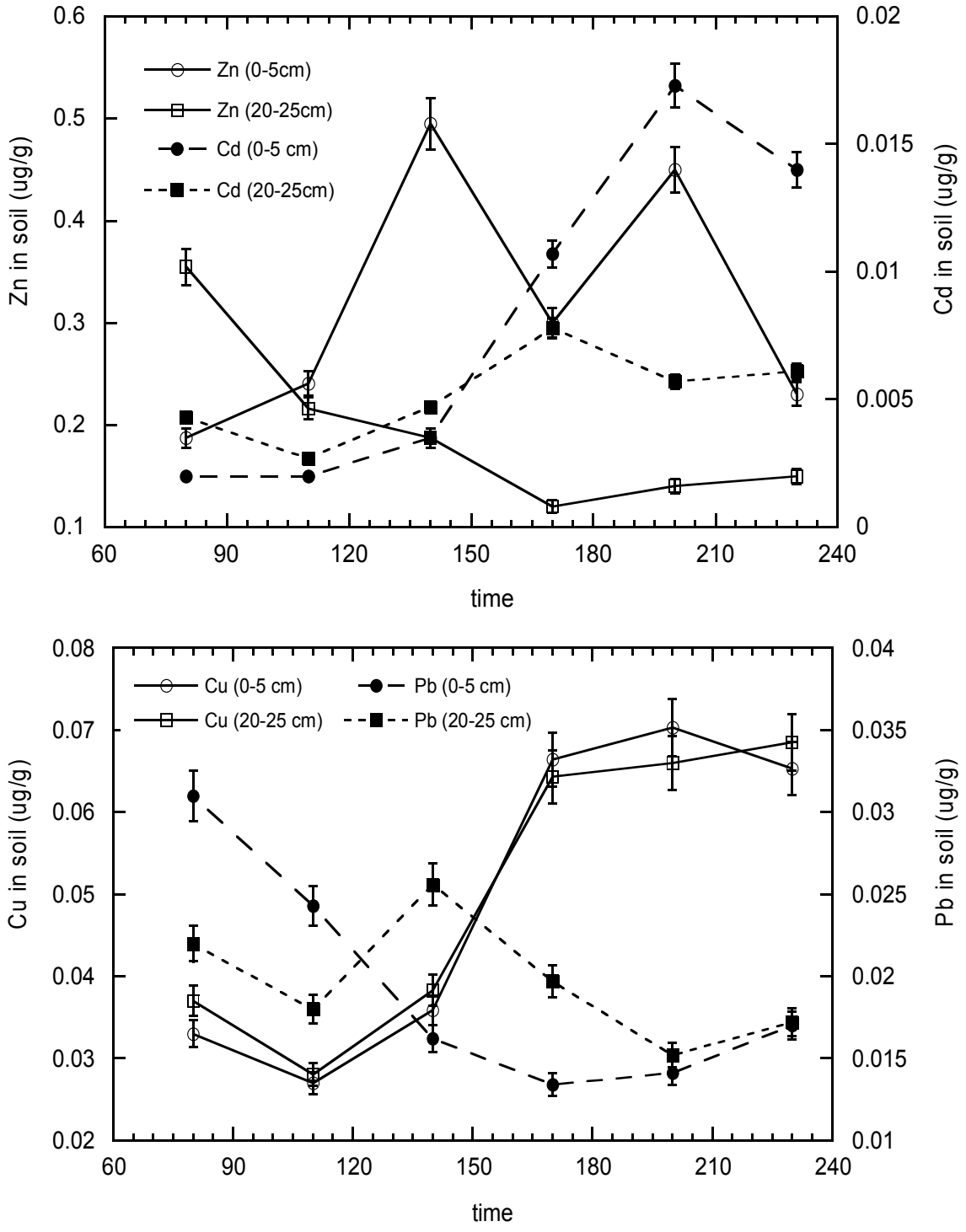


Figure 4.3-9. The concentration of $Sr(NO_3)_2$ extractable (bioavailable) metals in soil of pots with BC with high loading input

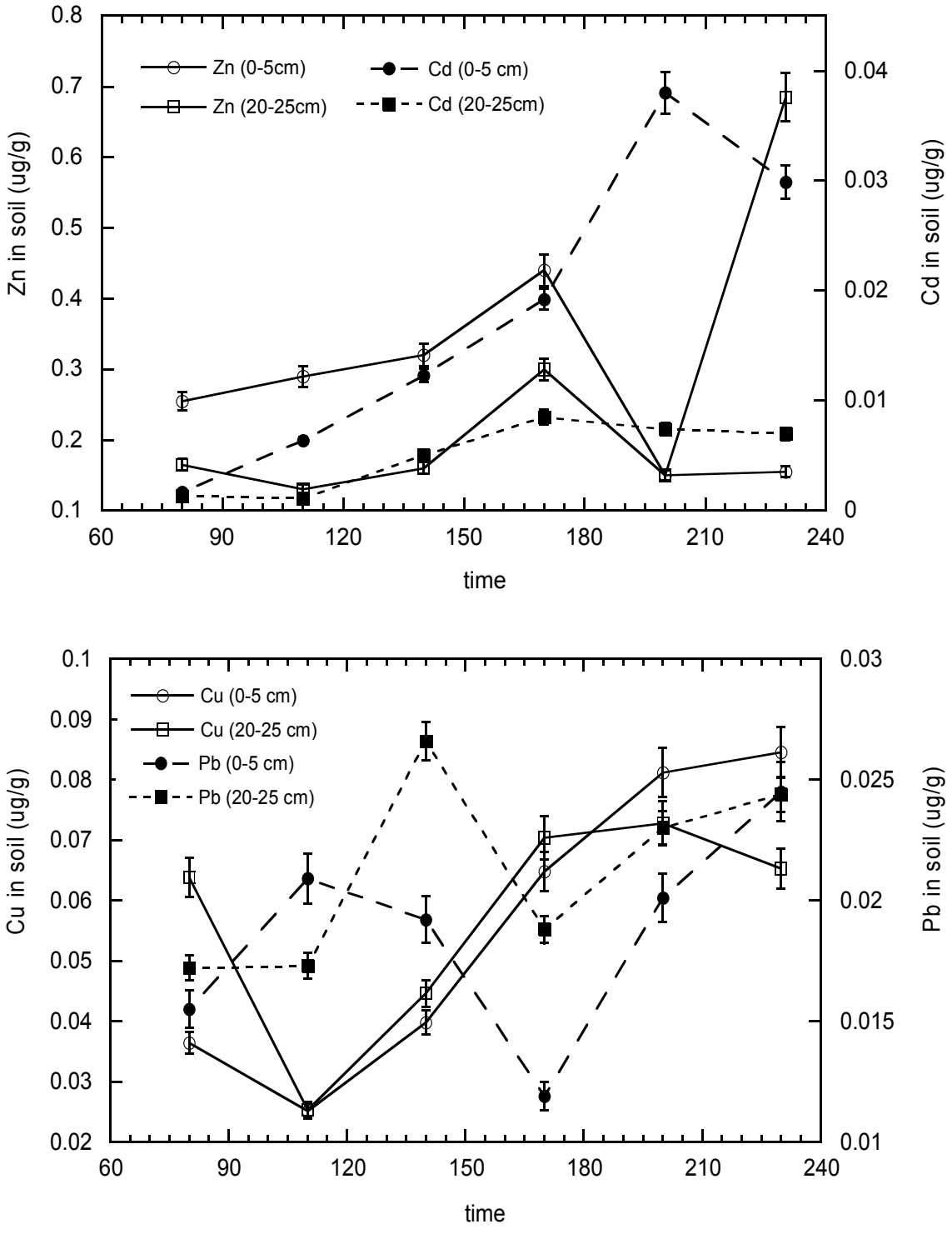


Figure 4.3-10. The concentration of $Sr(NO_3)_2$ extractable (bioavailable) metals in soil of control pots with high loading input

4.4 Relationship of metals in soil and plants

Curve estimation regression analysis was used to highlight the relationship between the trace metals in plant tissues and in soil. Trends in metal concentrations in plants as a function of metal content in soils can be described by three models: linear (constant partitioning model), plateau (saturated model), and the Langmuir sorption model (McBride, 1995). The relationship between the concentration of metals in the top soil layer soil and plant tissues are shown in Figures 4.4-1 to 4.4-8. The relationship between Zn, Cu, Pb and Cd in soil and tissues of plants are linear and positive except that for Cu and Cd in soil and shoots of BC with low load, and Cu in soil and shoots of PV with high loading.

Usually uptake of metals by plants did not occur in linear response to concentrations of the metals in soil, except at a low range of soil metal concentrations (Dudka et al., 1996). In the present experiments, the concentrations of the four metals in the soil are low compared to those in contaminated soils. Dudka et al. (1996) reported that Zn and Cd uptake by the studied plants could best be described by the plateau model using the concentration of Zn from 24.7 to 11375 mg/kg and Cd from 0.3 to 106.5 mg/kg in soil, while the correlation between Pb in soil and plants was linear because the concentration of Pb in soil was from 10.7 to 127.2 mg/kg, which is lower. Nan et al. (2002) reported that the relationships between Cd in corn grains and soil were linear, but Zn was not. Kim et al. (2003a) found a positive correlation coefficient between Zn, Cu and Pb contents in *P. thunbergii* plants tissues and those in the habitat soil. Herawati et al. (2000) found a significant positive correlation between Cd and Zn content in rice and in various soil types. All of the reports support the present results.

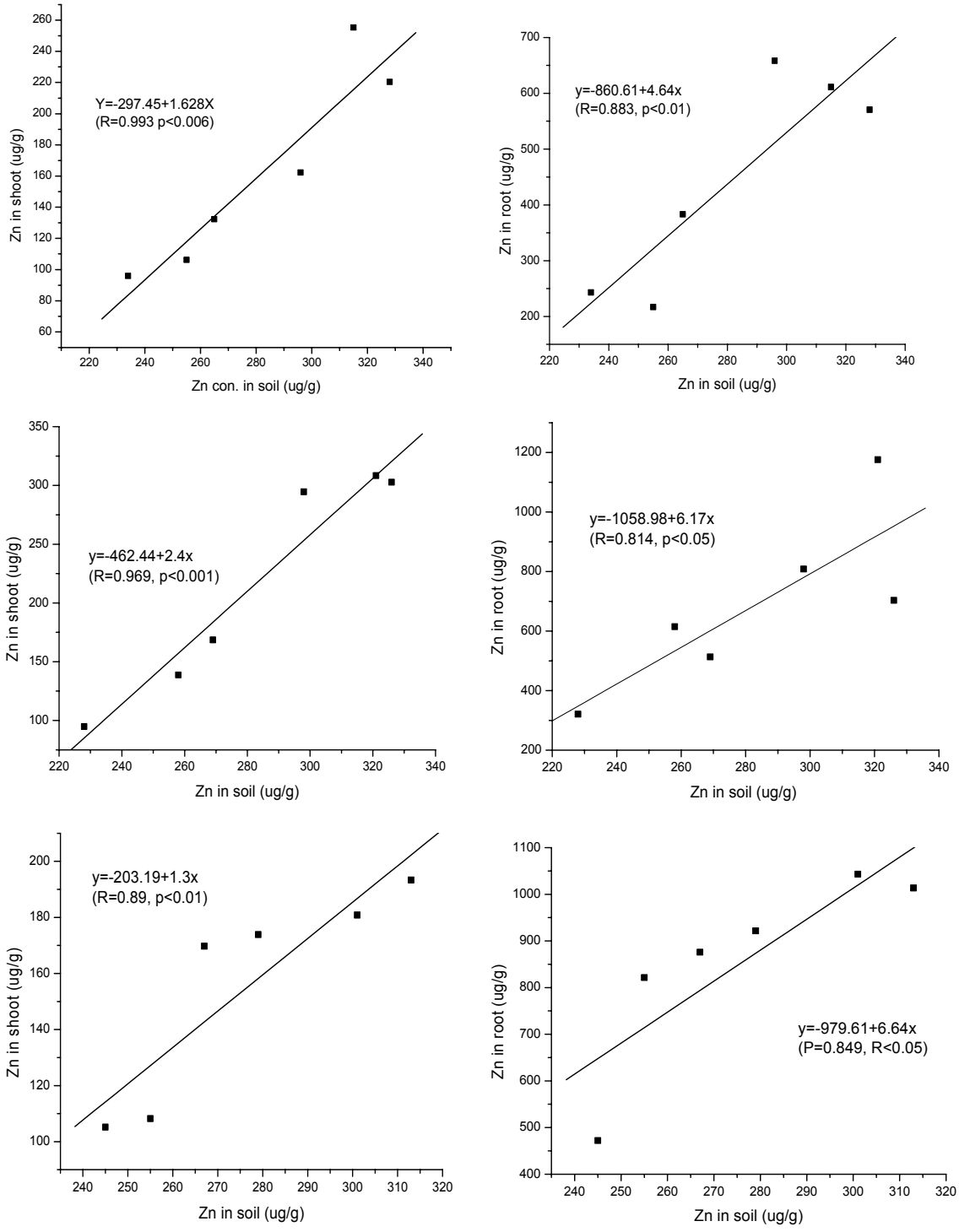


Figure 4.4-1. The relationship between concentrations of Zn in the surface soil and in the shoots and roots of PV (top), K-31 (middle) and BC (bottom) with low loading

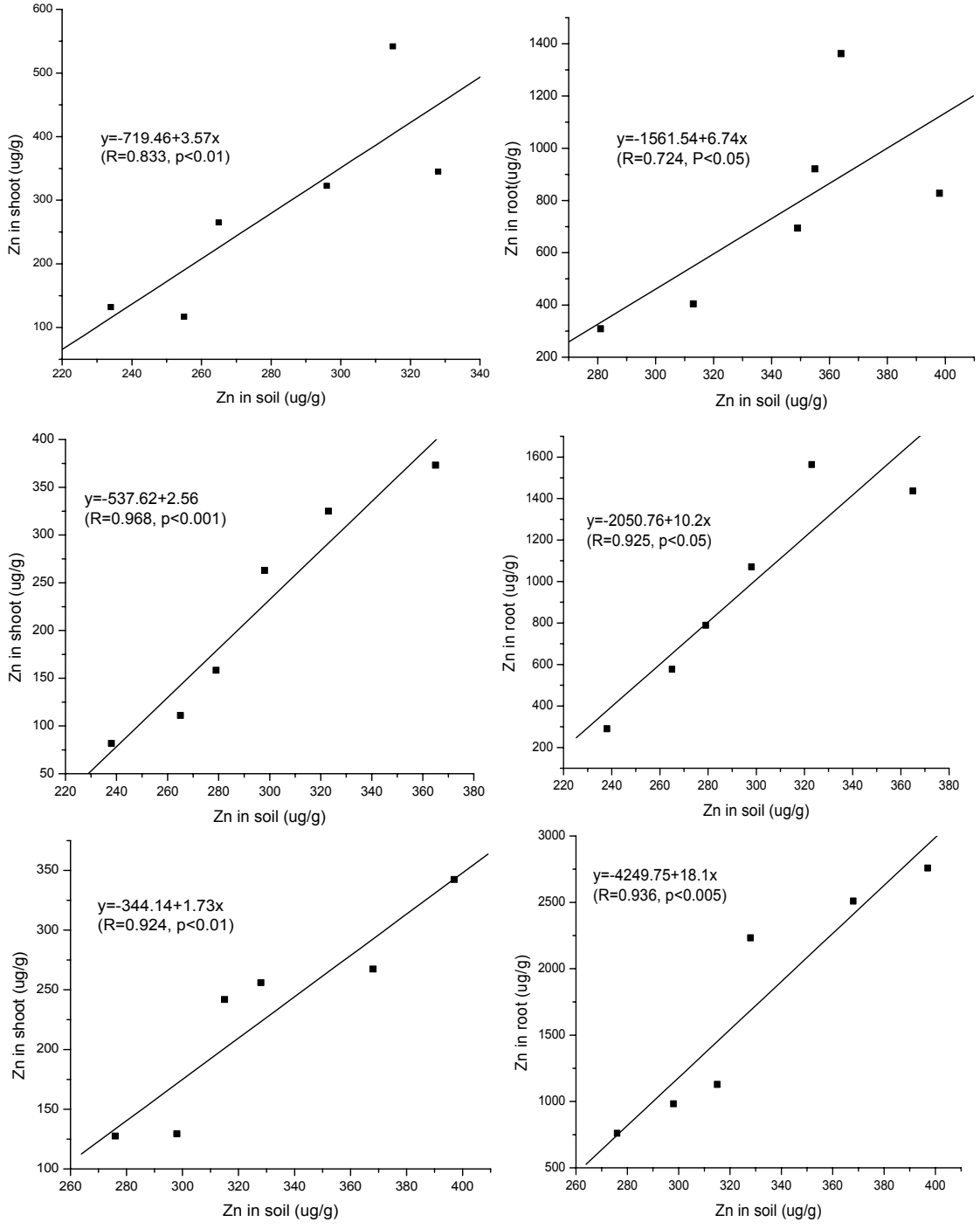


Figure 4.4-2. The relationship between concentrations of Zn in the surface soil and in the shoots and roots of PV (top), K-31 (middle) and BC (bottom) with high loading

