Biomass, Nutrient and Trace Element Dynamics in Cattail and Switchgrass during Wetland and Terrestrial Phytoremediation of Municipal Biosolids

by

Nicholson Ngoni Jeke

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Department of Soil Science
University of Manitoba
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ABSTRACT

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Advisor: Dr. Francis Zvomuya

Knowledge of nutrient accumulation and partitioning in plants is important to determine the optimum timing of harvesting during phytoremediation of biosolids. This research showed that a greater proportion of nitrogen (N) and phosphorus (P) absorbed by cattail and switchgrass was partitioned to the aboveground biomass (AGB), but this partition decreased after the onset of nutrient retranslocation to roots. Therefore, AGB should be harvested prior to retranslocation in order to optimize nutrient phytoextraction. Trace elements partitioned preferentially to the root biomass, indicating that AGB harvesting will have little impact on their phytoextraction. Net mineralized N concentration (N_{min}) in biosolids from the primary lagoon cell was optimized near field capacity [60% water filled pore space (WFPS) but changed little under drier conditions (30% WFPS). Under near-saturation conditions (90% WFPS), net N_{min} decreased with incubation time, likely due to reduced mineralization and denitrification. Available (Olsen) P concentration was not affected by moisture content.

FOREWORD

The thesis was prepared following guidelines set by the Department of Soil Science at the University of Manitoba. Chapter 1 briefly introduces the thesis with relevant literature and includes overall objectives of the study. The three research chapters of the thesis have been prepared and formatted for submission to the Journal of Environmental Quality. Chapter 2 is a laboratory incubation experiment, Chapters 3 and 4 are growth room experiments conducted at the University of Manitoba and Chapter 5 is an overall synthesis of the study. I designed all the experiments with the help of Dr. Francis Zvomuya. I was involved in setting up the experiments, watering, sampling, laboratory analyses, data processing and statistical analysis. I am the principal author of the three manuscripts based on this work.

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1. INTRODUCTION

1.1 Municipal Biosolids

Municipal biosolids are a nutrient rich organic material produced from the stabilization of sewage sludge that is generated from decomposition and settling of wastewater (Haynes et al., 2009; CCME, 2012). Nitrogen concentration in biosolids ranges from 10 to 80 g kg^{-1} dry biosolids while P concentration varies from 5 to 50 g kg^{-1} (LeBlanc 2008). The quantity of nutrients in biosolids varies with source of wastewater and the wastewater treatment process (Lu et al., 2012). Biosolids often contain trace elements such as zinc (Zn), copper (Cu), mercury (Hg), lead (Pb), cadmium (Cd), and chromium (Cr) that end up in sewage during wastewater treatment. Generally, lower trace element concentrations are expected in biosolids produced from domestic water effluent, while high concentrations are common in biosolids produced from industrial wastewater treatment (Haynes et al., 2009). In domestic wastewater, the bulk of Cu originates from piping while Zn comes from household products such as skin creams, deodorants, shampoos, and ointments (Comber and Gunn, 1996).

Many small rural municipalities use wastewater lagoons or stabilisation ponds for wastewater treatment because they are cheap to construct and operate and require low maintenance and minimal energy (Smith and Emde, 1999). In the late 1980's, over 1,000 waste stabilisation ponds accounted for half of the wastewater treatment systems in Canada (Townsend and Knoll, 1987; Heinke et al., 1991) with the majority in western and northern Canada (Heinke et al., 1991). As of 1994, 127 lagoons were in use in Manitoba, 278 in Alberta, 129 in Saskatchewan, and 128 in Ontario, and these accounted for 85%, 84%, 92%, and 33%, respectively, of the total wastewater treatment facilities in

these provinces (Smith and Emde, 1999). In the United States, over 7,500 wastewater stabilisation ponds have been built for rural communities with populations less than 10,000 (USEPA, 2002; Liu, 2007). Lagoons are decommissioned once they reach their lifespan, typically 20-30 years (Ross, 2003).

