STORMWATER BIORETENTION: NITROGEN, PHOSPHOROUS AND METAL REMOVAL BY PLANTS

by

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ABSTRACT

Stormwater Bioretention: Nitrogen, Phosphorous, and Metal Removal by Plants

by

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Stormwater runoff may contain high levels of pollutants and is regulated by the Federal National Pollution Discharge Elimination System (NPDES). Stormwater bioretention (BR) systems are often used to satisfy these regulations. BR systems collect accumulated runoff that leaches into groundwater. A greenhouse study evaluated nutrient and metal removal among plant species that are typically found growing in BR systems. A field demonstration study assessed citric acid enhanced metal bioaccumulation potential under typical BR system conditions.

The greenhouse experiment examined pollutant retention, and bioaccumulation potential for six plant species undergoing three hydraulic and pollutant loads. Results verified there was 98% recovery of total phosphorous over the study period. Biomass increased with higher hydraulic and pollutant loads for all species. Phragmites australis, Carex praegracilis, and Carex microptera took up significantly more total phosphorous and nitrogen mass into shoots than Typha latifolia, Scirpus validus, and Scirpus acutus.
This study also found that 89% of applied metals were removed within the top 27 cm of soil in all treatments. Similar results were found regarding copper, lead, and zinc concentrations and bioaccumulation. *Carex praegracilis*, and *Carex microptera* exhibited higher metal distribution in plant tissue and exfiltrate, and lower distribution in the soil media than the other species. This indicated species differences in biological and chemical processes taking place within the simulated BR systems.

The field experiment investigated citric acid enhanced metal bioaccumulation potential among three different plant species under representative BR conditions. Citric acid significantly increased metal concentrations in the soil pore water for the planted treatments, but this did not result in increased metal uptake into plant tissue. However, notable differences were found among species, where *Carex microptera* accumulated more Al, Cr, Cu, and Fe in the above ground tissue than *Helianthus maximiliani* and *Typha latifolia* (except for Cu in *Helianthus*). These results provide greater insight into the biological and chemical process that affect transport, uptake and translocation of nutrients and metals, and confirm the importance of species selection in BR systems to optimize nutrient and metal retention and recovery from stormwater runoff to minimize subsequent groundwater pollutant loading.
Nitrogen, Phosphorous, and Metal Removal by Individual Plant Species

in Stormwater Bioretention

Malgorzata Ryciewicz-Borecki

Stormwater runoff is an environmental concern. It increases the volume and velocity of surface water flow and contains high pollutant concentrations. High flows erode stream channels. Nutrients in the runoff cause eutrophication. Some metals from stormwater are toxic at high concentrations. For these reasons, stormwater runoff is regulated by the Federal National Pollution Discharge Elimination System (NPDES). Bioretention (BR) systems are often used in response to these regulations to retain and treat this stormwater runoff.

This study conducted greenhouse and field experiments, and evaluated differences in nutrient and metal removal among plant species that are typically found growing in BR systems but are not typically investigated in this detail. The extent of water quality improvement, the distribution of the nutrients and metals, and plant uptake potential were studied in an effort to better understand how individual plant species interact with, and influence nutrient and metal transport from the stormwater into the soil, plant root, and above ground plant tissue.

In the greenhouse experiment six plant species received three levels of water inundation and pollutant loads. Differences in pollutant retention, and plant uptake of nutrients and metals were studied. Biomass production increased with higher water and pollutant loads for all species. Common Reed, Common Field Sedge, and Smallwing Sedge were found to take up significantly more phosphorous and
nitrogen into harvestable tissue than Broadleaf Cattail, Soft-stem Bulrush, and Hard-stem Bulrush. More than 89% of copper, lead, and zinc from runoff accumulated in the top 27 cm of the soil. Continued accumulation can result in hazardous levels of metal concentrations. Common Field Sedge, and Smallwing Sedge exhibited higher metal distribution in the plant tissue and exfiltrate water, and lower distribution in the soil media than the other species. It was speculated that these Sedges allow metal uptake through biological and chemical processes.

The field BR experiment evaluated citric acid’s ability to increase metal uptake potential among three plant species. High citric acid application levels increased the concentrations of metals in the soil pore water, but did not increase plant metal uptake. However, notable differences were found among species, where Carex microptera accumulated more aluminum, chromium, and iron in the above ground tissue than Helianthus maximiliani and Typha latifolia.
DEDICATION

This dissertation is dedicated to my family: my grandparents, parents, husband, and daughters. All of you contributed to my success. Grandma and grandpa, you taught me the importance of perseverance. Mom and dad, you showed me that everything can be earned through hard work and diligence. Husband, you have loved me, accepted me, and believed in me; you are my strength. Emilia and Lilianna, you have provided me with perspective, laughter, unconditional love, and brutal honesty. This dissertation was possible because of all of you - my accomplishment is yours.
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CHAPTER 1
INTRODUCTION

1. Overview

Untreated stormwater runoff continues to detrimentally affect downstream water bodies despite United States Environmental Protection Agency (U.S. EPA) and state regulations to eliminate pollutant discharges from non-point sources such as stormwater runoff. Planted bioretention (BR) systems remove significantly more nutrients and metals from stormwater runoff than unplanted systems and are increasingly used in response to the U.S. EPA’s National Pollutant Discharge Elimination System (NPDES) regulations. This study, consisting of a greenhouse experiment and a field experiment, evaluated differences among species in uptake and mass distribution of Total Phosphorus (TP), Total Nitrogen (TN), and various metals and metalloids from a stormwater BR system.

In a survey of state officials directly involved in various aspects of the NPDES program (n=51) TN and TP were the two highest rated nutrients of concern (Collins et al. 2010). Other pollutants of concern include metals, such as chromium, copper, lead, and zinc (Cr, Cu, Pb, and Zn), which accumulate in soil environments, and potentially leach into the groundwater once the soil sorption capacity is reached. Pollutants that are not retained in the soils or leached down the soil profile, can be taken up into the plant roots and accumulate in root tissue or are translocated into the aerial plant tissue. This above ground plant biomass can be harvested and disposed of off-site, decreasing potentially hazardous buildup of pollutants in BR soils matrices.
There is significant evidence that certain species are more capable of surviving the stressful flood and drought conditions of a stormwater BR system (Brisson and Chazarenc 2009) and some are better accumulators of pollutants than others (Tanner 1996; do Nascimento and Xing 2006; Bratiers et al. 2008; Read et al. 2008). Most of these studies focus on plant variations in pollutant removal from the exfiltrate, and little work has been done to evaluate differences in nutrient and metal uptake potential that exist among species typically planted in stormwater BR systems. Additionally, these studies assume accurate measurements for each of the water, plant and/or soil compartments, completed without a mass balance, and implying nutrient and metal recoverability from start to end of the experiments.

Previous investigations indicate that more than 80% of the metal pollutants retained in BR systems accumulate in the soil (Sun and Davis 2007; Marchand et al. 2010). The sorption capacity of soils is finite, and continuous application of metals may increase the risk of toxic metal buildup and subsequent leaching to groundwater. Bioaccumulation is the process of absorbing and transporting compounds from the soil to the aerial parts of a plant, allowing for the harvest and removal of plants and sequestered contaminants. Bioaccumulation potential is contingent upon plant biomass yield and metal concentrations in the harvestable plant components (Meers et al. 2008; Sheoran et al. 2010). There are two basic strategies for metal bioaccumulation: natural bioaccumulation and enhanced-bioaccumulation (Salt et al. 1998; Meers et al. 2008). Natural bioaccumulation can use hyperaccumulators, but despite the plant’s ability to store high concentrations of metals and metalloids in their above ground tissue they are often an
inappropriate choice for bioaccumulation, because these plants do not produce significant biomass.

Enhanced bioaccumulation uses high biomass-producing plants in conjunction with chemical, biological and/or naturally occurring amendments in the soil, such as low molecular weight organic acids (LMWOA), to enhance metal solubility, thereby increasing uptake by the plant (Johnson and Singhal 2010). Most natural and enhanced bioaccumulation studies exploring non-hyperaccumulating species focus on the remediation of highly contaminated soils with high soil metal concentrations (Stottmeister et al. 2003; Wiessner et al. 2006; Marchand et al. 2010; Yadav et al. 2011; Narhi et al. 2012; Ladislas et al. 2013). Few studies have investigated the fate of pollutants in plant, soil and water compartments of BR systems, and even fewer studies have explored bioaccumulation and enhanced-bioaccumulation potential of plants grown in soils with continual application of low metal concentrations. This type of research is vital to understand how to reduce soil metal accumulation and the resulting formation of hazardous sites in urban stormwater treatment settings.

2. Research objectives

The overall research goal of this project was to determine differences in nutrient and metal distribution, and to evaluate citric acid induced-bioaccumulation potential of metals among individual plant species typically found within a stormwater BR system. Findings from this study will enable municipalities, institutions, and state governments to develop recommendations for the use of
specific plants to optimize management and harvest procedures for reducing stormwater impacts to surface and groundwater, and prevent potentially harmful metal buildup in the soil. The specific objectives were:

1) Determine the extent to which species, hydraulic loading, and pollutant loading affect nutrient distribution in the soil, aboveground tissue, belowground tissue, and exfiltrate water compartments of a BR system using a mass balance approach.

2) Evaluate species potential to reduce exfiltrate metal concentrations, quantify their metal bioaccumulation potential under three levels of stormwater pollutant loading, and evaluate species differences in metal accumulation mechanisms.

3) Assess citric acid-enhanced bioaccumulation as a stormwater management technique to increase metal and metalloid uptake into the harvestable aboveground plant tissue, and thereby slow the accumulation of metal deposition in the soil.

3. Experimental Design

Two studies were conducted. The first study, addressing Objectives 1 and 2, was performed in a greenhouse, which simulated stormwater BR systems. The second study, addressing Objective 3, was conducted at the existing Green Meadows BR field demonstration site. The greenhouse experiment studied six plant species typically found growing in stormwater BR systems, under three simulated hydraulic and pollutant loading regimes representing Logan, UT; Des Moines, IA; and Scranton,
PA (Figure 1). The species investigated included: *Phragmites communis* (Common Reed); *Typha latifolia* (Broadleaf Cattail); *Scirpus validus* (Soft-stem Bulrush); *Scirpus acutus* (Hard-stem Bulrush); *Carex praegracilis* (Common Field Sedge); *Carex microptera* (Smallwing Sedge); and Control - unplanted. The study was conducted from October 2010 through June 2011.

Figure 1: The three hydraulic and pollutant loads are based on three cities (Logan UT, Des Moines IA, and Scranton PA) located along the 41 north latitudinal coordinate, and 18° longitudinally apart. In the greenhouse microcosm treatments were grown in blue Sterilite containers simulating the three BR loads.

The field site, located in Logan, Utah, receives stormwater runoff from a surrounding residential subdivision and equally distributes it into treatment areas planted with one of three species: *Typha latifolia* (Broadleaf Cattail), *Carex microptera* (Smallwing Sedge), and *Helianthus maximilianii* (Maximilian Sunflower). Each treatment area was divided into one of three citric acid application levels,
using a triplicate split-block design (Figure 2). The study was conducted from May 2014 to October 2014.

Figure 2: Schematic diagram of the BR field site's flow pattern and randomly assigned citric acid concentrations in each treatment area.
CHAPTER 2
LITERATURE REVIEW

1. Introduction

Stormwater flows across urban environments, transporting pesticides, oils, heavy metals, nutrients and a variety of other contaminants (Bannerman et al. 1993; Flint and Davis, 2007). The runoff flows downstream, increases pollutant load and peak discharge into receiving water bodies, impacts water quality (nutrient and metal concentrations), and increases flooding potential. Since 1999, major industrial facilities, large and medium city storm sewers, and construction sites that disturb 5 or more acres are mandated by the U.S. Environmental Protection Agency (U.S. EPA) to reduce polluted runoff through the National Pollutant Discharge Elimination System (NPDES) water pollution control program (U.S. EPA 2005). The NPDES program is aimed at minimizing the environmental pollutants caused by urban and rural non-point sources. Unfortunately, the quality of U.S. fresh water has declined despite the NPDES water pollution control program (Scavia and Bricker 2006; Daloglu et al. 2012).

The NPDES regulations have accelerated installation of structural stormwater Best Management Practices (BMPs), as indicated by a study interviewing 23 northern Utah BMP site managers who were responsible for 84 BMP installations in Cache Valley since the regulations were put into place (Rycewicz-Borecki 2007). Constructed wetlands (CWs) and bioretention (BR)
systems are both documented to be natural alternatives to more mechanical methods of water treatment, and are two BMPs commonly used in response to this federal mandate.

BR systems are a type of BMP that use soil and plants to treat stormwater runoff from commercial, residential, and industrial areas. They allow the water to pond, and infiltrate into the underlying soils (U.S. EPA 1999). Similarly, constructed wetlands are vegetated systems that treat stormwater and/or wastewater, by slowing the flow and filtering the water. This settles out suspended solids, transforms pollutants into less soluble forms, and/or allows pollutants to be taken up by plants (U.S. EPA 2004). Many studies lump BR systems together with CWs as both are vegetated and have periods of inundation after a storm event. Therefore, this literature review focuses on studies using either CWs or BR systems to remove constituents from stormwater, wastewater, and agricultural runoff.

Plants are influential in removing pollutants in BR systems and CWs (Tanner 2001; Jing et al. 2001). Plants enhance pollutant removal by a variety of mechanisms, including stabilizing surface soils, enhancing vertical flow, producing organic matter, insulating the surface against frost, providing surfaces for microbial growth, and releasing oxygen into the root-zone (Brix 1997; Tanner 2001).

2. Pollutant Removal Efficiency

Plants grown in CWs and BR systems are continually stressed by inundation of water and exposure to drought, producing different uptake potential than plants grown under ideal conditions. Despite the stressful conditions, plants grown in BR
systems enhance water quality (influent vs. effluent) compared to non-vegetated systems.

It is well documented that CWs and BR systems increase the removal of total nitrogen (TN), total phosphorus (TP), copper (Cu), zinc (Zn), lead (Pb), and other metals from polluted water over unplanted detention systems (Brix 1997; Davis et al. 2001; Jing et al. 2001; Tanner 2001; Fraser et al. 2004; Read et al. 2008; Bratieres et al. 2008; Brisson and Chazarenc 2009; Tanner and Headley 2011; Houdeshel et al. 2012). For example, a summary by the National Pollutant Removal Performance Database Version 3 (Center for Watershed Protection 2007) reviewed 166 published studies and found that CWs remove a median of 24% TN, 48% TP, 47% Cu and 42% Zn, and BR systems perform better at removing TN (46%), Cu (81%), and Zn (79%) from the water, but remove less TP (5%). In these studies pollutant removal efficiency is significantly increased when plants are present, but the exfiltrate, plant, and soil compartments of the system remain an indiscriminate black box.

Some studies look further into this ‘black box’, examining discrepancies in water quality as a function of the individual planted species. Table A-1 shows the scientific names for the species studied individually in the following literature review. This list shows the variation of plant species studied in stormwater projects, and also highlights the lack of consistency in evaluating one species in various project designs, and hydraulic and loading conditions. *Typhus latifolia* is the most examined species (included in four studies), but as the following literature review presents, results regarding its nutrient removal performance are inconclusive due
to variations in project design, inflow volume and concentrations, plant stage of life, and growth media used.

The dearth of multiple studies using the same species under the same conditions prevents relative ranking of species’ nutrient removal performance. For example, two greenhouse studies in Australia found *Carex, Melaleuca,* and *Juncus* significantly reduced effluent TN and/or TP concentrations (Read et al. 2008; Bratieres et al. 2008). Fraser et al. (2004) found significant differences in effluent TN and TP concentrations from soil leachate in wetland microcosms among species, however, in this study, *Scirpus validus* was most effective and *Phalaris arundinacea* least effective. Milandri et al. (2012) found *Agapanthus, Pennisetum* and *Stenotaphrum* significantly reduced outflow concentration of orthophosphate (PO$_4^{3-}$), ammonia (NH$_3$) and nitrate (NO$_3^-$) from synthetic stormwater compared to unplanted controls and other species. With the exception of the two Australian studies, each study concluded that plants of different families provided the greatest removal performance.

These inconclusive results are supported in Brisson and Chazarenc’s (2009) literature review of peer-reviewed articles that measured water influent versus effluent, and the effect of species selection on pollutant removal in subsurface flow CWs. They examined 35 experiments with 48 plant species. Their results were also inconclusive as to which species performed best at nutrient and metal removal, and even the most tested species showed varied performance. For example, the most common pair of species examined for their relative efficiency was *Phragmites australis* and *Typha latifolia.* In two studies *P. australis* appeared more efficient than
T. latifolia at NO₃⁻ and NH₄ removal; T. latifolia was more efficient at TN and TP removal in one study, and no differences in TN and TP removal were found between the two species in two additional studies. Inconclusive results were also found between T. latifolia and Schoenoplectus validus, which were examined in four studies. In one study T. latifolia was found more efficient than S. validus at TKN removal; in two studies S. validus was more efficient at TN, NO₃⁻, NH₄, or TP removal; and in a fourth study no difference in TDN removal efficiency was found.

Two studies correlated general plant traits, including plant biomass, root surface and diameter, longest root length, specific leaf area, leaf area ratio and percent leaf mass, with N and P removal efficiencies. Jiang et al. (2011) used a hydroponic culture to investigate the biomass, root morphology and nutrient (N and P) uptake of 15 wetland species and found that the accumulation of N and P in plant tissue was significantly correlated with plant biomass and root surface. Read et al. (2010) correlated stormwater N and P removal in BR systems with root length and root mass, among other root characteristics. However, no significant correlations were found between any plant characteristics and effluent metal concentrations.

The presence of vegetation enhances physical and chemical/biological processes that often increase metal removal within BR and CW systems. Read et al. (2008), Davis et al. (2003), and Sun and Davis (2007) found correlations between increased hydraulic retention time within a system and improved removal efficiency of metals through various physical and chemical/biological processes including: 1) adsorption to sediment and organic matter, 2) precipitation, 3) absorption by plants and bacteria, and 4) suspended solids deposition. These mechanisms work together
to varying degrees depending on specific site environments, inlet metal concentrations, and hydraulic loading. The interactions of these mechanisms generate varying degrees of metal and metalloid removal efficiency in BR and CW systems, but removal rates are typically higher than 70% (Kropfelova et al. 2009; Marchand et al. 2010).

3. Metal Behavior in Soil

Soil metal contamination is a serious environmental problem requiring affordable strategies for remediation. Sorption capacity of soils is finite. Metals accumulate in stormwater runoff where they concentrate in soil environments, and potentially leach into the groundwater once the soil sorption capacity is reached. The International Stormwater BMP Database (Wright Water Engineers Inc. and Geosyntec Consultants 2010) provides a short list of 15 of the most commonly reported metals. Seven of these commonly found metals are discussed in this study: chromium (Cr), copper (Cu), lead (Pb), zinc (Zn), aluminum (Al), arsenic (As), and iron (Fe). Arsenic is technically a metalloid, having both metallic and non-metallic properties, but in this document the term metal will encompass all elements discussed.

All uncontaminated soils have naturally occurring trace levels of metals, directly linked to the geology of the soil’s parent material. Soil contamination results from the presence of anthropogenic metals in the soil. Anthropogenic metals, such as those collected in stormwater runoff, can: 1) be dissolved in the soil solution, 2) occupy exchange sites or be specifically adsorbed on inorganic soil minerals and soil
organic matter, 3) be incorporated into the structure of soil minerals or soil organic matter, or 4) be precipitated as a solid (Shuman 1991).

The interrelated processes of inorganic-organic complexation, and oxidation-reduction, precipitation-dissolution, and adsorption-desorption reactions control metal concentrations in the soil solution phase. Total dissolved metal concentrations are the sum of free metal ions, soluble complexes with (in) organic ligands, and metal associated with mobile complexes with (in) organic colloidal material (McLean and Bledsoe 1992).

The solubility of metals in soil environments varies with the individual metal. Of this group of metals Pb typically has the highest relative affinity to a variety of soils types. However, Pb also has a strong affinity for organic ligands, which when present, may substantially increase Pb mobility in soil. Similar to Pb, Cu has a high relative affinity to a variety of soils, but its high affinity for soluble organic ligands can greatly influence its mobility. Clay minerals, carbonates or Fe/Mn oxides easily adsorb Zn. As with all cationic metals, Zn adsorption to soil increases with increased pH, but the relatively high solubility of Zn minerals precludes its precipitation (McLean and Bledsoe 1992) unlike Pb and Cu that precipitate with phosphates, hydroxides, etc.

Redox conditions also affect metal mobility (Masscheleyn et al. 1991; Charlatchka and Cambier 2000; Frohnea et al. 2011). Reducing conditions may contribute to greater migration of metals in solution relative to oxidizing conditions. The reduction of Fe(III) to the very soluble Fe(II) generally releases Fe(II) and any
associated metals previously adsorbed to the ferric hydroxide surfaces (McLean and Bledsoe 1992).

Arsenic and Cr exist only as oxyanions in soil environments. Arsenic exists as either arsenate As(V), or arsenite As(III). Arsenite is more toxic and more mobile (Frumkin and Gerberding 2007). Arsenic's solubility is highly influenced by reductive dissolution of Fe and Mg oxides (Castaldi et al. 2013), and carbonate minerals (Meng 2015), ligand exchange (i.e. phosphate competition), redox and pH (McLean and Bledsoe 1992). Cr exists as the cationic, trivalent chromium, Cr(III), and the negatively charged oxyanions, hexavalent chromium, Cr(VI). Cr (III) is found in naturally in soil environments and is easily adsorbed by soils. Hexavalent chromium Cr (VI) is produced by industrial processes and is not expected at this site.

4. Plant-Metal Interactions, and Plant Uptake Mechanisms

Planted systems decrease the compaction of the soil, promoting faster infiltration rates along the root structures and increased organic matter production. It is speculated that although plants allow water to infiltrate more quickly down the soil column, metals complex with dissolved organic matter and increase metal bioavailability.

All plants require nine macronutrients (C, H, N, O, P, S, Ca, Mg, and K) and eight micronutrients (Cl, B, Cu, Fe, Mn, Mo, Ni, and Zn). Excessive levels of the micronutrients, or other non-essential elements (As, Cd, Cr, Ag, Au, Se, and Hg) are potentially toxic. For most species micronutrient and non-essential metal concentrations in plant tissue are low. This is not the case for hyperaccumulators,
which can accumulate metals at concentrations far greater than required for plant
growth (over 1,000 µg/g for Cu, Cr, Pb and Ni or 10,000 µg/g for Mn and Zn) (Baker
and Brooks 1989) and at levels exceeding those present in the soil through
distribution and sequestration of metals in the above ground tissue and by having
an exceptionally high tolerance to heavy metal concentrations (Sheoran et al. 2010).
At least 400 species from 45 plant families have been identified as
hyperaccumulators (Baker and Brooks 1989; Boyd and Martens 1998; Delorme et al.
2001) but they are relatively rare in the environment (Sheoran et al. 2010), and no
known hyperaccumulator species are typically found in BR systems.

Metal uptake into the above ground tissue differs among species, and
between soil and metal type. This is contingent upon the metal bioavailability in the
solid phase, the absorption of the metal by the plant roots, and the translocation of
the metal from the roots to the above ground tissue (Clemens et al. 2002; Bertin et al.
2003; Krämer et al. 2007). Intricate strategies have been developed by plants to
maintain metal homeostasis (extraction or avoidance to maintain stable internal
conditions) within their tissues. All plants have the ability to produce a variety of
root exudates in response to biotic and abiotic stresses (Bertin et al. 2003).
Exudates modify metal speciation and/or sorption characteristics of the soil media
(Meers et al. 2008; Marchand et al. 2010).

Additionally, root exudates mediate plant-microbial associations in the
rhizosphere (Bertin et al. 2003; Borymski and Piotrowska-Seget 2014). The
rhizosphere is the volume of soil, about 0 to 2 mm away from the root surface,
influenced by root activity (Hinsinger 1998). This area of soil contains large
concentrations of microorganisms, primarily bacteria and mycorrhizal fungi. These microorganisms can increase bioavailability of metal ions for plant uptake through the acceleration of redox transformations, and can exude organic compounds that increase metal bioavailability and enable absorption of Mn$^{2+}$, Cd$^{2+}$, and other metal ions in the roots (Sheoran et al. 2010).

The translocation of a metal from root to aboveground tissue controls metal concentrations, and is therefore, a principal parameter in a plant’s bioaccumulation potential. The process starts with the absorption of the metal from the soil-pore water into the root symplasm; release of metal into xylem vessels; and finally transport to the above ground tissue by the transpiration stream (Clemens et al. 2002). The circular plant root is divided into cortex (outer sphere) and stele (inner sphere), separated by the endodermis. Metal ions in the soil-pore water must pass the protective epidermis layer, and then travel via symplastic, apoplastic or transcellular routes across the Casparian strip (endodermis layer) to the vascular tissues of the root that contains the xylem cells. The Casparian strip prevents direct passage of toxic ions to the xylem by implementing selective ion uptake mechanisms (Johnson and Singhal 2010).

Divalent metal cations entering plant root cells are either 1) stabilized in the root by ligands such as phytochelatins or metallothioneins, or 2) complexed with soluble organic matter or ligands such as citrate and are routed into the xylem (Dhankhar et al. 2012; Krämer et al. 2007; Clemens et al. 2002). In the xylem, equilibrium between species in the transpiration stream (chelators, free metal
cations, metal/chelator complexes) and the fixed binding sites in the cell wall is pH dependent.

Once metals are translocated through the xylem to the above ground tissue, the plants use various biological and chemical metal tolerance strategies to detoxify or tolerate excess metals within their cells. These five strategies include: 1) ion sorption to cell walls, 2) active metal efflux from cells, 3) binding with phytochelatins in the cytoplasm, 4) absorption in vacuoles, or 5) increased protein production to chelate metals outside the cell wall (Hall 2002; Johnson and Singhal 2010).

5. Phytoremediation, Bioaccumulation and Enhanced Bioaccumulation

Phytoremediation is the in-situ practice of using plants to clean up contaminated environments, relying on a plant’s ability to intercept, extract and accumulate metals. It is a cost-effective, environmentally friendly alternative to the demanding practice of physically removing contaminated soils (Johnson and Singhal 2010). Phytoremediation can be divided into one of five different pollutant fates: phytostabilization, phytostimulation, phytovolatilization, phytoextractions (bioaccumulation), and phytodegradation (Pilon-Smits, 2005). Klassen et al. (2000) confirmed that different plant species use different phytoremediation strategies for heavy metal detoxification. Their study found that Carex microptera accumulated significantly higher concentrations of the non-essential metal in the plant tissue (bioaccumulation) than Betula occidentalis. However, B. occidentalis sequestered Pb in the root zone by precipitation with phosphorous (phytostabilization). Although
each of these processes contribute to the remediation of soil environments, this review focuses on bioaccumulation.