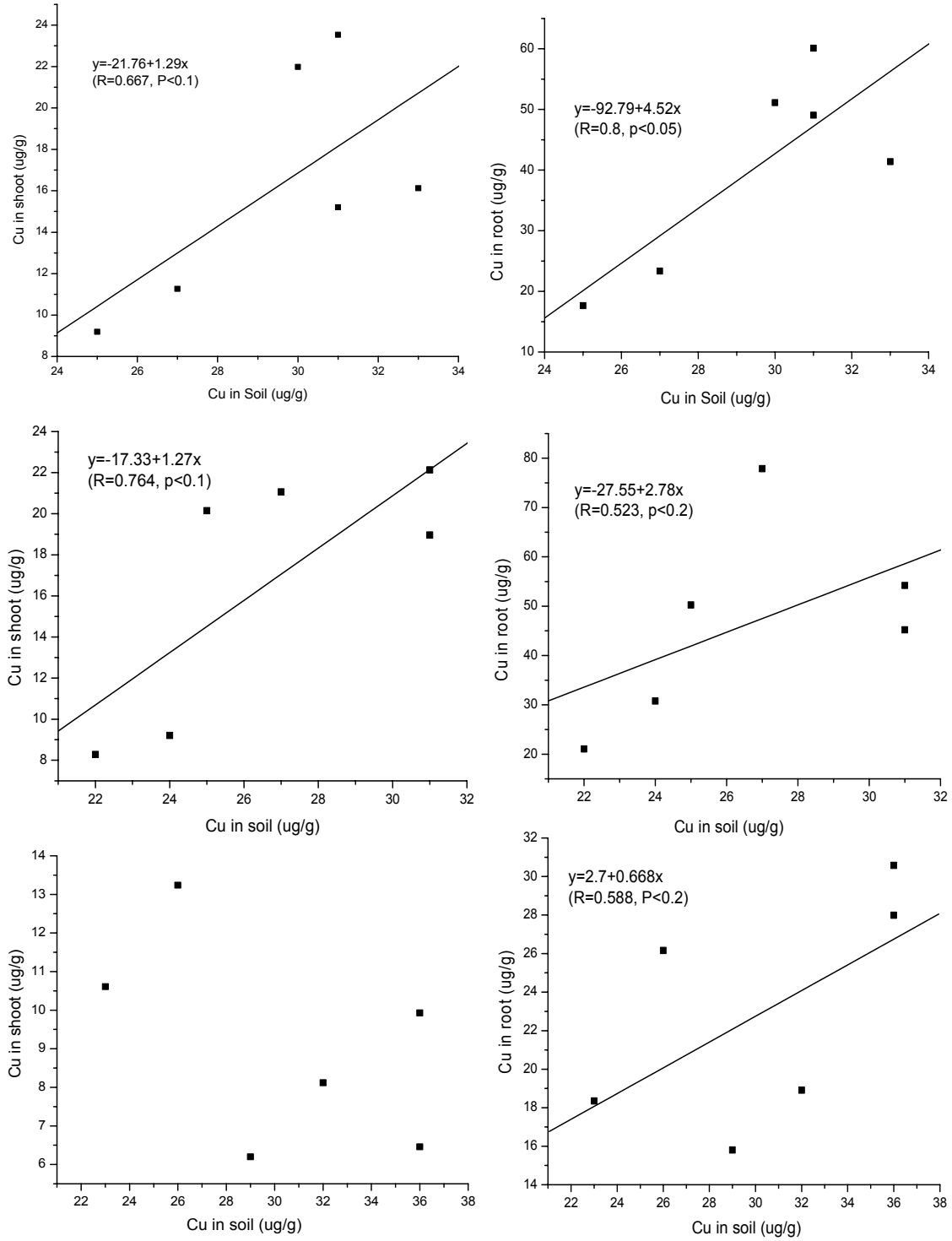


Figure 4.4-3. The relationship between concentrations of Cu in the surface soil and in the shoots and roots of PV (top), K-31 (middle) and BC (bottom) with low loading

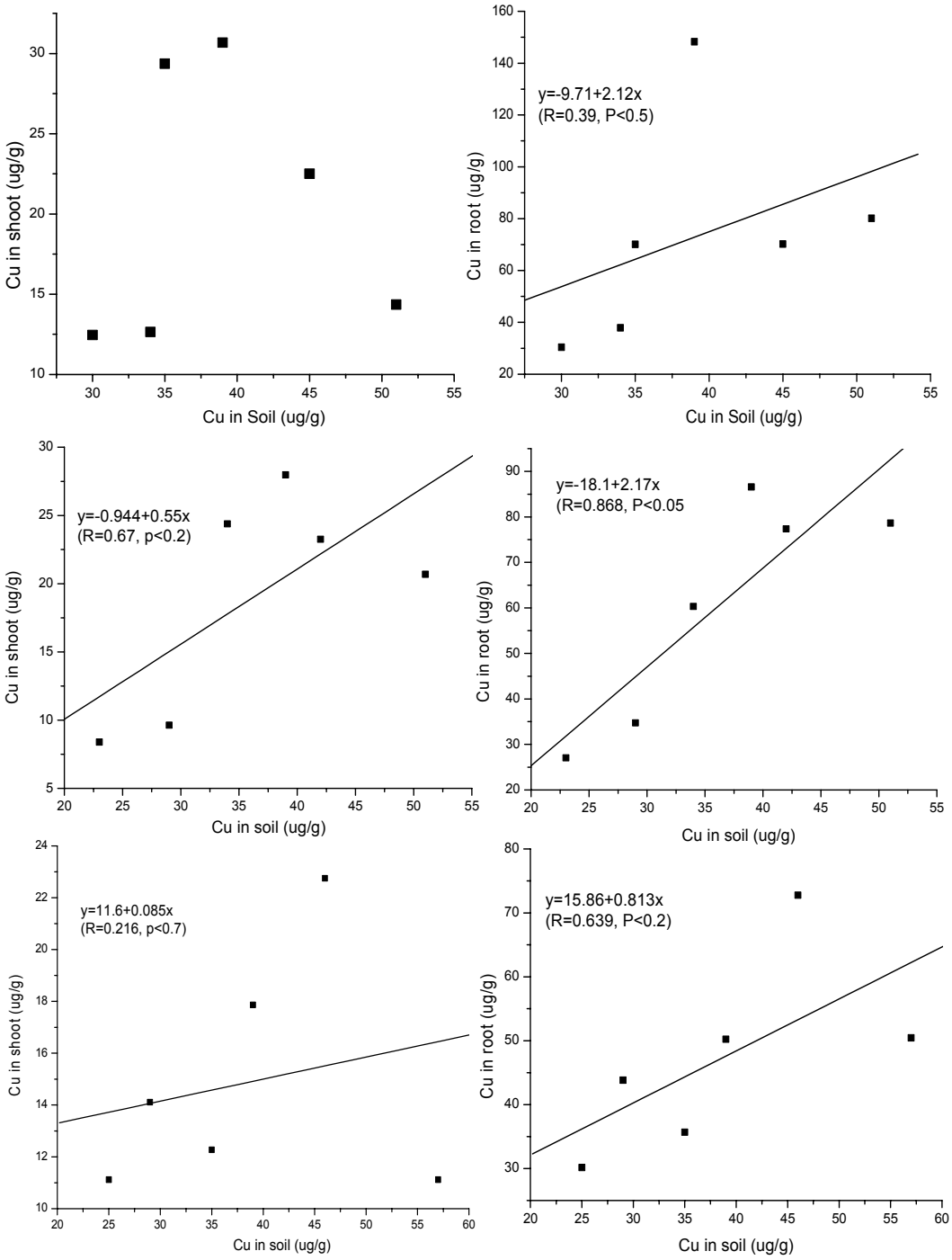


Figure 4.4-4. The relationship between concentrations of Cu in the surface soil and in the shoots and roots of PV (top), K-31 (middle) and BC (bottom) with high loading

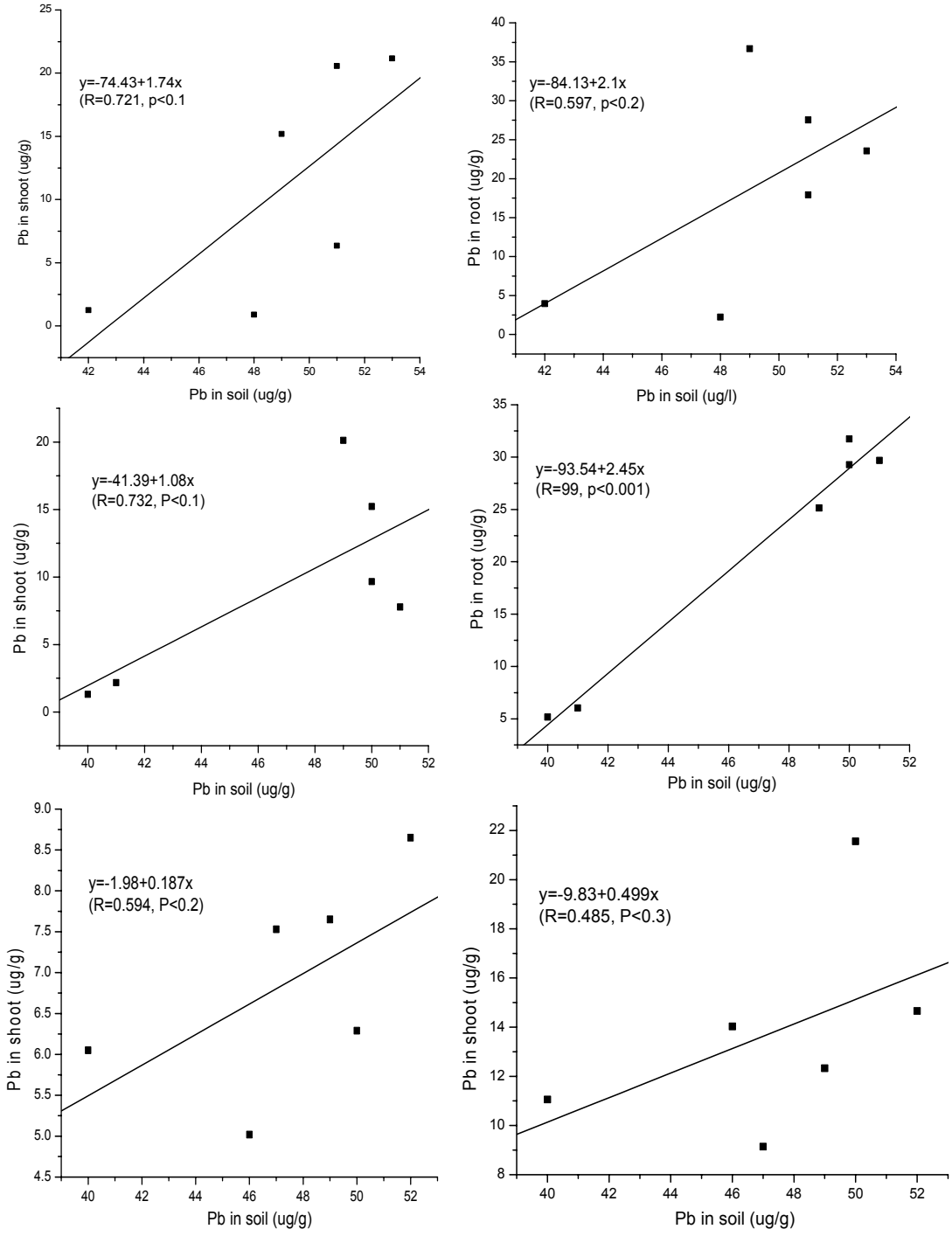


Figure 4.4-5. The relationship between concentrations of BC in the surface soil and in the shoots and roots of PV (top), K-31 (middle) and BC (bottom) with low loading

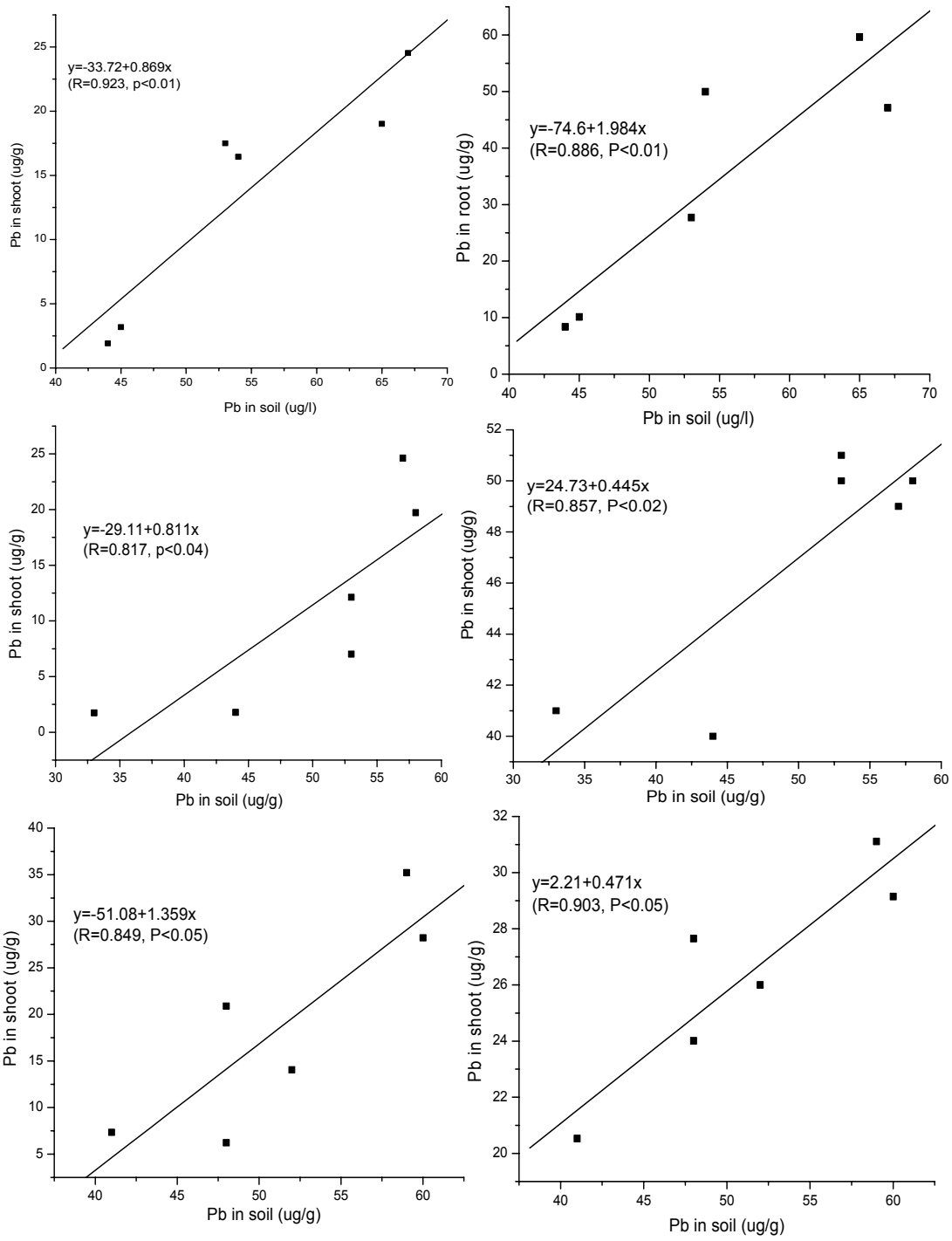


Figure 4.4-6. The relationship between concentrations of BC in the surface soil and in shoots and roots of PV (top), K-31 (middle) and BC (bottom) with high loading