Spreading biosolids on agricultural land is presently the most cost-effective and beneficial biosolids disposal option (Haynes et al., 2009; CCME, 2012). Biosolids provide nutrients, replenish soil organic matter, and improve soil structure for agricultural production. On a global scale, Vasilek (2007) reported that 75% of wastewater residuals were land-applied, while 7% were disposed of via landfilling and 12% were incinerated. In Canada and the United States, about 60% of biosolids generated are applied on land (Cogger et al., 2006; Pepper et al., 2008). This practice is even more common in western Canada, where about 80% of the biosolids are land-applied (City of Winnipeg, 2014).

Spreading of biosolids on land is currently the most cost-effective option for biosolids management (Campbell, 2000). For example, a small regional authority in Montgomery County, PA, USA has an award-winning biosolids recycling program with the following costs for the various recycling programs: $283 per dry tonne for agricultural use on a nearby farm, $820 per dry tonne for offsite dewatering and land application, $869 per dry tonnne for offsite lime stabilization and agricultural use, and $684 per dry tonne for landfill disposal (Vasileski, 2007). Evidently, land application of biosolids is the lowest cost biosolids management option for this municipality and likely to be reflective for many small municipalities.

Although spreading of biosolids on land is an attractive option, shortage of a suitable land base within economical distances may limit this biosolids management option. Application of biosolids based on agronomic phosphorus (P) rates is now a common practice to avoid excessive accumulation of P in soil from N-based rates, and this has resulted in an increase in the land base required to spread the same quantity of biosolids (Shober and Sims, 2003). In MB, spreading biosolids on agricultural land is only permitted on land on which cereals, forages, oil seeds, field peas, and lentils are grown three years following biosolids application (CCME, 2010). Cattle are not permitted on pasture land within three years of biosolids application (CCME, 2010). Contamination of food source, issues of emerging concerns such as pharmaceuticals and personal care products in biosolids, or banning of spreading on agricultural land may also restrict the use of biosolids on agricultural land. In European countries such as Denmark and Sweden, spreading of biosolids on agricultural land is now restricted (Wang et al., 2008). The City of Winnipeg, MB, Canada, stopped land application of biosolids in 2010 owing to more stringent provincial nutrient regulations under the Water Protection Act (City of Winnipeg, 2014). In Canada, regulators are adopting more stringent regulations such as those in Europe (Oleszkiewicz and Mavinic, 2002). With tightening of regulations, limitation of the biosolids content, and costs of disposal, disposal options will continue to be a challenge

Most of the land spreading occurs in small rural municipalities and any restrictions or limitations on land application of biosolids will be felt to a larger extent by these small rural communities. Where spreading on agricultural land is restricted, not feasible, or uneconomical, in situ phytoremediation of municipal biosolids is a promising alternative

for phytoremediation of end-of-life lagoons. During phytoremediation, plants take up available nutrients and other contaminants into the plant biomass, and harvesting the biomass removes the nutrients and other contaminants from the biosolids.

1.2 Nutrient Dynamics in Municipal Biosolids.

About 50–90% of N in biosolids is in the organic form (Sommers, 1977) while P is primarily found as inorganic phosphates (Maguire et al., 2000; Medeiros et al., 2005). Inorganic phosphates of aluminum (Al), iron (Fe) and calcium (Ca) are the dominant inorganic P forms (Lu et al., 2012). Mineralization of organic N and P and dissolution of P precipitates is important for N and P to be available for plant uptake (Petersen et al., 2003). Mineralization of nutrients such as N is an important prerequisite for phytoremediation not only for the uptake of N but also to support a healthy plant population that can produce high biomass yield and thus effectively function to remove contaminants from the biosolids.