Bioaccumulation is the process of absorbing and transporting potentially toxic compounds from the soil to the aerial parts of a plant, allowing for the harvest and removal of the plants and sequestered contaminants. Two advantages of bioaccumulation include the reclamation of economically important metals from harvested plant biomass, and the reduction of hazardous waste via postharvest treatments such as composting, compaction, and/or thermal treatment.

Metal removal via bioaccumulation is contingent upon plant biomass yield and metal concentrations in the harvestable plant parts (Meers et al. 2008; Sheoran et al. 2010). Initial research focused on the bioaccumulation potential of metal hyperaccumulator species. Despite the ability of hyperaccumulators to store high concentrations of metals and metalloids in their above ground tissue, they generally produce low levels of biomass. This often makes them inappropriate choices for bioaccumulation.

The bioaccumulation potential of non-hyperaccumulator species with high biomass production can match or exceed hyperaccumulator species (Souza et al. 2013; Meers et al. 2008; Johnson and Singhal 2010). Specifically, non-hyperaccumulator species typically grown in contaminated CW and BR systems can play an important role in phytoextracting metals (Sun and Davis 2007; Marchand et al 2010; Yadav et al. 2011; Ladislas et al. 2013). For example, Liu et al. (2007) found that among 19 species, capacity for Cd, Pb, and Zn accumulation in plant above
ground tissue differed by 47, 60 and 121 fold, and asserts that species selection in CWs significantly influences bioaccumulation potential.

There are two basic strategies for metal bioaccumulation: natural bioaccumulation and enhanced bioaccumulation (Salt et al. 1998; Meers et al. 2008). Bioaccumulation can be enhanced by the use of high biomass-producing plants in conjunction with chemical, biological and naturally occurring amendments in the soil, which enhance metal solubility, and result in increased uptake by the plant (Meers et al. 2008; Johnson and Singhal 2010).

Bioaccumulation can be enhanced by chemical, biological and bulk amendments. Bulk amendments include biosolids, minerals and processing by-products, which improve soil fertility and/or alter chemical speciation of a metal constituent. Biological amendments augment soil media with microbial populations to enhance constituent mobility. Recent biological amendment studies focus on enhanced metal phytoremediation including arbuscular mycorrhizal fungi, specific metal resistant bacteria, or other growth-promoting rhizobacteria (Hassan et al. 2013; Prapagdee et al. 2013; Rahman et al. 2013; Rojas-Tapias et al. 2012; Yang et al. 2013).

Chemical enhanced bioaccumulation can be further separated into six categories of chemical agents: 1) synthetic chelators, 2) organic acids, 3) amino acids, 4) surfactants, 5) phytohormones, and 6) inorganic amendments. Synthetic chelators and organic acids have been found to be very effective in increasing bioaccumulation in many plant species. Ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) are two of the widely researched
synthetic chelating agents proven to improve metal accumulation in above ground tissue (Sinha et al. 2010; Doumett et al. 2008; Seth et al. 2011; Ullah et al. 2011). However, these chelators have major drawbacks including potential risks of toxicity to plants, interference with natural plant uptake strategies, persistence in the environment as a result of slow biodegradability, and the potential for leaching of the mobilized metals into groundwater. For these reasons the wholesale use of synthetic chelating agents for enhanced bioaccumulation is not a defensible option, and further research toward more degradable alternatives such as low molecular weight organic acids (LMWOAs) is proposed (Meers et al. 2008; Sheoran et al. 2010; Souza et al. 2013).

LMWOAs increase constituent solubility by acidification, formation of organic-mineral complexes (Evangelou et al. 2007), and inhibition of metal hydroxide precipitation (Johnson and Loeppert 2006; Pérez-Esteban et al. 2013). Additionally, LMWOAs quickly biodegrade (Meers et al. 2005; Meers et al. 2008). Citric acid (CA) is a LMWOA gaining recognition as an environmentally friendly and cost effective option for increasing metal solubility and increasing bioaccumulation of Cu, Zn, Pb, Cd, and/or Mn for various species (do Nascimento and Xing 2006; Almaroai et al. 2012; de Araújo and do Nascimento 2010; Sinhal et al. 2010; Gao et al. 2012; Freitas et al. 2013; Perez-Esteban et al. 2013; Freitas et al. 2014). Some plants naturally exude CA (and other acids) from roots under phosphorus (P)- and iron (Fe)-deficient conditions (Poschenrieder and Barcelo 2002; Hens and Hocking 2002).
Despite CA’s rapid biodegradation and relatively weak complex stability, it is an effective soil acidifier, it acts as an organic-metal chelate (Martell and Smith 1977; Evangelou et al. 2007), and it inhibits metal hydroxide precipitation (Johnson and Loeppert 2006; Pérez-Esteban 2013). It has been found to be effective organic acid for increasing the metal solubility in soil pore water and uptake into plant tissue (de Araújo and do Nascimento 2010; Mihalík et al. 2011; Duarte et al. 2011; Freitas et al. 2013; Guo et al. 2014). Table 2-1 lists studies which focus on the solubility effects of citric acid and other LMWOAs additions to soil, the metals analyzed, and the concentrations utilized in the study.

Based on the information above, the focus of the greenhouse study was to determine nutrient (N and P) and metal accumulation potential (Cu, Pb, Zn) of species typically grown in BR systems under varying hydraulic and loading regimes. Additionally, the field study evaluated citric acid enhanced bioaccumulation potential for eight metals (Al, As, Ca, Cr, Cu, Fe, Mg, and Zn). This dissertation research attempts to evaluate the use of specific plant species to reduce pollutant accumulation in stormwater BR systems, and species differences of uptake and distribution.

In Chapter 3, species differences in metal accumulation mechanisms were evaluated based on bioaccumulation potential. Results show that differences among species exist in the biological and chemical processes that enhance or impede metal uptake, and that species selection can result in reduced site contamination.

Chapter 4 presents research findings from a simulated stormwater bioretention experiment, and proves that a complete mass balance of TP and TN
Table 2-1: List of studies testing citric acid enhanced metal mobility in soil and plants. Metals analyzed for increased availability as a result of citric acid addition are listed, as is the concentration of CA used in the study.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study focus</th>
<th>Analyzed metal</th>
<th>Species†</th>
<th>Citric Acid (mmol kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peréz-Esteban et al. (2013)</td>
<td>Soil pH 6.2, 5.5</td>
<td>Cu</td>
<td>-</td>
<td>≥ 45</td>
</tr>
<tr>
<td>Meers et al. (2005)</td>
<td>Soil calcareous</td>
<td>Cd, Cu, Pb, and Zn</td>
<td>-</td>
<td>10, 50, 250, 442, 500</td>
</tr>
<tr>
<td>Lesage et al. (2005)</td>
<td>Soil calcareous</td>
<td>Cd, Cu, Pb, and Zn</td>
<td>H. annus</td>
<td>10, 50, 250, 442, 500</td>
</tr>
<tr>
<td>Doumet et al.* (2008)</td>
<td>Potted Plant</td>
<td>Cu, Cd, Pb and Zn</td>
<td>P. tomentosa</td>
<td>1, 5, 10 (tartrate, glutamate)</td>
</tr>
<tr>
<td>Mihalík (2011)</td>
<td>Potted Plant</td>
<td>U, Ra, Fe</td>
<td>S. smithiana Helianthus sp.</td>
<td>25</td>
</tr>
<tr>
<td>Sinhal et al. (2010)</td>
<td>Potted Plant</td>
<td>Cu, Cd, Pb and Zn</td>
<td>T. erecta</td>
<td>10, 20 mg/L 7L every 5 days</td>
</tr>
<tr>
<td>de Araújo and do Nascimento (2010)</td>
<td>Potted Plant (acidic soil)</td>
<td>Pb</td>
<td>Z. mays</td>
<td>0, 5, 10, 30</td>
</tr>
<tr>
<td>Tapia et al. (2013)</td>
<td>Potted Plant</td>
<td>Cu, Fe, Mn</td>
<td>A. halimus R. officinalis</td>
<td>90</td>
</tr>
<tr>
<td>Freitas et al. (2013 and 2014)</td>
<td>Field Study</td>
<td>Pb</td>
<td>S. bicolor Z. mays C. zizanioides</td>
<td>40</td>
</tr>
<tr>
<td>Guo et al. (2014)</td>
<td>Potted Plant</td>
<td>Mn, Al, Fe</td>
<td>P. australis</td>
<td>10, 85, 160</td>
</tr>
</tbody>
</table>

*Doumet et al. used two LMWOAs, tartrate and glutamate, not citric acid.

† Species scientific and common names:

- **Helianthus annus** (sunflower)  
- **Paulownia tomentosa** (empress tree)  
- **Salix smithiana** (willow)  
- **Helianthus sp.** (sunflower)  
- **Tagetes erecta** (marigold)  
- **Zea mays** (maize)  

- **Atriplex halimus** (Mediterranean saltbush)  
- **Rosmarinus officinalis** (rosemary)  
- **Sorghum bicolor** (durra grass)  
- **Chrysopogon zizanioides** (vetiver grass)  

was feasible for six plant species, undergoing three hydraulic and pollutant loads.

Species differences in nutrient retention and uptake were evaluated.
Chapter 5 evaluated the extent of citric acid enhanced bioaccumulation among three plant species grown in a field-site bioretention system. This chapter also presents evidence of species specific sensitivity to citric acid enhanced metal toxicity, and ascertained that citric acid does not increase groundwater metal leaching potential at these CA doses.

Chapter 6 outlines the summary, conclusions, and recommendations derived from the dissertation research.
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CHAPTER 3

BIOACCUMULATION OF COPPER, LEAD, AND ZINC IN SIX MACROPHYTE SPECIES
GROWN IN SIMULATED STORMWATER BIORETENTION SYSTEMS

Abstract:

Stormwater bioretention (BR) systems collect runoff containing heavy metals, which can concentrate in soil environments and potentially leach into groundwater. This greenhouse experiment evaluated differences among six plant species undergoing three varying hydraulic and pollutant loads in their bioaccumulation potential when subjected to continual application of low metal concentrations as a means of preventing copper, lead, and zinc accumulation in the BR soil. Results show that >92% of metal mass applied to the treatments via synthetic stormwater was removed from the exfiltrate within 27 cm of soil depth. Compacted soil conditions of unplanted controls retained significantly more Cu, Pb, and Zn than Carex praegracilis, and Carex microptera treatments. Differences in above and below ground plant tissue concentrations differed among species, resulting in significant differences in mass accumulation. In the above ground tissue, from highest to lowest, Phragmites australis accumulated 8 times more Cu than Scirpus acutus, and Carex microptera accumulated 18 times more Pb, and 6 times more Zn than Scirpus validus. These results, and differences among species in mass distribution of the metals recovered at the end of the study, reveal various metal accumulation mechanisms.
1. Introduction:

Trace metals such as copper, lead, and zinc (Cu, Pb, and Zn) accumulate in stormwater runoff where they concentrate in soil environments, and potentially leach into the groundwater once the soil sorption capacity is reached. Bioretention (BR) systems are a type of stormwater best management practice (BMP) that utilize soil and plants to treat stormwater runoff from commercial, residential, and industrial areas by allowing stormwater to collect and infiltrate into the underlying soils (U.S. EPA 1999).

It is well documented that BR systems remove significant quantities of nutrients and metals from stormwater runoff (Tanner 1996; Fraser et al. 2004; Davis et al. 2006; Read et al. 2008; Trowsdale and Simcock 2011; Fassman 2012; Li et al. 2014). The efficiency of pollutant removal is often defined as the difference in pollutant concentrations, or mass, between the influent and exfiltrate water. This implies that metals are retained in the soil or plant components of these systems. Previous investigations indicate that more than 80% of the metal pollutants retained in BR systems accumulate in the soil (Sun and Davis 2007; Marchand et al. 2010). The sorption capacity of soils is finite, and continuous application of metals may increase the risk of toxic metal buildup and subsequent leaching to groundwater. Davis et al. (2003) estimated that in the BR system they studied, Pb and Zn accumulation from runoff would reach or exceed regulatory limits for biosolids application (U.S. EPA 1993) after 16 years of continuous use, and concluded that long-term accumulation of metals is an unintended consequence of treating stormwater in BRs.
BR systems are stressful environments for plant growth due to periods of flooding and pollutant loading, followed by long dry periods. Certain plant species are more capable of thriving in these hydraulic and pollutant loading extremes than others. Additionally, certain species contribute to higher levels of metal removal efficiencies under similar conditions (Read et al. 2008). Bioaccumulation potential for these plants is based on their ability to absorb and transport potentially toxic compounds, such as trace metals, from the soil to the aerial parts of a plant, allowing for the harvest and removal of the plant biomass which contains these toxic compounds. The effectiveness of metal uptake is contingent upon plant biomass yield and metal concentrations in the harvestable plant parts (Meers et al. 2008; Sheoran et al. 2011). Processes that influence accumulation include: 1) mobilization from soil, 2) uptake and sequestration in the roots, 3) efficiency of xylem transport, and 4) transport and storage into the aerial tissue (Clemens et al. 2002).

Most bioaccumulation studies focus on the remediation of highly contaminated soils at mining and industrial sites, with high soil metal concentrations (Stottmeister et al. 2003; Wiessner et al. 2006; Marchand et al. 2010; Yadav et al. 2011; Narhi et al. 2012; Ladislas et al. 2013). Liu et al. (2007) found that among 19 species, capacity for Cd, Pb, and Zn accumulation in aerial tissue differed by 47, 60, and 121 fold, respectively, and asserts that species selection in constructed wetlands significantly influences metal uptake (bioaccumulation) potential. However, these studies do not consider the bioaccumulation potential of plants grown in soils with continual application of low metal concentrations, nor do they assess bioaccumulation as a means of slowing that rate of metal accumulation.
in BMP soils. Research focusing on the bioaccumulation potential of plants in lower-level contamination conditions is vital to understand how to reduce, slow, and potentially prevent metal accumulation and the resulting formation of hazardous sites at BMPs.

Additionally, it is difficult to make definitive conclusions about the processes and mechanisms that affect BR system metal removal performance. Reasons for this include a lack of consistency in experimental methods, a wide variation in the reporting of results (load reduction versus concentration change), and a lack of explicit analysis of the fate of constituents in the plant, soil and water phases. Only two known studies investigated the fate of pollutants in plant, soil and water phases of BR systems (Sun and Davis 2007; Borin and Salvato 2012). Davis et al. (2009) concluded that despite the numerous studies being done regarding pollutant removal, many BMP design questions persist, such as which vegetative species provide the greatest metal bioaccumulation potential. Barrett et al. (2013) noted that it is important that any new research is conducted under controlled conditions and that detailed information be developed on the properties of the medium being tested.

A comprehensive exploration of Cu, Pb, and Zn bioaccumulation potential in this study investigates differences among six plant species undergoing three varying stormwater hydraulic and pollutant loading rates. The specific objectives of the study were to: 1) quantify differences in metal retention within simulated BR systems among six species undergoing hydraulic and metal loading typical of stormwater BR systems; 2) identify differences among species in the retention of Cu,
Pb, and Zn within the plant above ground (AG) and below-ground (BG) tissue based on biomass concentration and total harvested biomass measurements; and 3) evaluate differences in metal accumulation mechanisms used by these species in response to these hydraulic and pollutant loading conditions.

2. Materials and Methods:

2.1 Experimental Design

This study, conducted at Utah State University's Research Greenhouse from October 2010 through June 2011, used a randomized block design with six plant species and synthetic stormwater to represent three hydraulic, nutrient and metal loading regimes, in triplicate. The concentrations of the response factors (Cu, Pb, and Zn) were measured in the exfiltrate, soil, and above ground (AG) and below ground (BG) plant tissue.

Treatment containers were built in Sterilite® polypropylene and polyethylene 19 L containers (surface area of 0.143 m²). Each container was filled with 21 kg of soil consisting of 50% Kidman Sandy Loam (coarse-loamy, mixed, mesic Calcic Haploxeroll) and 50% sand, which enhanced water flow in this small-scale mesocosm study.

The six plant species most frequently found in constructed wetland BMPs (Brisson and Chazarenc 2009), and commonly identified in stormwater BMPs in Northern Utah (Rycewicz-Borecki and Winkler 2009) were chosen for this study. The species investigated included: Phr - *Phragmites australis* (Common Reed); Typ – *Typha latifolia* (Broadleaf Cattail); Scv - *Scirpus validus* (Soft-stem Bulrush); Sca -
Scirpus acutus (Hard-stem Bulrush); Cap - Carex praegracilis (Common field sedge); and Cam - Carex microptera (Smallwing sedge). Six plugs, obtained from Aquatics and Wetland Nursery, Ft. Lupton, Colorado, were planted equidistantly within each container. Due to the availability of plants from the nursery, treatment containers were constructed at two different time periods, 1 month apart. The controls for the study were non-vegetated containers filled only with the soil-sand mixture. Sunlight Supply’s 1000 W high-pressure sodium bulbs illuminated the greenhouse using a photoperiod of 12 hours per day.

Soil in each container was weighed prior to planting. Initial soil samples were collected from each container and analyzed for nutrient and metal concentrations during the establishment period, prior to synthetic stormwater application. Significant differences in background nutrient and metal concentrations were found between containers constructed during the two time periods (Table 3-1). As a result, each container’s individual initial and final constituent soil concentrations were used for all subsequent calculations. Plants were allowed to grow in the sand-soil mixture to establishment (rooted and producing new growth) for 6 months before synthetic stormwater was applied, and water sample collection began.

Each species was planted in triplicate containers under three hydraulic and metal loading regimes representing Logan, UT; Des Moines, IA; and Scranton, PA. These three cities are located along the 41°N latitude and are 18° longitudinally apart. Thus, the total number of containers monitored was 63 (6 x 3 x 3 + 9 = 63). Rainfall frequency, intensity and duration (hydraulic loading) were calculated from rainfall data from each city from 2005 to 2009 using the Driscoll method (Driscoll et al.
Table 3-1: Soil properties, and nutrient and metal concentrations (mg kg\(^{-1}\) dry soil) in the soil-sand mixtures used to construct test BR systems, along with selected properties of City of Logan tap water used in the study.

<table>
<thead>
<tr>
<th></th>
<th>SOIL-SAND MIXTURE</th>
<th>TAP WATER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactor Batch 1</td>
<td>Reactor Batch 2</td>
</tr>
<tr>
<td>pH</td>
<td>8.2 ± 0.03</td>
<td>7.3 ± 0.07</td>
</tr>
<tr>
<td>EC (µS cm(^{-1}))</td>
<td>630 ± 80</td>
<td>2,330 ± 100</td>
</tr>
<tr>
<td>Alkalinity (mg CaCO(_3) L(^{-1}))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CEC (meq 100g(^{-1}))</td>
<td>1.3 ± 0.1</td>
<td>1.6 ± 0.09</td>
</tr>
<tr>
<td>Organic Matter (%)</td>
<td>0.3 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Saturation (%)</td>
<td>25.6 ± 0.4</td>
<td>22.7 ± 1.1</td>
</tr>
<tr>
<td>Particle size distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand (%)</td>
<td>91.7 ± 0.3</td>
<td>88.7 ± 0.3</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>2.3 ± 0.3</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>6.0 ± 0.0</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td>Nutrient concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP (mg kg(^{-1}))</td>
<td>83.3 ± 5.0(^a)</td>
<td>142 ± 24(^a)</td>
</tr>
<tr>
<td>TN (mg kg(^{-1}))</td>
<td>476 ± 25(^a)</td>
<td>690 ± 26(^a)</td>
</tr>
<tr>
<td>TMetal concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu (mg kg(^{-1}))</td>
<td>1.1 ± 0.1(^a)</td>
<td>2.3 ± 0.13(^a)</td>
</tr>
<tr>
<td>Pb (mg kg(^{-1}))</td>
<td>0.9 ± 0.1(^a)</td>
<td>2.1 ± 0.1(^a)</td>
</tr>
<tr>
<td>Zn (mg kg(^{-1}))</td>
<td>6.2 ± 0.3(^a)</td>
<td>7.3 ± 0.3(^a)</td>
</tr>
</tbody>
</table>

mean ± SE; n=3 unless otherwise noted

\(^a\)Batch 1 n=22; Batch 2 n=7

1989). This method is used in the EPA Stormwater Best Management Practice (BMP) Design Guide (U.S. EPA 2004) to generate typical values of individual storm event statistics for 15 climate zones in the United States. The calculations carried out here are more specific to the study locations and use recent rainfall data rather than a region’s average values provided by GeoSyntec and ASCE (2002).

Pollutant total mass in the synthetic stormwater (Table 3-2) was based on the reported average pollutant event mean concentrations (EMCs) from three regional locations selected from the EPA BMP Design Guide (U.S. EPA 2004). As per general BMP guidelines (U.S. EPA 2002) the surface area of the study containers was...
Table 3-2: Synthetic Stormwater Total Mass Load for the Low, Medium, and High Loading Regimes, calculated over the experimental period of 27 weeks and the Regional EPA Event Mean Concentrations (EMCs) on which loading was based.

<table>
<thead>
<tr>
<th>Synthetic Stormwater</th>
<th>Regional EPA EMCs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>Total Mass Load</em> by Regime</em>*</td>
<td><strong>Logan</strong></td>
</tr>
<tr>
<td>Low (Logan)</td>
<td>Medium (Des Moines)</td>
</tr>
<tr>
<td>Total-P (mg)</td>
<td>300</td>
</tr>
<tr>
<td>Total-N (mg)</td>
<td>2,034</td>
</tr>
<tr>
<td>Cu (mg)</td>
<td>51.8</td>
</tr>
<tr>
<td>Pb (mg)</td>
<td>50.5</td>
</tr>
<tr>
<td>Zn (mg)</td>
<td>156</td>
</tr>
<tr>
<td>Tot. Water (L)</td>
<td>462</td>
</tr>
<tr>
<td>Event Volume (L)</td>
<td>14.4</td>
</tr>
<tr>
<td>No. of Events</td>
<td>32</td>
</tr>
</tbody>
</table>

*total applied mass after 27 weeks of synthetic stormwater application

set to 5% of an adjacent urban area, and the water volume for each rain event was calculated with a 50% impervious surface runoff coefficient.

Each rainfall event's constituent mass was applied to each container at the start of a rain event in a concentrated initial flush solution, simulating the ‘first flush’ of a rainfall event. The tap water (Table 3-1) was then added to the initial flush solution at the intensity and over the calculated duration of each storm to represent the remainder of each storm runoff volume. TN, TP, Cu, Pb and Zn concentrations in the tap water were measured and pollutant loading contributed by the tap water was added to the total mass input to each system. Total constituent input mass to each container was calculated as:

\[
M_{\text{in water}} = \left( \sum_{i=1}^{n} (C_{EMC} + C_{tap})V_i \right)
\]  

(1)
where \( M_{\text{in \, water}} \) is the metal mass input in the synthetic stormwater, \( C_{\text{EMC}} \) is the constituent EMC based on loading regime, \( C_{\text{tap}} \) is the concentration of the pollutant of interest in the greenhouse tap water, \( V_i \) is the input volume (L) applied for a given event, \( i \), and \( a \) is the number of runoff events for the loading regime based on rainfall duration and frequency calculations throughout the 27 week experiment (Table 3-2). Tap water concentrations (Table 3-1) were high in metal concentrations due to the age of the copper water supply lines in the research greenhouse. Table 3-2 presents a summary of the regional constituent EMCs, event volumes, and number of events for each region. Additionally, total mass of constituents, for each loading regime, is presented.

The highest EMCs for all constituents were reported for Logan, UT (Table 3-2). However, Logan also received the lowest rainfall intensity and frequency, producing the lowest total constituent mass loads. The total constituent mass (over the 27 week period) and hydraulic loading regimes (total water volume) for the three cities were categorized as Low (Logan), Medium (Des Moines), and High (Scranton). Only one constituent mass load did not follow this trend; Pb mass for the Medium Load was 118 mg, while it was 83.5 mg for the High Load (Scranton) based on EMC concentrations and runoff volumes for these respective regions.

Synthetic stormwater filtered through the soil-sand mixture and flowed out a half-inch diameter opening located at the bottom of each container. Drainage tubes (60-cm length) were installed at each opening to collect the excess water (exfiltrate) from the planted containers directly into a 38-L Sterilite® container during composite-sampling events. The volumes of the exfiltrate were recorded, and
composite subsamples of the exfiltrate were collected at the beginning (Weeks 1, 2, 3), middle (Weeks 14, 15, 16), and end (Weeks 25, 26, 27) of the study period. The percent water volume retained for each container for each of the species in the greenhouse study was calculated as:

\[
\% \text{ volume retained} = \left(1 - \left( \frac{\left(\sum_{j=1}^{9} V_j \ast a\right)}{\sum_{j=1}^{9} V_j} \right) \right) \ast 100
\]  

(2)

where \( V_j \) is the exfiltrate volume (L) measured at each sampling event, \( j \).

Percent mass retained in each container was calculated as:

\[
M_{\text{out water}} = \left( \frac{\sum_{j=1}^{9} (C_j V_j) \ast a}{9} \right)
\]  

(3)

\[
\% \text{ mass retained} = \left(1 - \left( \frac{M_{\text{out water}}}{M_{\text{in water}}} \right) \right) \ast 100
\]  

(4)

where \( M_{\text{out water}} \) is the total metal mass in the exfiltrate water, and \( C_j \) is the measured composite metal concentrations (mg L\(^{-1}\)) of the exfiltrate samples for each sampling event, \( j \).

At the end of the study all AG and BG plant material was harvested, and soil samples were collected. The AG samples consisted of various combinations of leaves, stems, flowers, seeds, etc., depending on the species being harvested. All BG tissue was thoroughly washed in sodium lauryl sulfate, followed by washing in 0.01M HCl, and finally a thorough rinse with deionized water. All plant tissue was oven dried (60°C) for >72 hours, weighed, and ground at the USU Greenville Research Farm. Total metal mass (mg) accumulated in the AG and BG tissue (\( M_{\text{AG}} \) and \( M_{\text{BG}} \)) was calculated as:
\[ M_{AG} = C_{AG} m_{AG} \]  
\[ M_{BG} = C_{BG} m_{BG} \]  

where \( C_{AG} \) and \( C_{BG} \) are metal concentrations (mg kg\(^{-1}\)) in the AG and BG tissue, respectively; and \( m_{AG} \) and \( m_{BG} \) are the corresponding total AG and BG dry weights (kg).