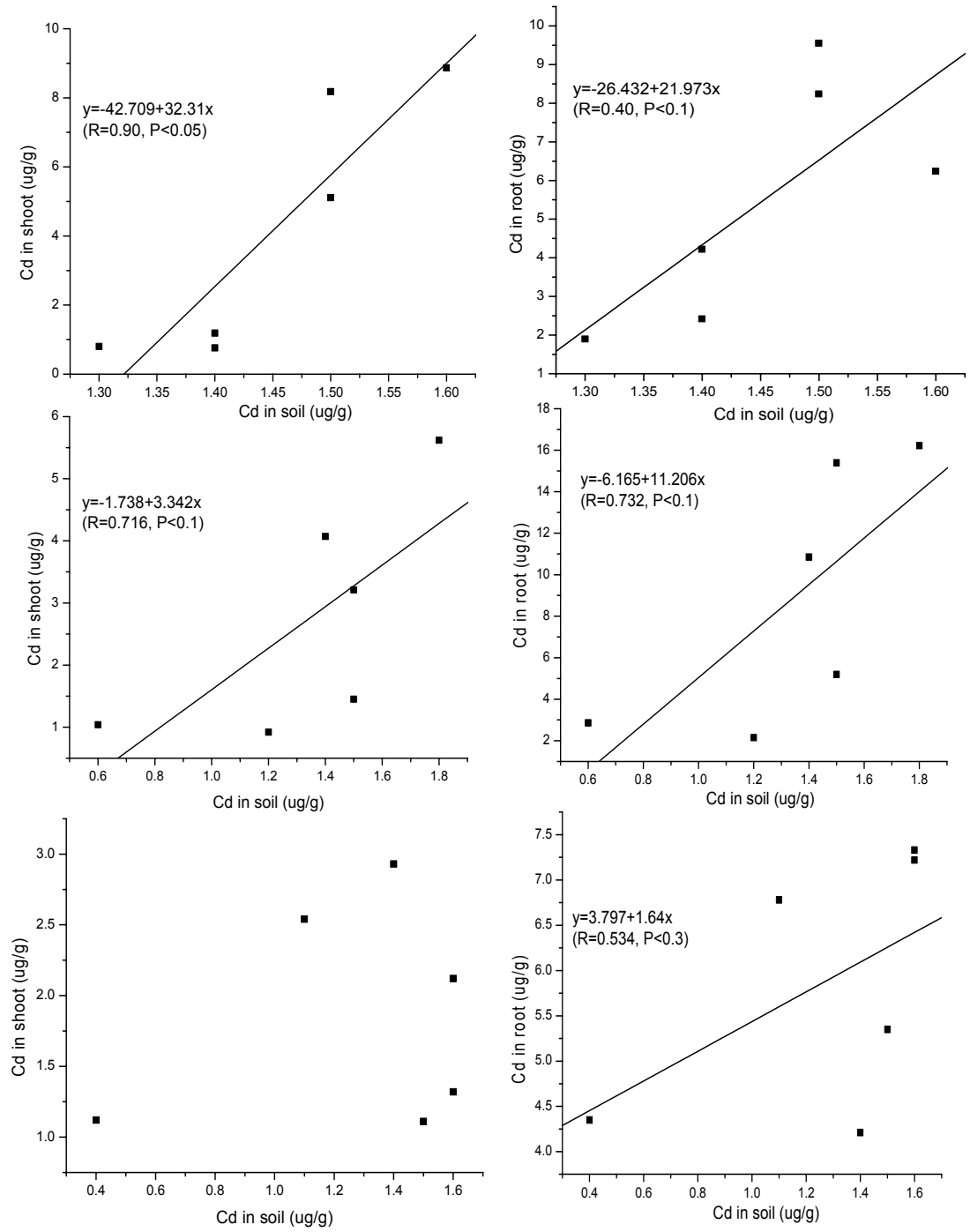


Figure 4.4-7. The relationship between concentrations of Cd in the surface soil and in the shoots and roots of PV (top), K-31 (middle) and BC (bottom) with low loading

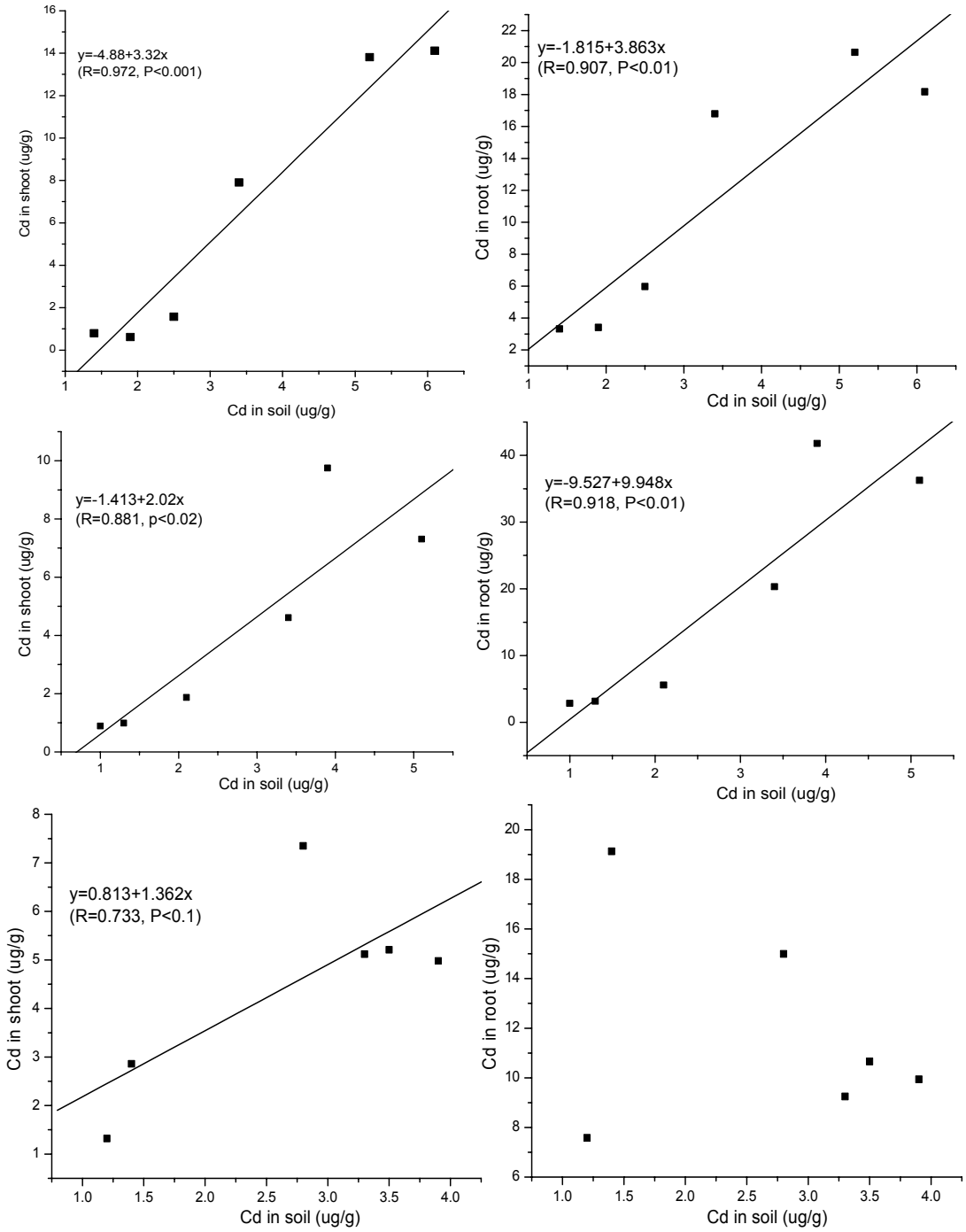


Figure 4.4-8. The relationship between concentrations of Cd in the surface soil and in the shoots and roots of PV (top), K-31 (middle) and BC (bottom) with high loading

4.5 Mass Balance of Metals

Based on the results of Sections 4.1 through 4.4, the final mass distributions of the input metals in different pots are shown in Figures 4.5-1 to 4.5-6. The percents of input metals uptaken by PV and K-31 are higher than those absorbed by BC, which is due to these grasses taking up high metal levels and also developing high biomass of shoots and roots. Therefore PV and K-31 will be better plants to remove metals from bioretention cells than BC.

Compared to the metals sorbed by the soil, the percentages of input metals uptaken by plants are relatively low and it seems that there are no advantages to use plants to remove metals accumulated in the soil and extend the lifetime metal burdens of bioretention facilities. As previously discussed, the concentrations of metals in these three plants are high, especially in roots, so the lower accumulations of total metals are mainly due to the lower biomass. From literature reports, PV, K-31 and BC can grow very well and yields of those plants are high under natural conditions. But in the present study, relatively low biomass was attained. The field aboveground yields of switch grass in Oklahoma have averaged about 1.8 kg/m^2 (DW) (18 t/ha) for the best cultivars (Fuentes et al., 2004), those of K-31 were 1.9 kg/m^2 (8.37 tons/acre) in the non- grazing stress areas at Lexington, Kentucky (Henning et al. 1999). However in this study, relatively low biomass was attained (0.2 kg/m^2 for PV and 0.22 kg/m^2 for K-31). The differences of biomass between lab and field may be due to physical parameters like insufficient nutrients and temperature.

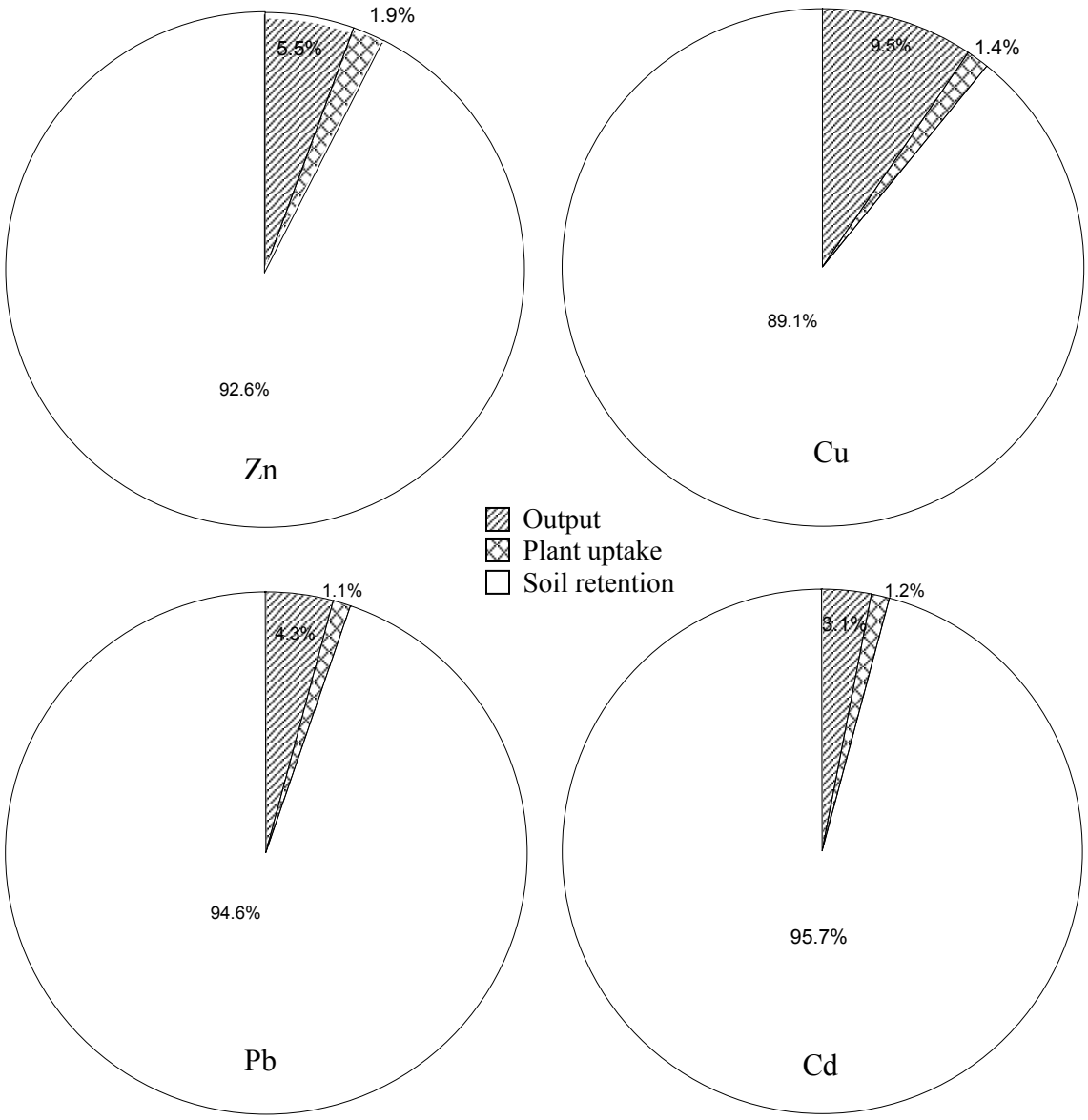


Figure 4.5-1. Distribution of metals input in pots with PV (low loading)

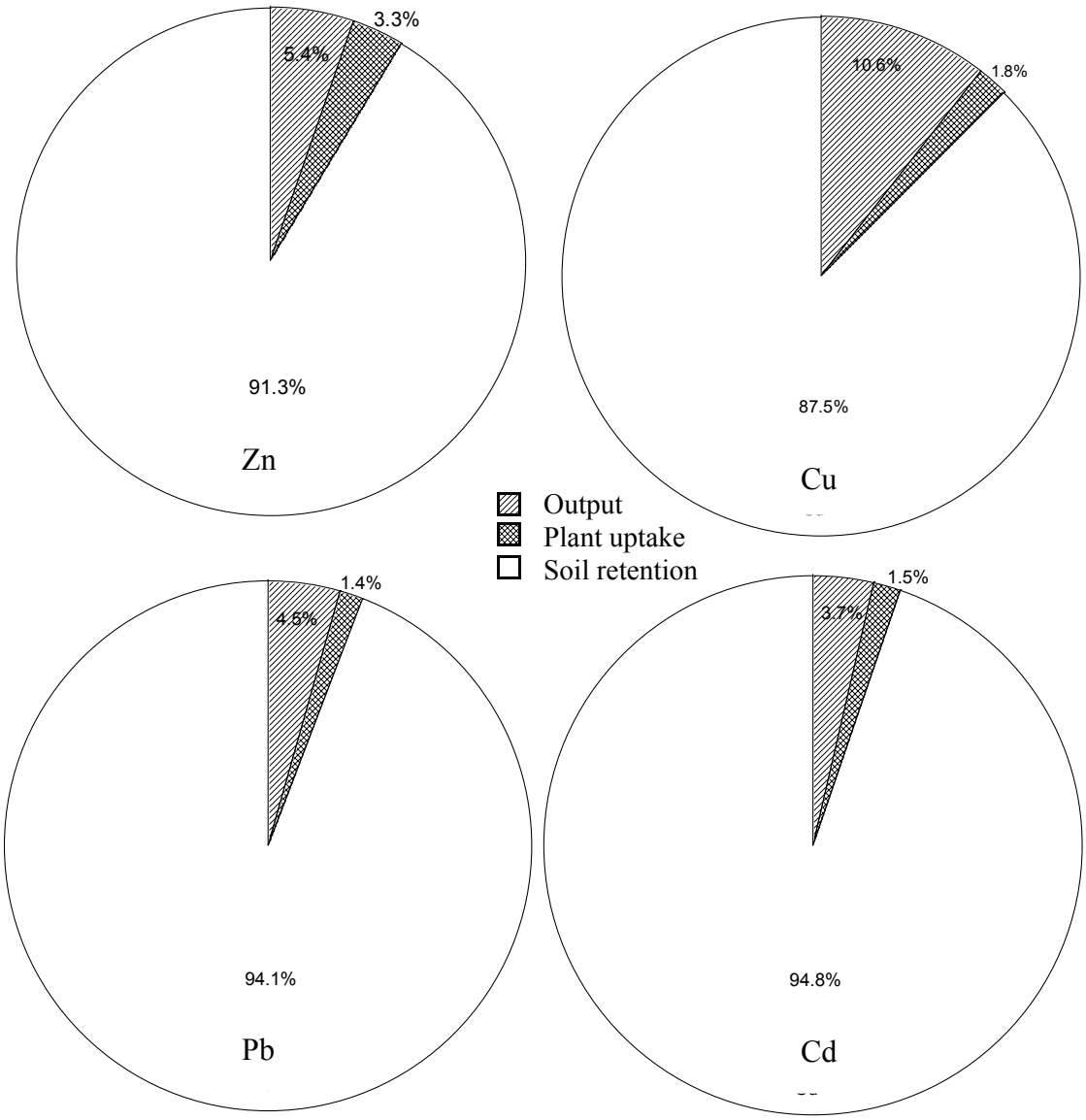


Figure 4.5-2. Distribution of metals input in pots with K-31 (low loading)