Phosphorus precipitates formed from soluble salts of Fe, Al or Ca deliberately added to remove P from wastewater reduce the phytoavailability of P in biosolids (O'Connor et al., 2004; Haynes et al., 2009). However, in natural wastewater stabilization ponds, these metal salts are not added, resulting in low concentrations of Fe from sources such as corrosion of pipes or Ca from human feces and thus more inorganic P is expected to be available for plant uptake (Haynes et al., 2009). Carliell and Wheatly (1997) reported 20% of total P being soluble in biological sludge while only 3% was soluble in sludge treated with ferric sulfate.

There is a wealth of knowledge on N dynamics in soils and soils amended with biosolids. In such cases, N dynamics in biosolids after incorporation in soil are deduced

from changes in the amended soil (Jaynes et al., 2003). Nitrogen release from biosolids is estimated from the difference in N mineralization between biosolids-amended soils and unamended soils. However, the difference in mineralisation cannot be attributed to biosolids alone because the addition of biosolids to soil interferes with the mineralization process, such as boosting microbial populations or making the soil environment to be more favourable for mineralization. Correa et al. (2001) reported mineralization ranging from 0 to 25% of organic N in biosolids incubated alone while 10-24% of organic N in the biosolids was mineralized in a Spodosol and 23-52% was mineralized in an Oxisol. Greater mineralization in the biosolids-amended soils was attributed to soil providing a more conducive environment for mineralization to take place. Therefore, the study of N and P availability in biosolids as substrate for plant growth is essential to predict nutrient availability in biosolids, a growth medium that has physicochemical conditions different from soil.

Mineralization of organic forms of N and P is a microbially-mediated process and is therefore affected by environmental conditions such as moisture and temperature that affect microbial activity. Lagoons are poorly drained due to the underlying clay material that functions to prevent seepage of contaminants to groundwater. Aerobic and anaerobic conditions are common in end-of-life lagoons as a result of ponding or saturation during snowmelt or heavy rains or drying during drought. Most studies on N and P availability have been conducted at moisture levels near field capacity (Gilmour et al., 2003; Gil et al., 2011; Corrêa et al., 2012). It is important to understand the effects of fluctuating moisture conditions typical of end-of-life lagoons to gain insights into potential impacts

on plant biomass yields and therefore the phytoextraction of nutrients and other contaminants.

1.3 Wetland Phytoremediation

Wetlands have been used widely in the treatment of domestic wastewater (Coleman et al., 2001; Karathanasis et al., 2003), municipal wastewater (Merlin et al., 2002; Ansola et al., 2003), effluent from livestock operations (Comeau et al., 2001), and industrial wastewater (Hadad et al., 2006; Di Luca et al., 2011). Wetlands use natural processes involving wetland plants, soils, and microbial populations to remove nutrients and metal contaminants from wastewater (Vymazal, 2007).

Nitrogen is removed from wetlands through denitrification, ammonia volatilization, microbial assimilation, and plant uptake (Sundaravadivel and Vigneswaran, 2001). Denitrification is an important N removal mechanism in wetlands (Bastviken et al., 2009) and accounts for greater than 50% of N removal in constructed wetlands (Søvik and Mørkved, 2008). Wetland plants enhance denitrification by supplying organic matter, which is utilized by denitrifying bacteria, or by lowering redox potential (Bachand and Horne, 1999; Søvik and Mørkved, 2008). Macrophytes also contribute to N removal by taking up N in the form of NH_4-N and NO_3-N, which can be removed from wetlands by harvesting aboveground biomass. Vymazal (2007) reported harvesting plants accounting for less than 10% of N removal from various constructed wetlands. Harvesting macrophytes is efficient in removing nutrients from wetlands receiving influent with low nutrient concentrations (Cicek et al., 2006).