Roots had penetrated throughout the container, so all soil below the top 3.8 cm were considered as rhizosphere soil. Surface soil (the upper 3.8 cm of soil) and rhizosphere soil (the lower 23.2 cm of soil) samples were composited from three randomly selected subsamples collected from their respective soil horizons. Moisture content of the soil samples was determined after oven-drying the samples at 103°C for >12 hours. Total metal mass (mg) accumulated in the soil-sand mixture \( (M_{soil}) \) was calculated as:

\[ M_{soil} = m_{soil} \left( \frac{3.8}{27} (C_{surf} - C_{initial}) + \frac{23.2}{27} (C_{rhizo} - C_{initial}) \right) \]

where \( m_{soil} \) total dry mass of soil-sand mixture (kg) in each container, and \( C_{initial} \), \( C_{surf} \) and \( C_{rhizo} \) are the metal concentrations (mg kg\(^{-1}\)) in the initial soil, surface soil, and rhizosphere soil, respectively.

Metal distribution within the BR systems was calculated using a mass balance approach:

\[ M_{in\,water} = M_{out\,water} + M_{AG} + M_{BG} + M_{soil} \]

where \( M_{in\,water} \) is the total metal mass input in the synthetic stormwater. The accumulated mass is the sum of mass in the exfiltrate \( (M_{out\,water}) \), AG tissue \( (M_{AG}) \), BG tissue \( (M_{BG}) \), and soil-sand mixture \( (M_{soil}) \), respectively.
High percent recoveries of applied mass (82.6% ± 12%, 100% ± 13%, and 90.6% ± 10%, for Cu, Pb, and Zn, respectively) confirm accuracy within measurements, sampling procedures, and laboratory analysis, and provide confidence in the results of this study.

2.2 Laboratory Analytical Methods:


All exfiltrate, AG and BG tissue, and soil samples were analyzed for metal concentrations at the Utah Water Research Laboratory (UWRL). Water samples were digested using the APHA hot block nitric acid digestion Method 3030E (APHA 1999) for total Cu, Pb, and Zn analysis. Soil samples were digested with nitric acid and hydrogen peroxide, and analyzed for total Cu, Pb, and Zn concentrations using a modified EPA Method 3050B for use with the Environmental Express HotBlock Digestion System (U.S. EPA 1996). Plant nitric acid digestion followed the Jones and Case (1990) method.

Once digested, analysis for water, plant and soil samples’ total metal concentrations was conducted on an Inductively Coupled Plasma Mass spectrometer (ICP-MS, Agilent 7500c) using SW-846 method 6020a (U.S. EPA 2007).
2.3 Statistical analysis:

Prior to one-way and two-way analysis of variance (ANOVA), data were log transformed to ensure a random distribution of residuals. The log transformed percent mass retained (Equation 4) values did not have a random residual distribution; therefore, these data were arcsine transformed. Post-hoc comparisons of means were done using the Tukey’s HSD test (p=0.05) on the transformed data. Statistical analyses were performed using the R statistical program (R Development Core Team 2013). Measurement variability is represented by the standard error of the means in tables and in graphical form throughout this paper.

3. Results and Discussion:

Stormwater BR systems are subjected to variable hydraulic and nutrient loads depending on size, design, and location. Nutrients and metals were applied under a range of three loading regimes, but this study focused on quantifying differences in metal retention, bioaccumulation, and metal distribution among six plant species. Analysis of results concluded that significant species differences sometimes occur within a given loading condition, but that an overall trend among species is most clearly observed when analyzing the results from all loads grouped together. For this reason, the results discussed below focus on species factor interactions for combined loading regimes. Differences in load interactions are discussed only when relevant.
3.1 Water volume and metal mass retention:

The percent water volume retained (Equation 2, Table 3-3) in the planted containers ranged from 17.0% to 33.3%, where the average for all planted treatments was approximately one-fourth of the input volume (averaging 23.2%), due to soil water exfiltration and/or evapotranspiration. The unplanted controls were highly susceptible to soil compaction, resulting in high levels of ponding (41.5% ± 4.2% volume retained). Due to the absence of roots in the unplanted controls, water surface evaporation increased and water movement through the soil decreased. This produced significantly higher percent water volume retention in the controls compared to all of the species, except Typ. The ponding water produced less exfiltrate, but increased the potential for anaerobic conditions in the saturated soils.

<table>
<thead>
<tr>
<th>Phr: Phragmites australis; Typ: Typha latifolia; Scv: Scirpus validus; Sca: Scirpus acutus; Cap: Carex praegracilis; Cam: Carex microptera;</th>
<th>Mean ± SE, n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different lower-case letters across the row indicate significant difference among species and control, based on Tukey HSD (P&lt;0.05). Values highlighted in blue (a) are significantly higher than values highlighted in peach.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Vol Retained</th>
<th>Phr</th>
<th>Typ</th>
<th>Scv</th>
<th>Sca</th>
<th>Cap</th>
<th>Cam</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.3 c ± 2.8</td>
<td>33.3 ab ± 4.8</td>
<td>17.0 b ± 1.1</td>
<td>20.8 c ± 2.0</td>
<td>23.3 bc ± 1.7</td>
<td>22.3 bc ± 1.6</td>
<td>41.5 a ± 4.2</td>
<td></td>
</tr>
<tr>
<td>% Cu Retained</td>
<td>97.5 abc ± 0.4</td>
<td>97.8 ab ± 0.5</td>
<td>97.9 ab ± 0.1</td>
<td>97.9 ab ± 0.3</td>
<td>96.5 c ± 0.4</td>
<td>96.6 bc ± 0.5</td>
<td>98.6 a ± 0.2</td>
</tr>
<tr>
<td>% Pb Retained</td>
<td>97.8 bcd ± 0.6</td>
<td>99.5 ab ± 0.1</td>
<td>99.2 ab ± 0.2</td>
<td>98.9 abc ± 0.4</td>
<td>95.4 d ± 1.7</td>
<td>96.5 cd ± 1.0</td>
<td>99.9 a ± 0.0</td>
</tr>
<tr>
<td>% Zn Retained</td>
<td>94.8 ab ± 1.2</td>
<td>95.8 a ± 0.7</td>
<td>96.3 a ± 0.4</td>
<td>95.7 a ± 1.1</td>
<td>91.7 c ± 1.8</td>
<td>92.3 b ± 1.5</td>
<td>97.6 a ± 0.4</td>
</tr>
</tbody>
</table>
The percent of Cu, Pb, and Zn mass retained (Equation 4) quantifies the reduction of metal mass in the exfiltrate as a function of the metal mass added in the synthetic stormwater (Table 3-3). Standard errors of all parameters were relatively small among the treatments, indicating consistency among the experiments. The metal retention in planted systems with Phr (with the exception of Pb), Typ, Scv and Sca were not different from the unplanted control whereas the two sedge species, Cap and Cam, retained less Cu, Pb and Zn.

Overall retention of Cu, Pb, and Zn was very high (96.5% to 98.6%, 95.4% to 99.9%, and 91.7% to 97.6%, respectively), indicating a high affinity of these three metals to the soil media and the plant biomass within the planted systems. The high metal removal efficiencies agree with those reported by Davis et al. (2003), Weiss, et al. (2006), and Murakami et al. (2008). They are higher, however, than results from published studies reviewed by the Center for Watershed Protection (2007), which reported BR removal efficiency for Cu averaging 81% and 79% for Zn (n=5, no data were presented for Pb). It is speculated that these lower removal efficiencies are due to differences in sampling procedures (effluent surface water sampling versus exfiltrate sampling), soil type, soil adsorption capacity, and the design of BR units.

The general trend of percent retention in the BRs was Pb>Cu>Zn, which is supported by the weaker association of Zn to soil than Cu and Pb (McLean and Bledsoe 1992). This is different than results reported by Davis et al. (2003), and Sun and Davis (2007), where Cu retention in the BR was lower than Pb and Zn based on influent and effluent concentrations. We speculate that this difference is due to the differences of metal concentration inputs into the system, and their use of an EMC
efficiency measurement, where this study bases efficiency on mass retention measurements.

3.2 Plant Biomass

The dry biomass of the six species harvested at the end of the study was compared as a function of loading and species (Figure 3-1). AG biomass was significantly different among species, and among loading regimes, where the high-loading regime produced significantly more biomass than the medium and low loadings. This suggests that plant growth positively correlates with increases in water and nutrient load, and indicates that the added metal loads were within tolerance limits of these species.

Among species, Phr produced significantly more biomass than all of the other species, and Cap and Cam produced significantly more biomass than Scv and Sca, confirming our hypothesis that some species, in this case Phr, Cap, and Cam, thrive more than others in the increased hydraulic, nutrient, and metal loading regimes common in BR systems. Unlike AG biomass production analysis of BG biomass production showed no significant differences among species, with an average biomass of 176.4 ± 10.0 g across all species. Significant differences were only found among loading regimes, where the low loading regime produced significantly less BG biomass than the medium and high regimes (data not shown).
Figure 3-1: AG biomass production of different plant species at the low, medium and high pollutant loading regimes. Mean AG biomass increases with hydraulic and constituent loading, as reflected in the positive slope of each of the trend lines. Error bars represent the standard error (n=3).

Phr: Phragmites australis; Typ: Typha latifolia; Scv: Scirpus validus; Sca: Scirpus acutus; Cap: Carex praegracilis; Cam: Carex microptera.

Lower-case letters refer to Tukey HSD post-hoc comparison (P<0.05) among species for all loadings.

Upper-case letters refer to Tukey HSD post-hoc comparison (P<0.05) among loading regimes for all species.

3.3 Plant metal concentrations and bioaccumulation

Metal uptake into plant tissue is known to differ among species, and between soil and metal type. The BG and AG tissue concentrations of Cu, Pb, and Zn for each species are presented in Figure 3-2a and 3-2b. AG concentrations ranged from 13.3 to 56.4 mg kg\(^{-1}\) for Cu; 5.5 to 44.5 mg kg\(^{-1}\) for Pb; and 51.5 to 166 mg kg\(^{-1}\) for Zn. BG tissue concentrations were higher, ranging from 19.5 to 130 mg kg\(^{-1}\) for Cu; from 5.2 to 24.2 mg kg\(^{-1}\) for Pb; and from 60.7 to 240 mg kg\(^{-1}\) for Zn. These concentrations are
well below the defined hyperaccumulator concentrations of >1,000 mg kg\(^{-1}\) for Cu and Pb, and >10,000 mg kg\(^{-1}\) for Zn. However, the concentrations are within the ranges reported for plants grown on contaminated soils (Yoon et al. 2006; Liu et al. 2007). For example, the native species in Yoon et al. (2006) were found to have AG and BG tissue concentrations of Cu, Pb, and Zn of 6 to 460, 20 to 1183, and 17 to 598 mg kg\(^{-1}\), respectively. Previous studies regarding metal concentrations in plants grown on metal-laden soils also found higher concentrations of metals in the roots than in the AG tissue (Dahmani-Muller et al. 2000; Pilon-Smits and Pilon 2002; MacFarlane, et al. 2003; Xia 2004) supporting current findings, with the exception of Pb.

Bioaccumulation is affected by metal bioavailability in the soil, rate of absorption by the plant roots, and translocation from the roots to the AG tissue (Clemens et al. 2002; Bertin, et al 2003; Krämer, et al. 2007). Most species utilize root bioactivation mechanisms to enhance root absorption (Salt and Rauser 1995; Khan et al. 2000). These mechanisms include acidification by the rhizosphere (Bernal et al. 1994; Clemens, et al. 2002; Ghosh and Singh 2005); secretion of organic acids, metal chelates, or enzymes to increase available ion concentrations (Cakmak et al. 1996; Yang and Roemheld 1999; Ma, et al. 2001); and/or promotion of microorganism growth.

Phr, Cap, and Cam have significantly higher (approximately twice as high) root concentrations than Scv, Sca, and Typ. It is speculated that Phr, Cap, and Cam either activate root mechanisms to enhance metal mobilization, uptake and sequestration into the root cells, or at a minimum, do not restrict metal uptake.
Figure 3-2: Concentrations of Cu, Pb, and Zn in the BG (a) and AG tissues (b) and harvestable mass (c) in the AG tissue for low, medium and high loadings. Phr: *Phragmites australis*; Typ: *Typha latifolia*; Scv: *Scirpus validus*; Sca: *Scirpus acutus*; Cap: *Carex praegracilis*; Cam: *Carex microptera*.

Error bars are the standard error, n=9. Values within a row followed by the same lower-case letter are not significantly different among species (P<0.05), based on Tukey HSD. Note different scales on y-axis.
Alternatively, Typ has the lowest metal root concentrations, significantly lower than all other species, indicating that Typ possibly implements selective ion uptake mechanisms to restrict metal ion transport across the root epidermis and entry into the root apoplast.

The AG tissue concentrations also differ among species. Interestingly, despite low BG concentrations Typ’s AG concentrations are among the highest, where Typ, Cap, and Cam have significantly higher concentrations than Scv and Sca. It is speculated that Typ actively restricts metal uptake into its roots, but is highly efficient at the transport of ions inside the xylem and their storage in the AG tissue. In contrast, Phr BG concentrations are considerably higher than its AG concentrations for Cu and Zn, indicating a higher tendency to uptake and sequester metals in the root, and less capacity for xylem transport and storage in the AG tissue.

Overall, more metal mass was stored in the BG than AG tissue, with the exception of Typ. However, making beneficial use of metal bioaccumulation depends on the metal uptake into the AG plant tissue (Equation 5), and its harvest and removal off-site. Roots are typically not harvested in order to support yearly plant regrowth. For this reason, total bioaccumulation of metal mass in AG is presented in Figure 3-2c, where Phr, Typ, Cap, and Cam consistently accumulated significantly more metals in the AG tissue than Scv and Sca. Mass accumulation differences (from highest to lowest) were eight times higher for Phr than Sca for Cu; Cam accumulated 18 times more Pb than Scv; and Cam accumulated six times more Zn than Scv, highlighting differences in bioaccumulation potential among species.
3.4 Metal distribution at end of study

Based on an analysis of metal mass in each compartment at the beginning and end of the study it was shown that metal mass from the synthetic stormwater adsorbed to the surface soil, traveled down through the BR media into the rhizosphere soil layer, was taken up into the plant tissue, and/or moved down through the BR system via the exfiltrate.

Movement through the soil layers was confirmed with analysis of surface and rhizosphere soil concentrations (B-1a), which showed increased metal concentrations from the beginning to the end of study. Differences were also found among species in Cu and Pb rhizosphere soil concentrations (Table B-1b), indicating that root-soil interactions, which affect metal bioavailability to the various species, impact metal concentrations that develop within the soils.

Analyzing the fate of metals within BR systems can illuminate differences in a plant’s uptake mechanisms, and bioaccumulation potential. The percent distribution of accumulated metals recovered at the end of the study is presented in Table 3-4. For the planted systems, the majority of total metals were captured in the soil-sand mixture (64.9-97.0%), 0.0-10.8% passed through the system via the exfiltrate, 1.3-25.8% accumulated in the BG tissue, and 0.3-5.8% accumulated in the harvestable AG tissue. The unplanted control treatments captured a significantly higher
Table 3-4: Percent distribution of recovered Cu, Pb, and Zn mass at the end of study among the exfiltrate, above ground, below ground, and soil compartments for the six species and unplanted control. Phr: *Phragmites australis*; Typ: *Typha latifolia*; Scv: *Scirpus validus*; Sca: *Scirpus acutus*; Cap: *Carex praegracilis*; Cam: *Carex microptera*. Mean ± SE, n=9.

<table>
<thead>
<tr>
<th></th>
<th>Copper Distribution (%)</th>
<th>Lead Distribution (%)</th>
<th>Zinc Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phr</td>
<td>Typ</td>
<td>Scv</td>
</tr>
<tr>
<td>Exfiltrate</td>
<td>2.5 a ± 1.3</td>
<td>9.2 a ± 14.9</td>
<td>3.1 a ± 1.2</td>
</tr>
<tr>
<td>AG</td>
<td>3.6 a ± 2.6</td>
<td>3.6 a ± 2.1</td>
<td>0.7 b ± 1.3</td>
</tr>
<tr>
<td>BG</td>
<td>18.6 ab ± 7.9</td>
<td>7.8 b ± 7.8</td>
<td>9.9 b ± 7.6</td>
</tr>
<tr>
<td>Soil</td>
<td>75.3 bc ± 9.2</td>
<td>79.4 bc ± 23.3</td>
<td>86.2 ab ± 7.8</td>
</tr>
</tbody>
</table>

Values within a row followed by the same lower-case letter are not significantly different among species (P<0.05), based on Tukey HSD. Values shaded in blue are significantly higher than values shaded in peach within each row.
percentage of metals (96.1-99.9%) in the soil-sand mixture than Cap and Cam (except Cu for Cap). Scv, Cap, and Cam also had higher percentages of Pb (also Zn for Cam) in the exfiltrate than the unplanted controls, which were more susceptible to soil compaction than planted systems, as discussed in Section 3.1.

The percent mass distributions measured in the plant (AG plus BG) compartment in this study (10.6-30.4% Cu, 2.4-8.7% Pb, 8.5-21.9% Zn) are higher than reported in Sun and Davis (2007), where only 0.6-1.8% Cu, 0.5-1.4% Pb, and 1.4-3.3% Zn accumulated in the whole plant. It is speculated that these differences are due to the addition of leaf mulch in the Sun and Davis soil media, which may have provided even higher adsorption capacity than that of native soil. In addition, Sun and Davis evaluated three grass species (*Panicum virgatum, Kentucky-31, and Bromus ciliatus*) with low biomass density (177-263 g m\(^{-2}\)) compared to this study's six macrophyte species typically found in BR systems with higher biomass (291-1203 g m\(^{-2}\)) densities.

Differences in metal distribution in the soil, plant, and exfiltrate among the plant species evaluated in this study support the hypothesis that these species implement various biological and chemical mechanisms to facilitate or restrict their metal uptake. In general, the two sedge species, Cap and Cam, appear to increase metal solubility in the soil, leading to the increase of metal mass in both the AG and BG tissue relative to the other species (Table 3-4). Increased metal mobility is further supported by higher exfiltrate concentrations compared to the control treatments (Table B-1c, except Cu for Cap).
4. Summary and Conclusions:

Phytoremediation is commonly practiced on heavily contaminated soils, and utilizes plants that can attain high pollutant concentrations in their AG tissue with low to moderate biomass production (hyperaccumulators), or species with high biomass production that can attain moderate AG tissue concentrations (bioaccumulators). This study shows that certain species commonly grown in stormwater BMPs, undergoing continual but relatively low pollutant application, can be used to reduce future site contamination by harvesting their biomass after having bioaccumulated heavy metals loaded to these BMPs.

The results of this study confirmed that more than 92% of input metals were removed within 27 cm of soil depth in planted and unplanted treatments. Biomass production of the harvestable AG tissue increased with increased water, nutrient and metal loading, indicating that at the concentrations used in this study, metal toxicity limits were not reached. Significantly more biomass was produce by Phr, Cap and Cam than Scv and Sca.

Differences among species were found for AG and BG tissue concentrations, AG metal uptake, and metal distribution, indicating that species selection can play an important role in metal harvestability from BR systems. Based on the three loading regimes and six species evaluated in this study, it was found that the Phr, Typ, Cap and Cam species typically yielded higher concentrations and mass accumulation of Cu, Pb, and Zn in the AG tissue than Scv and Sca.

The total accumulated metal mass in the harvestable AG plant tissue was significantly lower than adsorption to soil media (Table 3-4). This suggests that
simply harvesting vegetation may not be a viable metal control option unless BR sites are managed for optimal biomass yields (Sun and Davis 2007). In a separate field study conducted by the authors of this article, vegetation was successfully managed for high biomass density, with significant (≥50%) increases of biomass yield each year over a 4-year observation period (Figure B-1). Additionally, induced bioaccumulation (e.g., various acidifying agents, fertilizer salts, or chelating agents) can increase metal solubility in the soil-pore water and may increase metal concentrations in the plant, potentially allowing for greater metal mass uptake and removal via AG biomass harvesting.

Potential differences in biological and chemical processes developed by species to allow or avoid metals were also found. Cap and Cam species are speculated to promote metal mobilization as suggested by higher metal distributions in the AG and BG tissue and exfiltrate, and lower distribution in the soil media, whereas Phr showed a tendency to uptake and sequester metals in the root cells, but inhibited translocation of the metals into the AG tissue. In contrast, Typ is speculated to restrict metal uptake as evidenced by low concentrations of Cu, Pb, and Zn in the BG tissue, but effectively facilitated translocation of metals from the BG to AG tissue.

Phr is listed as an invasive weed species by the Utah Department of Natural Resources, and is a species-specific target of control and containment efforts in the State of Utah (Berger 2009). Typ tends to invade stressed native plant communities, and can out compete native species under stress (Stevens and Anderson 2006). Overall, Phr and Typ biomass and uptake results were not consistent, and Sca and
Scv species consistently provided among the lowest bioaccumulation potential of the species tested. For these reasons, Phr, Typ, Sca and Scv are not recommended if metal bioaccumulation and recovery from stormwater BR systems is a management goal. However, Cam and Cap consistently provided among the highest biomass, AG tissue concentrations and metal uptake of Cu, Pb, and Zn of all species tested. Additionally, Cam and Cap are among the easiest species to harvest, and when managed for optimal biomass production, can play an important role in reducing metal accumulation in BR systems under these low-level contamination conditions.

5. Acknowledgments:

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CHAPTER 4

NITROGEN AND PHOSPHORUS MASS BALANCE, RETENTION AND UPTAKE IN SIX PLANT SPECIES GROWN IN SIMULATED STORMWATER BIORETENTION SYSTEMS

Abstract

Stormwater runoff contains high levels of nutrients, and is regulated by the Federal National Pollution Discharge Elimination System (NPDES) to protect surface water quality. Stormwater bioretention (BR) systems are increasingly used to address these regulations. Planted BR systems remove significantly more pollutants than unplanted systems, but most studies do not attempt to verify a pollutant mass balance and seldom evaluate differences in nutrient uptake among species. This greenhouse experiment proved that an overall 98% recovery of Total Phosphorus (TP) mass over the study period was feasible for six plant species, ensuring accuracy of measurements and analyses. Additionally, it was found that *Phragmites australis*, *Carex praegracilis*, and *Carex microptera* uptake significantly more TP and Total Nitrogen (TN) mass into harvestable tissue than *Typha latifolia*, *Scirpus validus*, and *Scirpus acutus*. These results confirm that species selection can optimize nutrient retention and recovery from stormwater and decrease pollutant discharge to surface waters.
1. Introduction

As much as half of the US surface waters have excess nutrients disrupting aquatic life (U.S. EPA 2002). The Federal National Pollution Discharge Elimination System (NPDES) Stormwater Program dictates that states must regulate stormwater runoff, including the regulation of nutrient discharge into surface water bodies, in an effort to reduce eutrophication (U.S. EPA 2005). Total Nitrogen (TN) and Total Phosphorous (TP) are the two highest rated nutrients of concern in a survey of state officials directly involved in various aspects of the NPDES program (n=51) conducted by Collins et al. (2010). Stormwater best management practices (BMPs), such as bioretention (BR) systems, are low cost alternatives for nutrient management, which can serve as a means of meeting NPDES requirements.

Planted BR systems have been proven to remove significantly more pollutants from stormwater runoff than unplanted systems (Tanner 2001; Stottmeister et al. 2003; Fraser, Carty and Steer 2004; Wiessner et al. 2006; Milandri et al. 2012). Plants, which contribute to nutrient retention through plant uptake, maintenance of soil porosity, and the influence of soil microbial communities (Read et al. 2008), are increasingly being used to address NPDES regulations.

Stormwater pollutants entering a BR system are retained in the soil media via sedimentation, filtration, and sorption on mulch and soil layers, may be biodegraded by soil microorganisms, or mobilized and sequestered in the root cells, and/or taken up into the aerial portions of plants (Davis et al. 2001). The pollutants stored in the above ground biomass can be harvested and disposed of off-site, preventing the seasonal re-release of pollutants. The literature also provides
evidence that certain species are more capable of surviving the stressful flood and
draught conditions of a stormwater BR system than others (Brisson and Chazarenc
2009), and that some of these species are better accumulators of pollutants than
others (Tanner 1996; do Nascimento and Xing 2006; Bratiers et al. 2008, Read et al.
2008). Most of these studies focus on plant variations in pollutant removal from the
exfiltrate, and little work has been done to evaluate differences in nutrient uptake
potential that exist among plant species typically planted in stormwater BR systems.
Additionally, these studies assume accurate measurements for each of the water,
plant and/or soil compartments, without a complete mass balance, implying, but not
providing, explicit nutrient recoverability from start to end of the experiments.

This experiment used six plant species typically found in stormwater BMPs
undergoing pollutant loading and hydraulic stresses typical of stormwater BR
systems. The results provide stormwater BMP managers data necessary to make
more informed choices when selecting vegetative species, and when considering
BMP management options, such as plant harvesting, to optimize nutrient removal
from urban runoff and prevent pollution of sensitive downstream surface water
bodies. This study had three primary objectives:

1) to provide a mass balance of N and P at the start and end of the study to
   ensure the accuracy of measurements and analyses,

2) to calculate mass distribution at the beginning and end of the study to
   explore differences in the extent of nutrient uptake and soil sequestration,
   and
3) to assess nutrient retention efficiency, plant biomass production, and uptake by the six plant species.

With this information, relevant facility design and maintenance procedures can be incorporated into future stormwater management systems to optimize pollutant retention and decrease nutrient discharge into downstream surface water.

2. Materials and Methods

2.1 Experimental Design

This greenhouse study used a randomized block design with six plant species and three hydraulic, nutrient and metal loading regimes in triplicate, as previously described in detail (Ryczewicz-Borecki et al. 2015). Briefly, the fate of the response factors (TN, and TP) was measured in the exfiltrate, soil, above ground (AG) and below ground (BG) plant tissue. Fate of metals (Cu, Pb, and Zn) were also evaluated but reported elsewhere (Ryczewicz-Borecki et al. 2015). The study was conducted at Utah State University’s Research Greenhouse from October 2010 through June 2011. Plastic Sterilite 19 L containers were filled with a 21 kg mixture of half Kidman Sandy Loam soil (coarse-loamy, mixed, mesic Calcic Haploxeroll) and half sand. Containers were constructed at one of two time periods, 1 month apart, in response to plant availability. Significant differences in the initial soil properties and pollutant concentrations were found between containers constructed during the two time periods (Ryczewicz-Borecki et al. 2015; Chapter 3). For this reason, each individual container’s initial and final constituent soil concentrations were used for all subsequent calculations. The six plant species investigated included: Phr -
Phragmites australis (Common Reed); Typ – Typha latifolia (Broadleaf Cattail); Scv - Scirpus validus (Soft-stem Bulrush); Sca - Scirpus acutus (Hard-stem Bulrush); Cap - Carex praegracilis (Common field sedge); Cam - Carex microptera (Smallwing Sedge); and an unplanted, soil only control. Plugs, obtained from the Aquatics and Wetland Nursery, Ft. Lupton, Colorado, were planted equidistantly within each container. Plants were allowed to root and produce new growth for 6 months before synthetic stormwater application and water-sample collection began. Nine non-vegetated containers filled only with the soil-sand mixture served as the controls.