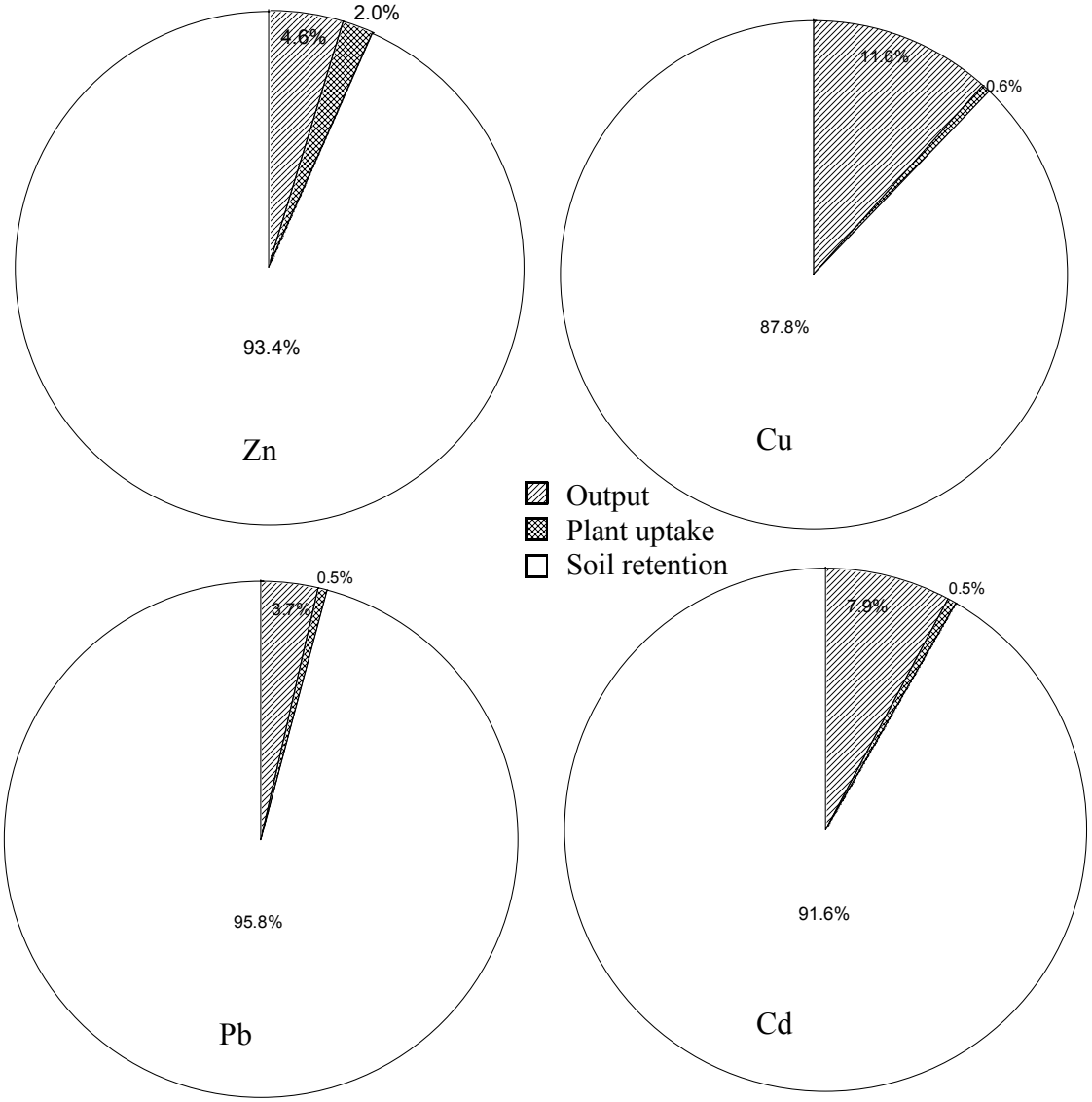


Figure 4.5-3. Distribution of metals input in pots with BC (low loading)

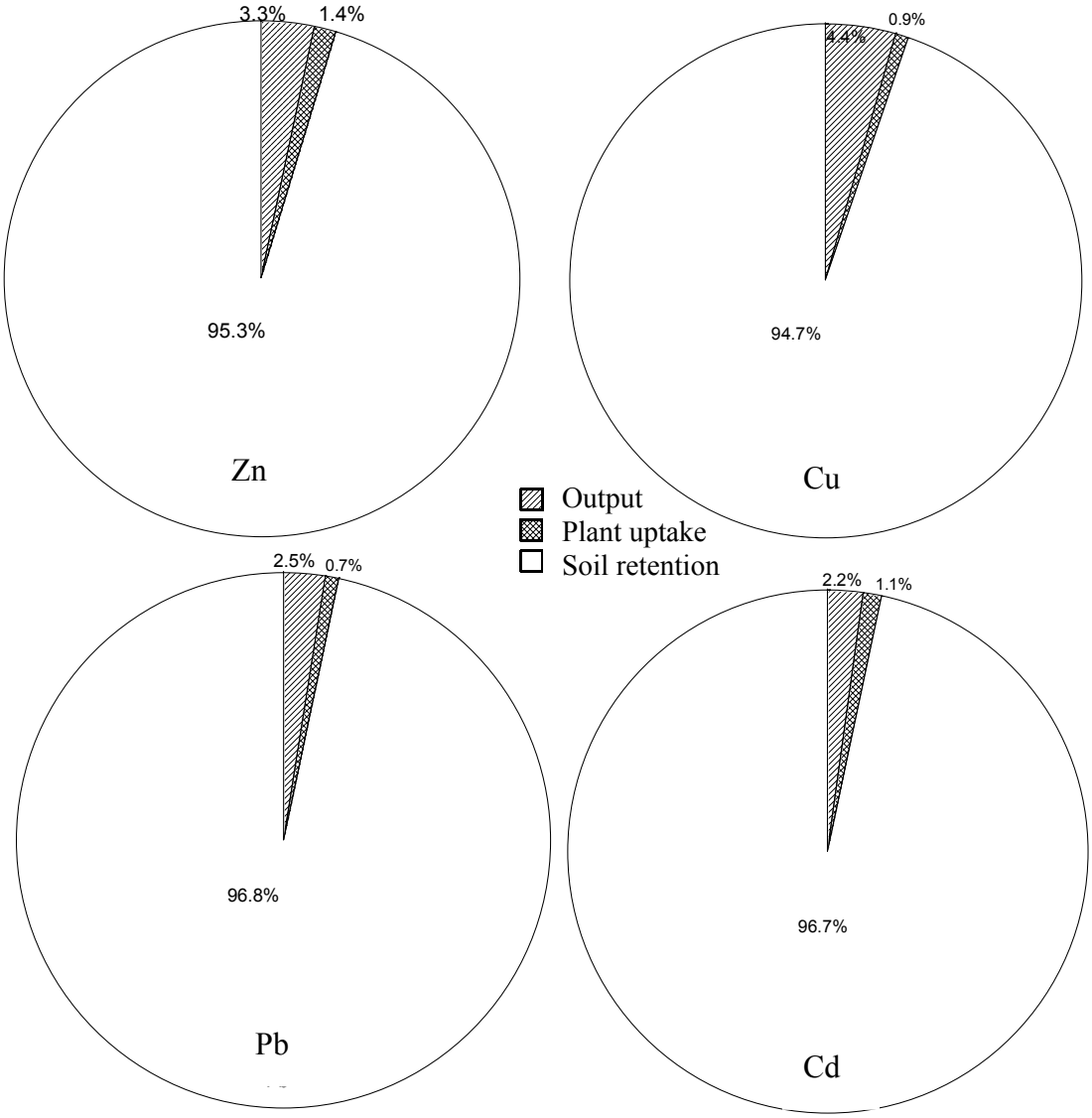


Figure 4.5-4. Distribution of metals input in pots with PV (High loading)

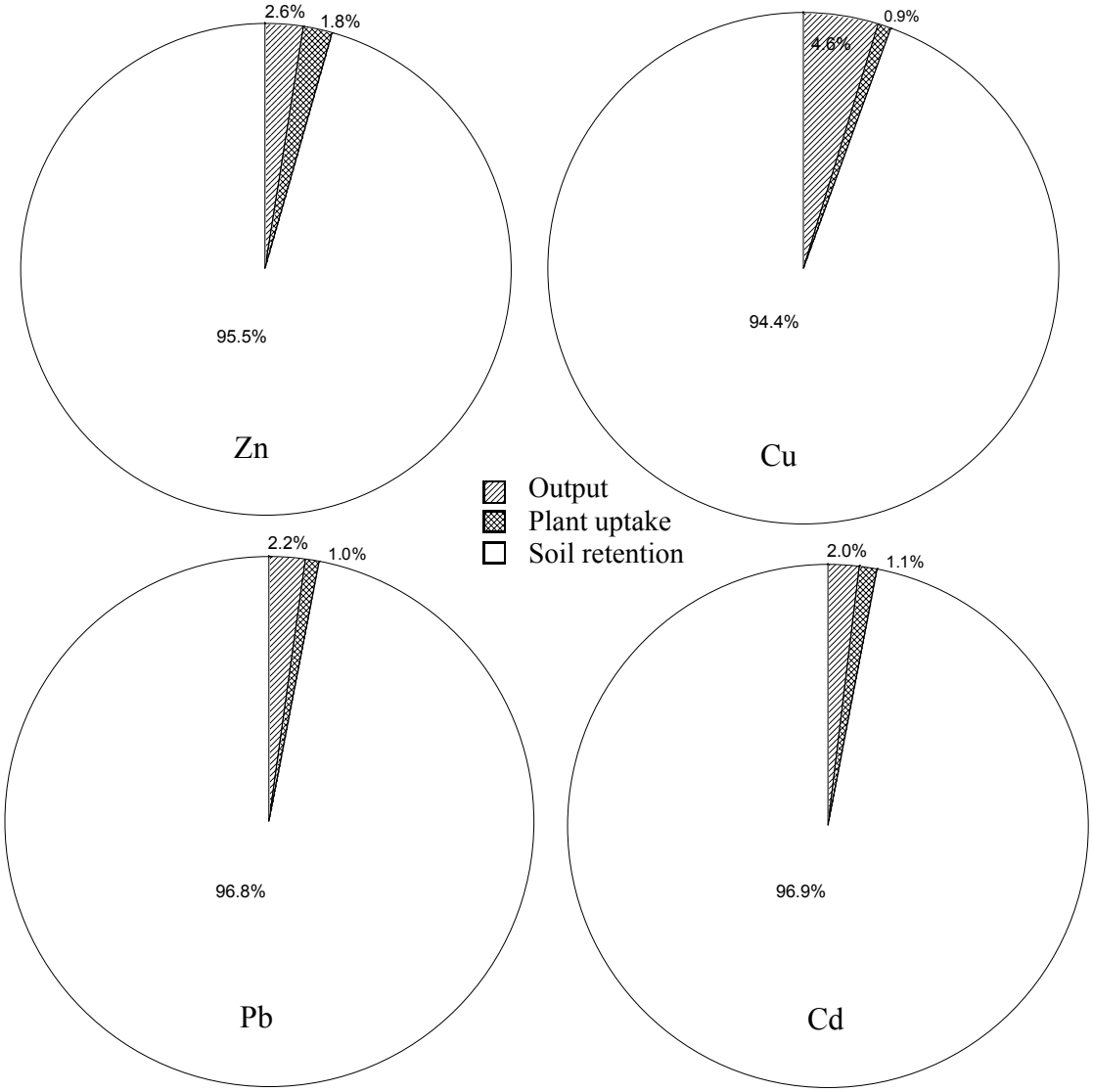


Figure 4.5-5. Distribution of metals input in pots with K-31 (High loading)

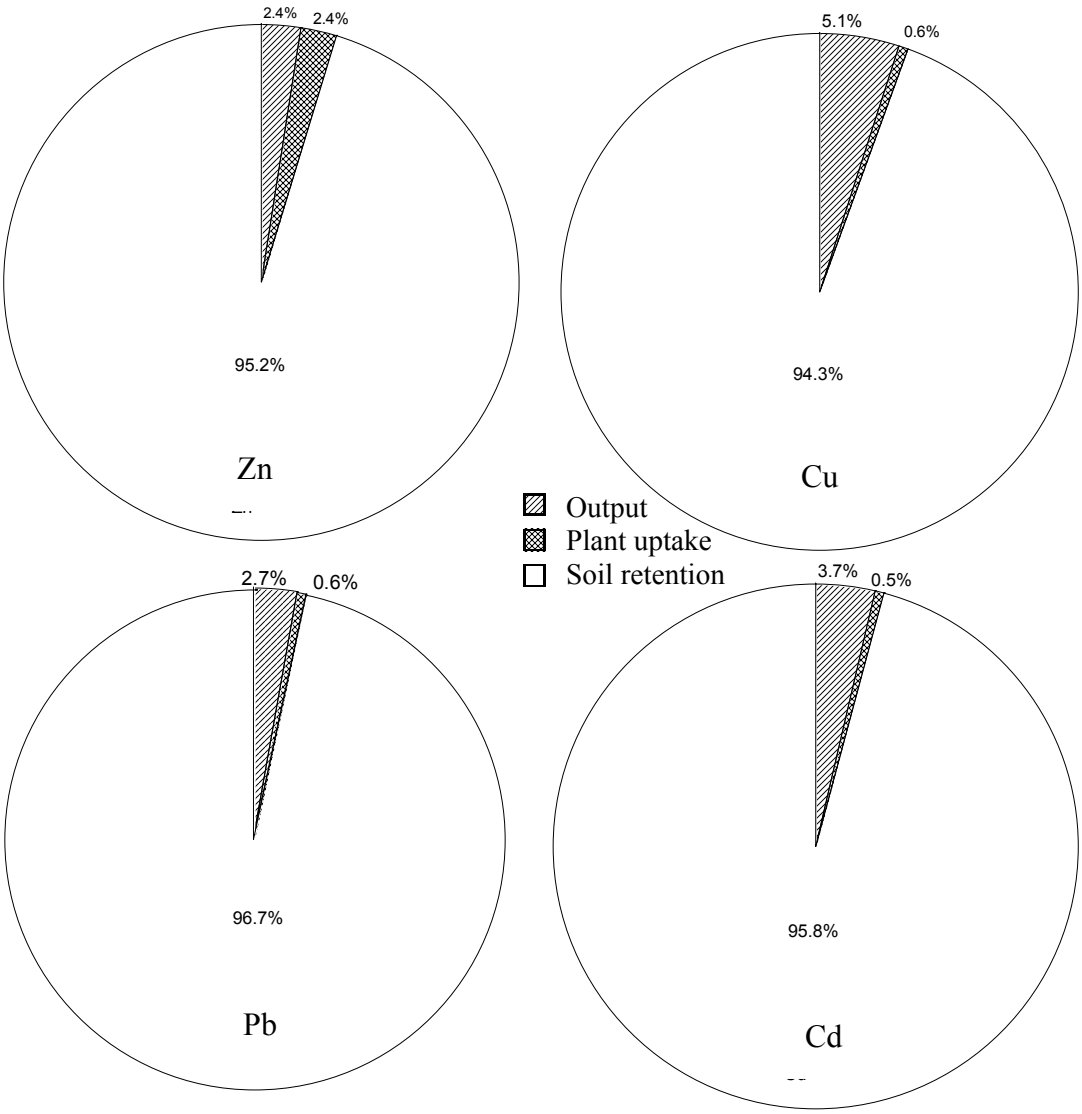


Figure 4.5-6. Distribution of metals input in pots with BC (High loading)

In order to estimate the removal of metals by plants in the field and evaluate the roles of PV, K-31 and BC correctly, a sample calculation is conducted. The assumptions deployed are:

1. The pollutant loading in the real bioretention cells is the same as used for the low loading used in present lab experiment (Table 4.5-1) which is similar the metals loading .
2. The removal efficiencies of the metals in the field are the same to those in the lab experiment. Experiments have shown metal removal efficiencies in the field and lab are similar (Davis *et al*., 2003).
3. The concentrations of metals in the plant tissues do not change if the experiments are conducted in the field instead of lab.
4. The ratios of underground and aboveground biomass of plants in the field are the same as those found in the lab.

Based on these assumptions, the biomass of different plants required to remove 10%, 20% and 30% of metals are estimated and shown in Table 4.5-2. Comparing these calculated biomass requirements to the yields of these grasses discussed above, it appears possible and practical to achieve removals of Zn, Cu, Pb and Cd of 20% by PV, 15% by K-31 and 10% by BC, respectively if the plants are harvested annually. Therefore PV is the best among these three grasses to be used to remove metals through periodic cutting and removal of plant tissues in bioretention facilities. The lifetime of bioretention facilities can be extended more than 20 years compared to those in which no metals are removed by plants, based on the estimation and if the lifetime of bioretention facilities are 100 years. Therefore vegetation management

represents a possible technology to remove metals in order to prolong the lifetime of bioretention facilities.

Table 4.5-1. Metals required to be uptaken by plants according the assumed removal efficiencies (g/m^2).

Metals	Input	Output	Soil retention	Assumed removal efficiencies		
				10%	20%	30%
Zn	3200	180	3100	320	640	930
Cu	350	33	320	35	66	100
Pb	340	14	320	34	68	100
Cd	110	3	100	11	22	33

Table 4.5-2. Biomass required to remove 10%, 20% and 30% metals from bioretention facilities respectively (g/m^2 DW).

Removal		10%			20%			30%		
Plants		Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
PV	Zn	563	326	888	1125	652	1777	1688	977	2665
	Cu	789	457	1246	914	1579	2493	2367	1371	3738
	Pb	925	536	1461	1071	1850	2921	2778	1608	4386
	Cd	829	480	1309	960	1658	2618	2491	1443	3934
K-31	Zn	495	134	628	989	267	1257	2931	5063	7994
	Cu	1287	348	1635	2574	696	3270	4112	7102	11215
	Pb	1203	325	1528	2405	650	3055	4825	833	5658
	Cd	1369	370	1739	2738	740	3479	4328	7474	11802
BC	Zn	556	200	756	1112	399	1511	8794	15188	23981
	Cu	3023	1085	4109	6046	2171	8217	12337	21307	33644
	Pb	1714	616	2330	3429	1231	4660	14476	2499	16975
	Cd	2661	956	3617	5322	1911	7233	12983	22423	35405

4.6 Crop Disposal

Phytoextraction is a promising and cost-effective method for remediating soils contaminated with toxic metals (Flathman et al., 1998). It includes two processes: the accumulation of metals in shoot tissue and harvesting of shoot biomass (Blaylock and Huang, 2000). Metal accumulation in plant shoots brings along the risk of wildlife ingestion, and any increase in metal accumulation via biotechnology will lead to a proportional increase of this risk (Elizabeth, 2002). Therefore crop disposal after phytoextraction is important. Some limited research has addressed these problems and suggested a few methods to treat the crops. If a site can be cleaned in a shorter time, the duration of exposure may be reduced. The risk of metal ingestion by wildlife may be minimized by fencing off the area, using deterrents such as periodic noise, and the use of less palatable plant species (Elizabeth, 2002). After harvesting the root and/or shoot biomass, the plant material may be ashed, followed by recycling of the metals if economically feasible (Chaney et al., 1997), or disposal of the ash in a landfill. Alternatively, the plant material may be used for non-food purposes, for example, cardboard or wood products (Elizabeth, 2002). Kumar et al. (1995) suggested that the dried, ashed, or composted plant residues highly enriched in heavy metals may be isolated as hazardous waste or recycled as metal ore.