Phosphorus in wastewater treatment wetlands is removed from wastewater by sedimentation of particulate P, adsorption of soluble P onto clay particles, precipitation,

complexation, and plant uptake (Reddy and DeLaune, 2008). Adsorption of P by sediment due to reactions with Fe, Al or Ca is the main P removal mechanism in wastewater treatment wetlands (Comeau et al., 2001; Vymazal, 2007). Harvesting plants is an important process to remove P from sediments and thus it is important to harvest plants at peak P accumulation to maximize P removal. In northern climates, wetland plants such as cattail go through an annual cycle in which growth is maximized during spring and early summer followed by reduced growth during retranslocation of nutrients and photoassimilates, such as carbohydrates produced during photosynthesis, to rhizomes (Vymazal, 2007). Retranslocation of nutrients and photoassimilates vary among species but may reach more than 50% of the aboveground nutrients and photoassimilates (Vymazal, 1995). From a phytoremediation perspective, harvesting aboveground biomass before nutrients and contaminants are retranslocated to belowground biomass is essential to maximize phytoremediation potential.

Similar to P, trace elements are removed from sediments through harvesting aboveground biomass. Unfortunately, trace element translocation from roots to shoots is very limited in wetland plants due to an exclusion mechanism to protect aerial parts from metal toxicity, resulting in ineffective removal of trace elements by harvesting aboveground biomass (Batty and Younger, 2004; Deng et al., 2004). Phytoextraction of trace elements and removal by harvesting can be practical with use of high biomass plants (Keller et al., 2003; Chen et al., 2012). High biomass plants such as cattail and reeds have been effectively used for phytoremediation of zinc and lead (Ye et al., 1997a; Ye et al., 1997b).

Macrophytes play an important role in contaminant removal in wetlands, including taking up contaminants, enhancing decomposition of pollutants by transporting oxygen for aerobes to the roots, providing attachment surfaces for microorganisms, and aiding in settling of suspended solids (Maddison et al., 2005; Yang et al., 2007). Contaminant removal achieved by harvesting plants depends on biomass yield and contaminant concentrations in the plant biomass (Alkorta et al., 2004; Vymazal, 2007), thus effecting the importance of choice of plant species for successful phytoremediation. Cattail, a large emergent plant found in wetlands across North America, has been extensively used to remove N, P, and trace elements in wastewater treatment wetlands (Ciria et al., 2005; Maddison et al., 2005; Cicek et al., 2006). Cattail is a high biomass plant that accumulates high nutrient concentrations in the biomass and is tolerant of metals (Grosshans, 2014). Another important attribute of harvested cattail biomass is its potential usefulness as a feedstock for bioenergy production (Cicek et al., 2006; Grosshans, 2014).

Although wetlands have gained acceptance as an efficient, cost-effective treatment alternative, research has focused primarily on wastewater treatment. There is a need to assess the potential use of biosolids as substrates for wetland creation, which would provide an opportunity for treating the biosolids in situ. For small communities with limited funds for disposal of biosolids, constructed wetlands would cost considerably less than conventional remediation approaches to return land to productive use. In addition to their proven effectiveness in contaminant removal in wastewater treatment, wetlands blend into the natural landscape setting, adding aesthetic value and providing habitat for birds and wildlife, thereby creating recreational opportunities (Knight, 1997).

1.4 Terrestrial Phytoremediation

Terrestrial phytoremediation is a cost-effective method that reduces, removes, or renders harmless contaminants in unsaturated environments (Van Der Lelie et al., 2001; Lasat, 2002; Pilon-Smits, 2005). The global phytoremediation market was estimated at $55–$103 million in 2000 and was expected to reach $214–$370 million in 2005 (Glass, 1999). In the United States alone, the estimated remediation cost of engineered remediation methods such as excavation, pump and treat systems, and soil washing in the early 2000s was approximately $7 to $8 billion per year (Bennett et al., 2003).

The selection of plant species that are capable of establishing and surviving in the contaminated medium is essential for phytoremediation (Nedunuri et al., 2009). High biomass yielding plants that accumulate large concentrations of contaminants in the biomass under terrestrial environments are crucial for the success of terrestrial phytoremediation. Phytoremediation timelines often span decades (USEPA, 2012), thus reducing the attractiveness of adoption of this strategy. Harvesting plants to coincide with maximum nutrient and contaminant accumulation is crucial to enhance contaminant removal in harvested aboveground biomass. Biomass production for bioenergy or some other land use functions to generate economic or other benefits during the phytoremediation process may help alleviate drawbacks from long timeframes typical of phytoremediation projects.