Each species was planted in triplicate containers under three hydraulic and nutrient loading regimes representing Logan, UT; Des Moines, IA; and Scranton, PA. These three inland cities are located 18° longitudinally apart, on the 41°N latitude. Rainfall frequency, intensity and duration (hydraulic loading) were calculated based on rainfall data from each city from 2005 to 2009 using the Driscoll method (Driscoll et al. 1989), rather than using the more generalized region’s average values (GeoSyntec and ASCE 2002).

Synthetic runoff was applied to each container at the start of each rainfall event in a concentrated initial flush solution, simulating the storm’s ‘first flush’. Pollutant total mass in the synthetic stormwater, as described in Ryczewicz-Borecki et al. (2015), was based on the locations’ regionally reported average pollutant event mean concentrations (EMC) in the EPA BMP Design Guide (2004).

Logan City tap water was added to the initial flush solution and was used to represent the remainder of the storm runoff volume. Constituent concentrations in the tap water were measured as: pH=7.6; EC=285 µs cm⁻¹; alkalinity=166 mg CaCO₃
L⁻¹; TP= 0.05mg L⁻¹; TN=0.40 mg L⁻¹; and 64.4, 3.2, and 67.3 µg L⁻¹ for Cu, Pb, and Zn, respectively. Pollutant loading contributed by the tap water was added to the total mass input of the system. Total input constituent mass was calculated as:

\[ M_{\text{in water}} = \left( \sum_{i=1}^{a} (C_{\text{EMC}} + C_{\text{tap}})V_i \right) \] (1)

where \( M_{\text{in water}} \) is the constituent mass applied in the synthetic runoff (mg), \( C_{\text{EMC}} \) is the constituent EMC based on loading regime (mg L⁻¹), \( C_{\text{tap}} \) is the concentration of the pollutant of interest in the greenhouse tap water (mg L⁻¹), \( V_i \) is the input volume (L) applied for a given event, \( i \), and \( a \) is the number of runoff events for the loading regime based on rainfall duration and frequency calculations throughout the 27 week experiment.

Table 4-1 presents the number of events for each region, event volumes, and total mass of constituents applied in the synthetic stormwater (\( M_{\text{in water}}; \) TN, TP, Cu, Pb and Zn), for each loading regime. The constituent mass and hydraulic loading regimes for the three cities were categorized into Low (Logan), Medium (Des Moines), and High (Scranton). Logan, UT received the lowest rainfall intensity and frequency, producing the lowest total constituent mass loads.

Synthetic runoff filtered through the soil-sand mixture and the volumes of the exfiltrate (excess water draining from the bottom of each container) were recorded. Composite subsamples of the exfiltrate were collected at the beginning (weeks 1, 2, 3), middle (weeks 14, 15, 16), and end (weeks 25, 26, 27) of the study period.
Table 4-1: Calculated rainfall events, event volume, total water, and total mass load for the low, medium, and high loading regimes

<table>
<thead>
<tr>
<th></th>
<th>Low (Logan)</th>
<th>Medium (Des Moines)</th>
<th>High (Scranton)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Events</td>
<td>32</td>
<td>47</td>
<td>63</td>
</tr>
<tr>
<td>Tot. Water (L m⁻²)</td>
<td>3,231</td>
<td>9,580</td>
<td>16,594</td>
</tr>
<tr>
<td>Total-P (mg m⁻²)</td>
<td>2,098</td>
<td>3,532</td>
<td>5,790</td>
</tr>
<tr>
<td>Total-N (mg m⁻²)</td>
<td>14,223</td>
<td>26,539</td>
<td>37,664</td>
</tr>
<tr>
<td>Cu (mg m⁻²)</td>
<td>362</td>
<td>755</td>
<td>1,448</td>
</tr>
<tr>
<td>Pb (mg m⁻²)</td>
<td>353</td>
<td>825</td>
<td>584</td>
</tr>
<tr>
<td>Zn (mg m⁻²)</td>
<td>1,091</td>
<td>2,462</td>
<td>3,224</td>
</tr>
</tbody>
</table>

*total applied mass m⁻² after 27 weeks of synthetic stormwater application*

All AG and BG plant material was harvested, and soil samples were collected at the end of the study. The AG samples consisted of various combinations of leaves, stems, flowers, seeds, etc., depending on the species being harvested. All BG tissue was thoroughly washed in sodium lauryl sulfate, followed by washing in 0.01M HCl, and a thorough rinse with deionized water. All plant tissue was oven dried (60°C) for >72 hours, weighed, and ground at the USU Greenville Research Farm.

Roots had penetrated throughout the container, so all soil below the top 3.8 cm was considered as rhizosphere soil. Surface soil (the upper 3.8 cm of soil) and rhizosphere soil (the lower 23.2 cm of soil) samples were composited from three randomly selected subsamples collected from their respective soil horizons. Dry weight moisture content of the soil samples was determined after oven-drying the samples at 103°C for >12 hours.
2.2 Laboratory Analytical Methods


Exfiltrate, AG and BG tissue, and soil samples were analyzed for nutrient concentrations at the Utah Water Research Laboratory (UWRL) unless otherwise noted. Undigested water samples were filtered and analyzed for NH\textsubscript{3}-N and NO\textsubscript{3}-N on the Seal Analytical AQ+2 discrete analyzer (Mequon, WI), using Methods 350.1 and 353.2, respectively (U.S. EPA 1993 a,b). In all samples, NH\textsubscript{3}-N levels were at or below the method detection limit (MDL) (0.017 mg L\textsuperscript{-1}) and the NO\textsubscript{3}-N results were not significantly different from TN values. Therefore, only the TN results are presented. This suggests that any absorbed NH\textsubscript{3}-N is nitrified to NO\textsubscript{3}-N during the aerobic conditions between rain events (Hatt et al. 2007; Cho et al. 2009).

Water samples were digested using a persulfate oxidation method for the simultaneous determination of TN and TP, modified from Valderrama (1981). Digested water samples were analyzed for TN according to Method 353.2, and TP analysis was carried out according to Method 365.1 (U.S. EPA 1993b, 1993c).

Soil samples were digested with nitric acid and hydrogen peroxide, and analyzed for TP concentrations using an adaptation of EPA Method 3050B for use with the Environmental Express HotBlock Digestion System (U.S. EPA 1996). Plant
nitric acid digestion and analysis for TP concentrations followed the Jones and Case (1990) method. Once digested, analysis for plant and soil samples’ TP concentrations was conducted on an AQ+2 instrument using the EPA ascorbic reduction method 365.1 (EPA 1993c). Plant and soil subsamples were also sent to the Utah State University Analytical Laboratories, where combustion analysis was conducted to quantify the percent TN in these samples using a LECO TruSpec C/N analyzer (St Joseph, MI).

2.3 Statistical analysis

Prior to one-way and two-way analysis of variance (ANOVA) data were log transformed to meet the assumptions of normality and homogeneity of variance, and to ensure a random distribution of residuals. Percent retention values did not meet these assumptions after log transformation; therefore, these data were arcsine square root transformed (Sokal and Rohlf 1995) to meet the normality and homogeneity of variance assumptions. Post-hoc comparisons of means were done using the Tukey’s HSD test (P=0.05) on the transformed data. Statistical analyses were performed using the R statistical program (R Development Core Team 2013). Measurement variability is represented by the standard error of the means in tables and in graphical form.

3. Results and Discussion

This study aimed to quantify differences among six plant species undergoing three loading regimes in their nutrient mass distribution, retention efficiency, and
bioaccumulation potential as a means to increase nutrient removal from stormwater. Analysis of these data concluded that significant species differences occur for each of these analyses, and the predominant trend among species is most clearly observed when analyzing the results combining all loading regimes. For this reason, the results focus on species effects on the fate of TN and TP in BR systems.

3.1 Mass Balance Percent Recovery

This study calculated total percent recovery to ensure accountability of metal mass at the start and end of the study:

\[
\% \text{ recovery} = \frac{M_{\text{in water}} + M_{\text{soil start}}}{M_{\text{out water}} + M_{\text{AG}} + M_{\text{BG}} + M_{\text{soil end}}} \times 100
\]  

(2)

where \( M_{\text{in water}} \) is the applied mass of the nutrient (mg) in the synthetic stormwater runoff (Equation 1), and \( M_{\text{out water}} \) is the total nitrogen or phosphorous mass in the exfiltrate (mg), calculated as:

\[
M_{\text{out water}} = \left( \frac{\sum_{j=1}^{9} (C_j V_j)}{9} \right) \times a
\]  

(3)

where \( C_j \) is the measured composite nutrient concentrations (mg L\(^{-1}\)) of the exfiltrate samples, and \( V_j \) is the exfiltrate volume (L) measured at each sampling event, \( j \). The total constituent mass (mg) in the soil-sand mixture at the start (\( M_{\text{soil start}} \)) and end of the study (\( M_{\text{soil end}} \)) were calculated as:

\[
M_{\text{soil start}} = m_{\text{soil}} (C_{\text{initial}})
\]  

(4)

\[
M_{\text{soil end}} = m_{\text{soil}} \left( \frac{3.8}{27} (C_{\text{surf}}) + \frac{23.2}{27} (C_{\text{rhizo}}) \right)
\]  

(5)

where \( m_{\text{soil}} \) total dry mass of soil-sand mixture (kg) in each container, and \( C_{\text{initial}}, C_{\text{surf}}, \) and \( C_{\text{rhizo}} \) are the nutrient concentrations (mg kg\(^{-1}\)) in the initial soil, surface soil, and
rhizosphere soil, respectively; and the total constituent mass (mg) accumulated in the AG and BG tissue ($M_{AG}$ and $M_{BG}$) was calculated as:

$$M_{AG} = C_{AG} m_{AG} \quad (6)$$

$$M_{BG} = C_{BG} m_{BG} \quad (7)$$

where $C_{AG}$ and $C_{BG}$ are nutrient concentrations (mg kg\(^{-1}\)) in the AG and BG tissue, respectively, and $m_{AG}$ and $m_{BG}$ are the corresponding total AG and BG dry weights (kg).

There are no known studies of stormwater BR systems that quantify the constituent mass percent recoveries to ensure confidence in the study results. Borin and Salvato (2012) published a paper that used a mass balance approach to quantify denitrification in constructed wetland mesocosms, and Sun and Davis (2007) presented mass distribution of four metals using a mass balance approach, but neither provided evidence of total percent recovery of the constituent mass between the beginning and end of study. As such, it is notable to achieve an overall 98.1% ± 2% recovery for TP (Equation 2, Table 4-2). Overall recovery was lower for TN (54.7% ± 2%), and was hypothesized to be due to the denitrification process during periods of soil saturation.

TP and TN percent recovery results for each of the species are presented in Table 4-2. The percent recovery of TP ranged from 91% to 115%, with no significant differences among treatments. This confirms accuracy within measurements, sampling procedures, and laboratory analysis, and provides confidence in the results.
Table 4-2: Start and Final TP (a) and TN (b) Percent Recovery and Mass Distribution (mg m⁻²).

**a) TP (mg/m²)**

<table>
<thead>
<tr>
<th>recovery</th>
<th>Applied</th>
<th>+</th>
<th>Soil at start</th>
<th>=</th>
<th>Exfiltrate</th>
<th>+</th>
<th>AG</th>
<th>+</th>
<th>BG</th>
<th>+</th>
<th>Soil at end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phr 115% a</td>
<td>3,811</td>
<td>12,245</td>
<td>b ± 202</td>
<td>1,186 b ± 296</td>
<td>1,471 a ± 116</td>
<td>1,416 ab ± 69</td>
<td>14,546 b ± 1,001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typ 99% a</td>
<td>3,811</td>
<td>20,196</td>
<td>b ± 97</td>
<td>1,193 b ± 180</td>
<td>440 c ± 85</td>
<td>1,975 b ± 231</td>
<td>20,715 a ± 3,363</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scv 94% a</td>
<td>3,811</td>
<td>12,541</td>
<td>b ± 203</td>
<td>1,702 ab ± 301</td>
<td>261 c ± 49</td>
<td>1,414 ab ± 145</td>
<td>12,098 b ± 973</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sca 97% a</td>
<td>3,811</td>
<td>12,613</td>
<td>b ± 198</td>
<td>1,667 ab ± 288</td>
<td>249 c ± 47</td>
<td>1,300 b ± 185</td>
<td>12,881 b ± 1,053</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cap 95% a</td>
<td>3,811</td>
<td>12,200</td>
<td>b ± 101</td>
<td>1,283 b ± 252</td>
<td>947 b ± 76</td>
<td>1,507 ab ± 134</td>
<td>11,454 b ± 1,138</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cam 94% a</td>
<td>3,811</td>
<td>12,249</td>
<td>b ± 65</td>
<td>1,385 ab ± 256</td>
<td>1,298 a ± 116</td>
<td>1,085 b ± 144</td>
<td>11,385 b ± 1,115</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctrl 93% a</td>
<td>3,811</td>
<td>19,755</td>
<td>a ± 212</td>
<td>1,866 ab ± 244</td>
<td>3,014 b ± 509</td>
<td>3,730 c ± 616</td>
<td>20,209 a ± 1,686</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**b) TN (mg/m²)**

<table>
<thead>
<tr>
<th>recovery</th>
<th>Applied</th>
<th>+</th>
<th>Soil at start</th>
<th>=</th>
<th>Exfiltrate</th>
<th>+</th>
<th>AG</th>
<th>+</th>
<th>BG</th>
<th>+</th>
<th>Soil at end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phr 62% a</td>
<td>26,084</td>
<td>66,700</td>
<td>e ± 1,153</td>
<td>13,202 bc ± 2,188</td>
<td>4,525 b ± 369</td>
<td>10,040 a ± 408</td>
<td>31,140 a ± 8,185</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typ 56% a</td>
<td>26,084</td>
<td>78,719</td>
<td>a ± 472</td>
<td>11,799 c ± 1,157</td>
<td>3,777 bc ± 837</td>
<td>8,118 a ± 892</td>
<td>36,585 a ± 6,854</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scv 54% a</td>
<td>26,084</td>
<td>71,595</td>
<td>b ± 1,158</td>
<td>17,054 a ± 2,565</td>
<td>2,453 c ± 359</td>
<td>10,597 a ± 1,045</td>
<td>24,075 a ± 4,814</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sca 58% a</td>
<td>26,084</td>
<td>72,005</td>
<td>a ± 1,133</td>
<td>17,586 a ± 2,832</td>
<td>2,627 c ± 435</td>
<td>10,007 a ± 1,071</td>
<td>28,191 a ± 5,106</td>
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<td></td>
</tr>
<tr>
<td>Cap 55% a</td>
<td>26,084</td>
<td>69,648</td>
<td>bc ± 575</td>
<td>14,614 abc ± 2,005</td>
<td>6,476 a ± 639</td>
<td>8,151 a ± 528</td>
<td>24,562 a ± 3,803</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cam 56% a</td>
<td>26,084</td>
<td>69,925</td>
<td>bc ± 371</td>
<td>13,797 bc ± 1,986</td>
<td>7,213 a ± 546</td>
<td>8,412 a ± 857</td>
<td>25,344 a ± 3,562</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctrl 42% a</td>
<td>26,084</td>
<td>76,993</td>
<td>a ± 1,033</td>
<td>16,007 ab ± 1,469</td>
<td>16,007 ab ± 1,469</td>
<td>16,007 ab ± 1,469</td>
<td>28,033 a ± 4,846</td>
<td></td>
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</tr>
</tbody>
</table>

Phr: Phragmites australis; Typ: Typha latifolia; Scv: Scirpus validus; Sca: Scirpus acutus; Cap: Carex praegracilis; Cam: Carex microtera; Ctrl: unplanted control. Mean ± SE, n=9. Different letters in each column indicate significant differences among species at P=0.05 by Tukey HSD test, analyzed by one-way analysis of variance (ANOVA).

Values in blue (a) are significantly higher than values in peach.

of this study. Furthermore, this establishes that TN’s lower recovery percentages (ranging from 42% to 62% among treatments) are due to nitrification and denitrification processes occurring in the soil-sand media during wetting and drying cycles. Borin and Salvato (2012) calculated gaseous nitrogen loss at 16.6% to 37% among their five vegetated treatments (no data were presented for the unplanted control treatment). These lower estimated gaseous losses are speculated to be due to differences in experimental setup, and higher nitrogen loading; where this study applied a maximum of 38 g m⁻² of TN over 27 weeks, the Borin study applied 308 g m⁻² over two growing seasons.
3.2 Nutrient Mass Distribution

The nutrients applied in the synthetic runoff were lost in the exfiltrate, taken up into the AG or BG plant tissue, or captured by the soil-sand mixture. The nutrient mass distribution (Table 4-2) for each of the seven treatments was calculated using a mass balance approach:

\[ M_{\text{in\_water}} + M_{\text{soil\_start}} = M_{\text{out\_water}} + M_{\text{AG}} + M_{\text{BG}} + M_{\text{soil\_end}} \]  

where \( M_{\text{in\_water}} \) is the total nutrient mass applied (mg); and the accumulated mass is the sum of mass (mg) in the exfiltrate (\( M_{\text{out\_water}} \)), AG tissue (\( M_{\text{AG}} \)), BG tissue (\( M_{\text{BG}} \)), and soil-sand mixture (\( M_{\text{soil\_start}} \) and \( M_{\text{soil\_end}} \)), respectively.

TP distribution (Table 4-2a) results show differences between the control and three of the species (Phr,Typ,Cap) in exfiltrate mass, where the control allowed more TP mass to pass through the system. Differences among species were also found in the AG and BG distribution. Phr, Cap, and Cam sequestered significantly more TP mass in the AG tissue than the other species, where Phr’s mass uptake was almost six times higher than Sca. In the BG tissue Typ sequestered significantly more TP than Sca and Cam. Translocation of TP from BG to AG tissue varies among species, as demonstrated by Cam’s significantly lower uptake of TP in the BG tissue than Typ, but significantly higher uptake in the AG tissue than Typ. TP distribution in the soil at the end of the study shows that Typ and the control had significantly more mass than the other treatments, which is expected, due to these same differences in TP mass in the soil at the start of the study.

Significant differences among species for TN mass distribution (Table 4-2b) were found in the exfiltrate and AG tissue distribution. Scv and Sca had significantly
higher TN mass in the exfiltrate than Phr, Typ, and Cam. Distribution of TN in the AG tissue mass followed a similar trend to TP’s AG distribution, where Cap, Cam, and Phr have a significantly higher percent mass than Scv, and Sca. Cam sequestered 2.5 times the amount of harvestable TN than Scv (highest to lowest values). No differences were found among species in the BG tissue, indicating that although TN uptake into the roots was consistent, the translocation of the TN into the AG tissue was significantly different among species. Additionally, the lack of significant differences in soil distribution at the end of study, despite differences in TN mass in the initial soil, suggests that TN soil levels were equalized via gaseous losses into the atmosphere by the end of the study.

3.3 Nutrient Retention Efficiency

Many studies focus on water quality, using pollutant retention efficiency (RE) to estimate the efficacy of a system. RE is often calculated with event mean concentrations (EMCs), which is relatively easy to measure and calculate, but does not account for water volume (or mass) leaving the system. REs can also be calculated based on total mass, which accounts for both the incoming and outgoing loads. This study uses a mass RE calculation:

$$RE = \left(1 - \left(\frac{M_{\text{out \ water}}}{M_{\text{in \ water}}}\right)\right) \times 100$$  \hspace{1cm} (9)

which is considered more accurate than the EMC calculation (Fraley-McNeal et al. 2007).

Cumulative RE values (Figure 4-1) for the six species range from 57% to 73% for TP, and 48% to 52% for TN. This TP cumulative RE value is significantly higher
than results from published studies reviewed by the Center for Watershed Protection (Fraley-McNeal et al. 2007) where bioretention system median RE values of only 5% for TP (n=10) were reported. The TN cumulative RE value observed in this study is similar, however, to the reported RE values for TN (46%, n=5). We speculate that the lower RE values for TP are due the various study designs, measurement procedures, and RE calculations within the ten studies included in the CfWP database.

The unplanted controls’ RE values (48% for TP, and 34% for TN) are significantly lower than Phr, Typ, and Cam (and Cap for P only), but not significantly different than Scv and Sca. This indicates that the Phr, Typ, and Cam have nutrient uptake mechanisms that are well adapted to the physical and biological environment of this BR system, providing higher TN and TP mass retention, despite the control’s higher levels of soil compaction and lower exfiltrate volumes.

The cumulative RE values found in this study (57% to 73% for TP, and 48% to 52% for TN) are within the result range found in Lucas and Greenway (2008). Lucas and Greenway observed 44, 67 and 92% retention of cumulative TP mass loads, and 40, 51, and 76% TN retention for vegetated treatments grown in gravel, sand and loam media, respectively. Bratieres et al. (2008) also found Carex appressa to have a similar TN retention (from 56% to 70%), and >77% TP retention, which they contributed to this species’ extensive root system and root hairs. Lucas and Greenway (2008) also found significantly lower RE values for the soil only treatments (14 to 56% for TP, and 7 to 18% for TN) than their vegetated treatments.
Figure 4-1: Percent Retention Efficiency of TN and TP. Different letters indicate significant differences at P=0.05 by the Tukey’s HSD test, analyzed by one-way analysis of variance (ANOVA). Phr: Phragmites australis; Typ: Typha latifolia; Scv: Scirpus validus; Sca: Scirpus acutus; Cap: Carex praegracilis; Cam: Carex microptera. Mean ± SE, n=9.

This study found no differences among the planted treatments for TP RE’s, however significant differences were found among the species for TN RE’s, where Phr and Typ had significantly higher RE values than Scv and Sca. These results agree with Read et al. (2008) and Bratieres et al. (2008) that vegetation selection significantly affects nutrient retention performance possibly due to physiological, chemical and morphological variations.

Exfiltrate concentrations (Table C-1) also confirmed significant differences among planted systems and the unplanted controls. The TP exfiltrate concentrations ranged from 0.16 to 0.22 mg L⁻¹ for the planted systems, while the control concentrations were significantly higher at 0.46 mg L⁻¹. These TP concentrations are similar to those found in Bratieres et al. (2008) of 0.03 to 0.09 mg L⁻¹, and Read et al.
Exfiltrate TN concentrations ranged from 1.83 to 2.41 mg L$^{-1}$ for the planted systems, while the control treatments had a significantly higher average concentration (3.57 mg L$^{-1}$). Exfiltrate TN concentrations reported in Read et al. (2008) were comparable to this study's at 1.69 to 2.18 mg L$^{-1}$. However, the Bratieres (2008) study found a broader range in TN concentrations (0.79 to 7.66 mg L$^{-1}$) showing retention in some of the planted treatments, but net accumulation (higher concentrations in the outflow than in the inflow) for other treatments.

### 3.4 Biomass Production

Nutrient load reduction requires species with high biomass production that can attain moderate AG tissue concentrations. Biomass production (Table 4-3a) in the AG plant tissue differed significantly among species, with the highest biomass (g m$^{-2}$) produced by Phr, Cap, and Cam, and the least produced by Scv and Sca. Every species saw higher BG biomass production than AG mass, ranging from 1,080 to 1,405 g m$^{-2}$. Although there were no significant differences among species in BG biomass, the mass ratio of AG to BG shows significant differences among species, where Phr, Cap, and Cam had higher ratios than the other species. The ratio for all species is less than one, indicating that all of the species support BG root growth prior to AG biomass production during their first year of growth.

AG and BG biomass production are lower than those reported in Borin and Salvato (2012), which ranged from 1,057 to 8,240 g m$^{-2}$ for the AG tissue of 5 tested wetland species (Carex, Juncus, Typhoides, Phragmites, and Typha); and 2,395 to
Table 4-3: AG and BG plant biomass, AG/BG ratio, TN and TP concentrations, and ratios of harvested\textsuperscript{a}/retained\textsuperscript{b} and whole plant\textsuperscript{c}/retained\textsuperscript{a} (mean ± SE).

<table>
<thead>
<tr>
<th>(a) Biomass</th>
<th>AG mass (g m\textsuperscript{-2})</th>
<th>BG mass (g m\textsuperscript{-2})</th>
<th>Mass ratio (AG/BG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phr</td>
<td>885±95</td>
<td>1,246 ± 81.4</td>
<td>0.71 ± 0.1</td>
</tr>
<tr>
<td>Typ</td>
<td>429 bc ± 73</td>
<td>1,092 ± 206</td>
<td>0.39 ± 0.3</td>
</tr>
<tr>
<td>Scv</td>
<td>283 c ± 44</td>
<td>1,405 ± 209</td>
<td>0.20 d ± 0.4</td>
</tr>
<tr>
<td>Sca</td>
<td>284 c ± 45</td>
<td>1,347 ± 219</td>
<td>0.21 d ± 0.5</td>
</tr>
<tr>
<td>Cap</td>
<td>571 b ± 52</td>
<td>1,080 ± 86.5</td>
<td>0.53 ab ± 0.2</td>
</tr>
<tr>
<td>Cam</td>
<td>570 b ± 50</td>
<td>1,230 ± 188</td>
<td>0.46 ab ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(b) Total P</th>
<th>AG (mg g\textsuperscript{-1})</th>
<th>BG (mg g\textsuperscript{-1})</th>
<th>TP (harvested/retained)</th>
<th>TP (plant/retained)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phr</td>
<td>1.78 b ± 0.19</td>
<td>1.13 bc ± 0.05</td>
<td>63% a ± 9.1%</td>
<td>118% a ± 15%</td>
</tr>
<tr>
<td>Typ</td>
<td>1.00 bc ± 0.07</td>
<td>2.00 a ± 0.16</td>
<td>18% c ± 2.6%</td>
<td>111% ab ± 13%</td>
</tr>
<tr>
<td>Scv</td>
<td>0.93 c ± 0.08</td>
<td>1.09 bc ± 0.09</td>
<td>12% c ± 1.4%</td>
<td>84% bc ± 7%</td>
</tr>
<tr>
<td>Sca</td>
<td>0.85 c ± 0.05</td>
<td>1.02 bc ± 0.07</td>
<td>11% c ± 1.4%</td>
<td>76% c ± 9%</td>
</tr>
<tr>
<td>Cap</td>
<td>1.69 a ± 0.08</td>
<td>1.40 b ± 0.08</td>
<td>41% b ± 3.3%</td>
<td>106% ab ± 10%</td>
</tr>
<tr>
<td>Cam</td>
<td>2.31 a ± 0.18</td>
<td>0.93 c ± 0.07</td>
<td>57% a ± 3.7%</td>
<td>105% ab ± 8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(c) Total N</th>
<th>AG (mg g\textsuperscript{-1})</th>
<th>BG (mg g\textsuperscript{-1})</th>
<th>TN (harvested/retained)</th>
<th>TN (plant/retained)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phr</td>
<td>5.3 d ± 0.35</td>
<td>8.3 a ± 0.56</td>
<td>37% b ± 3.8%</td>
<td>122% a ± 12%</td>
</tr>
<tr>
<td>Typ</td>
<td>8.3 c ± 0.48</td>
<td>8.2 a ± 0.67</td>
<td>30% b ± 4.4%</td>
<td>111% a ± 20%</td>
</tr>
<tr>
<td>Scv</td>
<td>9.2 c ± 0.72</td>
<td>8.3 a ± 0.84</td>
<td>28% b ± 3.6%</td>
<td>152% a ± 20%</td>
</tr>
<tr>
<td>Sca</td>
<td>9.4 bc ± 0.42</td>
<td>9.3 a ± 2.32</td>
<td>30% b ± 3.7%</td>
<td>159% a ± 23%</td>
</tr>
<tr>
<td>Cap</td>
<td>11.5 ab ± 0.61</td>
<td>7.7 a ± 0.36</td>
<td>60% a ± 5.0%</td>
<td>139% a ± 12%</td>
</tr>
<tr>
<td>Cam</td>
<td>12.9 b ± 0.82</td>
<td>7.3 a ± 0.60</td>
<td>62% a ± 3.7%</td>
<td>138% a ± 14%</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Harvested is the mass of AG nutrient mass.
\textsuperscript{b} Retained nutrient mass calculated as the difference between $M_{\text{in water}}$ and $M_{\text{out water}}$.
\textsuperscript{c} Whole plant is the sum of AG and BG nutrient mass.