The crop volumes should be reduced and excess water should be removed to lower the cost of transportation to the treatment or disposal site (Sas-Nowosielska et al., 2004). Composting, compaction or pyrolysis were used to reduce the volume of contaminated plant biomass as shown in Figure 4.6-1. After the volumes of crops were reduced, some treatment methods could be used for disposal.

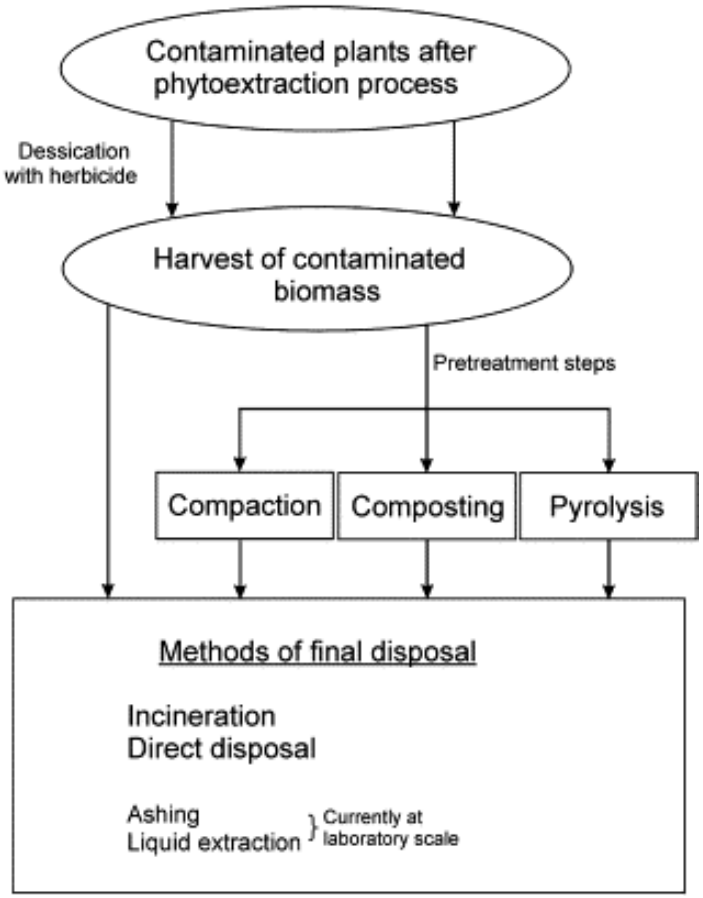


Figure 4.6-1. Comparison of pretreatment methods for crops volume reduction (Sas-Nowosielska et al., 2004).

Phytotoxic levels of metals are important factors for risk assessment of crops and the disposal method elected. Table 4.6-1 shows the maximum levels tolerated by livestock (Madejón, 2002). The maximum metal contents in the shoots of PV, K-31 and BC are shown in Table 4.6-2. Comparing the concentrations in the shoots to the recommended toxic levels for livestock, the results show that maximum contents of Zn, Cu and Pb do not exceed the toxic levels recommended for livestock forage, except those for sheep, for which the levels are relatively strict. However the maximum contents of

Cd are far above the maximum levels tolerated by livestock (0.5 mg/kg). K-31 and BC should not be fed to the livestock because of the relative high concentrations of Cd.

Although the concentrations in roots of the crops are high, they are not the food of livestock. Therefore there are no risks to livestock.

For wild animals, to date there are no regulation and toxic levels existing; therefore it is difficult to perform the risk assessment. But based on the previous discussion for livestock, the concentration of Cd in the shoots may be a problem as well. Further investigation is required to evaluate this.

Table 4.6-1. Normal ranges in plants, phytotoxic concentrations and toxic levels for livestock of several trace elements; levels in parentheses were estimated (by NRC) by extrapolating between animal species (Madejón, 2002).

Element	Normal levels (mg/kg dry foliage)	Phytotoxic levels (mg/kg dry foliage)	Maximum levels tolerated by livestock (mg/kg dry diet)			
			Cattle	Sheep	Swine	Chicken
Cd	0.1-1	5-700	0.5	0.5	0.5	0.5
Cu	3-20	25-40	100	25	250	300
Fe^{+2}	30-300	-	1000	500	3000	1000
Mn	15-150	400-2000	1000	1000	400	2000
Ni	0.1-5	50-100	50	50	100	300
Pb	2-5	-	30	30	30	30
Zn	15-150	500-1500	500	300	1000	1000

Table 4.6-2. Maximum metal contents in the shoots of different plants

Metals (µg/g)		Zn	Cu	Pb	Cd
PV	Low loading	255±14	24±3.5	21±3	8.9±1
	High loading	542±24	30.7±1.3	25±1.1	14±3
K-31	Low loading	308±5	22±3.7	20±2.8	5.6±1.1
	High loading	373±10	28±3.5	25±2.3	9.8±2.4
BC	Low loading	193±11	13±4	8.7±2.3	2.9±1.1
	High loading	342±21	23±3.3	35±2.2	7.4±3

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

Bioretention is a best management practice (BMP) which utilizes soils and both woody and herbaceous plants to remove pollutants from storm water runoff and enhance the quality of downstream water bodies through physical, chemical and biological processes. In this study, three types of grasses were investigated: *Panicum virgatum* (switch grass*), Kentucky-31* and *Bromus ciliatus.* These three species are erect, coarse, perennial grasses with high biomass and potential for phytoremediation.

More than 90% of input metals are removed within the top 25 cm of bioretention depth. The high contaminant loading has high removal efficiencies for the same metals. Therefore bioretention is a good way to treat storm runoff, which agrees with previous results.

The metal concentrations in the plant tissues showed large temporal and spatial variation, as summarized in Table 5.1. The concentrations of Zn, Cu, Pb and Cd in different grass species are different. The accumulation patterns of Zn, Cu, Pb and Cd in whole plants of PV, K-31 and BC increased in the order of Zn>Cu>Pb>Cd with both low and high loading, which matched the concentrations in the synthetic runoff. Higher contaminant loading can enhance metal uptake by the plant. The relationships between the concentrations of Zn, Cu, Pb and Cd in the shoots and roots were positive although there were a few exceptions.

Nearly all of the concentrations of four metals decreased with height in the order of (*Root) >(0-15cm) >(15-25 cm) > (>25 cm).* The exception was Cd, in which concentration in the root of PV was less than that in the 0-15 cm shoot. For the same

height, generally, the concentrations of Zn, Cu, Pb and Cd in all three grasses with low loading were less than those with high loading.

Table 5.1 Summery ranges of concentration in different plants

Plants			Concentrations (μg/g)			
			Zn	Cu	Pb	Cd
PV	Low loading	Shoot	96 ~ 255	9.2 ~ 24	0.9 ~ 21	0.8 ~ 8.9
		Root	217 ~ 658	18 ~ 60	2.2 ~ 37	1.9 ~ 9.6
	High loading	Shoot	117 ~ 543	12.5 ~ 31	1.9 ~ 25	0.6 ~ 14
		Root	136 ~ 309	30 ~ 148	8.4 ~ 60	3.3 ~ 21
K-31	Low loading	Shoot	95 ~ 308	8.3 ~ 22	1.3 ~ 20	0.9 ~ 5.6
		Root	321 ~ 1176	21 ~ 78	5.2 ~ 32	2.2 ~ 16
	High loading	Shoot	82 ~ 373	8.4 ~ 28	1.8 ~ 25	0.9 ~ 10
		Root	290 ~ 1564	27 ~ 83	8.3 ~ 83	2.9 ~ 42
BC	Low loading	Shoot	105 ~ 193	6.2 ~ 13	5 ~ 8.7	1.1 ~ 2.9
		Root	472 ~ 1043	16 ~ 31	9.1 ~ 22	4.2 ~ 7.3
	High loading	Shoot	128 ~ 342	11 ~ 23	6.2 ~ 35	1.3 ~ 7.4
		Root	760 ~ 2758	30 ~ 73	21 ~ 31	7.6 ~ 19

Based on the values of BCF (C_{shoot}/C_{root}), the PV has lowest uptake capacities for Zn and Cd under both low and high loading conditions, and BC has lowest absorption capacities for Cu and Pb, and has highest capacities for Zn and Cd with both loadings. The transfer ratio (TR) of the plants showed time-dependent variation as did the BCF. TR values for different metals in these three plants were less than 1 and most of them were less than 0.5, which demonstrated that the metals were retained by the roots and translocation of those four metals from roots to shoot is rather slow.

Vertical distributions of metals in the soil profile showed relatively high metal concentrations within the top 5 cm and then a sharp decline with depth. Most input metals are captured by the top layer. Regression analysis shows the relationship between Zn, Cu, Pb and Cd in soil and tissues of plants are linear and positive, except for Cu and Cd in soil and shoots of BC with low loading, and Cu in soil and shoots of PV with high loading in which there is no any relationship between concentration of Cu in soil and shoots.

The results of mass balance calculations show the fates of input metals are 87.5-96.9% captured in soil media, 0.5-3.3% accumulated in plants and 2.0-11.6% not captured by bioretention media. Compared to the metals sorbed by the soil, the percentages of input metals uptaken by plants are relatively low due to low biomass yields, which are 0.2 kg/m^2 for PV, 0.22 kg/m^2 for K-31, and 0.16 kg/m^2 for BC, respectively. However, some literature reported that the above ground biomass yields of PV and K-32 in fields were high (1.8 kg/m^2 for PV and 1.9 kg/m^2 BC). Based on the field yields and metal concentrations in the tissues of the plants exposed to the low loading, the assumed accumulations of metals by the plants were estimated. It appeared possible and practical to achieve removals of Zn, Cu, Pb and Cd of 20% by PV, 15% by K-31 and 10% by BC, respectively. If 20% input metals are removed by plants, the life time of bioretention facilities will be extended one-fourth comparing to those that no any metals are untaken by plants. Therefore if the life time of bioretention cell is 20 years, the life time will be extended 5 years. PV, K-31 and BC can possibly be utilized as high accumulators to remove the metals in bioretention and extend the lifetime of bioretention facilities. PV is the best one based on the calculation results.

The goal of this research was to verify if the plants can uptake metals from bioretention media so as to prolong the life time of the bioretention facilities. The results showed that the concentrations in the tissues of PV, K-31 and BC are higher. Biomass assumptions and calculations demonstrated that it may be possible and practical to achieve removals of the metals. Therefore, further research should investigate the metal uptake process in real bioretention cells, comparing results to the laboratory results, to evaluate the role of plants.

APPENDIX

Note:

(1) The A, B, C, D, E, G and F are pots with different plants with low loading. The A and D, B and E, C and G are the pots with same plants, respectively, and F is the control pot.

(2) The H, I, J, K, L, M and N are pots with different plants with high loading. The H and K, I and L, J and G are are the pots with a same plants, respectively and N is the control pot.

(3) After time 4, the A and D, B and E, C and G come from same pots, respectively.

(4) For appendix 1.1 to 1.4, the 100ml samples are digested and diluted to 50 ml solution. In input column, upper values in one raw are for A, B, D, E, F, H, I, K, L, and N, the bottom values are for C, G, J and M.

Appendix 1.1-A. Zn concentrations in the effluent of pots (low loading)

Runoff	Input	A	B	C	D	E	G	F1	F2
1		0.119	0.126	0.091	0.188	0.098	0.086	0.091	0.099
	(0.616±0.023 mg/l)	0.073	0.085	0.098	0.069	0.081	0.057	0.048	0.052
	(0.576±0.063 mg/l)	0.066	0.055	0.125	0.047	0.079	0.178	0.063	0.068
		0.044	0.042	0.064	0.090	0.057	0.062	0.061	0.066
2		0.060	0.047	0.096	0.033	0.043	0.061	0.069	0.075
	(0.414±0.005 mg/l)	0.044	0.041	0.069	0.023	0.059	0.104	0.104	0.113
	(0.593±0.016 mg/l)	0.065	0.075	0.049	0.052	0.068	0.022	0.060	0.065
		0.058	0.072	0.080	0.062	0.060	0.075	0.068	0.074
3		0.046	0.085	0.029	0.039	0.040	0.037	0.038	0.041
	(0.551±0.02mg/l)	0.044	0.071	0.076	0.047	0.077	0.039	0.045	0.049
	(0.512±0.05 mg/l)	0.041	0.041	0.120	0.075	0.057	0.040	0.021	0.023
		0.033	0.057	0.052	0.053	0.036	0.289	0.052	0.057
4		0.041	0.067	0.035	0.051	0.041	0.038	0.018	0.020
	(0.661±0.08 mg/l)	0.024	0.066	0.048	0.026	0.031	0.032	0.043	0.047
	(0.541±0.064 mg/l)	0.028	0.014	0.081	0.047	0.019	0.051	0.020	0.022
		0.028	0.021	0.043	0.024	0.023	0.177	0.008	0.008
5		0.039	0.050	0.132	0.060	0.042	0.090	0.020	0.022
	(0.657±0.011 mg/l)	0.057	0.037	0.143	0.042	0.044	0.088	0.024	0.026
	(0.858±0.029 mg/l)	0.091	0.034	0.161	0.024	0.013	0.082	0.041	0.045
		0.020	0.032	0.082	0.050	0.014	0.021	0.060	0.065
		0.048	0.048	0.132	0.324	0.057	0.089	0.037	0.041
6	(0.751±0.089 mg/l)	0.161	0.025	0.125	0.086	0.016	0.110	0.127	0.138
	(0.719±0.056 mg/l)	0.059	0.122	0.187	0.055	0.066	0.099	0.078	0.085
		0.028	0.065	0.111	0.024	0.124	0.097	0.035	0.038
7		0.074	0.105	0.079	0.063	0.068	0.073	0.066	0.072
	(0.576±0.063 mg/l)	0.078	0.024	0.027	0.059	0.061	0.021	0.021	0.023
	(0.711±0.006 mg/l)	0.042	0.060	0.049	0.091	0.046	0.060	0.024	0.026
8									
	(0.593±0.016 mg/l)	0.055	0.068	0.057	0.031	0.029	0.074	0.036	0.039
	(0.773±0.171 mg/l)	0.045	0.041	0.067	0.055	0.032	0.063	0.028	0.030
9		0.135	0.236	0.088	0.022	0.021	0.097	0.043	0.047
	(0.512±0.05 mg/l)	0.246	0.035	0.056	0.031	0.321	0.056	0.057	0.062
	(0.755±0.126mg/l)	0.261	0.037	0.051	0.039	0.020	0.088	0.035	0.038
10		0.098	0.118	0.087	0.026	0.024	0.068	0.047	0.051
	(0.541±0.064 mg/l)	0.257	0.045	0.090	0.032	0.129	0.051	0.059	0.064
	(0.721±0.017 mg/l)	0.081	0.041	0.061	0.044	0.025	0.077	0.040	0.044
11		0.191	0.204	0.108	0.133	0.119	0.051	0.113	0.123
	(0.858±0.029 mg/l)	0.187	0.178	0.097	0.189	0.213	0.123	0.118	0.129
	(0.64±0.09 mg/l)	0.107	0.166	0.090	0.163	0.112	0.075	0.087	0.095
12		0.115	0.189	0.089	0.123	0.090	0.097	0.150	0.163
	(0.719±0.056 mg/l)	0.175	0.168	0.045	0.156	0.078	0.056	0.118	0.129
	(0.781±0.147 mg/l)	0.124	0.156	0.061	0.185	0.102	0.088	0.231	0.252
13		0.083	0.093	0.058	0.097	0.029	0.073	0.035	0.038
	(0.711±0.006 mg/l)	0.024	0.113	0.049	0.105	0.074	0.021	0.088	0.096
	(0.641±0.113 mg/l)	0.020	0.125	0.061	0.058	0.117	0.060	0.094	0.102
14		0.055	0.050	0.058	0.099	0.060	0.047	0.093	0.102
	(0.773±0.171 mg/l)	0.060	0.067	0.049	0.071	0.066	0.032	0.060	0.066
	(0.715±0.054 mg/l)	0.073	0.057	0.061	0.081	0.080	0.039	0.070	0.077
15		0.065	0.048	0.034	0.035	0.056	0.037	0.098	0.107
	(0.755±0.126mg/l)	0.058	0.062	0.059	0.047	0.084	0.064	0.084	0.092
	(0.749±0.13mg/l)	0.068	0.071	0.103	0.067	0.047	0.112	0.079	0.086
16		0.057	0.054	0.084	0.056	0.065	0.092	0.107	0.116
	(0.721±0.017 mg/l)	0.069	0.063	0.061	0.087	0.087	0.067	0.084	0.091
	(0.712±0.12 mg/l)	0.071	0.074	0.049	0.063	0.085	0.054	0.076	0.083
17		0.098	0.116	0.065	0.100	0.085	0.071	0.058	0.063
	(0.64±0.09 mg/l)	0.117	0.141	0.027	0.089	0.080	0.029	0.084	0.092
	(0.63±0.03 mg/l)	0.093	0.141	0.046	0.098	0.083	0.050	0.091	0.099
18		0.054	0.051	0.046	0.058	0.056	0.050	0.108	0.117
	(0.781±0.147 mg/l)	0.067	0.055	0.074	0.047	0.084	0.081	0.084	0.092
	(0.779±0.159 mg/l)	0.064	0.071	0.068	0.078	0.047	0.075	0.071	0.077
19		0.047	0.070	0.068	0.097	0.029	0.075	0.040	0.044
	(0.641±0.113 mg/l)	0.032	0.042	0.051	0.105	0.074	0.056	0.080	0.087
	(0.651±0.113 mg/l)	0.021	0.038	0.078	0.058	0.117	0.085	0.062	0.068
20		0.047	0.070	0.042	0.045	0.032	0.046	0.023	0.025
	(0.715±0.054 mg/l)	0.032	0.042	0.051	0.065	0.041	0.056	0.066	0.072
	(0.7075±0.06mg/l)	0.021	0.038	0.032	0.047	0.062	0.035	0.033	0.036