Different lower-case letters down each column indicate significant difference among species, based on Tukey HSD ($P=0.05$). Values in blue (a) are significantly higher than values highlighted in peach.

6,367 g m\textsuperscript{2} for the BG tissue. Borin and Salvatos’s higher production of similar plant species is likely due to their higher nutrient loading and harvest after 3 years of growth compared to this study’s total growth of one year. However, biomass production was similar to those found in a Lenhart and Hunt’s (2008) study of wetland plants (\textit{Pontederia}, \textit{Saururus}, \textit{Scirpus}, \textit{Sagittaria}, and \textit{Schoenoplectus}) harvested after 6 years of field growth in a stormwater treatment wetland, which...
ranged from 228 to 1,847 g m⁻² in the AG tissue, and 132 to 1,693 g m⁻² in the BG tissue. Both studies found higher AG:BG ratios than our one-season growth study, indicating stabilization of BG biomass production, and an increase in the AG production in subsequent years of plant growth.

3.5 Plant Nutrient Uptake

RE focuses on pollutant removal from the exfiltrate water, without discerning if plants directly take up the constituents, or if they are simply adsorbed to the soil media. Houdeshel et al. (2012) confirmed that plants in BR systems take up nutrients directly from polluted stormwater using isotopic labeling (¹⁵N tracers). This verified that inorganic nitrogen applied to the system in the synthetic runoff was directly captured within the AG plant tissue through N assimilation.

Different species are capable of nutrient uptake to varying levels, and this is apparent in the AG and BG tissue TP concentrations shown in Table 4-3b. Retained nutrient mass is calculated as the difference between $M_{\text{in water}}$ and $M_{\text{out water}}$. TP concentrations differed significantly among species. Phr, Cap and Cam had higher AG concentrations and higher percent of harvestable mass over retained mass than Scv and Sca. AG tissue can be harvested, allowing for removal of the nutrients from the treatment site. Harvestable mass (mg) is the product of shoot concentration and biomass production, and it represented 11% (Sca) to 63% (Phr) of the TP retained, where the Phr percentage was almost six times higher than for Sca, indicating a large variability among species in the potential for TP uptake and off-site nutrient
removal. The BG TP concentrations ranged from 0.93 to 2.0 mg g\(^{-1}\), where Typ, had significantly higher BG concentrations than all of the other species.

Phr had the highest percentage (118%) of TP mass contained in the whole plant over the retained mass and Sca the lowest (76%). Nutrient stores in the BG tissue provide temporary immobilization, but these stores can become an internal nutrient load if re-discharged into the environment (Sartoris et al. 2000). However, average percentages over 100 reveal that the plants can take up more TP than was retained from the runoff, indicating that the plants potentially also take up TP originally adsorbed to the soil-sand mixture.

The AG tissue’s TN concentrations (Table 4-3c) ranged from 5.3 to 12.9 mg g\(^{-1}\), where concentrations were significantly higher for Cap and Cam than Phr, Typ, and Scv. Unlike TP concentration results, Phr (5.3 mg g\(^{-1}\)) had the lowest TN concentrations of all the species, and despite Phr producing significantly higher biomass (885 g m\(^{-2}\)), its percent TN harvested over retained mass is significantly lower than Cap and Cam. Cap and Cam’s percent TN harvested over retained mass (60% and 62%, respectively) was approximately twice as high as the other species. No significant differences were found among species for BG tissue concentrations (7.3 to 9.3 mg g\(^{-1}\)), or percent TN mass in whole plant over retained mass (111% to 159%). Percentages over 100 reveal that plants uptake more TN mass into their tissues than mass retained from the runoff, indicating active uptake from the soil-sand environment. This last observation is again supported by the net loss of nitrogen from the soil compartment from the beginning to the end of the study (Table 4-2b).
The AG and BG tissue TN concentrations where similar to concentrations found in other studies. Borin and Salvato (2012) found AG and BG tissue TN concentrations ranged from 8 to 18 mg g\(^{-1}\) and 11 to 16 mg g\(^{-1}\), respectively. Lenhart and Hunt (2008) found that AG tissue TN concentrations in four species growing in a North Carolina stormwater treatment wetland ranged from 3.1 to 12.7 mg g\(^{-1}\), while the BG tissue had TN concentrations ranging from 2.4 to 13.0 mg g\(^{-1}\). A study of periphyton growing in a stormwater treatment wetland (Debusk et al. 2004) also produced similar TN and TP mean concentrations (7.8 mg g\(^{-1}\) and 0.41 mg g\(^{-1}\), respectively). As stated earlier, this study found lower AG biomass production and similar TN concentrations to the Borin and Salvato (2012) study. However, despite lower application levels (this study applied a maximum of 38 g m\(^{-2}\) of TN over 27 weeks, the Borin study applied 308 g m\(^{-2}\) over two growing seasons), the portion of harvested to retained N were similar in both studies, indicating a steady rate of TN uptake from the applied runoff.

4. Conclusion

BR systems are increasingly being used to remove nutrients from stormwater runoff. The first objective of this study was to determine if a complete recovery of constituent mass at the end of the study was feasible for six plant species when undergoing pollutant loading and hydraulic stresses typical of stormwater BR systems. Our results showed an overall average 98% recovery of TP, ensuring accuracy of measurements and analysis, and providing confidence in the
distribution, retention efficiency, and plant nutrient uptake results. TN recovery was lower (54%) due to denitrification and loss of N into the atmosphere.

The second objective of this study was to calculate the TP and TN mass distribution at the beginning and end of study. Differences were found among the treatments in the exfiltrate mass, and in AG tissue mass for both TP and TN. This shows that species selection affects the amount of nutrient retained in the treatment system, as well as the potential for nutrients to be taken up into the harvestable plant tissue.

Differences among species in their ability to retain and take up nutrients were supported by a detailed evaluation of the nutrient retention efficiency, and plant nutrient uptake (objective three). TN and/or TP discharge is regulated in many urban environments, and RE values for Phr, Typ and Cam were found to be significantly higher than the unplanted controls, indicating that planted systems can optimize nutrient removal from stormwater BR systems. Phr, Cap, and Cam also had significantly higher distribution of mass in the AG tissue, and a higher ratio of harvestable mass to retained mass, indicating that these species are capable of not only taking up a larger mass of nutrients, but they also have the highest potential for plant harvesting and off-site nutrient disposal. Off-site disposal decreases the possibility of nutrients being re-distributed back into the soil environment. These results are critical for BMP managers to make informed choices regarding plant species selection, and confirm the benefits of harvesting AG plant tissue to prevent net accumulation of nutrients over time.
5. Acknowledgments

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U.S. EPA. (2002). Considerations in the design of Treatment Best Management Practices (BMPs) to Improve Water Quality. EPA/600/R-03/103, Cincinnati, OH.


CHAPTER 5

ANALYSIS OF CITRIC ACID ENHANCED METAL AND METALLOID BIOACCUMULATION IN STORMWATER BIORETENTION SYSTEMS

Abstract

Citric acid is a low cost, biodegradable organic acid proven to increase uptake of certain metals from highly contaminated soils in a variety of plant species. This stormwater bioretention field experiment investigated if citric acid enhanced bioaccumulation among three plant species in calcareous soil systems of low-level, continually applied contamination. Results indicated that citric acid significantly increased metal concentrations in the soil pore water for the planted treatments, but did not result in increased metal uptake into the plant tissue. However, notable differences among species were found, where Carex microptera accumulated more Al, Cr, Cu, and Fe in the above ground tissue than Helianthus maximiliani and Typha latifolia (except Cu in H. maximiliani). Results also showed that citric acid does not promote significant leaching of solubilized metals into the groundwater. This information provides insight into the limitations of citric acid enhanced bioaccumulation and the role of species selection in the design of optimal systems to effectively slow metal accumulation in calcareous soils, optimize metal recovery, and lengthen the operating life of stormwater bioretention (BR) systems.
1.0 Introduction

Soil metal contamination is a serious environmental problem requiring affordable strategies for remediation; one such strategy is enhanced bioaccumulation in plants. Citric Acid (CA) has recently gained attention as a biodegradable, low cost option for enhanced bioaccumulation that successfully increases uptake of certain metals in a variety of plant species (Römkens et al. 2002; Qu et al. 2011; Mihalík et al. 2011; Almaroai et al. 2012; Tapia et al. 2013; Freitas et al. 2013; Freitas et al. 2014). The extent of bioaccumulation enhancement by citric acid in three plant species grown in a stormwater bioretention (BR) system is presented in this paper.

Stormwater runoff retained in BR systems often contains elevated levels of lead (Pb), copper (Cu), zinc (Zn) and other trace metals and metalloids from roof shingles, motor vehicles, soil erosion, corroding metal surfaces, and combustion processes (U.S. EPA 2003; Davis 2001). Although metal and metalloid concentrations in stormwater runoff are relatively low compared to industrial waste and mine tailings, stormwater is regulated by Environmental Protection Agency’s (EPA) National Pollutant Discharge Elimination System (NPDES).

Metals are a concern because of their toxicity and persistence in the environment. Arsenic (As) is technically a highly toxic metalloid, having both metallic and non-metallic properties (ATSDR 2007). In this document the term metal will encompass discussion regarding As. It is well documented that BRs remove significant quantities of metals from runoff (Read et al. 2008; Trowsdale and Simcock 2011; Fassman 2012; Li et al. 2014; Liu et al. 2014). This implies that
metals are retained in the soil or plant components of these systems, and previous investigations indicate that more than 80% of the metals retained in BR systems accumulate in the soil (Sun and Davis 2007; Marchand et al. 2010).

All soils have naturally occurring trace levels of metals directly linked to the geology of the soil’s parent material. Soil metal contamination results from the presence of anthropogenic metals in the soil. Anthropogenic metals, such as those collected in stormwater runoff, can: 1) be dissolved in the soil solution, 2) occupy exchange sites or be specifically adsorbed on inorganic soil minerals and soil organic matter, 3) be incorporated into the structure of soil minerals or soil organic matter, or 4) be precipitated as a solid (Shuman 1991).

The sorption capacity of soils is finite, and continuous application of metals may increase the risk of toxic metal buildup and subsequent leaching to groundwater. For this reason, the long-term fate of metals in BR facilities is of concern. Davis et al. (2003) used nationally averaged stormwater metal concentrations to estimate that continual metal accumulation from runoff will lead to reaching the EPA regulatory limits for soil contamination after 15-20 years of deposition.

Bioaccumulation is the process of using plants to absorb and transport potentially toxic compounds from the soil, allowing for the harvest and removal of plants and sequestered contaminants. Processes that influence plant accumulation of metals include: 1) metal mobilization from soil and pore water, 2) metal uptake and sequestration into the root, 3) metal xylem transport, and 4) metal transfer and storage into aerial plant tissue (Clemens et al. 2002).
Metal removal effectiveness via bioaccumulation is contingent upon plant biomass yield and metal concentrations in the harvestable plant components (Meers et al. 2008; Sheoran et al. 2010). There are two basic strategies for metal bioaccumulation: natural bioaccumulation and enhanced bioaccumulation (Salt et al. 1998; Meers et al. 2008). Natural bioaccumulation using hyperaccumulators is often an inappropriate choice for bioaccumulation, despite the plant’s ability to store high concentrations of metals in their above ground tissue, because these plants often produce low levels of biomass. Enhanced bioaccumulation uses high biomass-producing plants in conjunction with chemical, biological and/or naturally occurring amendments in the soil, such as low molecular weight organic acids (LMWOA), to enhance metal solubility and result in increased metal uptake by the plant (Johnson and Singhal 2010).

Most studies exploring non-hyperaccumulating species have focused on the remediation of highly contaminated soils (Stottmeister et al. 2003; Wiessner et al. 2006; Marchand et al. 2010; Yadav et al. 2011; Narhi et al. 2012; Ladislas et al. 2013). Liu et al. (2007) found that among 19 species, capacity for cadmium (Cd), Pb, and Zn accumulation in aerial tissue differed by 47, 60, and 121 fold, respectively, in a small-scale constructed wetland receiving simulated industrial wastewater. These studies assert that species selection in constructed wetlands significantly influences metal bioaccumulation potential. However, the enhanced bioaccumulation potential of plants grown in soils with continual application of low metal concentrations has not been considered in these studies, nor has CA enhanced bioaccumulation been assessed as a means of slowing the rate of metal accumulation in BR soils. This type
of research is vital to understand how to reduce soil metal accumulation and the resulting formation of hazardous sites in urban stormwater treatment settings.

CA is a LMWOA gaining recognition as an environmentally friendly and cost effective option for increasing metal solubility in soils. Plants have been found to naturally exude CA (and other acids) from roots under phosphorus (P)- and iron (Fe)-deficient conditions (Dinkelaker et al. 1988; Poschenrieder and Barcelo 2002; Hens and Hocking 2002). Unlike synthetic chelating agents, such as ethylenediaminetetraacetic acid (EDTA), CA rapidly biodegrades, mineralizing within a period of weeks (Meers et al. 2005), effectively eliminating the risk of accelerated pollutant leaching to groundwater. Despite CA’s rapid biodegradation and relatively weak complex stability, it is an effective soil acidifier, it acts as an organic-metal chelate (Martell and Smith 1977; Evangelou et al. 2007), and it inhibits metal hydroxide precipitation (Johnson and Loeppert 2006; Pérez-Esteban 2013). It has been found to be an effective organic acid for increasing metal solubility in soil pore water and uptake into plant tissue (de Araújo and do Nascimento 2010; Mihalík et al. 2011; Duarte et al. 2011; Freitas et al. 2013; Guo et al. 2014).

CA not only increases constituent solubility by acidification but also by the formation of organic-metal chelates. Log K values for citrate and seven metals (Fe, As, aluminum (Al), Chromium (Cr), Cu, Zn, Pb) taken from Martell and Smith (1977) are listed in Table 5-1. The stability constants provided are for each element’s strongest complex.
Table 5-1: List of Log K values for citric acid with seven common metals (Martell and Smith, 1977; Jean et al. 2007). All stability constants are given for temperatures of 20°C and a solution ionic strength of 0.1, unless otherwise noted.

**Stability Constants (Log K)**

<table>
<thead>
<tr>
<th></th>
<th>Fe$^{3+}$</th>
<th>As(III)</th>
<th>Al</th>
<th>Cr (III)</th>
<th>Cu$^{2+}$</th>
<th>Zn$^{2+}$</th>
<th>Pb$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Citric Acid (CA)</strong></td>
<td>11.5$^b$</td>
<td>9.3$^d$</td>
<td>9.1$^c$</td>
<td>8.7$^b$</td>
<td>5.9$^b$</td>
<td>5.9$^{ae}$</td>
<td>6.1$^{ef}$</td>
</tr>
</tbody>
</table>

$^a$ T=25°C, I =0.5  
$^b$ Metal (Me) + L = MeL  
$^c$ Me + L + H = MeHL  
$^d$ Me + L + (OH)$_2$ = Me(OH)$_2$L  
$^e$ Me + L$^2$ = MeL$_2$  
$^f$ T=25°C, I =2.0  

CA stability constants for these seven metals range from 11.5 (Fe$^{3+}$) to 5.9 (Cu$^{2+}$ and Zn$^{2+}$). These values are relatively low compared to synthetic chelators, such as Cu-EDTA (Log K=18.8). As expected, Almaroai et al. (2012) observed that CA solubilized significantly less Cu than EDTA, nitrilotriacetic acid, or oxalic acid at 2.5 and 5 mmol kg$^{-1}$ concentrations. However, their study also found that Cr and As (oxidation states were not mentioned in this article) were solubilized more effectively with CA than with the synthetic chelators. Trivalent chromium [Cr(III)] is found in natural sources. Hexavalent chromium Cr (VI) is produced by industrial processes and is not expected at this field site.

In nature, arsenic (III) commonly exists as an oxyanion, and would therefore not complex with CA. Instead, arsenic (As(V), As(III)) strongly associates with Fe oxides, and As solubility is controlled by the dissolution of Fe oxyhydroxides (Castaldi et al. 2013). CA causes dissolution of Fe oxides through acidification of the soil and complexation of the Fe, releasing As to solution.
Research focusing on enhanced bioaccumulation in systems of low-level contamination, but with continual application such as BRs, is needed to assess the possibility to slow, and potentially prevent soil metal accumulation, and the resulting formation of hazardous sites. This field experiment investigates the potential for and extent of CA enhanced bioaccumulation among three different plant species (*Typha latifolia, Carex microptera, Helianthus maximiliani*) as a stormwater management technique, based on testing four null hypotheses:

1) Adding CA to a stormwater BR system will not increase soluble metal concentrations in the soil pore water,

2) CA application will not enhance bioaccumulation potential by increasing metal uptake into the harvestable plant tissue,

3) Metal uptake and bioaccumulation potential are species independent, and therefore species selection does not influence bioaccumulation potential, and

4) The addition of CA will promote the risk of soluble metals leaching into the groundwater.

Determining the extent of CA enhanced bioaccumulation as a function of individual species will optimize management and maintenance strategies for metal management, and reduce the risk of toxic metal buildup in the soil and subsequent leaching to groundwater.

### 2.0 Materials and Methods

#### 2.1 Field Site Experimental Design
The field study was conducted at the existing Green Meadows BR field demonstration site in Logan, Utah (41° 43’ 15.05”N; 111° 52’ 30.76”W) from May 2014 to October 2014. The field site, built in partnership with the City of Logan, was initially planted in Fall 2010. Stormwater runoff flows from the surrounding residential subdivision into a flow distribution box, channels, and weirs designed to equally distribute water into each of 24 treatment bays. Water from the treatment bays can overflow into the remaining (original) bioretention basin.

Two plant species were chosen for evaluation at this field demonstration site based on preliminary biomass production results from a greenhouse study conducted by the authors (Rycewicz-Borecki et al. 2015) prior to field site establishment: *Typha latifolia* (Broadleaf Cattail), and *Carex microptera* (Smallwing Sedge). *Helianthus maximiliani* (Maximilian Sunflower) is the third species utilized at the site. It is commonly found in BR systems, is native to the semi-arid west, and is in the same genus as a well-studied metal accumulator, *Helianthus annuus*. All species were originally planted in six replicate 6.97 m² bays, each bay initially planted with 60 plugs that were obtained from Aquatics and Wetland Nursery, Ft. Lupton, Colorado, or seeded with sunflower seeds obtained from Mountain Valley Seed Co., Salt Lake City, Utah. All bays received compost (0.34 m³) prior to tilling of the soil and planting, and mulch (0.17 m³) from the Logan Landfill after initial planting was complete. Twelve of the 24 original bays were used for the CA study. Each bay utilized a triplicate split-block design, to ensure replicates of each species and to accommodate three levels of CA application. A diagram of the randomly assigned species locations and the CA application is provided in Figure 5-1.
2.2 Initial Measurements (2012-2014)

Water, plant, and soil were sampled from 2012 to 2013, and served as background data for the study. Stormwater runoff samples from the flow distribution box and at the bay overflows were analyzed at the UWRL in 2012 and no significant differences in TN, TP, Cu, Pb, Zn were found between inflow and overflow. Runoff samples continued to be sampled at the flow distribution box in 2013 and 2014 to monitor event mean concentrations (EMC) entering the site (Table 5-2), and to calculate the runoff mass loading.

It is speculated that the runoff EMC values increase from 2012 to 2014 as a result of aging asphalt and building materials, and increased automobile activity. Despite the gradual increase, Cu, Pb, and Zn concentrations remained low. For example, the BR Cu, Pb, and Zn EMCs were similar to tap water concentrations.
Table 5-2: Event mean concentration (EMC) (µg L⁻¹), and runoff mass load (mg meter⁻² year⁻¹) for various metals in the stormwater runoff at Green Meadows field site (2012, 2013, and 2014). Values are mean ± standard error; n=84 in 2012; n=29 in 2013; n=42 in 2014. Concentrations of local tap water, drinking water MCLs, and regional EMCs are provided for comparison.

<table>
<thead>
<tr>
<th>Bioretention Field Site</th>
<th>Runoff EMC (µg L⁻¹)</th>
<th>Comparative Concentrations</th>
<th>Total Runoff Mass Load (mg m⁻² year⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2012</td>
<td>2013</td>
<td>2014</td>
</tr>
<tr>
<td></td>
<td>135 ± 32</td>
<td>1,114 ± 171</td>
<td>2,863 ± 723</td>
</tr>
<tr>
<td></td>
<td>1,0 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>4.8 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>99 ± 23</td>
<td>742 ± 117</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>135 ± 32</td>
<td>1,114 ± 171</td>
<td>2,863 ± 723</td>
</tr>
<tr>
<td></td>
<td>675 ± 104</td>
<td>449 ± 71</td>
<td>1,366 ± 361</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>60 ± 14</td>
<td>449 ± 71</td>
<td>1,366 ± 361</td>
</tr>
</tbody>
</table>

measured in a greenhouse study in Logan, Utah (Ryciewicz-Borecki et al. 2015; Table 5-2), but EMCs for Al, As, Cr, and Fe were significantly higher. However, only Al and Fe concentrations in the BR runoff were higher than national drinking water standards (U.S. EPA 2009; Table 5-2). These values were significantly lower than regional EMC concentrations posted for urban areas in the EPA BMP Design Guide (U.S. EPA 2004) indicating relative low levels of contaminant accumulation in the field site stormwater runoff.

The average total annual runoff volume (L) that enters the field site per year is 168,843 L year⁻¹ (Equation 1):
\[
V_{BR/\text{year}} = \frac{V_{\text{rain/\text{year}}}}{A_{\text{drainage}}} \times \%_{\text{runoff}} \times Z
\]  

(1)

where \( V_{\text{rain}} \) (362.8 mm) is the average volume of rain per year in 2013 and 2014 as measured on a Cambel Scientific Weather Station located on site, \( A_{\text{drainage}} \) (93,078 m\(^2\)) is the area of the subdivision that drains to the BR, \( \%_{\text{runoff}} \) is the percent of rainfall estimated to reach the BR (assumed to be 50%), and \( Z \) is the conversion factor (0.01) to yield runoff volume in L.

Total metal mass loading (mg m\(^{-2}\) year\(^{-1}\)) in the stormwater runoff was calculated using Equation 2:

\[
\text{Metal Load in Runoff} = \frac{V_{BR/\text{year}}}{C_{\text{runoff}}} \times \frac{1}{A_{\text{BR}}}
\]  

(2)

where \( V_{BR} \) is the total annual runoff volume of the BR (L), \( C_{\text{runoff}} \) is the average concentration of the metal in the stormwater (given in Table 5-2), and \( A_{\text{BR}} \) is the area of the BR system (278.7 m\(^2\)). The calculated loading (mg m\(^{-2}\) year\(^{-1}\)), based on 2012, 2013, and 2014 average total concentrations (µg L\(^{-1}\)) are also presented in Table 5-2.

The soils at the field site are classified as Roshe Springs Silt Loam (fine-loamy, carbonatic, mesic Typic Calciaquolls). These soils are classified as poorly drained, non-saline to very slight saline, with an average water capacity of 27.4 cm, and a CaCO\(_3\) equivalent ≤ 55% (USDA 2013). The USDA CaCO\(_3\) level was verified using composite soil samples collected from the field site in October 2013. The field soil samples were found to have 30.73% calcium carbonate, which is within the soil survey reported 55% maximum, but relatively high compared to soils in many other
regions of the world. Organic matter percent (8.83 ± 0.8%) is high for Utah soils due to the addition of compost and tilling of the soil prior to planting, and decomposition of a final mulch cover layer since its placement in 2010. These and additional results for the field site’s calcareous soil are also presented in Table 5-3.

Pollutants from stormwater runoff are deposited in the BR system and accumulate in the soil. Many local and national regulatory limits are based on total soil metal concentrations; therefore, both the bioavailable and total surface soil (the upper 3.8 cm of soil) concentrations were analyzed. Samples were taken in triplicate in April and October 2012 from each bay. No significant differences in concentrations were found among triplicate subsamples. Therefore, composite

Table 5-3: Characteristics of surface soil samples (0 - 3.8 cm depth) taken at the Green Meadows field site. Samples were taken randomly across the site. Mean ± SE. n=3 unless otherwise noted.

<table>
<thead>
<tr>
<th>Water Soluble Extraction (mg kg⁻¹)</th>
<th>DTPA Extraction (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013 October</td>
<td></td>
</tr>
<tr>
<td>pH 8.23 ± 0.1</td>
<td>Ca 58.0 ± 21</td>
</tr>
<tr>
<td>CEC* (cmol kg⁻¹) 14.8 ± 3.7</td>
<td>Mg 66.9 ± 13</td>
</tr>
<tr>
<td>calcium carbonate (%) 30.7 ± 10</td>
<td>Nitrate-Nitrogen (mg L⁻¹) 1.21 ± 0.7</td>
</tr>
<tr>
<td>organic matter (%) 8.83 ± 0.8</td>
<td>Carbonate (mmol L⁻¹) 3.46 ± 2.0</td>
</tr>
<tr>
<td>sulfate-sulfur (mg kg⁻¹) 31.6 ± 12</td>
<td>Bicarbonate (mmol L⁻¹) 9.97 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Chloride (mg L⁻¹) 260 ± 86</td>
</tr>
<tr>
<td></td>
<td>S 41.6 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>K 74.1 ± 18</td>
</tr>
<tr>
<td></td>
<td>Na 127 ± 38</td>
</tr>
<tr>
<td></td>
<td>Al &lt;dl</td>
</tr>
<tr>
<td></td>
<td>As 3.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Cr 0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Cu 3.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Fe 104 ± 47</td>
</tr>
<tr>
<td></td>
<td>Pb 2.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Zn 12 ± 1.2</td>
</tr>
</tbody>
</table>

n=3 unless otherwise noted.

*CEC = cation exchange capacity
surface soil samples were taken from each bay the following year (October 2013). October 2014 samples include the control (no citric acid addition) treatments only.

Metal soil concentrations showed a gradual, but insignificant increase from 2012 to 2013, and from 2013 to 2014, based on an analysis of variance. However, continual metal soil deposition from runoff is an unintended consequence of treating stormwater in BRs (Davis 2003). Measured GM total soil metal concentrations are presented in Table 5-4 compared to total concentrations used for US EPA (2002) soil screening levels and NYS DEC (2006) residential use regulatory limits. Average yearly increases in total metal concentrations are also presented.

Table 5-4: U.S. EPA soil screening levels and NYS DEC chronic human health-based soil cleanup objectives, for select metals based on total concentrations. Total soil metal concentrations at the field site, 2012, 2013, and 2014 (October). Values are means ± standard error.

<table>
<thead>
<tr>
<th>US EPA screening level</th>
<th>NYS DEC screening level</th>
<th>FIELD SITE SOIL CONCENTRATIONS</th>
<th>average increase mg kg⁻¹ year⁻¹</th>
<th>years (to reach NYS DEC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (mg kg⁻¹)</td>
<td>Residential use</td>
<td>2012 oct</td>
<td>2013 oct</td>
<td>2014 oct</td>
</tr>
<tr>
<td>Al</td>
<td>-</td>
<td>Al</td>
<td>4,556 ± 157</td>
<td>6,020 ± 339</td>
</tr>
<tr>
<td>As</td>
<td>0.4</td>
<td>As</td>
<td>9.0 ± 0.5</td>
<td>11 ± 1.0</td>
</tr>
<tr>
<td>Ca</td>
<td>-</td>
<td>Ca</td>
<td>114,000 ± 3,500</td>
<td>150,000 ± 4,900</td>
</tr>
<tr>
<td>Cr ([III])</td>
<td>120,000</td>
<td>Cr</td>
<td>7.3 ± 0.2</td>
<td>12 ± 0.9</td>
</tr>
<tr>
<td>Cu</td>
<td>-</td>
<td>Cu</td>
<td>12 ± 0.8</td>
<td>11 ± 0.8</td>
</tr>
<tr>
<td>Fe</td>
<td>-</td>
<td>Fe</td>
<td>5,727 ± 175</td>
<td>6,453 ± 280</td>
</tr>
<tr>
<td>Mg</td>
<td>-</td>
<td>Mg</td>
<td>33,900 ± 1,100</td>
<td>42,900 ± 1,500</td>
</tr>
<tr>
<td>Pb</td>
<td>400</td>
<td>Pb</td>
<td>6.9 ± 0.4</td>
<td>7.9 ± 0.7</td>
</tr>
<tr>
<td>Zn</td>
<td>23,600</td>
<td>Zn</td>
<td>55 ± 3.3</td>
<td>49 ± 3.1</td>
</tr>
</tbody>
</table>

a US. EPA Ecological Screening Level Narratives are available (2003b, 2003c, and 2007b) for these elements.
Davis et al. (2003) calculated that Cu, Pb, and Zn would exceed EPA Part 503 biosolids regulations in 77, 16 and 16 years, respectively, based on typical concentrations in urban runoff. This study used the average yearly increase in soil total metal concentrations (mg kg\(^{-1}\) year\(^{-1}\)) from 2012 to 2014 to estimate years to reach the NYS DEC regulatory limits at this rate of increase. Under these conditions Cu was calculated to reach regulatory limits after 30 years of accumulation, which is sooner than Davis et al.’s estimation, but Pb and Zn accumulation would take longer at 312 and 57 years, respectively. Cr is estimated to reach these limits in 5.5 years.

Soil arsenic levels are currently well above the 0.21 mg kg\(^{-1}\) NYS DEC residential use concentration limits. Natural arsenic levels in soil areas near geological deposits of arsenic-rich minerals, such as many areas in Cache Valley (Meng, 2015), can range from 1 to 40 mg kg\(^{-1}\), with a mean of 5 mg kg\(^{-1}\) (U.S. Department Of Health And Human Services 2007).

2.3 Citric Acid Enhanced Bioaccumulation Study

CA application design was based on a study by Freitas (2013) where CA was applied to the soil, and extraction performance of Pb was assessed for two plant species. This study utilized the existing planted bays (\(T.\, latifolia\), \(C.\, microptera\), \(H.\, maximiliani\), and unplanted), in triplicate, and further divided each 6.97 m\(^2\) bay into three 2.32 m\(^2\) sections. Two pieces of 20.3 cm length sheet metal were installed vertically; 16.5 cm below ground level, leaving 3.8 cm above ground level. This was done to ensure separation of vegetative root systems between treatments and to prevent cross contamination of irrigation and soil pore water. Prior to installation,
sheet metal was painted with Krylon® Paint with Durable Covermax® Technology to impede metal leaching from the sheet metal into the soil (Figure 5-2).

Soil pore water ceramic suction cup samplers ( SoilMoisture Equipment Corp. Models 1900 and 1911 ) were installed per manufacture specifications with a vacuum-pack tightness at 7.6 cm (3-inch) and 15.2 cm (6-inch) depths, respectively (Figure 5-2). Prior to installation, all suction cup samplers were flushed with a nutrient solution mimicking anticipated field study concentrations to prevent adsorption of citrate, nitrogen and phosphorous to the ceramic cup during sampling ( ASTM D4696–92, 2008 ). N and P analyses were completed for these samples, but is outside the scope of this paper and will be discussed in a separate publication. Metal adsorption to the ceramic cup is not preventable with flushing and a separate sampling procedure was used for metal soil pore water analysis.

Figure 5-2: Images of the planted bays (September 2013), a treatment area separator, and a soil pore water suction cup sampler installed at 15.2 cm.
Each treatment area was randomly assigned one of three CA levels. The three levels, chosen on the basis of prior research reported in the literature and a preliminary CA study done at the UWRL, were control (0 mmol kg$^{-1}$), low (10 mmol kg$^{-1}$), and high (50 mmol kg$^{-1}$) loading treatments. An RL Flo-Master backpack pump sprayer was used to apply 30 L of food grade, anhydrous CA solution at three different concentrations (0 g L$^{-1}$, 36.3 g L$^{-1}$, or 181.7 g L$^{-1}$) to the treatment areas, followed by 180 L of Logan City potable water to ensure soil saturation to a 19-cm depth. A diagram of the randomly assigned CA application levels is presented in Figure 5-1. During this CA application study, treatment bay inlet weirs were raised to prevent inundation of treatment areas with runoff.

### 2.4 Soil pore Water, Plant, and Soil Sampling

Each 2.32 m$^2$ treatment area was divided into 25, 930-cm$^2$ sections. Suction cup samplers, soil, and plant sample locations were randomly chosen from within one of the nine interior 930-cm$^2$ sections (Figure 5-3).

Soil pore water and soil samples were collected 24 hours after CA application. Soil pore water samples were collected from each treatment area at two depths (7.6 cm and 15.2 cm) to assess if CA addition affected metal leaching potential to the shallow groundwater (21 to 46 cm depth) at this field site.

Two soil pore water sampling methods were used. The first method utilized suction cup samplers to collect samples for pH and citrate analyses. The second method utilized soil samples collected with a 5.1 cm diameter auger to a depth of 19.05 cm. Each of these soil samples was segregated into three sections: surface soil
Figure 5-3: A schematic of the interior nine sections used for randomly locating suction cup samplers, soil auger samples, and sampling AG and BG tissue. The image on the right shows the portable sampling structure used in the field.

(0-3.8 cm), 7.6 cm soil depth (3.8-11.4 cm), and 15.2 cm soil depth (11.4-19.1 cm). Soil from the 7.6 cm and 15.2 cm depths was centrifuged to extract the pore water from the soil. This centrifuged pore water was used for metal analyses. Surface soil samples (0-3.8 cm depth) were analyzed for both bioavailable and total metals.

Above ground (AG) and below ground (BG) plant tissue samples were taken 5 days after CA application, shortly after the highest expected levels of metal solubility (Freitas et al. 2013; Souza et al. 2013; de Araújo and do Nascimento, 2010; Meers, 2005). AG tissue was harvested at ground level. Representative BG samples were taken from the same 930-cm² sections, with a 5.1 cm diameter auger. These samples were brought to the UWRL to separate BG plant tissue from the soil by washing with tap water over a 1-mm sieve to fully remove all non-plant tissue. BG tissue was rinsed with deionized water. AG and BG samples were dried, ground, and analyzed for total biomass production, and total metals. Results of AG tissue
analyses were used to calculate the uptake of total metals into the harvestable plant material. BG metal mass accumulation was also calculated. Soil pore water, soil and plant samples (without additional CA application) were collected again in October at a time when all CA should theoretically be biodegraded.

2.5 Laboratory Analytical Methods:


All soil pore water, plant, and soil samples were analyzed for metal concentrations at the Utah Water Research Laboratory (UWRL). Ceramic suction cup pore water samples were analyzed for pH within 1 hour of collection. Citrate analysis of these samples used the Dionex Application Note 123 (Dionex Corporation 2006) and analyzed on an ICS-3000 ion chromatograph. Centrifuged soil pore water samples were filtered through a 0.45 µm filter postulating that these metals are available for plant uptake, and directly analyzed for metals. Soil samples were digested with nitric acid and hydrogen peroxide, and analyzed for total metal concentrations using an adaptation of EPA Method 3050B for use with the Environmental Express HotBlock Digestion System (U.S. EPA 1996). Plant nitric acid digestion and analysis for metal concentrations followed the Jones and Case (1990)
method. Soil samples were additionally analyzed for extractable Al, As, Ca, Cr, Cu, Fe, Mg, and Zn using the DTPA-AB extraction method (Reed and Martens 1996).

All metal analyses of undigested, extracted, and digested samples were conducted on an Agilent 7700x series Inductively Coupled Plasma Mass Spectrometry (ICP-MS) instrument using SW-846 method 6020a (U.S. EPA 2007a).

2.6 Statistical Analysis

Statistical analyses were performed using the R statistical program (R Development Core Team 2013). Non-transformed data were evaluated and met the assumptions of normality, homogeneity of variance, and a random distribution of residuals. One-way and two-way analysis of variance (ANOVA) were conducted on the data, and post-hoc comparisons of means were done using the Tukey’s HSD test (P=0.05). Measurement variability was represented by the standard error of the means in tables and in graphical form.

3.0 Results and Discussion

3.1 Preliminary Citric Acid Soil Investigation:

Prior to field site CA application, preliminary investigations verified CA’s ability to increase metal solubility and potentially increase plant uptake under the site’s specific soil conditions (pH 8.23 ± 0.06). In a preliminary laboratory study, five levels of CA (5, 50, 100, 250, 500 mmol kg⁻¹ soil) were added to soil subsamples from the field site (Table D-1). Soil began to visibly produce CO₂ gas at 100 mmol kg⁻¹ CA concentrations. Pb soil concentrations measured in this initial laboratory
experiment were all below the method detection limit and are not presented in the results discussed below.

Soil pore water concentrations of metals are controlled by pH dependent cation-exchange processes and complexation to DOC (McBride, 1994). It was found that with CA addition, soil pH decreased and water extractable concentrations (mg kg\(^{-1}\) soil) of Al, As, Cr, Cu, Fe, Mg, and Zn significantly increased (Figure D-1). To demonstrate the range of metal solubility behavior in response to CA addition or simple pH adjustment with HCl, soil pore water concentrations of two metals (Fe and Mg) are shown in Figure 5-4a and 5-4b, where Fe and Mg concentrations increased as a function of increased CA. Calcium (Ca) showed initial potential to increase in solubility at low levels of CA addition (10-200 mmol kg\(^{-1}\)), but dropped to an average concentration of 2.24 ± 0.33 g kg\(^{-1}\) at higher CA doses (Figure 5-4c). The Ca release with citrate was expected to follow the same pattern as Mg. The non release of Ca at higher CA concentrations was unexpected, but preliminary MINTEQ modeling investigations show Ca-citrate precipitate forms with CA at the concentrations and pH range studied (Figure D-2). Additionally, Dinkelaker et al. (1988) found Lupinus albus L. growing in P deficient calcareous soils excreted citric acid, also leading to the dissolution of CaCO\(_3\) and precipitation of calcium citrate.

Hydrochloric acid (HCl, a monovalent, non-chelating, mineral acid) was added in concentrations to match the resulting pH values of soils treated with CA, 24 hours after application (Figure 5-4 and Table 5-5) to assess the extent to which pH alone affected metal availability in the BR soil, and to distinguish between
Figure 5-4: Fe (a), Mg (b), and Ca (c) concentrations in soil pore water as a function of citric acid (CA) addition. Fe, Mg and Ca concentrations are also shown as a function of hydrochloric acid (HCl) addition (see text for mmol kg⁻¹ equivalent concentration details).

acidification and chelation effects. Mg concentrations in the HCl addition samples (Figure 5-4b) increased proportionally with the CA addition samples indicating that observed Mg solubility is the result of pH control only, as observed by Meer et al 2005. This was not true for Fe (Figure 5-4a), or Al, As, Cr, Cu, and Zn (Figure D-1), which undergo greater solubility with CA addition than HCl addition. This indicates significant CA chelation for these metals, and/or the release of metal ions, such as As, with the dissolution of Fe oxides, rather than simply a pH affect. Calcium increased
in solubility with HCl addition (Figure 5-4c), supporting the speculation that Ca precipitated as calcium citrate at high levels of CA addition.

Table 5-6 lists six studies that focus on solubility effects of CA and other LMW0As additions to soil, the metals analyzed, species analyzed (if applicable), and the CA concentrations utilized in the study. Most studies conducted experiments on soils with neutral to low pH. Lesage et al. (2005) and Meers et al. (2005) studied CA addition in calcareous soils and found that CA dissolved carbonates, increased soil compaction, and metal solubility and plant uptake increased only after exceeding soil buffering capacity (<442 mmol kg⁻¹ soil).

Based on the laboratory CA experiment solubility results, calcium citrate precipitation above 200 mmol kg⁻¹, observed CO₂ production at 100 mmol kg⁻¹, and economic restraints of CA costs, the CA enhanced bioaccumulation potential was evaluated 0, 10, and 50 mmol kg⁻¹, at levels similar to the studies presented in Table 5-6, but below the buffering capacity of the field site calcareous soils.
Table 5-6: List of studies testing citric acid enhanced metal mobility in soil and plants. Metals analyzed for increased availability as a result of citric acid addition are listed, as is the concentration of CA used in the study.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study focus</th>
<th>Analyzed metal</th>
<th>Species†</th>
<th>Citric Acid (mmol kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peréz-Esteban et al. (2013)</td>
<td>Soil pH 6.2, 5.5</td>
<td>Cu</td>
<td>-</td>
<td>≥ 45</td>
</tr>
<tr>
<td>Meers et al., (2005)</td>
<td>Soil calcareous</td>
<td>Cd, Cu, Pb, and Zn</td>
<td>-</td>
<td>10, 50, 250, 442, 500</td>
</tr>
<tr>
<td>Doumet et al.* (2008)</td>
<td>Potted Plant</td>
<td>Cu, Cd, Pb and Zn</td>
<td><em>P. tomentosa</em></td>
<td>1, 5, 10 (tartrate, glutamate)</td>
</tr>
<tr>
<td><em>Mihalík</em> (2011)</td>
<td>Potted Plant</td>
<td>U, Ra, Fe</td>
<td><em>S. smithiana</em> <em>Helianthus</em> <em>sp.</em></td>
<td>25</td>
</tr>
<tr>
<td>Sinhal et al. (2010)</td>
<td>Potted Plant</td>
<td>Cu, Cd, Pb and Zn</td>
<td><em>T. erecta</em></td>
<td>10, 20 mg/L 7L every 5 days</td>
</tr>
<tr>
<td>de Araújo and do Nascimento, (2010)</td>
<td>Potted Plant (acidic soil)</td>
<td>Pb</td>
<td><em>Z. mays</em></td>
<td>0, 5, 10, 30</td>
</tr>
<tr>
<td>Tapia et al. (2013)</td>
<td>Potted Plant</td>
<td>Cu, Fe, Mn</td>
<td><em>A. halimus</em> <em>R. officinalis</em></td>
<td>90</td>
</tr>
<tr>
<td>Freitas et al., (2013 and 2014)</td>
<td>Field Study</td>
<td>Pb</td>
<td><em>S. bicolor</em> <em>Z. mays</em> <em>C. zizanioides</em></td>
<td>40</td>
</tr>
</tbody>
</table>

*Doumet et al. used two LMWOAs, tartrate and glutamate, not citric acid.

† Species scientific and common names:

- *Paulownia tomentosa* (empress tree)
- *Salix smithiana* (willow)
- *Helianthus sp.* (sunflower)
- *Helianthus annuus* (sunflower)
- *Tagetes erecta* (marigold)
- *Zea mays* (maize)
- *Atriplex halimus* (Mediterranean saltbush)
- *Rosmarinus officinalis* (rosemary)
- *Sorghum bicolor* (durra grass)
- *Chrysopogon zizanioides* (vetiver grass)
- *Phragmites australis* (common reed)
3.2 Field Site Confirmation of Citric Acid Enhanced Metal Solubility

3.2.1 Citrate recovery for unplanted treatments

To ensure recovery of CA in the field site experiment soil pore water was sampled and analyzed for citrate. Figure 5-5 illustrates the increase of measured citrate (mmol L\(^{-1}\)) with the applied CA doses (mmol kg\(^{-1}\)) for the unplanted controls at 7.6 cm and 15.2 cm depths, 24 hours after CA application in the August sampling event. Soil pore water citrate concentrations produced a significant linear increase with CA dosage in the unplanted treatments at both depths measured (Fig 5-5). This indicates increased recovery of citrate for the unplanted controls, as a function of CA application, and an even distribution throughout the soil profile.

3.2.2 Citrate recoverability for planted systems

No significant differences were found among species for measured citrate at either sample depth, therefore, all planted treatments were grouped together as planted systems and analyzed against the unplanted controls. At the 7.6 cm depth, no differences were found in the measured citrate concentrations among the planted and unplanted treatments. However citrate concentrations for the 50 mmol kg\(^{-1}\) dose at the 15.2 cm depth in the planted systems were found to be significantly lower than concentrations in the 7.6 cm depth and the unplanted treatments at both depths (Table 5-7). This indicates that as water flows down through the soil profile at the high CA dose, the plants and their rhizosphere (root zone) environment interact with the citric acid. In addition to sorption to the soil, other possible processes for decreased citrate levels include plant uptake, and microbial enhanced
Figure 5-5 Citrate concentrations, at 7.6 cm and 15.2 cm soil pore water depths, for the unplanted controls, at the field site, 24 hours after CA application in the August sampling event (n=3 at each dose).

CA transformation and/or mineralization (Römkens et al. 2002; Meers et al. 2005) in the rhizosphere. CA can be utilized as a carbon source by many different types of microorganisms (Chen et al. 2006), and it is therefore speculated that the rhizosphere’s microbial activity is responsible for the significant degradation of

Table 5-7: Measured citrate concentrations (mmol L⁻¹) of the soil pore water, at the 7.6 cm and 15.2 cm depths, for unplanted and planted treatments for the 10 and 50 mmol kg⁻¹ CA doses.

<table>
<thead>
<tr>
<th></th>
<th>Measured Citrate Concentrations</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mmol kg⁻¹</td>
<td>50 mmol kg⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(mmol L⁻¹)</td>
<td>(mmol L⁻¹)</td>
<td></td>
</tr>
<tr>
<td>unplanted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.6 cm</td>
<td>3.86 a ± 3.97</td>
<td>13.4 A ± 3.73</td>
<td></td>
</tr>
<tr>
<td>15.2 cm</td>
<td>2.55 a ± 1.44</td>
<td>8.03 A ± 4.65</td>
<td></td>
</tr>
<tr>
<td>planted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.6 cm</td>
<td>3.01 a ± 3.01</td>
<td>12.3 A ± 13.0</td>
<td></td>
</tr>
<tr>
<td>15.2 cm</td>
<td>0.84 a ± 1.17</td>
<td>0.49 B ± 0.82</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± standard error, unplanted n=3, planted n=9. Different letters, in columns per treatment, indicate significant differences according to Tukey's HSD test (p ≤ 0.05).
citric acid at the 50 mmol kg\(^{-1}\) dose, 15.2 cm depth. It is speculated that these processes are also occurring at the 10 mmol kg\(^{-1}\) dose, but significant differences are lost in the variability of the lower values of CA concentrations at this lower CA dose.

3.2.3 Citric acid enhanced metal solubility

CA increases metal solubility in soil pore water by decreasing pH, forming organic-metal complexes, and for some metals, through the promotion of ligand exchange, the solubilization of Fe and Mn oxides and/or carbonates, and stimulated microbial activity. No significant differences in pH were found among the CA application treatments and the controls in August. Additionally, no significant differences in pH were found among individual species at the 7.6 cm or 15.2 cm depths (Table D-2). It is speculated that the soil pore water pH was not affected due to the high buffering capacity of these calcareous soils. However, differences were found when species data from all CA levels were combined as planted and compared to the unplanted control, as a function of depth (Table 5-8).

In August, significantly higher pH values were found in the planted 7.6 cm depth samples than the planted 15.2 depth samples. These shallow soil depth pH values are not statistically different for the October samples, despite measured citrate concentrations indicating complete degradation of the CA by this time. It is speculated that in these calcareous soils pH is significantly influenced by the root environment’s microbial activity, and not by CA dose.
Previous studies found increased metal solubility in soils with CA application. For example, Mandiwana et al. (2007) demonstrated that soil pore water solubility of Cr (III) was directly proportional to applied CA concentrations. De Araújo and do Nascimento (2010) found a linear relationship between CA and the concentrations of solubilized Pb brought into solution at CA concentrations of 5 to 30 mmol kg$^{-1}$. Tapia et al. (2013) found that daily CA application to pruning waste and biosolids compost for 60 days, totaling approximately 90 mmol kg$^{-1}$ of CA, increased soluble Fe concentrations. Pérez-Esteban et al. (2013) showed that higher concentrations of CA (≥45 mmol kg$^{-1}$) increased Cu and Zn desorption from contaminated mine soils, and proposed that CA can facilitate enhanced bioaccumulation for these metals.

For the August sampling event a few sporadic significant differences were found for soil pore water metal concentrations among species and CA application dose at either sample depth, but no consistent trends were discernable (Table D-3). Therefore, the three species were grouped together as a planted system and analyzed against the unplanted controls. Arsenic is the only metal where a significant species influence was found, and will be discussed in detail in Section 3.6.
For consistency in this analysis, As concentrations were also grouped as planted and unplanted.

Figure 5-6 shows the field site’s pore water Al, As, Ca, Cr, Cu, Fe, Mg, and Zn concentrations at the 7.6 cm depth as a function of CA dose for the planted (green) and unplanted controls (brown) treatments at the 0, 10, and 50 mmol kg⁻¹ CA application doses. Although the CA doses in the field experiment are low compared to the CA doses in the laboratory experiment, pore water concentrations for all metals presented (except Zn) increase with CA dose for the planted treatments, and for Al, Ca, Cr, Cu, and Mg for the unplanted treatments, with size-of-variance similar to laboratory experiment. This trend decreases, but continues to be visible in the 15.2 depth samples (Figure D-3).

Soluble metal concentrations in the unplanted treatments (n=8) are generally lower than concentrations in planted treatments (n=26). This lower solubility at the 10 and 50 mmol kg⁻¹ CA doses is within range of the laboratory experiments results at the low CA doses. It is speculated that CA stimulated microbial and root exudate production in the planted treatments, resulting in a net increase of metal solubility. Mg and Ca have the highest relative metal concentrations in the soil pore water (Figure 5-6). In August average concentrations range from 585 ± 273 to 64 ± 12 mg L⁻¹ for Mg, and 306 ± 228 to 50 ± 9 mg L⁻¹ for Ca at the three CA doses. These concentrations are up to four orders of magnitude greater than Cr and Cu (67 ± 44 to 2.4 ± 1.6 µg L⁻¹ and 31 ± 21 to 6.0 ± 3.3 µg L⁻¹, respectively), emphasizing the relative abundance of soluble Mg and Ca over other metals under these field site conditions.
Figure 5-6: Box plots of Al, As, Ca, Cr, Cu, Fe, Mg, and Zn soil pore water concentrations at 7.6 cm depth, as a function of CA dose for planted and unplanted treatments. Planted n=26, Unplanted n=8. Note different scales on the y-axis for different metal species.

3.3 Groundwater Leaching Potential

The use of synthetic chelators, such as EDTA, has been found to be effective at increasing metal solubility and bioaccumulation potential, but have also been shown to pose unnecessary risk to the environment due to increased metal leaching and potential groundwater contamination (Evangelou et al. 2007; Doumett et al.)
2008; Meers et al. 2008; Karczewska et al. 2011). CA rapidly biodegrades within a period of weeks (Meers et al. 2005). Table 5-9 presents the citrate and soluble metal concentrations for treatments with combined CA application doses to assess CA’s potential for leaching metals to the groundwater. Again, the three individual species were grouped together as a planted system and analyzed against the unplanted controls, due to no discernable predominant trends for all metals except As, as discussed in detail in Section 3.6.

Planted treatments had significantly higher citrate, Al, Cr, Cu, Mg, and Zn concentrations at the 7.6 cm depth than 15.2 cm depth in August. For the unplanted treatments, the only significant differences between the 7.6 cm and 15.2 cm depth samples in August were for Zn. The August citrate and soluble metal concentrations at the 15.2 depth were not statistically different than concentrations measured 6 weeks after CA application (October) in both the planted and unplanted treatments. These field measurements confirm that CA application increased metal solubility at the 7.6 cm soil depth, but its influence was quickly reduced as it traveled down the soil profile to a depth of 15.2 cm. CA is quickly biodegraded, and does not pose a risk of leaching soluble metals into the shallow (21 to 46 cm depth) groundwater at this BR field demonstration site.

3.4 Citric Acid-Enhanced Bioaccumulation Potential

Bioaccumulation is affected by metal bioavailability in the soil, rate of absorption by the plant roots, and translocation from the roots to the AG tissue.
Table 5-9: Citrate, Al, As, Ca, Cr, Cu, Fe, Mg, and Zn concentrations for planted and unplanted treatments at low (10 mmol kg\(^{-1}\)) and high (50 mmol kg\(^{-1}\)) CA doses combined. Samples were taken 24 hours after CA application (August), and after CA biodegradation (October).