Appendix 1.1-B. Zn concentrations in the effluent of pots (high loading)

Runoff	Input	H	I	J	K	L	M	N1	N2
1	(1.454±0.024 mg/l)	0.077	0.078	0.119	0.115	0.083	0.202	0.089	0.097
	(1.571±0.281 mg/l)	0.086	0.063	0.128	0.078	0.068	0.216	0.029	0.031
		0.087	0.074	0.081	0.057	0.072	0.156	0.063	0.068
		0.094	0.081	0.305	0.085	0.072	0.259	0.086	0.093
2	(1.454±0.024 mg/l)	0.082	0.262	0.024	0.085	0.101	0.097	0.038	0.042
	(1.30±0.154 mg/l)	0.061	0.029	0.030	0.060	0.039	0.065	0.040	0.044
		0.043	0.049	0.104	0.037	0.048	0.114	0.041	0.045
		0.056	0.035	0.087	0.072	0.239	0.079	0.052	0.056
3	(1.278±0.023 mg/l)	0.054	0.020	0.075	0.162	0.030	0.128	0.034	0.037
	(1.465±0.036 mg/l)	0.004	0.015	0.077	0.020	0.024	0.025	0.011	0.011
		0.024	0.030	0.020	0.026	0.006	0.022	0.069	0.075
		0.008	0.013	0.023	0.022	0.020	0.019	0.030	0.033
4	(1.329±0.064 mg/l)	0.054	0.061	0.081	0.107	0.069	0.097	0.036	0.038
	(1.398±0.086 mg/l)	0.064	0.086	0.056	0.095	0.035	0.045	0.057	0.061
		0.067	0.059	0.031	0.078	0.065	0.037	0.072	0.078
		0.060	0.069	0.027	0.061	0.077	0.051	0.075	0.081
5	(1.392±0.043 mg/l)	0.047	0.075	0.126	0.059	0.021	0.150	0.065	0.071
	(1.451±0.043 mg/l)	0.037	0.098	0.112	0.050	0.055	0.156	0.071	0.077
		0.048	0.056	0.126	0.049	0.028	0.137	0.068	0.074
		0.057	0.060	0.178	0.034	0.051	0.180	0.049	0.053
6	(1.798±0.012 mg/l)	0.101	0.105	0.136	0.076	0.068	0.165	0.079	0.085
	(1.383±0.027 mg/l)	0.099	0.069	0.121	0.402	0.105	0.167	0.121	0.131
		0.064	0.291	0.116	0.205	0.128	0.116	0.100	0.108
		0.071	0.043	0.189	0.073	0.063	0.123	0.084	0.090
7	(1.571±0.281 mg/l)	0.071	0.073	0.047	0.160	0.067	0.052	0.049	0.053
	(1.51±0.049mg/l)	0.050	0.231	0.073	0.298	0.080	0.028	0.064	0.070
		0.298	0.071	0.051	0.081	0.045	0.065	0.047	0.051
8	(1.30±0.154 mg/l)	0.077	0.031	0.105	0.034	0.021	0.138	0.021	0.023
	(1.417±0.106mg/l)	0.040	0.033	0.096	0.035	0.012	0.137	0.066	0.072
		0.068	0.071	0.085	0.070	0.092	0.107	0.084	0.090
9	(1.465±0.036 mg/l)	0.076	0.070	0.121	0.149	0.132	0.106	0.073	0.079
	(1.516±0.112mg/l)	0.050	0.036	0.132	0.053	0.042	0.093	0.200	0.216
		0.036	0.115	0.109	0.033	0.039	0.089	0.035	0.037
10	(1.398±0.086 mg/l)	0.077	0.074	0.114	0.114	0.123	0.112	0.068	0.074
	(1.439±0.192mg/l)	0.054	0.041	0.067	0.069	0.112	0.101	0.124	0.134
		0.045	0.089	0.098	0.056	0.089	0.087	0.054	0.058
11	(1.451±0.043 mg/l)	0.156	0.161	0.045	0.136	0.159	0.061	0.158	0.172
	(1.447±0.105mg/l)	0.129	0.103	0.059	0.109	0.143	0.086	0.164	0.178
		0.182	0.147	0.037	0.130	0.141	0.051	0.142	0.154
12	(1.383±0.027 mg/l)	0.186	0.156	0.114	0.132	0.156	0.106	0.190	0.206
	(1.427±0.116mg/l)	0.132	0.112	0.135	0.106	0.158	0.093	0.150	0.162
		0.195	0.135	0.128	1.470	0.114	0.089	0.120	0.130
13	(1.51±0.049mg/l)	0.039	0.038	0.049	0.055	0.042	0.052	0.060	0.066
	(1.374±0.057mg/l)	0.043	0.052	0.077	0.048	0.031	0.028	0.048	0.052
		0.037	0.049	0.061	0.055	0.055	0.065	0.037	0.041
14	(1.417±0.106mg/l)	0.133	0.126	0.049	0.103	0.145	0.053	0.110	0.119
	(1.387±0.064mg/l)	0.103	0.125	0.077	0.078	0.112	0.034	0.101	0.109
		0.086	0.097	0.061	0.125	0.095	0.058	0.089	0.097
15	(1.516±0.112mg/l)	0.112	0.112	0.046	0.098	0.116	0.050	0.109	0.119
	(1.52±0.22mg/l)	0.134	0.156	0.097	0.077	0.098	0.106	0.102	0.110
		0.100	0.145	0.139	0.082	0.066	0.152	0.136	0.148
16	(1.439±0.192mg/l)	0.174	0.124	0.078	0.105	0.108	0.085	0.116	0.126
	(1.457±0.184mg/l)	0.132	0.155	0.103	0.099	0.097	0.112	0.094	0.102
		0.142	0.078	0.099	0.088	0.058	0.108	0.131	0.141
17	(1.447±0.105mg/l)	0.068	0.053	0.120	0.069	0.055	0.131	0.041	0.045
	(1.438±0.115mg/l)	0.010	0.055	0.074	0.014	0.042	0.081	0.066	0.072
		0.064	0.036	0.084	0.047	0.038	0.092	0.110	0.120
18	(1.427±0.116mg/l)	0.141	0.142	0.110	0.098	0.116	0.121	0.136	0.148
	(1.411±0.117mg/l)	0.125	0.156	0.087	0.112	0.098	0.096	0.133	0.145
		0.107	0.142	0.089	0.082	0.066	0.098	0.100	0.108
19	(1.374±0.057mg/l)	0.071	0.041	0.110	0.055	0.042	0.121	0.059	0.063
	(1.38±0.057mg/l)	0.064	0.063	0.080	0.048	0.031	0.087	0.050	0.054
		0.042	0.055	0.125	0.055	0.055	0.137	0.045	0.049
20	(1.387±0.064mg/l)	0.074	0.041	0.065	0.055	0.052	0.071	0.068	0.074
	(1.381±0.076mg/l)	0.065	0.063	0.097	0.048	0.061	0.106	0.066	0.072
		0.059	0.055	0.061	0.055	0.048	0.058	0.072	0.078

Appendix 1.2-A. Cu concentrations in the effluent of pots (low loading)

runoff	Input	A	B	C	D	E	G	F1	F2
1		30.6	60.8	23.9	31.2	39.9	29.9	42.2	45.9
	(75.44±4.58 □g/l)	31.5	26.4	39.8	30.3	24.2	49.9	42.3	46.1
	(78.54±4.58 □g/l)	26.9	18.0	40.3	29.4	21.8	50.6	39.5	43.0
		27.9	24.0	45.3	29.7	32.6	56.9	27.5	29.9
2		23.7	34.5	38.6	26.8	22.2	26.3	31.8	34.6
	(74.22±2.32 □g/l)	22.7	18.5	15.7	33.7	17.3	18.4	36.1	39.3
	(70.13±1.72□g/l)	21.6	16.2	12.9	20.3	20.6	8.0	29.2	31.7
		19.9	20.8	6.0	16.6	20.1	1.7	20.0	21.7
3		17.5	41.3	5.9	26.2	20.8	7.0	34.4	37.5
	(69.88±5.61 □g/l)	35.4	56.4	5.9	51.5	44.6	7.0	46.7	50.8
	(78.62±4.93□g/l)	22.1	42.2	3.4	25.8	35.0	4.1	16.2	17.6
		22.8	23.3		21.1	39.7		20.6	22.5
4		12.8	29.0	20.1	11.6	12.2	11.2	12.1	13.1
	(76.11±3.12 □g/l)	8.6	10.5	15.8	21.2	10.9	11.9	12.3	13.3
	(78.62±4.93□g/l)	8.4	8.3	11.3	37.0	14.3	13.3	8.7	9.4
		16.7	9.5	20.4	12.1	10.5	10.2	8.2	8.9
5		17.1	14.8	19.6	9.9	4.6	35.7	9.2	10.0
	75.64±5.12 □g/l)	13.2	18.3	30.5	18.3	9.4	38.2	12.0	13.0
	(69.26±12.82□g/l)	25.4	14.1	24.2	20.3	18.1	17.6	15.1	16.4
		11.1	12.6	28.3	10.0	11.5	29.1	13.7	14.9
6		16.2	13.9	20.2	15.6	26.9	13.3	19.9	21.6
	(69.52±9.22 □g/l)	11.0	11.3	20.1	10.9	9.7	12.5	9.1	10.0
	(78.15±6.32□g/l)	10.7	10.1	23.5	10.4	9.1	19.2	10.0	10.9
7		21.5	26.9	18.9	27.0	33.7	19.5	14.6	15.9
	(78.54±4.58 □g/l)	16.1	18.9	1.6	20.2	23.8	9.5	18.3	19.9
	(71.07 ±14.21□g/l)	20.9	32.3	13.5	26.2	40.5	19.0	12.5	13.6
8		9.9	11.8	15.2	12.4	11.3	21.6	6.8	7.4
	(70.13±1.72□g/l)	9.8	9.6	23.3	8.4	9.0	18.7	7.5	8.2
	(68.88±12.41□g/l)	6.7	10.6	11.9	7.2	8.4	16.4	12.6	13.7
9		18.6	11.5	4.6	13.6	22.0	8.1	11.8	12.8
	(78.62±4.93□g/l)	12.4	15.9	6.2	18.8	14.7	6.5	9.7	10.5
	(68.21±5.26□g/l)	12.9	12.9	3.3	15.3	15.2	4.1	8.4	9.2
10		21.1	12.3	17.2	18.9	12.4	26.3	23.6	25.7
	(78.62±4.93□g/l)	15.4	12.0	14.6	11.2	22.1	18.4	19.3	21.0
	(74.11±14.21□g/l)	12.4	13.3	11.0	15.3	14.5	8.0	16.9	18.4
11		31.3	33.1	15.3	14.8	24.1	13.8	39.2	42.7
	(69.26±12.82□g/l)	16.6	28.1	12.7	20.6	20.6	26.9	21.2	23.0
	(52.31±16.54□g/l)	19.1	27.2	15.1	23.7	23.7	14.2	28.6	31.2
12		19.2	12.2	12.1	16.3	22.1	20.1	30.0	32.6
	(78.15±6.32□g/l)	17.9	18.7	11.4	15.4	19.7	14.7	19.3	21.0
	(72.14±10.54mg/l)	16.3	22.3	10.5	14.2	25.2	10.4	23.6	25.7
13		18.3	18.1	9.9	15.6	2.2	26.3	8.8	9.6
	(71.07 ±14.21□g/l)	8.1	19.4	12.4	13.6	21.3	18.4	17.6	19.2
	(65.83±6.32□g/l)	3.0	19.3	10.2	14.0	17.5	8.0	17.0	18.5
14		12.4	11.3	9.9	14.1	11.4	12.4	21.1	23.0
	(68.88±12.41□g/l)	19.3	27.7	12.4	18.5	19.5	9.7	15.1	16.5
	(70.12±8.52□g/l)	17.0	23.0	10.2	11.7	11.0	8.3	11.8	12.9
15		4.9	5.2	12.3	6.3	3.9	13.5	4.5	4.9
	(68.21±5.26□g/l)	3.7	4.2	16.3	3.9	4.8	17.8	4.0	4.4
	(71.23±6.58□g/l)	2.3	4.0	17.5	2.2	3.6	19.2	3.5	3.9
16		10.1	10.2	19.4	12.4	11.3	21.2	11.4	12.4
	(74.11±14.21□g/l)	9.6	10.4	10.3	8.4	9.0	11.3	11.9	12.9
	(75.23±11.58□g/l)	7.6	10.3	12.0	7.2	8.4	13.1	10.1	10.9
17		16.3	12.7	8.9	13.5	13.4	9.8	11.8	12.9
	(52.31±16.54□g/l)	13.4	19.2	10.2	12.6	11.8	11.1	11.4	12.5
	(58.69±17.69□g/l)	13.9	19.7	4.8	13.7	13.0	5.3	11.9	13.0
18		8.2	11.8	13.3	12.4	9.8	14.5	7.1	7.8
	(75.36±9.87mg/l)	10.1	9.6	16.5	8.4	6.5	18.1	7.7	8.4
	(79.64±8.52mg/l)	5.4	10.6	11.8	7.2	7.1	12.9	9.7	10.6
19		10.2	8.5	12.4	12.4	11.3	13.6	9.8	10.7
	(69.81±11.36□g/l)	9.3	9.7	8.4	8.4	9.0	9.2	10.9	11.9
	(70.33±8.14□g/l)	6.2	11.3	15.7	7.2	8.4	17.2	11.8	12.9
20		10.2	8.5	14.6	11.2	10.2	16.0	10.8	11.7
	(71.89±9.78□g/l)	9.3	9.7	18.7	9.4	9.7	20.4	9.9	10.8
	(68.93±11.54□g/l)	6.2	11.3	13.0	8.1	8.1	14.2	9.8	10.7

Appendix 1.2-B. Cu concentrations in the effluent of pots (high loading)