<table>
<thead>
<tr>
<th></th>
<th>Citrate (mmol L(^{-1}))</th>
<th>Al (mg L(^{-1}))</th>
<th>As (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planted</td>
<td>7.6 cm</td>
<td>7.88 a ± 10.5</td>
<td>11.9 a ± 14.3</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>0.65 b ± 0.98</td>
<td>2.53 b ± 5.80</td>
</tr>
<tr>
<td></td>
<td>7.6 cm</td>
<td>0.001 b ± 0.004</td>
<td>0.31 b ± 0.50</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>0.000 b ± 0.001</td>
<td>1.05 b ± 1.79</td>
</tr>
<tr>
<td>Unplanted</td>
<td>7.6 cm</td>
<td>8.63 a ± 6.25</td>
<td>2.75 ab ± 4.72</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>5.29 ab ± 4.30</td>
<td>1.54 b ± 2.93</td>
</tr>
<tr>
<td></td>
<td>7.6 cm</td>
<td>0.001 b ± 0.002</td>
<td>1.16 b ± 0.84</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>0.000 b ± 0.000</td>
<td>0.43 b ± 0.32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Ca (mg L(^{-1}))</th>
<th>Cr (µg L(^{-1}))</th>
<th>Cu (µg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planted</td>
<td>7.6 cm</td>
<td>228 a ± 193</td>
<td>42.7 a ± 40.8</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>123 ab ± 138</td>
<td>11.7 b ± 14.7</td>
</tr>
<tr>
<td></td>
<td>7.6 cm</td>
<td>62.0 b ± 17.6</td>
<td>1.46 b ± 1.08</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>69.6 b ± 31.7</td>
<td>1.66 b ± 2.28</td>
</tr>
<tr>
<td></td>
<td>7.6 cm</td>
<td>127 ab ± 119</td>
<td>26.5 ab ± 19.9</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>114 ab ± 110</td>
<td>19.0 ab ± 25.3</td>
</tr>
<tr>
<td></td>
<td>7.6 cm</td>
<td>62.4 b ± 18.2</td>
<td>1.67 b ± 1.02</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>55.7 b ± 24.5</td>
<td>1.05 b ± 0.62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fe (mg L(^{-1}))</th>
<th>Mg (mg L(^{-1}))</th>
<th>Zn (µg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planted</td>
<td>7.6 cm</td>
<td>8.57 a ± 11.8</td>
<td>470 a ± 273</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>2.94 ab ± 8.62</td>
<td>236 b ± 171</td>
</tr>
<tr>
<td></td>
<td>7.6 cm</td>
<td>0.41 b ± 0.45</td>
<td>209 b ± 102</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>1.04 b ± 1.13</td>
<td>148 b ± 51.8</td>
</tr>
<tr>
<td></td>
<td>7.6 cm</td>
<td>0.73 b ± 1.02</td>
<td>230 b ± 123</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>0.52 b ± 0.75</td>
<td>145 b ± 114</td>
</tr>
<tr>
<td></td>
<td>7.6 cm</td>
<td>0.87 b ± 0.45</td>
<td>90.8 b ± 20.2</td>
</tr>
<tr>
<td></td>
<td>15.2 cm</td>
<td>0.43 b ± 0.32</td>
<td>81.8 b ± 24.9</td>
</tr>
</tbody>
</table>

Mean ± SE; Planted n=18, Unplanted n=6
Different letters indicate significant difference in concentrations between planted and unplanted treatments at the two sampling times (two-way ANOVA), based on Tukey’s HSD (P<0.05).

(Clemens et al. 2002; Bertin et al. 2003; Krämer et al. 2007). It is contingent upon plant biomass yield and metal concentrations in the harvestable plant components.
CA was theorized to increase biomass production due to the dissolution of Fe and Mn oxides (Pérez-Esteban et al. 2013), and the resulting increases in P mobilization (Hens and Hocking, 2002; Johnson and Loeppert, 2006). In previous studies on highly contaminated soils, CA has also been found to increase metal solubility in soil pore water and uptake into harvestable plant tissue (Kim and Lee, 2010; Duarte et al. 2011; de Araújo and do Nascimento 2010; Pérez-Esteban et al. 2013; Tapia et al. 2013; Freitas et al. 2013; Guo and Cutright, 2014).

This study applied CA to an existing stormwater BR system with soil metal concentrations significantly lower than EPA’s levels of concern for human health for all metals except As (Table 5-3). Despite a measurable increase in Al, Ca, Cr, Cu, and Mg concentrations in planted and unplanted treatments in the 7.6 cm depth soil pore water samples (Figure 5-6), no significant differences in DTPA extractable or total soil concentrations were found as a function of CA dose for any of the metals, and therefore are not discussed in this paper.

Only six significant interactions were found between CA application and metal uptake in the AG and BG tissue for any individual species sampled in August and October (out of 108 possible total interactions), with no predominant trend (Table D-4). Therefore, uptake of metals into harvestable plant tissue cannot be statistically increased with the use of CA in BR systems in the soils and at the metal loadings evaluated in this study. Consequently, CA application does not delay the need for soil remediation under these conditions.

The limited effect of CA found in this field demonstration site study is speculated to be due to the high buffering capacity of the calcareous soils, the low
starting metal concentrations, as well as the high variability inherent to field site conditions, which indicates a need for more replicates with more intensive sampling.

3.5 Plant Species Differences in Bioaccumulation Potential

Analysis of CA application results showed sporadic significant differences among treatment levels for plant AG and BG biomass, concentrations, and mass uptake per unit area, with no predominant trend (Figure D-3). Although Ca and Mg uptake did not show significant differences with CA dose, it is speculated that the relative abundance of Ca and Mg in the pore water outcompetes other metal for uptake into the plant tissue. However, significant differences were found among species for each of these parameters, indicating that species selection is more important under these test conditions than exogenous enhancements for uptake of metals. For this reason the results discussed below focus on species factor interactions that were shown to impact metal uptake during this field study.

3.5.1 Biomass production

AG and BG biomass did not increase between August and October for *C. microptera*, *H. maximiliani*, and *T. latifolia* (Figure 5-7), indicating that growth plateaued shortly after midsummer for these test species. The only significant change from August to October was that *H. maximiliani* AG biomass significantly decreased, which was unexpected. Although CA application levels were not a factor in AG biomass production for any of the species, the accelerated die-back of *H.*
maximiliani, clearly visible 17 days after CA application, is speculated to be due to a species-specific sensitivity to CA application and the resulting increase of toxic levels of As H. maximiliani was exposed to after CA addition. This is discussed in detail in Section 3.6.

Differences in AG and BG biomass production were significant among species (Figure 5-7). In August H. maximiliani produced significantly more AG biomass than C. microptera and T. latifolia, but produced significantly less BG biomass than C. microptera. This highlights species differences in the allocation of resources and plant growth patterns. C. microptera supplied a greater portion of resources to BG
tissue production, generating three to four times as much BG biomass than AG tissue. *H. maximiliani* generated slightly more AG tissue than BG biomass. In October no differences were seen among species in AG biomass, but again, *H. maximiliani* produced significantly less BG tissue than *C. microptera* and *T. latifolia*.

### 3.5.2 Metal bioaccumulation potential

Although *C. microptera’s* AG biomass was significantly lower than *H. maximiliani* in August and not different than *T. latifolia*, *C. microptera* had significantly higher Al, Cr, Cu, and Fe concentrations in its AG biomass than both these species and took up significantly more Al, Cr, Cu, and Fe (except Cu in *H. maximiliani*). *H. maximiliani* had higher As, Ca, and Mg AG tissue concentrations and AG bioaccumulation than the other species (except As concentration in *C. microptera*) in August (Figure 5-8). These field data confirm that various species are able to take up higher amounts of metal than others, despite equal or lower biomass production.

In addition to species selection, time-of-harvest can significantly increase bioaccumulation potential. Al, Cr, Cu, and Fe concentrations and uptake significantly decreased from August to October for *C. microptera*, and As, Ca, Cu, Mg and Zn accumulation decreased for *H. maximiliani*, suggesting that uptake of these metals culminates at the end of summer. Additionally, it is speculated that CA application earlier in the growing season, corresponding to the most rapid yearly growth stage, would show greater increases in metal uptake potential.
Above Ground Plant Tissue

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Uptake</th>
<th>Concentration</th>
<th>Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al (mg g⁻¹)</td>
<td>Ca (mg g⁻¹)</td>
<td>Cu (mg g⁻¹)</td>
<td>Mg (mg g⁻¹)</td>
</tr>
<tr>
<td>Al (μg g⁻¹)</td>
<td>Fe (mg g⁻¹)</td>
<td>Cu (μg g⁻¹)</td>
<td>Fe (mg g⁻¹)</td>
</tr>
<tr>
<td>Al (log g⁻¹)</td>
<td>Zn (μg g⁻¹)</td>
<td>Al (mg g⁻¹)</td>
<td>Zn (μg g⁻¹)</td>
</tr>
</tbody>
</table>

Figure 5-8: Plant AG metal concentration and accumulation. Mean ± SE, n=27. Note various scales on y-axis. Different upper-case letters indicate significant difference in AG concentration among species and harvest dates (two-way ANOVA), based on Tukey’s HSD (P<0.05).
Different lower-case letters indicate significant difference in AG accumulation among species and harvest dates (two-way ANOVA), based on Tukey’s HSD (P<0.05).
Cam = Carex microptera; Heli = Helianthus maximiliani; Typ = Typha latifolia

Significant differences where found in BG concentration among species

(Figure 5-9). In August T. latifolia had significantly higher Al and Mg BG concentrations than C. microptera, but C. microptera had significantly higher Zn concentrations than both of the other species. However, C. microptera sequestered
higher concentrations of As, Ca, Fe, Mg, and Zn than one or both of the other species in October. BG uptake also differed among species. In August *C. microptera* had significantly higher Al, Cr, Fe, and Zn mass uptake than *H. maximiliani*. In October this trend is even more evident, as *C. microptera* had significantly more BG accumulation of all eight metals than *H. maximiliani* and *T. latifolia* (except Mg in *T. latifolia*). Both *H. maximiliani* and *T. latifolia* did not show any differences in BG metal accumulation from August to October. These data confirm species differences in metal bioaccumulation potential, which is not only correlated to biomass production, but also to AG tissue concentrations, and emphasize the impact of species selection in optimizing the pollutant removal performance of stormwater BR systems.

Bioaccumulation efficiency also relies on the translocation of metals from the BG tissue to sequester them in the AG plant tissue (Usman and Mohamed 2009). Ryczewicz-Borecki et al. (2016) found that *Typha latifolia* restricts Cu, Pb, and Zn uptake into the BG tissue, but effectively translocated these metals to the AG tissue, whereas *Phragmites australis* showed a tendency to uptake and sequester metals in the root cells, but inhibited translocation of the metals into the AG tissue. In this field study, all three species, including *T. latifolia*, showed higher concentrations in the BG tissue than in the AG tissue, similar to *P. australis*. No significant differences in translocation of metals among species were found.
3.6 Helianthus maximiliani’s Speculated Arsenic Toxicity

Although biomass production for *H. maximiliani* was not statistically related to CA dose, there was visible dieback of *H. maximiliani* after CA application for both the low and high CA treatments. All measured pH values after CA application in August continued to be neutral to alkaline (8.2 ± 0.45 at 7.6 cm depth and 7.7 ± 0.36 at 15.2 cm depth), indicating the high buffering capacity of this soil. For this
reason pH was not considered a factor in *H. maximiliani’s* accelerated death. Figure 5-10 shows *H. maximiliani* at time of CA application, and the noticeable die back 17 days later.

Drinking water standards for As are 10 μg L⁻¹ regardless of oxidation state (US EPA, 2001c). In a related study, total As concentrations of stormwater runoff entering this field site from 5/26/12 to 9/26/14 were well below the drinking water limit at 1.4 ± 0.11 μg L⁻¹ (n=119). In this CA application study As speciation was not measured, but the total soil pore water As concentrations were more than an order of magnitude higher than drinking water standards for all of the treatments (Table 5-10). Arsenic concentrations ranging from 16 μg L⁻¹ to 94 μg L⁻¹ were also found in 68% of Logan City Landfill monitoring wells and piezometers installed by the Utah Water Research Laboratory in 2008 up-gradient of the Logan Landfill in the center of the Cache Valley (Meng 2015). The potential source of these
high levels of As is speculated to be arsenic sulfide minerals deposited onto surface soils from eroded tertiary sedimentary and volcanic rock of the Salt Lake Formation (Meng 2015). For these reasons it is speculated that the source of As at this field site is the surface soil, and is not associated with the stormwater runoff accumulation.

Arsenate [As (V)] and arsenite [As (III)] are the primary forms of As in soils, where the fully protonated As (III) is the more toxic oxidation state, and is more soluble than the partially protonated As (V) (Frumkin and Gerberding 2007). Arsenic in the soil can be released into solution when changes in redox or pH occur (McLean and Bledsoe 1992).

Interestingly, As soil pore water concentrations for *H. maximiliani* were significantly higher than the *C. microptera* at both soil pore water sampling depths and the unplanted treatment at the 7.6 cm depth in August (Table 5-10). The concentrations of As were consistent between the two sample depths for all treatments. In October *H. maximiliani* was the same as *C. microptera* but different from *T. latifolia* and the control at 7.6 cm and from the control at 15.2 cm. Arsenic is one of only three elements for which *H. maximiliani* had significantly higher AG mass uptake than *C. microptera* and *T. latifolia* (Figure 5-10).

This suggests a species influence over As solubility, indicating that *H. maximiliani*’s chemical and microbial rhizosphere environment solubilizes As more effectively than the other species, allows for transport into and movement through the root tissue (as suggested by the low BG mass uptake), and efficiently transports As to the AG plant material. This high AG uptake and lower BG uptake is in contrast
Table 5-10: Arsenic soil pore water concentrations (µg L\(^{-1}\)), at the 7.6 cm and 15.2 cm depths, for the individual plant species for August and October.

<table>
<thead>
<tr>
<th>Species</th>
<th>As (Aug) (µg L(^{-1}))</th>
<th>As (Oct) (µg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.6 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carex</td>
<td>190 b ± 127</td>
<td>364 AB ± 363</td>
</tr>
<tr>
<td>Helianthus</td>
<td>615 a ± 288</td>
<td>826 A ± 733</td>
</tr>
<tr>
<td>Typha</td>
<td>437 ab ± 355</td>
<td>210 B ± 205</td>
</tr>
<tr>
<td>Control</td>
<td>177 b ± 97.0</td>
<td>109 B ± 48.9</td>
</tr>
<tr>
<td>15.2 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carex</td>
<td>112 b ± 72.1</td>
<td>208 AB ± 192</td>
</tr>
<tr>
<td>Helianthus</td>
<td>601 a ± 653</td>
<td>766 A ± 1026</td>
</tr>
<tr>
<td>Typha</td>
<td>253 ab ± 190</td>
<td>135 AB ± 61.4</td>
</tr>
<tr>
<td>Control</td>
<td>149 ab ± 199</td>
<td>92.6 B ± 45.0</td>
</tr>
</tbody>
</table>

Values are mean ± standard error, unplanted n=9. Different letters, in columns per treatment, indicate significant differences according to Tukey’s HSD test (p ≤ 0.05) for a two-way ANOVA factor interaction (species x depth).

to studies on As uptake reported in the literature that indicate that As predominantly accumulates in the root tissues of non-hyperaccumulator species (Frumkin and Gerberding 2007; Garg and Singla 2011; Finnegan and Chen 2012).

Turgut et al. (2004) reported that higher doses of natural organic acids may be toxic to certain plant species due to toxic levels of increased metal uptake. Lesage et al. (2005) reported >80% decreased growth of Helianthus annuus at CA doses above 50 mmol kg\(^{-1}\). Arsenic is a non-essential metalloid, and toxic to plants. Arsenic inhibits root production and growth. In the AG tissue, As inhibits plant growth, compromises plant reproductive capacity, and/or interferes with critical metabolic process (Finnegan and Chen 2012). It is speculated that H. maximiliani’s accelerated death is correlated to species-specific sensitivity to CA application, which resulted in toxic levels of As uptake. H. maximiliani As concentrations in the AG tissue at the
high CA dose (1.8 mg kg\(^{-1}\)) were not different than the low CA dose concentrations (1.3 mg kg\(^{-1}\)), but were significantly higher than its control CA treatment (1.0 mg kg\(^{-1}\)), and all other treatments, including all of the other two-way factor interactions of species and CA doses (ranging from 0.4 to 1.1 mg kg\(^{-1}\)), and As concentrations in \(H.\) maximiliani from previous year’s analysis (average of 0.4 mg kg\(^{-1}\)). These concentrations are much lower than the tolerance limits of known As hyperaccumulators, such as the \(Pteris\) genus, which are between 5,000 and 10,000 mg kg\(^{-1}\) (Tu and Ma 2002; Zhao et al. 2009). More research is needed to better understand the extent of the species enhanced CA’s effect on As solubility, uptake, and toxicity limits for \(H.\) maximiliani.

### 4.0 Summary and Conclusions

CA is an effective LMWOA shown to increase metal bioavailability and bioaccumulation potential of various plant species when grown on contaminated soils. This study evaluated the extent of CA’s enhancement of bioaccumulation in three plant species grown in a field demonstration BR system, where the soils undergo continual application of polluted stormwater runoff.

An initial laboratory study of the field soil demonstrated that Al, As, Cr, Cu, Fe, and Zn were solubilized proportionally to CA addition (0-500 mmol kg\(^{-1}\)). Next, at the field site, measured citrate recovery in the soil pore water was found to linearly increase with CA dose for unplanted control treatments at both the 7.6 cm and 15.2 cm depths. Measured citrate levels at the 15.2 cm depth, 50 mmol kg\(^{-1}\) CA dose, were
significantly lower for the planted treatments, suggesting increased CA degradation when in contact with the plant’s root zone microbial environment.

At the field site, CA doses (0, 10, 50 mmol kg\(^{-1}\)) corresponded to the lower range of CA additions in the initial laboratory study. At these low CA concentrations pH was not influenced by CA dose, but was significantly influenced by the root environment’s microbial activity. Metal pore water concentrations tended to increase with CA dose for the planted and unplanted treatments at both depths, with the planted system having higher metal solubility than the unplanted. It was speculated that CA stimulated microbial and root exudate production in the planted treatments, increasing the net metal solubility above the unplanted systems. The relative abundance of soluble Mg and Ca was also noted in these calcareous soils.

After the August CA application, significantly lower citrate, Al, Cr, Cu, Mg, and Zn concentrations were found in the 15.2 cm depth soil pore water than in the 7.6 cm depth for the planted samples. These 15.2 depth values were statistically the same as the values measured after CA was fully biodegraded (October). This indicated that the addition of CA did not promote significant risk of leaching solubilized metals into the groundwater for the planted systems.

No significant differences in plant metal uptake were found as a function of CA dose. However, significant differences were found in AG biomass production, metal concentrations, and metal uptake among species. *H. maximilianii* produced the highest amount of AG biomass, however, *C. microptera* accumulated significantly more Al, Cr, Cu, and Fe in the AG tissue than *H. maximilianii* and *T. latifolia* (except Cu in *H. maximilianii*), and significantly more of these metals accumulated in *C.*
microptera’s BG tissue than in H. maximiliani’s BG tissue. This verifies that selecting species with high biomass production and the ability to store high concentrations of metals in the AG tissue can significantly influence bioaccumulation potential. Additionally, C. microptera saw a significant decrease in uptake of these metals from August to October, indicating that time of harvest significantly affected bioaccumulation potential. It was speculated that harvesting earlier in the growing season, corresponding to the plant’s highest rates of growth, would also increase metal uptake. H. maximiliani was speculated to have a species-specific sensitivity to CA application which resulted in toxic As concentrations in its AG tissue, further emphasizing the importance of species selection in optimizing BR metal recovery.

The results of this study show that in these calcareous soils, under low existing soil metal concentrations, soil pore water metal concentrations increase with CA addition. However, this increase was not significant enough to affect bioaccumulation potential in the field BR test plots. Instead, bioaccumulation potential was significantly influenced by species, with C. microptera taking up significantly more Al, Cr, Cu, and Fe into its AB biomass than either T. latifolia or H. maximiliani. With this new information, stormwater managers can better understand the important role of species selection to promote metal uptake, and will aid in the design of BR systems to effectively slow soil metal accumulation, optimize metal recovery, and lengthen their operating life.
5.0 Acknowledgments

This study has been carried out with the financial support of the Utah Mineral Lease Fund, the Utah State University Research Catalyst Grant, and the FY 2010 Source Reduction Assistant Grant Program EPA-HQ-OPPT-2010-02. The authors are grateful to the City of Logan who provided the field site location, and provided labor for the initial construction of the experimental field demonstration site.

6.0 References


CHAPTER 6
SUMMARY, ENGINEERING SIGNIFICANCE

1. Summary

Stormwater runoff contains high levels of nutrients and metals, which detrimentally affect downstream water bodies and/or accumulate in soil environments. Many case studies evaluate pollutant removal efficiencies of BR systems, but the ability to extrapolate a particular study’s results to a location with different temperature, rainfall, and pollutant loads is highly limited. This dissertation’s research design utilized a holistic approach to study pollutant removal efficiency, distribution, and bioaccumulation potential in stormwater BR systems undergoing three hydraulic and pollutant loads. Additionally, the capacity of a low cost, biodegradable organic acid to enhance metal bioaccumulation was examined to increase metal uptake and subsequent removal, and slow metal accumulation in the soil.

This investigation compiled data from past studies, expanded the plant species typically reported in the literature, and provided unprecedented insight into distribution differences among these species. The six species investigated in the greenhouse study were *Phragmites australis* (Common Reed); *Typha latifolia* (Broadleaf Cattail); *Scirpus validus* (Soft-stem Bulrush); *Scirpus acutus* (Hard-stem Bulrush); *Carex praegracilis* (Common Field Sedge); and *Carex microptera* (Smallwing Sedge).
The greenhouse experiment established that an overall 98% recovery of total phosphorous mass over the study period was achievable in Chapter 4, ensuring accuracy of measurements and analyses. *P. australis, C. praegracilis*, and *C. microptera* were found to uptake significantly more total phosphorous and total nitrogen mass into their harvestable tissue than *S. validus*, and *S. acutus* (Table 4-3). These results confirmed that species selection optimizes nutrient retention and recovery from stormwater and decreases nutrient discharge to surface waters.

Metals persist in the environment, and soil metal contamination is a serious problem requiring affordable remediation strategies. In Chapter 3, the greenhouse study confirmed that more than 89% of copper, lead, and zinc accumulated in the soil for all treatments. Bioaccumulation potential is based on a plant’s ability to absorb and transport potentially toxic compounds from the soil, through the roots, and to the aerial parts of a plant. Metal removal via bioaccumulation is contingent upon biomass yield and metal concentrations in the above ground plant tissue. This allows for the harvest and removal of the contaminated plant biomass. However, all plants have a metal toxicity limit that results in growth reduction or death. Toxicity limits were not reached at the pollutant loads typical of stormwater BR systems, as evidenced by increased biomass production with increased hydraulic, nutrient and metal loading. Differences among species were found for above ground and below ground tissue metal concentrations, above ground constituent mass uptake, and mass distribution. In general, *C. praegracilis*, and *C. microptera* increase metal solubility in the soil, leading to the increase of metal mass in the plant tissue relative to the other species.
It is speculated that differences in biological and chemical processes that enhance or prevent metal uptake are the reason for these differences among species. These mechanisms include acidification by the rhizosphere, secretion of organic acids, metal chelates, or enzymes to increase available ion concentrations, and/or promotion of microorganism growth. These results indicated that species selection plays an important role in metal harvestability from BR systems, which can result in reduced site contamination.

Comparisons of biomass and metal uptake between the greenhouse study and a field experiment located in a BR system in Logan, Utah, show similar mass per unit area results. *C. microptera* (Cam) and *T. latifolia* (Typ) were planted in both experiments. Figure 6-1 shows species differences in the greenhouse biomass, where *P. australis* (Phr) produced significantly more biomass than all of the other species except *C. microptera* (Cam).

In the field biomass significantly increased every year from 2011 to 2013 for the *C. microptera*, *H. maximiliani*, and *T. latifolia* species. From 2013 to 2014 biomass did not significantly increase for any of the species, indicating that all species reached full establishment by their third year of growth, and resulted in a plateau in biomass production at or slightly above the growth noted in the greenhouse.

Cu and Zn uptake for the greenhouse and field-site are presented in Figure 6-2 (a and b, respectively). In the greenhouse, mass uptake followed a similar trend as biomass production. *P. australis*’ Cu uptake was significantly higher than *S. validus*, *S. acutus*, and *T. latifolia*. Cu uptake in the field was noticeably lower in 2011, but
Figure 6-1: Plant biomass production in the greenhouse (left most, 6 species) and in the field (3 species, 2001 to 2014). Significant differences are found among species in the greenhouse study, and biomass significantly increased from 2011 to 2013 year for *C. microptera* (Cam), *H. maximiliani* (Heli), and *T. latifolia* (Typ) in the field. Different lower and upper case letters denote differences within the greenhouse and field study, respectively. Values followed by the same letter are not significantly different among species (P<0.05), based on Tukey HSD, analyzed by one-way analysis of variance (ANOVA).

Cam = *C. microptera*  
Sca = *S. acutus*  
Heli = *H. maximiliani*  
Cap = *C. praegracilis*  
Scv = *S. validus*  
Phr = *P. australis*  
Typ = *T. latifolia*

increased significantly in 2012, and leveled out (*C. microptera* and *H. maximiliani*) or continued to increase (*T. latifolia*) in 2013. In 2014, the *C. microptera* and *T. latifolia* Cu uptake matched the greenhouse mass uptake, indicating that after establishment, the uptake values of the plants at the field-site corresponded to the controlled laboratory results.
Figure 6-2: Mass uptake of Cu and Zn (a and b, respectively) for the Greenhouse and Field projects. Error bars are the standard error; n=3 for the Greenhouse; n=6 for 2011, n=12 for 2012 to 2014 for *C. microptera*, *H. maximiliani*, and *T. latifolia*; and n=6 (2012 to 2014). Different lower and upper case letters denote differences within the greenhouse and field study, respectively. Values followed by the same letter are not significantly different among species (P<0.05), based on Tukey HSD, analyzed by one-way analysis of variance (ANOVA). Note different scales on y-axis.

Cam = *C. microptera*  
Cap = *C. praegracilis*  
Phr = *P. australis*  
Sca = *S. acutus*  
Scv = *S. validus*  
Heli = *H. maximiliani*  
Typ = *T. latifolia*
In 2014 the field experiment was also used to investigate citric acid enhanced bioaccumulation potential among three different plant species in typical BR conditions with calcareous soil, as presented in Chapter 5. The species included in this study were *Carex microptera, Helianthus maximiliani,* and *Typha latifolia.* Many studies of highly contaminated sites have proven the effectiveness of citric acid at increasing metal solubility in soil pore water and the resultant uptake of metals into plant tissue. The total soil concentrations in the field were significantly below the NYS DEC chronic human health-based soil cleanup objectives for residential areas limits for aluminum, copper, iron, lead, and zinc, slightly below for chromium (15 mg kg\(^{-1}\), Oct 2014), but above these levels for arsenic (18 mg kg\(^{-1}\), Oct 2014). Currently no studies are available that evaluate citric acid’s enhanced bioaccumulation potential in these calcareous soils with lower level soil metal concentrations.

In this field study, significantly lower citrate and metal concentrations were found at the 15.2 cm depth and in soil pore water than at the 7.6 cm depth for the planted plots, confirming that citric acid easily biodegrades under these conditions, and did not promote significant risk of leaching solubilized metals into the groundwater.

Measured citrate concentrations significantly decreased 24 hours after application in the planted treatments, as pore water traveled down the soil profile through the root zones. It is speculated that consumption of citric acid by microorganisms reduced measured citrate concentrations in the soil pore water, but the increased microbial growth resulted in enhanced microbial exudate production,
and a subsequent net increase of metal solubility. This speculation is validated by increased metal pore water concentrations with CA dose at the 7.6 and 15.2 cm depths.

The increase of metals in the pore water did not correspond with increased metal uptake in the plant tissue. Instead, significant differences were found in AG biomass production, metal concentrations, and metal uptake among species. Significantly more Al, Cr, Cu, and Fe was accumulated in the AG tissue of *C. microptera* than *H. maximiliani* and *T. latifolia* (except Cu in *H. maximiliani*). Additionally, *C. microptera* saw a significant decrease in uptake of these metals from August to October. This verifies that time of harvest and species selection can significantly influence bioaccumulation potential.

Interestingly, a species-specific sensitivity to citric acid application was suspected to augment arsenic uptake into *H. maximiliani*, and resulted in arsenic toxicity, and subsequent accelerated die-back. Arsenic concentrations in the above ground tissue (1.8 mg kg\(^{-1}\)) at the high citric acid dose were significantly higher than the control treatment concentrations (1.0 mg kg\(^{-1}\)) and concentrations from previous year’s analysis (average of 0.4 mg kg\(^{-1}\)).

### 2. Engineering Significance

This dissertation study provided a more detailed understanding of individual plant species influence over constituent removal efficiency, end distribution, transport, and bioaccumulation potential for species typically grown in stormwater BR systems. Biomass and metal uptake were compared between the greenhouse and
field studies and provided comparable results. The field study also provided evidence that citric acid application is not warranted as an enhancement tool for metal bioaccumulation under site conditions with calcareous soil and low soil metal concentrations.

The greenhouse investigation provided mass balance recovery, and insight into pollutant distribution differences among six species. This new information filled existing gaps in understanding the fate of nutrients and metals within water, soil, root, and above ground plant tissue compartments of a BR system. Differences in constituent transport tendencies from the water compartment to plant tissue, and bioaccumulation potential of metals among various species typically grown in stormwater BR systems. Potential differences in biological and chemical processes developed by species to allow or avoid metals were also found. *C. praegracilis* and *C. microptera* species are speculated to promote metal mobilization, and lower distribution in the soil media, whereas *P. australis* showed a tendency to uptake and sequester metals in the root cells, but inhibited translocation of the metals into the above ground tissue.

The results of this study are critical for environmental managers and other decision makers to allocate resources more strategically to meet Total Maximum Daily Load regulations, to make informed choices regarding plant species selection and harvesting procedures of above ground plant tissue as a means of optimizing bioaccumulation potential. These results can also aid stormwater managers in slowing nutrient and metal accumulation in the soil, and effectively controlling contaminant migration from BR systems. These more informed actions would
lengthen the operating life of stormwater BR systems and reduce unintended environmental contamination.

Further investigation into these physical, chemical, and biological processes involved in the uptake of constituents into plant roots, and the translocation to the above ground tissue is needed to more fully understand species differences in nutrient and metal bioaccumulation potential. More research is needed to determine toxicity limits, and to better evaluate the full bioaccumulation potential of this species.
Table A-1: Scientific names of 97 species individually studied for pollutant removal. Bolded species are those also used in the greenhouse study described herein.

<table>
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<th>Scientific name</th>
<th>Citation</th>
<th>Scientific name</th>
<th>Citation</th>
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<td>Arund donax</td>
<td>Jiang 2011</td>
<td>Pennisetum purpureum</td>
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<td>Baumea articulata</td>
<td>in Brisson 2009</td>
<td>Phragmites sp.</td>
<td>Milandri 2012</td>
</tr>
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<td>Betula occidentalis</td>
<td>Klassen 2000</td>
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<td>in Brisson 2009</td>
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<td>Bolboschoenus fluviatilis</td>
<td>in Brisson 2009</td>
<td>Phragmites vallatoria</td>
<td>in Brisson 2009</td>
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<tr>
<td>Canna generalis</td>
<td>Jiang 2011</td>
<td>Poo labillardierei Steud.</td>
<td>Read 2008 &amp; 2010</td>
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<td>Pomaderris paniculosa</td>
<td>Read 2008 &amp; 2010</td>
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<td>Pragmites mauritianus</td>
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<td>Reineckia carnea</td>
<td>Jiang 2011</td>
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<td>Carex proagracilis</td>
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<td>Sagittaria latifolia</td>
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<td>Carex rostrata</td>
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<td>Saururus cernus</td>
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Table B-1: (a) Total metal concentrations in the soil at the beginning and end of the study (end rhizosphere and end surface soil layers); (b) Total metal concentrations in the rhizosphere soil layer at the end of the study by species; (c) and composite metal concentrations in the exfiltrate throughout the study.

(a) Soil concentrations (mg kg$^{-1}$) at beginning and end of study

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<td>beginning$^a$</td>
<td>end rhizo$^b$</td>
<td>end surface$^c$</td>
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<td>end rhizo$^b$</td>
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<td>Cu</td>
<td>1.1 ± 0.1</td>
<td>3.4 ± 0.4</td>
<td>12.7 ± 1.1</td>
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<td>Pb</td>
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<td>Zn</td>
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<td>7.3 ± 0.3</td>
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(b) Rhizosphere soil concentrations (mg kg$^{-1}$) at end of study by species$^e$

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<td>2.5 ± 0.5 b</td>
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<tr>
<td>Zn</td>
<td>6.1 ± 1.1 a</td>
<td>2.9 ± 0.4 b</td>
<td>2.5 ± 0.5 b</td>
<td>3.7 ± 0.6 ab</td>
<td>2.4 ± 0.4 b</td>
<td>3.4 ± 0.5 ab</td>
</tr>
</tbody>
</table>

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>19.6 ± 3.6 a</td>
<td>12.0 ± 1.6 a</td>
<td>11.1 ± 2.0 a</td>
<td>13.7 ± 1.8 a</td>
<td>10.3 ± 1.6 a</td>
<td>13.2 ± 2.3</td>
</tr>
<tr>
<td>Pb</td>
<td>15.0 ± 2.4 a</td>
<td>11.1 ± 1.6 a</td>
<td>10.5 ± 1.6 a</td>
<td>13.5 ± 2.0 a</td>
<td>12.0 ± 1.5 a</td>
<td>12.5 ± 1.4 a</td>
</tr>
<tr>
<td>Zn</td>
<td>13.6 ± 1.8 ab</td>
<td>10.9 ± 1.6 bc</td>
<td>12.3 ± 2.2 abc</td>
<td>21.1 ± 3.3 a</td>
<td>20.1 ± 2.7 ab</td>
<td>14.1 ± 2.0 abc</td>
</tr>
</tbody>
</table>

(c) Composite exfiltrate concentrations (mg L$^{-1}$) by species$^f$

<table>
<thead>
<tr>
<th></th>
<th>BATCH 1</th>
<th></th>
<th></th>
<th>BATCH 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td></td>
<td>Phr</td>
<td>Scv</td>
<td>Sca</td>
<td>Cap</td>
<td>Cam</td>
</tr>
<tr>
<td>Pb</td>
<td>2.5 ± 0.3 ab</td>
<td>2.1 ± 0.2 ab</td>
<td>2.2 ± 0.4 ab</td>
<td>3.8 ± 0.4 a</td>
<td>3.6 ± 0.3 ab</td>
<td>2.8 ± 0.7 ab</td>
</tr>
<tr>
<td>Zn</td>
<td>13.6 ± 1.8 ab</td>
<td>10.9 ± 1.6 bc</td>
<td>12.3 ± 2.2 abc</td>
<td>21.1 ± 3.3 a</td>
<td>20.1 ± 2.7 ab</td>
<td>14.1 ± 2.0 abc</td>
</tr>
</tbody>
</table>

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>2.5 ± 0.3 ab</td>
<td>2.1 ± 0.2 ab</td>
<td>2.2 ± 0.4 ab</td>
<td>3.8 ± 0.4 a</td>
<td>3.6 ± 0.3 ab</td>
<td>2.8 ± 0.7 ab</td>
</tr>
<tr>
<td>Pb</td>
<td>1.4 ± 0.3 abc</td>
<td>0.6 ± 0.2 bc</td>
<td>0.8 ± 0.3 abc</td>
<td>2.5 ± 0.7 a</td>
<td>2.1 ± 0.6 ab</td>
<td>0.4 ± 0.1 bc</td>
</tr>
<tr>
<td>Zn</td>
<td>13.6 ± 1.8 abc</td>
<td>10.9 ± 1.6 bc</td>
<td>12.3 ± 2.2 abc</td>
<td>21.1 ± 3.3 a</td>
<td>20.1 ± 2.7 ab</td>
<td>14.1 ± 2.0 abc</td>
</tr>
</tbody>
</table>

Mean ± SE; $^a$ n=15; $^b$ n=6; $^c$ n=45; $^d$ n=18; $^e$ n=9; $^f$ n=27
Values within a row followed by the same lower-case letter are not significantly different among species (P<0.05), based on Tukey HSD within batches.
Figure B-1: Biomass density over a 4-year observation period at Green Meadows Bioretention Site, Logan, Utah. Error bars represent standard error; n=6 in 2011, n=18 in 2012 and 2013, n=54 in 2014.
Table C-1: Exfiltrate TDN and TDP concentrations (mean ± SE).

<table>
<thead>
<tr>
<th>Exfiltrate</th>
<th>TDN (mg L⁻¹)</th>
<th>TDP (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phr</td>
<td>1.83 c ± 0.6</td>
<td>0.18 b ± 0.13</td>
</tr>
<tr>
<td>Typ</td>
<td>2.22 bc ± 1.1</td>
<td>0.22 b ± 0.12</td>
</tr>
<tr>
<td>Scv</td>
<td>2.22 bc ± 0.6</td>
<td>0.20 b ± 0.04</td>
</tr>
<tr>
<td>Sca</td>
<td>2.41 b ± 0.7</td>
<td>0.21 b ± 0.05</td>
</tr>
<tr>
<td>Cap</td>
<td>2.22 bc ± 0.8</td>
<td>0.16 b ± 0.05</td>
</tr>
<tr>
<td>Cam</td>
<td>1.91 c ± 0.7</td>
<td>0.20 b ± 0.11</td>
</tr>
<tr>
<td>Ctrl</td>
<td>3.57 a ± 1.5</td>
<td>0.46 a ± 0.27</td>
</tr>
</tbody>
</table>

Phr: *Phragmites australis*; Typ: *Typha latifolia*; Scv: *Scirpus validus*; Sca: *Scirpus acutus*; Cap: *Carex praegracilis*; Cam: *Carex microptera*.
Different lower-case letters down each column indicate significant difference among species, based on Tukey HSD (P=0.05). Values in blue (a) are significantly higher than values highlighted in peach.
Figure D-1: Al, As, Cr, Cu, Fe, and Zn concentrations, in the soil pore water, as a function of citric acid (CA) addition and hydrochloric acid (HCl) addition (see text for mmol kg⁻¹ equivalent concentration details).
Figure D-2: MINTEQ model results of calcium citrate precipitation (earlandate), calcium citrate, calcite and Ca\(^{2+}\) concentrations (mol L\(^{-1}\)) at increasing citric acid dose (mol L\(^{-1}\)) at pH 7.2.
Figure D-3: Soluble metal concentrations in the 15.2 cm depth pore-water samples, as a function of CA dose for planted and unplanted treatments. Planted n=26, Unplanted n=8. Note different scales on the y-axis.
Table D-1: Laboratory Experiment’s citric and hydrochloric acid soil extraction details; soil mass (wet and dry), acid concentrations (mmol kg\(^{-1}\)), and pH values.

### Citric Acid (CA) extraction (20 mL CA:10 g wet soil)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Soil wet (g)</th>
<th>Soil dry (g)</th>
<th>CA (mg)</th>
<th>pH Ca (next day)</th>
<th>SEM</th>
<th>pH Ca (next day)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>52.54</td>
<td>10.58</td>
<td>10.01</td>
<td>6.80</td>
<td>0.02</td>
<td>6.80</td>
<td>0.02</td>
</tr>
<tr>
<td>2nd</td>
<td>52.53</td>
<td>10.58</td>
<td>10.01</td>
<td>6.80</td>
<td>0.02</td>
<td>6.80</td>
<td>0.02</td>
</tr>
</tbody>
</table>

### HCl extraction (20 mL HCl:10 g wet soil)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Soil wet (g)</th>
<th>Soil dry (g)</th>
<th>CA (mg)</th>
<th>pH Ca (next day)</th>
<th>SEM</th>
<th>pH Ca (next day)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>52.54</td>
<td>10.58</td>
<td>10.01</td>
<td>6.80</td>
<td>0.02</td>
<td>6.80</td>
<td>0.02</td>
</tr>
<tr>
<td>2nd</td>
<td>52.53</td>
<td>10.58</td>
<td>10.01</td>
<td>6.80</td>
<td>0.02</td>
<td>6.80</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Note:** SEM values are calculated for each sample.
Table D-2: August soil pore water pH among CA application dose and individual species for the 7.6 cm sampling depth.

<table>
<thead>
<tr>
<th>pH (Aug)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mmol/kg</td>
<td>8.07 a ±</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mmol/kg</td>
<td>8.06 a ±</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mmol/kg</td>
<td>7.83 a ±</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carex</td>
<td>8.7 A ±</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helianthus</td>
<td>8.2 A ±</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhus</td>
<td>8.1 A ±</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil only</td>
<td>8.1 A ±</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± standard error, unplanted n=3. Lower case letters indicate significant differences according to Tukey’s HSD test (p ≤ 0.05) for a one-way ANOVA factor interaction (CA dose). Upper case letters indicate significant differences according to Tukey’s HSD test (p ≤ 0.05) for a one-way ANOVA factor interaction (species).
Table D-3: August soil pore water concentrations (mg L\(^{-1}\) or µg L\(^{-1}\), as noted) among individual species and CA application dose for the 7.6 cm and 15.2 cm sampling depths.

<table>
<thead>
<tr>
<th>Species</th>
<th>Control</th>
<th>10 mmol kg(^{-1})</th>
<th>50 mmol kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typha</td>
<td>0.51 b 5.37</td>
<td>61.8 a 49.6</td>
<td>9.37 b 17.6</td>
</tr>
<tr>
<td>Heli</td>
<td>0.06 b 43.0</td>
<td>13.3 b 18.9</td>
<td>13.3 a 18.9</td>
</tr>
<tr>
<td>Carex</td>
<td>0.37 b 316</td>
<td>32.2 a 15.6</td>
<td>32.2 a 15.6</td>
</tr>
<tr>
<td>Unplanted</td>
<td>0.09 b 44.2</td>
<td>5.32 b 59.8</td>
<td>5.32 b 59.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Control</th>
<th>10 mmol kg(^{-1})</th>
<th>50 mmol kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typha</td>
<td>0.23 b 240</td>
<td>0.79 b 180</td>
<td>1.23 b 250</td>
</tr>
<tr>
<td>Heli</td>
<td>0.27 b 375</td>
<td>1.55 b 190</td>
<td>1.55 b 190</td>
</tr>
<tr>
<td>Carex</td>
<td>0.63 b 850</td>
<td>1.13 a 175</td>
<td>1.13 a 175</td>
</tr>
<tr>
<td>Unplanted</td>
<td>0.18 b 277</td>
<td>0.83 b 404</td>
<td>0.83 b 404</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Control</th>
<th>10 mmol kg(^{-1})</th>
<th>50 mmol kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typha</td>
<td>3.40 b 19.9</td>
<td>0.24 b 5.3</td>
<td>0.24 b 5.3</td>
</tr>
<tr>
<td>Heli</td>
<td>19.9 b 33.7</td>
<td>2.43 b 5.1</td>
<td>2.43 b 5.1</td>
</tr>
<tr>
<td>Carex</td>
<td>0.66 b 3.4</td>
<td>0.66 b 3.4</td>
<td>0.66 b 3.4</td>
</tr>
<tr>
<td>Unplanted</td>
<td>1.87 b 3.4</td>
<td>1.11 b 3.4</td>
<td>1.11 b 3.4</td>
</tr>
</tbody>
</table>

Values are mean ± standard error, unplanted n=3. Different letters, at each depth for each metal, indicate significant differences according to Tukey’s HSD test (p ≤ 0.05) for a two-way ANOVA factor interaction (species x CA dose).
### Table D-4: Biomass (g m⁻²), and Al, As, Cr, Cu, Fe, Pb, and Zn uptake (mg m⁻²) in above ground and below-ground plant tissue.

#### Above Ground

<table>
<thead>
<tr>
<th>Month</th>
<th>Biomass (g m⁻²)</th>
<th>Al (mg m⁻²)</th>
<th>Cu (mg m⁻²)</th>
<th>Fe (mg m⁻²)</th>
<th>Mg (mg m⁻²)</th>
<th>Zn (mg m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct</td>
<td>Ø 503 c ± 230</td>
<td>0.69 ± 0.55</td>
<td>5.29 ± 0.12</td>
<td>2.52 ± 1.4</td>
<td>3.661 ± 1.332</td>
<td>1.4 ± 0.61</td>
</tr>
<tr>
<td>low</td>
<td>721 ab ± 173 c</td>
<td>0.91 ± 0.27</td>
<td>9.67 ± 0.19</td>
<td>3.42 ± 1.218</td>
<td>4.219 ± 0.926</td>
<td>3.4 ± 0.99</td>
</tr>
<tr>
<td>high</td>
<td>619 c ± 131 a</td>
<td>0.76 ± 0.29</td>
<td>7.95 ± 1.54</td>
<td>2.935 ± 1.228</td>
<td>2.88 ± 0.87</td>
<td>3.8 ± 1.7</td>
</tr>
<tr>
<td>Aug</td>
<td>Ø 857 abc ± 152</td>
<td>0.79 ± 0.46</td>
<td>2.23 ± 2.175</td>
<td>5.579 ± 0.383</td>
<td>4.22 ± 0.49</td>
<td>2.7 ± 1.5</td>
</tr>
<tr>
<td>low</td>
<td>1287 a ± 835 c</td>
<td>1.34 ± 0.77</td>
<td>1.57 ± 0.45</td>
<td>12.794 ± 0.283</td>
<td>0.51 ± 0.25</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>high</td>
<td>1255 ab ± 457 c</td>
<td>2.2 ± 1.3</td>
<td>2.1 ± 1.215</td>
<td>13.887 ± 5.528</td>
<td>0.74 ± 0.45</td>
<td>4.7 ± 2.9</td>
</tr>
<tr>
<td>Sep</td>
<td>Ø 491 c ± 374 c</td>
<td>0.86 ± 0.96</td>
<td>1.47 ± 1.24</td>
<td>2.586 ± 0.844</td>
<td>0.22 ± 0.25</td>
<td>1.2 ± 0.46</td>
</tr>
<tr>
<td>low</td>
<td>569 c ± 276 c</td>
<td>0.75 ± 0.25</td>
<td>0.58 ± 0.53</td>
<td>2.185 ± 1.212</td>
<td>0.25 ± 0.27</td>
<td>0.6 ± 0.60</td>
</tr>
<tr>
<td>high</td>
<td>654 bc ± 342 c</td>
<td>0.46 ± 0.45</td>
<td>1.6 ± 1.26</td>
<td>4.322 ± 2.096</td>
<td>0.36 ± 0.28</td>
<td>0.9 ± 0.80</td>
</tr>
</tbody>
</table>

#### Below Ground

<table>
<thead>
<tr>
<th>Month</th>
<th>Biomass (g m⁻²)</th>
<th>Al (mg m⁻²)</th>
<th>Cu (mg m⁻²)</th>
<th>Fe (mg m⁻²)</th>
<th>Mg (mg m⁻²)</th>
<th>Zn (mg m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct</td>
<td>Ø 464 ab ± 194 c</td>
<td>0.88 ± 0.24</td>
<td>1.88 ± 0.19</td>
<td>2.935 ± 1.045</td>
<td>0.29 ± 0.16</td>
<td>2.2 ± 0.75</td>
</tr>
<tr>
<td>low</td>
<td>612 a ± 204 c</td>
<td>0.39 ± 0.15</td>
<td>3.82 ± 0.78</td>
<td>1.513 ± 3.788</td>
<td>0.57 ± 1.0</td>
<td>3.4 ± 2.4</td>
</tr>
<tr>
<td>high</td>
<td>540 ab ± 129 b</td>
<td>0.40 ± 0.30</td>
<td>4.95 ± 1.99</td>
<td>3.977 ± 1.114</td>
<td>0.71 ± 0.40</td>
<td>5.4 ± 1.4</td>
</tr>
<tr>
<td>Aug</td>
<td>Ø 446 ab ± 250 b</td>
<td>0.41 ± 0.27</td>
<td>2.41 ± 0.90</td>
<td>9.99 ± 1.321</td>
<td>0.62 ± 0.44</td>
<td>1.4 ± 0.80</td>
</tr>
<tr>
<td>low</td>
<td>541 ab ± 205 b</td>
<td>0.87 ± 0.36</td>
<td>1.06 ± 0.94</td>
<td>7.328 ± 1.977</td>
<td>0.53 ± 0.26</td>
<td>1.3 ± 0.60</td>
</tr>
<tr>
<td>high</td>
<td>611 a ± 148 b</td>
<td>1.3 ± 1.04</td>
<td>1.57 ± 8.13</td>
<td>8.079 ± 2.038</td>
<td>0.48 ± 0.22</td>
<td>1.9 ± 1.11</td>
</tr>
<tr>
<td>Sep</td>
<td>Ø 388 ab ± 134 b</td>
<td>0.42 ± 0.18</td>
<td>0.93 ± 0.44</td>
<td>2.585 ± 1.044</td>
<td>0.30 ± 0.15</td>
<td>1.9 ± 0.63</td>
</tr>
<tr>
<td>low</td>
<td>256 b ± 63 b</td>
<td>0.24 ± 0.17</td>
<td>1.63 ± 2.25</td>
<td>1.85 ± 0.314</td>
<td>0.21 ± 0.10</td>
<td>0.61 ± 0.19</td>
</tr>
<tr>
<td>high</td>
<td>371 ab ± 104 a</td>
<td>0.48 ± 0.43</td>
<td>0.51 ± 0.40</td>
<td>2.337 ± 0.760</td>
<td>0.27 ± 0.20</td>
<td>0.72 ± 0.38</td>
</tr>
</tbody>
</table>

#### Mean ± SE, n=9

**Helianthus maximiliani; Typha; Typha latifolia; Carex; Carex microptera.**

Lower-case letters refer to Tukey HSD post-hoc comparison (P<0.05) for a two-way ANOVA factor interaction (species x CA dose). Values shaded in blue are significantly higher than values shaded in peach within that particular species.
CURRICULUM VITAE

MALGORZATA RYCEWICZ-BORECKI
margie.borecki@aggiemail.usu.edu

EDUCATION

2015  PhD of Environmental Engineering, Utah State University, CEE
      Advisor: Dr. R. Ryan Dupont (gpa 3.98/4.0)
      “Stormwater Bioretention: Nitrogen, Phosphorus, and
      Metal Removal by Plants”

2005  Master of Landscape Architecture, University of Michigan, SNRE
      Advisor: B. Grese
      “The Tsar's Hunting Palace Garden in Białowieża, Poland”

1997  Bachelor of Landscape Architecture
      University of Illinois, School of Fine Arts

1995  Instrument & Private Pilot Licenses
      University of Illinois, Department of Aviation

1993  Military Occupational Specialty School, NAS Memphis, TN

1992  U.S. Marine Corps Boot Camp, Parris Island, SC

PROFESSIONAL EXPERIENCE

2007-2014  Track Co-chair, Council of Educators in Landscape Architecture
            Conference Committee

2005-2009  Assistant Professor, Department of Landscape Architecture and
            Environmental Planning, Utah State University

2004-2005  Graduate Student Instructor, Department of Landscape Architecture,
            School of Natural Resources, University of Michigan

2000-2002  Landscape Architect & Project Manager, The Lakota Group, Chicago, IL


1994-1998  Corporal, Non-Commissioned Officer, United States Marine Corps
            Reserves, Glenview, IL and Fort Worth, TX
AWARDS AND ACKNOWLEDGMENTS

2013  UPR’s Contemporary Western Woman Interview, by Elaine Thatcher
2012  Women Tech Awards Finalist for Academic Excellence
2012  UPR’s Morning Addition Feature, by Storee Powell
2012  CELA Outstanding Communications Award for the CELA Track Chairs System
2012  3rd place, Fresh Ideas Poster Contest, AWWA Intermountain Section
2006  USU Outstanding Faculty Service to First Year Students Award Nomination

REGISTRATIONS

2010  American Water Works Association, State of Utah Member
2001  Registered Landscape Architect, State of IL, License No. 157.001079

MAJOR RESEARCH TOPICS AND FUNDING SOURCES

Spring 15  USU PhD Completion Scholarship, College of Engineering (Amount $20,000); individual
Spring 10  EPA’s FY 2010 Source Reduction Assistant Grant Program: “Optimization of Nitrogen and Phosphorus Removal by Vegetated Stormwater Detention Basins” (Amount $25,107); Co-PI with Dr. R. R. Dupont and Joan E. McLean
Spring 09  USU Research Catalyst Grant: “Uncovering the State of the Intermountain West’s Wet Structural Stormwater BMPs” (Amount $19,940); principal investigator
Summer 07  Advance at USU Collaborative Research Grant: “Functionality Assessment of Cache Valley Stormwater Best Management Practices Based on Plant Community Composition” (Amount $8,237); principal investigator
Spring 06  USU New Faculty Grant 2006-2007: “Storm Water Best Management Practices (BMPs) in Northern Utah: Catalog of Sites Utilizing BMPs and their Perceived Effectiveness” (Amount: $12,970); individual
Spring 99  University of Illinois Ryerson Traveling Fellowship: “The Branicki Garden in Bialystok, Poland; A Parallel of Poland’s Past” (Amount: $6,000); individual


Book Reviews:


Invited Articles:


PRESENTATIONS