Runoff	Input	H	I	J	K	L	M	N1	N2
1		30.56	40.41	45.82	34.24	25.31	57.53	34.98	37.90
	(175.32±6.23 □g/l)	24.53	19.82	34.37	21.34	19.23	43.15	16.42	17.78
	(187.2.72±3.65 □g/l)	47.18	27.01	42.21	15.28	15.69	52.99	24.23	26.25
		21.75	19.32	32.66	36.39	18.59	41.01	17.40	18.84
2		26.64	23.53	3.20	22.94	24.73	11.04	28.16	30.50
	(149.12±16.54 □g/l)	41.00	20.65	14.93	22.72	19.51	8.03	22.63	24.51
	(145.75±22.09□g/l)	18.99	15.85	35.01	15.86	23.32	25.08	15.84	17.16
		18.34	20.04	16.51	26.80	37.05	17.85	18.36	19.88
3		20.10	19.63	3.10	20.15	17.57	3.67	13.13	14.23
	(136.01±26.23 □g/l)	15.60	18.03	4.33	17.12	20.35	5.12	20.37	22.07
	(178.56±6.41 □g/l)	17.79	27.99	13.42	22.58	13.76	15.88	42.73	46.29
		23.30	16.27	13.23	19.33	14.23	14.35	16.01	17.35
4		15.88	18.51	25.42	18.08	37.11	28.54	17.43	18.89
	(156.96± 28.04 □g/l)	13.50	13.16	21.32	19.72	11.35	21.30	13.89	15.05
	(178.56±6.41 □g/l)	18.71	14.40	19.68	13.85	19.83	24.32	11.97	12.97
		11.15	15.07	28.96	7.36	10.46	16.98	12.15	13.17
5		33.37	53.03	20.02	33.25	28.63	30.96	8.57	9.28
	(214.4 ±7.36 □g/l)	21.84	48.79	30.27	31.23	30.01	21.96	25.93	28.09
	(178.34±5.25 □g/l)	57.31	17.21	22.08	12.62	13.78	14.78	10.37	11.23
		22.49	16.25	17.25	14.68	15.87	18.93	19.52	21.14
6		14.73	19.69	25.42	19.61	20.14	25.14	8.71	9.44
	(189.12±8.34 □g/l)	22.51	25.83	36.28	17.02	25.34	16.36	20.26	21.94
	(186.32±5.14 □g/l)	28.82	17.39	12.30	16.12	15.84	15.63	11.64	12.60
		9.03	14.13	18.92	10.96	16.84	19.25	8.38	9.08
7		32.98	29.85	22.83	41.40	37.48	21.50	11.04	11.97
	(187.2.72±3.65 □g/l)	17.88	18.62	29.82	22.45	23.37	18.34	9.43	10.22
	(154.32±9.63□g/l)	39.10	21.54	19.03	49.09	27.05	22.13	31.55	34.17
8		18.86	14.98	18.50	2.40	12.33	16.81	3.18	3.44
	(145.75±22.09□g/l)	14.16	10.68	12.69	12.76	11.24	14.28	5.15	5.57
	(171.67±10.28□g/l)	13.93	19.56	12.89	14.56	32.90	11.28	21.50	23.30
9		15.11	16.21	3.65	19.18	17.87	3.02	9.09	9.84
	(178.56±6.41 □g/l)	16.22	20.83	7.52	24.64	19.18	1.78	11.16	12.09
	(170.12±4.71□g/l)	8.69	15.75	10.23	18.63	10.28	18.64	13.75	14.90
10		12.36	17.89	J	15.62	16.98	M	18.17	19.69
	(178.56±6.41 □g/l)	19.25	19.68	10.87	20.13	25.12	11.04	22.32	24.18
	(155.04±16.87 □g/l)	18.69	20.25	14.21	15.63	13.63	8.03	27.50	29.80
11		23.96	23.07	12.93	19.71	23.28	12.29	21.53	23.33
	(178.34±5.25 □g/l)	15.20	24.33	11.73	20.61	15.01	15.80	12.31	13.33
	(147.69±14.58□g/l)	16.82	9.85	10.75	12.22	12.55	19.67	14.67	15.89
12		23.96	25.68	10.17	19.62	25.32	11.04	22.29	24.15
	(186.32±5.14 □g/l)	21.56	29.63	10.24	12.03	16.25	8.03	15.60	16.90
	(171.29±15.67mg/l)	19.63	19.56	21.75	11.11	11.55	25.08	16.20	17.54
13		17.15	18.77	9.65	14.52	27.91	11.04	23.16	25.10
	(154.32±9.63□g/l)	16.32	15.53	12.37	15.62	22.54	8.03	17.64	19.10
	(146.21±11.24□g/l)	14.69	15.40	14.23	14.45	14.14	25.08	16.92	18.32
14		26.52	13.18	9.65	18.54	16.95	12.39	19.69	21.33
	(171.67±10.28□g/l)	12.44	11.64	12.37	16.15	13.46	9.36	13.02	14.10
	(172.34±13.14□g/l)	12.60	11.36	14.23	12.35	16.11	15.34	15.70	17.00
15		8.65	8.54	19.61	1.68	6.47	21.47	5.46	5.92
	(170.12±4.71□g/l)	4.25	2.57	17.56	1.91	1.49	19.22	7.28	7.88
	(173.22±3.24□g/l)	10.36	10.32	22.12	8.90	13.70	24.21	6.28	6.80
16		18.97	11.24	21.34	11.24	12.33	23.36	17.52	18.98
	(160.11±17.45 □g/l)	15.49	12.54	29.68	12.76	11.24	32.49	16.84	18.24
	(159±14.5 □g/l)	14.28	13.69	23.45	14.56	17.97	25.67	15.72	17.04
17		11.66	11.31	18.39	11.57	14.08	20.13	10.54	11.42
	(147.69±14.58□g/l)	10.71	11.41	20.08	16.64	10.64	21.99	15.05	16.31
	(149.71±18.47□g/l)	10.25	10.47	22.21	11.24	12.94	24.32	16.57	17.95
18		18.86	14.98	29.81	10.54	11.02	32.64	14.63	15.85
	(179.24±13.68mg/l)	14.16	10.68	24.02	11.78	10.69	26.29	10.44	11.30
	(181.54±10.39mg/l)	13.93	19.56	27.68	15.69	18.79	30.31	17.82	19.30
19		14.56	11.02	19.17	2.40	12.33	20.99	6.12	6.62
	(159.34±10.25□g/l)	13.65	11.32	12.12	12.76	11.24	13.27	12.08	13.08
	(164.23±8.42□g/l)	15.21	15.47	21.43	14.56	32.90	23.46	16.65	18.03
20		14.56	11.02	11.15	12.36	11.32	12.21	9.97	10.81
	(173.88±11.26□g/l)	13.65	11.32	12.10	10.32	14.36	13.25	12.73	13.79
	(172.17±12.97□g/l)	15.21	15.47	10.81	11.25	10.21	11.84	14.73	15.95

Appendix 1.3-A. Pb concentrations in the effluent of pots (low loading)

Runoff	Input	A	B	C	D	E	G	F1	F2
1		1.26	1.45	6.94	4.91	0.20	9.16	0.45	0.49
	(60.59±9.62 □g/l)	0.69	1.82	9.11	1.00	0.97	5.70	0.45	0.49
	(60.33±10.12□g/l)	3.32	1.27	14.80	0.72	0.72	7.52	0.60	0.65
		2.10	2.53	0.92	3.16	3.16	6.98	1.47	1.60
2		5.12	1.78	7.42	3.17	2.95	8.13	1.04	1.14
	(65.27±8.48 □g/l)	5.18	0.68	12.20	2.95	2.88	6.55	6.24	6.79
	(148.31±14.23□g/l)	4.01	1.66	8.46	2.48	4.24	4.13	1.90	2.07
		5.55	9.96	7.06	3.84	5.59	2.70	3.51	3.82
3		14.66	15.77	4.42	8.57	9.61	4.15	7.86	8.56
	(58.21±12.35 □g/l)	7.02	11.36	7.02	6.15	15.31	7.33	8.67	9.44
	(66.17±4.56□g/l)	7.93	11.23	10.36	11.43	9.47	7.30	7.99	8.70
		11.13	7.53	4.05	9.63	12.26	8.59	7.35	8.00
4		15.60	22.12	6.99	14.50	9.11	9.16	8.54	9.29
	(74.47±1.20 □g/l)	11.88	12.20	9.21	15.13	10.44	5.70	11.01	11.99
	(75.12±5.21□g/l)	19.12	12.70	10.32	9.84	12.43	7.52	9.59	10.44
		9.13	9.14	4.21	11.19	27.44	6.98	7.23	7.87
5		7.85	6.14	4.42	3.35	1.12	7.73	4.86	5.29
	(67.6±10.12 □g/l)	2.88	5.04	5.53	3.07	3.84	7.82	6.61	7.19
	(65.12±8.81□g/l)	9.90	3.06	9.99	2.72	13.86	6.10	9.95	10.83
		7.51	4.70	7.09	4.46	6.05	11.25	6.24	6.79
6		2.27	6.94	5.21	8.05	9.31	7.37	4.70	5.12
	(77.21±13.18 □g/l)	5.45	6.45	3.24	7.32	5.61	6.36	8.17	8.89
	(74.01±5.69□g/l)	4.20	2.19	5.24	17.10	9.45	6.99	2.93	3.19
7		9.99	8.25	5.74	5.41	5.81	6.68	6.85	7.46
	(60.33±10.12□g/l)	4.75	5.30	1.82	5.33	6.32	1.89	8.86	9.65
	(65.12±11.54□g/l)	9.64	7.26	5.33	7.05	8.74	9.32	5.11	5.56
8		4.90	4.28	7.85	6.34	3.90	13.27	3.24	3.53
	(65.27±10.24□g/l)	2.90	4.01	9.23	3.94	4.79	9.23	1.65	1.80
	(67.18±6.56□g/l)	1.97	3.77	5.32	2.21	3.60	7.88	5.11	5.56
9		10.70	6.14	7.21	9.08	9.44	9.16	4.29	4.67
	(66.17±4.56□g/l)	3.44	5.59	4.56	7.57	4.40	5.70	5.88	6.40
	(64.87±5.32□g/l)	4.65	2.94	7.21	6.74	7.36	7.52	5.47	5.95
10		4.21	8.12	7.21	5.32	5.81	9.16	8.84	9.62
	(75.12±5.21□g/l)	4.32	6.21	5.64	5.27	6.32	5.70	5.05	5.50
	(75.29±5.64□g/l)	8.52	7.32	5.32	6.09	8.74	7.52	5.96	6.49
11		6.29	5.91	6.77	6.64	6.44	6.84	8.13	8.85
	(65.12±8.81□g/l)	4.41	9.96	7.69	10.03	4.50	4.23	5.89	6.41
	(61.17±9.41□g/l)	4.45	7.67	8.53	8.58	8.39	10.21	5.33	5.80
12		9.21	8.25	8.14	5.41	6.33	8.13	6.96	7.58
	(74.01±5.69□g/l)	10.21	6.23	10.23	5.33	5.24	6.55	8.03	8.74
	(70.11±12.34mg/l)	9.64	7.12	7.68	6.32	7.52	4.13	4.15	4.52
13		10.61	7.15	5.87	5.64	2.42	6.68	3.09	3.37
	(65.12±11.54□g/l)	1.97	5.72	3.69	7.25	5.15	1.89	4.85	5.28
	(62.12±7.45□g/l)	1.83	5.55	4.21	5.04	7.22	9.32	5.89	6.41
14		10.88	7.04	5.87	8.82	9.80	5.34	23.26	25.32
	(67.18±6.56□g/l)	10.72	10.81	3.69	6.96	6.73	2.98	5.28	5.74
	(68.97±11.21□g/l)	9.65	10.42	4.21	5.35	7.14	8.14	4.48	4.88
15		4.56	6.25	6.88	5.41	5.81	7.53	6.84	7.44
	(64.87±5.32□g/l)	3.24	4.28	4.43	5.33	6.32	4.85	8.17	8.89
	(67.84±4.21□g/l)	5.26	7.12	2.38	7.05	8.74	2.60	6.09	6.63
16		7.58	4.25	4.54	5.41	5.81	4.97	6.00	6.53
	(75.29±5.64□g/l)	5.12	6.32	4.90	5.33	6.32	5.37	8.36	9.10
	(74.13±6.66□g/l)	10.23	7.12	8.04	7.05	8.74	8.80	8.84	9.62
17		9.24	8.25	9.16	8.40	8.82	10.03	5.77	6.29
	(61.17±9.41□g/l)	9.00	9.89	6.59	8.32	7.96	7.22	7.23	7.88
	(68.14±2.36□g/l)	10.32	13.11	5.43	11.29	8.60	5.95	8.49	9.24
18		4.90	4.28	5.47	6.34	4.02	5.99	4.50	4.90
	(70.11±12.34mg/l)	3.85	4.01	6.92	3.94	4.21	7.57	5.06	5.51
	(72.64±9.56mg/l)	2.69	3.77	2.89	2.21	3.98	3.16	4.59	5.00
19		3.15	6.49	4.07	5.64	2.42	4.45	5.96	6.49
	(62.12±7.45□g/l)	2.98	5.78	5.09	7.25	5.15	5.57	4.93	5.37
	(65.43±6.67□g/l)	5.41	6.12	6.50	5.04	7.22	7.11	6.13	6.68
20		3.15	6.49	12.08	4.65	3.21	13.23	7.88	8.58
	(68.97±11.21□g/l)	2.98	5.78	5.91	6.97	5.01	6.47	5.96	6.49
	(69.12±8.69□g/l)	5.41	6.12	4.58	5.12	6.34	5.01	5.64	6.13

Appendix 1.3-B. Pb concentrations in the effluent of pots (high loading)

Runoff	Input	H	I	J	K	L	M	N1	N2
1		11.81	13.49	12.99	11.18	10.64	10.55	10.04	10.88
	(156.36±12.14□g/l)	12.72	10.78	6.00	6.48	4.35	9.41	6.94	7.52
	(147.63±8.26 □g/l)	12.70	6.52	9.13	12.12	5.82	14.73	11.33	12.27
		8.66	3.57	9.48	18.88	4.81	10.32	9.71	10.51
2		7.70	7.22	2.90	5.39	5.24	3.02	6.03	6.53
	(170.12±9.65 □g/l)	7.93	7.19	6.53	14.06	7.11	1.78	13.23	14.33
	(66.17±4.56□g/l)	6.20	4.60	15.68	1.77	1.94	18.64	8.65	9.37
		5.34	4.25	18.45	7.70	2.24	18.34	3.96	4.28
3		6.12	11.93	10.06	18.85	9.10	5.02	8.75	9.47
	(127.35±21.36 □g/l)	3.34	6.48	5.44	11.82	14.68	6.91	7.57	8.21
	(148.84±7.89□g/l)	6.42	10.50	5.56	5.12	6.65	9.52	15.34	16.62
		6.43	8.45	9.95	11.23	6.13	6.39	7.63	8.27
4		48.00	44.04	11.00	35.47	9.52	10.55	30.96	33.54
	(185.21± 7.58 □g/l)	28.31	20.97	7.35	32.09	15.72	9.41	23.79	25.78
	(187.96±2.13□g/l)	14.23	12.58	8.52	5.42	8.48	14.73	10.97	11.88
		19.73	12.34	8.78	17.56	10.93	10.32	10.44	11.31
5		19.98	15.72	6.75	11.39	17.04	9.13	17.15	18.58
	(141.8±14.25□g/l)	20.17	24.19	6.14	11.45	11.70	16.05	11.14	12.06
	(161.12±16.77□g/l)	7.62	27.49	6.73	5.64	7.73	5.73	5.87	6.36
		7.35	7.89	5.82	7.40	8.04	5.70	5.72	6.20
6.00		6.41	10.34	8.56	5.55	6.29	9.66	5.53	5.99
	(182.5±6.33 □g/l)	6.28	7.56	7.54	6.44	7.09	9.25	6.50	7.04
	(183.7±8.93□g/l)	6.74	6.56	9.26	7.10	5.21	10.65	5.94	6.43
		6.75	5.97	10.24	8.48	7.01	11.23	6.39	6.92
7		9.96	7.98	9.18	13.02	4.31	13.39	3.26	3.53
	(147.63±8.26 □g/l)	4.79	4.37	10.76	3.96	4.67	21.71	5.42	5.87
	(167.12±12.52□g/l)	11.50	6.45	9.10	19.32	4.75	12.22	14.14	15.32
8		8.00	7.73	15.77	1.68	6.47	12.23	1.85	2.00
	(148.31±14.23□g/l)	2.46	1.70	7.42	1.91	1.49	10.60	1.63	1.77
	(156.97±10.35□g/l)	12.51	12.90	6.49	8.90	13.70	5.86	6.48	7.02
9		8.68	7.09	10.97	8.65	9.20	10.55	5.78	6.26
	(148.84±7.89□g/l)	7.38	7.45	7.56	6.20	7.18	9.41	6.16	6.67
	(170.69±5.87□g/l)	7.20	6.87	8.24	6.30	6.93	14.73	5.87	6.36
10		10.21	6.32	10.87	13.02	4.31	10.55	6.93	7.50
	(187.96±2.13□g/l)	9.65	7.56	6.97	3.96	4.67	9.41	6.68	7.24
	(185.89±7.68 □g/l)	10.63	10.32	8.99	19.32	4.75	14.73	9.91	10.74
11		8.15	7.53	8.30	5.76	7.78	7.26	10.08	10.92
	(161.12±16.77□g/l)	7.43	14.56	7.21	6.72	6.37	10.47	6.73	7.29
	(141.85±15.98□g/l)	6.05	6.44	8.62	5.17	7.32	6.76	13.73	14.87
12		10.24	9.52	9.16	10.32	8.37	3.02	11.87	12.85
	(183.7±8.93□g/l)	8.56	9.63	8.48	9.37	7.37	7.89	9.27	10.04
	(165.89±18.67mg/l)	11.24	8.56	11.96	10.23	8.97	18.64	8.03	8.70
13		7.53	9.19	10.54	9.07	10.20	13.39	9.44	10.23
	(167.12±12.52□g/l)	7.35	7.77	6.87	9.36	9.78	21.71	13.10	14.20
	(142.11±13.24□g/l)	7.62	10.55	8.97	7.95	11.72	12.22	6.74	7.30
14		1.93	9.10	10.54	9.55	9.68	11.21	16.98	18.40
	(156.97±10.35□g/l)	6.15	5.61	6.87	10.77	6.42	10.24	7.98	8.65
	(159.67±12.69□g/l)	7.54	6.19	8.97	5.70	5.73	10.39	5.17	5.60
15		10.12	8.25	8.28	13.02	4.31	9.07	9.96	10.80
	(170.69±5.87□g/l)	5.24	5.63	7.56	3.96	4.67	8.28	9.25	10.03
	(169.25±5.79□g/l)	6.31	7.24	7.81	19.32	4.75	8.55	10.82	11.72
16		10.24	7.14	4.54	13.02	4.31	4.97	6.99	7.57
	(185.89±7.68 □g/l)	8.54	5.87	5.45	3.96	4.67	5.97	6.61	7.17
	(181.74±6.64 □g/l)	6.29	7.26	7.51	19.32	4.75	8.22	10.19	11.03
17		7.04	7.23	5.11	6.87	6.97	5.60	6.07	6.58
	(141.85±15.98□g/l)	6.48	7.39	7.56	2.88	6.54	8.28	8.58	9.30
	(143.85±11.69□g/l)	8.65	8.59	8.68	7.49	7.05	9.51	7.88	8.53
18		8.47	7.73	9.44	4.69	6.47	10.34	10.91	11.81
	(165.89±18.67mg/l)	7.89	4.26	6.97	8.41	5.86	7.63	10.44	11.30
	(164.33±11.45mg/l)	10.69	12.90	4.66	8.90	12.53	5.10	8.88	9.62
19		7.98	5.87	5.28	9.07	10.20	5.78	9.48	10.26
	(142.11±13.24□g/l)	6.32	6.98	8.09	9.36	9.78	8.86	10.79	11.69
	(141.47±11.69□g/l)	8.21	7.59	9.03	7.95	11.72	9.88	8.20	8.88
20		7.98	5.87	10.01	8.12	9.65	10.96	11.87	12.85
	(159.67±12.69□g/l)	6.32	6.98	10.79	8.57	10.32	11.81	13.64	14.78
	(157±11.36□g/l)	8.21	7.59	8.02	7.24	8.25	8.78	9.16	9.92

Appendix 1.4-A. Cd concentrations in the effluent of pots (low loading)

Runoff	Input	A	B	C	D	E	G	F1	F2
1		0.95	1.53	1.14	2.42	2.07	1.06	1.34	1.46
	(27.18±4.28 mg/l)	1.81	0.93	1.46	1.67	0.84	0.75	1.08	1.17
	(23.32±5.47□g/l)	1.44	0.75	1.18	1.83	0.67	1.20	0.67	0.73
		2.06	4.93	0.92	3.10	3.09	3.85	7.52	8.18
2		1.70	1.81	2.86	12.46	1.40	1.30	8.92	9.71
	(19.24±4.48 □g/l)	1.22	0.77	1.51	18.74	0.96	0.96	12.17	13.25
	(23.36±1.85□g/l)	0.69	0.93	0.59	1.77	0.49	0.52	0.76	0.82
		0.62	0.63	0.49	0.98	1.16	0.14	0.49	0.53
3		4.85	17.08	0.84	0.36	20.35	0.18	3.20	3.49
	(19.19±1.56 □g/l)	3.41	1.87	2.61	0.36	10.06	0.51	0.63	0.69
	(20.62±0.894□g/l)	2.37	20.03	0.72	4.27	3.12	0.78	1.10	1.20
		10.47	15.48	0.54	20.18	4.96	0.23	9.12	9.93
4		1.28	0.93	1.25	0.94	1.21	0.24	1.34	1.46
	(23.01±2.45 □g/l)	1.65	0.90	1.98	1.62	0.82	0.95	0.77	0.83
	(21.66±0.93□g/l)	0.59	1.22	0.65	0.76	0.50	0.65	0.65	0.70
		0.94	1.25	1.97	0.85	0.70	0.54	0.48	0.52
5		1.20	0.54	1.89	1.29	0.68	4.74	0.56	0.61
	(23.91±2.12 □g/l)	0.57	0.77	5.93	1.00	0.47	7.53	0.52	0.56
	(24.15±2.81□g/l)	0.33	0.14	11.80	0.06	0.32	2.44	0.33	0.36
		0.13	0.16	6.91	0.12	0.31	7.77	0.28	0.31
6		0.25	0.61	0.70	0.35	0.80	1.02	0.43	0.47
	(23.25±4.98 □g/l)	0.51	0.47	1.35	0.33	0.28	1.02	1.09	1.19
	(22.55±2.13□g/l)	1.70	1.69	1.57	0.80	0.39	1.00	0.78	0.85
		0.11	0.69	2.36	0.97	4.59	2.31	0.78	0.85
7		5.64	0.39	0.82	0.46	0.88	0.90	0.30	0.33
	(23.32±5.47□g/l)	0.34	0.02	0.11	0.41	1.11	0.54	0.32	0.35
	(19.12±2.19□g/l)	0.64	0.64	0.51	0.39	1.08	0.67	7.84	8.54
8		0.36	0.51	1.12	0.45	0.52	3.21	0.34	0.37
	(23.36±1.85□g/l)	0.23	0.63	1.66	0.34	0.83	0.77	0.13	0.14
	(17.21±6.11□g/l)	0.58	2.40	1.73	0.10	0.47	1.59	0.44	0.47
9		1.98	0.58	2.31	0.62	0.71	1.54	0.38	0.41
	(20.62±0.894□g/l)	1.55	0.47	1.69	0.45	3.66	1.69	0.40	0.44
	(22.17±2.68□g/l)	0.30	0.23	1.21	0.33	0.59	1.71	0.29	0.31
10		1.64	1.27	0.98	0.89	0.54	1.06	2.27	2.47
	(21.66±0.93□g/l)	1.25	0.58	0.65	0.65	2.31	0.75	2.44	2.65
	(21.32±7.13□g/l)	0.56	0.96	0.76	0.87	1.24	1.20	1.18	1.29
11		4.38	3.71	0.69	6.76	3.14	0.67	11.46	12.48
	(24.15±2.81□g/l)	1.87	7.93	0.46	12.40	1.77	1.06	19.32	21.03
	(21.11±1.24□g/l)	2.80	2.86	0.42	7.18	5.33	0.46	3.03	3.30
12		3.22	1.20	1.58	0.65	1.02	1.30	0.95	1.03
	(22.55±2.13□g/l)	1.23	1.02	2.14	0.69	1.00	0.96	0.84	0.91
	(22.12±3.58mg/l)	1.25	0.37	0.89	0.79	0.65	0.52	1.18	1.29
13		0.89	0.93	1.87	0.75	0.17	0.90	0.34	0.37
	(19.12±2.19□g/l)	0.11	0.96	0.84	0.74	0.76	0.54	0.71	0.77
	(17.84±2.45□g/l)	0.17	6.08	0.64	0.99	0.80	0.67	0.67	0.73
14		1.63	1.42	1.87	0.65	0.97	1.23	3.03	3.30
	(17.21±6.11□g/l)	1.33	1.62	0.84	2.33	3.22	1.21	1.04	1.13
	(19.58±2.59□g/l)	0.91	1.10	0.64	0.76	0.67	0.87	0.81	0.88
15		0.45	0.62	0.67	0.65	0.98	0.73	1.09	1.19
	(22.17±2.68□g/l)	0.65	0.98	1.62	1.54	1.36	1.77	1.32	1.43
	(23.14±2.14□g/l)	0.42	1.21	1.31	2.14	1.58	1.44	0.94	1.02
16		0.87	0.65	1.56	0.46	0.88	1.71	0.85	0.93
	(21.32±7.13□g/l)	0.84	0.37	1.46	0.41	1.11	1.60	0.92	1.00
	(24.31±7.23□g/l)	0.54	0.49	0.68	0.39	1.08	0.75	0.55	0.60
17		0.65	1.01	1.67	0.69	0.60	1.83	0.73	0.79
	(21.11±1.24□g/l)	0.73	0.89	0.30	0.57	0.44	0.33	0.46	0.50
	(23.45±1.32□g/l)	0.42	0.59	0.86	0.41	0.50	0.94	0.41	0.45
18		0.56	0.51	1.24	0.45	0.54	1.35	0.41	0.45
	(22.12±3.58mg/l)	0.43	0.63	0.40	0.34	0.87	0.44	0.84	0.91
	(24.31±4.75mg/l)	0.55	2.40	0.51	0.10	0.69	0.56	0.56	0.61
19		0.87	1.11	0.68	0.75	0.17	0.75	0.82	0.89
	(17.84±2.45□g/l)	0.21	1.32	0.78	0.74	0.76	0.85	1.21	1.32
	(18.97±6.94□g/l)	0.54	2.31	0.61	0.99	0.80	0.67	1.09	1.19
20		0.87	1.11	0.44	0.87	0.57	0.48	1.20	1.30
	(19.58±2.59□g/l)	0.21	1.32	1.65	0.65	0.88	1.81	0.98	1.07
	(21.11±3.14□g/l)	0.54	2.31	0.29	0.89	1.11	0.31	0.99	1.08

Appendix 1.4-B. Cd concentrations in the effluent of pots (high loading)

Runoff	Input	H	I	J	K	L	M	N1	N2
1		18.42	18.68	3.50	15.63	15.13	0.86	11.37	12.31
	(64.58±7.90 □g/l)	9.09	3.28	0.62	2.21	5.56	1.24	1.30	1.41
	(56.98±2.29 □g/l)	18.70	2.22	0.84	3.62	0.97	1.17	4.84	5.24
		17.19	14.58	0.47	20.05	21.75	3.25	1.96	2.12
2		0.84	0.57	1.23	0.74	1.34	0.32	0.81	0.88
	(46.29±7.80 □g/l)	3.05	1.62	2.32	4.14	3.97	0.60	4.11	4.45
	(48.42±5.16□g/l)	0.30	0.96	2.10	0.58	1.69	1.23	0.38	0.41
		0.72	1.22	5.26	0.74	0.65	0.99	0.40	0.43
3		13.77	1.77	3.50	18.72	13.49	0.90	10.68	11.58
	(41.04±7.30 □g/l)	0.75	0.71	1.27	8.71	7.15	1.54	34.17	37.01
	(52.4±1.25 □g/l)	4.37	10.74	3.13	1.69	12.32	1.23	10.57	11.45
		12.36	18.01	1.25	14.95	3.26	3.25	16.60	17.98
4		1.23	0.84	2.69	1.52	2.11	1.21	1.00	1.09
	(42.17± 2.10 □g/l)	0.85	0.77	5.32	0.92	0.86	1.32	0.82	0.88
	(55.04±6.89 □g/l)	0.85	0.44	2.31	0.22	0.40	2.12	0.48	0.52
		0.51	0.37	2.65	0.11	0.66	2.31	0.68	0.73
5		0.26	1.37	1.58	0.16	0.14	2.05	0.19	0.20
	(i36.72±4.25□g/l)	0.22	0.26	12.35	0.30	0.19	17.88	0.21	0.23
	(41.64±4.21□g/l)	0.78	0.29	6.47	0.31	0.27	5.02	0.30	0.33
		0.51	4.37	0.30	0.24	2.60	0.31	0.50	0.54
6		0.73	0.57	4.32	0.78	0.58	1.27	0.60	0.66
	(56.56±0.94 □g/l)	0.53	0.46	2.40	0.56	0.66	2.66	0.78	0.84
	(50.72±5.97□g/l)	0.64	0.52	1.30	0.50	0.35	4.33	0.60	0.65
7		1.44	1.06	0.71	1.83	1.33	1.32	0.30	0.32
	(56.98±2.29 □g/l)	0.29	0.28	1.32	0.42	0.42	0.74	0.17	0.18
	(45.31±3.21□g/l)	7.08	1.69	0.62	0.34	0.68	0.51	0.32	0.34
8		1.04	1.02	2.68	1.21	3.58	4.05	1.18	1.28
	(48.42±5.16□g/l)	0.11	2.65	1.20	2.21	5.46	4.41	2.08	2.25
	(40.33±8.27□g/l)	1.07	3.56	1.65	1.27	3.55	2.31	0.66	0.71
9		1.54	1.06	2.58	0.88	1.55	1.03	0.54	0.59
	(52.4±1.25 □g/l)	1.27	1.03	2.47	0.86	0.90	2.13	0.95	1.03
	(46.28±6.21□g/l)	5.42	1.69	3.59	0.55	0.55	1.26	0.92	0.99
10		1.54	0.89	2.14	0.89	2.54	0.86	2.22	2.40
	(55.04±6.89 □g/l)	1.68	3.54	2.13	1.25	1.36	1.24	2.16	2.34
	(54.37±12.29 □g/l)	2.31	6.36	1.86	0.69	3.24	1.17	0.94	1.02
11		10.80	3.53	2.32	0.87	12.32	0.65	1.74	1.88
	(41.64±4.21□g/l)	2.84	14.77	2.11	0.92	0.96	0.14	3.60	3.90
	(52.23±2.97□g/l)	0.46	0.85	1.02	0.14	0.24	2.14	0.38	0.41
12		1.25	2.36	1.23	1.99	1.37	3.68	2.30	2.49
	(50.72±5.97□g/l)	2.36	0.99	2.32	1.55	1.59	0.60	3.55	3.84
	(47.25±8.88mg/l)	2.59	2.36	2.10	1.65	0.96	1.23	3.13	3.39
13		0.78	0.66	1.17	1.62	1.88	1.32	0.69	0.75
	(45.31±3.21□g/l)	0.67	0.71	1.39	0.63	0.66	0.74	0.76	0.82
	(43.69±9.41□g/l)	0.54	1.38	0.94	0.40	1.06	0.51	0.66	0.72
14		0.52	2.15	1.17	1.17	2.34	1.12	2.20	2.39
	(40.33±8.27□g/l)	0.88	1.37	1.39	1.36	0.84	1.07	1.54	1.67
	(47.26±3.27□g/l)	3.58	4.06	0.94	3.73	5.67	0.99	0.87	0.95
15		1.98	2.34	2.11	2.48	3.12	2.31	1.18	1.28
	(46.28±6.21□g/l)	2.36	2.68	1.96	1.25	4.21	2.14	2.27	2.45
	(47.98±6.671□g/l)	2.14	1.59	1.84	2.69	3.01	2.02	1.19	1.29
16		1.54	1.87	1.86	1.83	1.33	2.04	1.81	1.97
	(54.37±12.29 □g/l)	1.63	1.56	4.48	0.42	0.42	4.91	2.48	2.68
	(53.69±10.11 □g/l)	2.87	1.98	2.17	0.34	0.68	2.37	2.53	2.75
17		1.02	1.11	1.41	1.22	0.98	1.54	1.18	1.28
	(52.23±2.97□g/l)	0.99	1.03	1.65	1.54	1.65	1.81	2.58	2.80
	(50.47±3.12□g/l)	1.65	2.31	1.88	1.27	1.75	2.06	2.28	2.46
18		1.04	2.13	1.29	1.21	3.58	1.41	2.05	2.23
	(47.25±8.88mg/l)	1.69	2.65	0.93	2.21	4.11	1.02	2.08	2.25
	(46.37±9.14mg/l)	1.57	3.56	2.34	1.27	3.69	2.56	4.19	4.53
19		1.24	0.79	2.03	1.62	1.88	2.23	0.94	1.02
	(43.69±9.41□g/l)	2.13	1.36	2.07	0.63	0.66	2.27	1.50	1.62
	(47.98±6.87□g/l)	0.89	1.54	1.79	0.40	1.06	1.96	1.81	1.97
20		1.24	0.79	1.88	1.24	1.54	2.06	1.19	1.29
	(47.26±3.27□g/l)	2.13	1.36	1.48	0.87	1.32	1.62	1.52	1.65
	(45.67±2.12□g/l)	0.89	1.54	2.01	1.23	0.85	2.20	1.63	1.77

Appendix 1.5. The volumes of the effluents (ml)

	A	D	B	E	C	G	F
Runoff	low loading						
1	12530	12296	12297	12319	12882	13012	14321
2	14214	14291	13596	13506	13603	13876	15647
3	14087	13942	13987	14629	14478	14573	15670
4	13830	13533	14012	14109	14529	14032	14914
5	14705	14302	14890	15128	14019	14987	14778
6	16729	15971	15772	16369	16486	14762	16868
7	14713	12997	12566	14050	13896	14327	14155
8	14409	14213	13755	14214	14139	14282	13799
9	13977	12997	13990	12775	14244	13757	12736
10	13877	12904	13983	12792	14212	13811	12816
11	14079	12288	13614	12464	13238	13548	13761
12	14085	12295	13598	12486	13264	13565	13776
13	13818	13181	13891	13920	13628	14034	13421
14	13649	13521	13933	13898	13551	13914	13581
15	13655	13543	13963	13902	13571	13908	13594
16	13835	13950	14022	13966	14007	13976	13851
17	12651	12937	13672	14456	14295	13519	13420
18	13278	13521	13933	13898	13551	13914	13581
19	13818	13181	13891	13920	13628	14034	13421
20	13798	13201	13891	13890	13588	14134	13411
Runoff	high loading						
	H	K	I	L	J	M	N
1	12963	13243	13098	12310	13426	13021	14354
2	13897	13897	14021	13672	14444	14562	15675
3	14597	13626	13765	14895	14276	13789	15185
4	14476	14532	15675	14928	14779	14567	15789
5	14348	14532	14782	14928	14779	14876	14819
6	14354	14603	14271	14937	14812	14562	14799
7	14438	14622	14193	13956	14574	13296	13687
8	14047	14461	13619	13358	13918	14420	13913
9	13922	13741	13315	13976	13132	13326	13011
10	13897	13751	13399	13955	13162	13421	13302
11	14651	14109	13546	14485	13337	13144	13632
12	14667	14176	13504	14512	13376	13211	13625
13	13777	13730	13837	14238	13789	13297	13812
14	13677	13589	13652	13942	13780	13852	13812
15	13677	13572	13713	13744	13807	13798	13876
16	13964	14051	14014	14071	14097	14008	14010
17	13848	14032	13610	12900	13379	13044	13455
18	13677	13589	13652	13942	13780	13852	13812
19	13775	13730	13837	14238	13789	13297	13819
20	13761	13742	13797	14225	13699	13317	13782

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