Switchgrass, a C4 perennial grass, is drought tolerant, adapted to a wide range of conditions, and produces high biomass yields (Parrish and Fike, 2005), making it a noteworthy plant species of choice for terrestrial phytoremediation (Alkorta et al., 2004; Ashworth, 2010; Silveira et al., 2013). Hassan (2014) showed that switchgrass yielded

more biomass and accumulated more nutrients and trace elements than cattail in a microcosm study on terrestrial phytoremediation of municipal biosolids.

1.5 Thesis Objectives

The overall objective of this thesis was to examine biomass, nutrient (N and P), and trace element accumulation in aboveground and belowground biomass in cattail and switchgrass to determine the harvest stage that maximizes uptake of these elements during in situ wetland and terrestrial phytoremediation of municipal biosolids. The specific objectives of the thesis were to: (a) determine the effects of moisture levels (30, 60, and 90% water filled pore space) on N and P availability in primary biosolids and secondary biosolids (Chapter 2); (b) characterize the accumulation and partitioning of biomass, nutrients, and trace elements (Zn. Cu, Cd and Cr) between aboveground and belowground plant tissues in cattail grown in primary biosolids under a wetland system (Chapter 3), and (c) characterize the accumulation and partitioning of biomass, nutrients, and trace elements between aboveground and belowground plant tissues in switchgrass grown in secondary biosolids under a terrestrial system (Chapter 4).Chapter 5 provides an overall synthesis of findings from these experiments.

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2. MOISTURE EFFECTS ON NITROGEN AND PHOSPHORUS AVAILABILITY IN MUNICIPAL BIOSOLIDS FROM END-OF-LIFE MUNICIPAL LAGOONS

2.1 Abstract

Nitrogen (N) and phosphorus (P) availability affects plant biomass yields and hence phytoextraction of nutrients and trace element contaminants during phytoremediation of end-of-life municipal lagoons. End-of-life lagoons are characterized by fluctuating moisture conditions, whose effects on biosolids N dynamics have not been adequately characterized. This 130-d laboratory incubation investigated effects of three moisture levels [30%, 60%, and 90% water-filled pore space (WFPS)] on plant available N and bicarbonate extractable P (Olsen P) in biosolids from a primary (PB) and a secondary (SB) municipal lagoon cell. Results showed a net increase in N mineralization (N_{min}) with time at 60% WFPS and a net decrease at 90% WFPS in PB, while N_{min} at 30% WFPS did not change significantly. By comparison, moisture level and incubation time had no significant effect on N_{min} in SB. Nitrogen mineralization rate in PB was described by a three-half order kinetic model. Potential mineralizable N (N_0) in PB was significantly greater at 60% WFPS (222 mg kg^{-1}) than at 30% WFPS (30 mg kg^{-1}), but rate constants did not differ significantly between the two moisture levels. Nitrogen mineralization in SB followed first order kinetics, with N_0 significantly greater at 60% WFPS (68.4 mg kg^{-1}) and 90% WFPS (94.1 mg kg^{-1}) than at 30% WFPS (32 mg kg^{-1}). The rate constant in SB was significantly greater at 30% WFPS than at 60% and 90% WFPS. Moisture level and incubation time had no significant effect on Olsen P concentration in both PB and SB. Net Olsen P concentrations decreased with incubation time in both biosolids. Low N mineralization in SB may provide insufficient N, resulting

in low biomass yield, hence low contaminant phytoextraction. Our results indicate that while high N mineralization in PB provides sufficient N to support a healthy plant population, phytoextraction potential would be reduced under dry and near-saturated conditions. The decrease in Olsen P concentration over time, probably a result of P fixation, reduces the uptake of available P for plant growth and reduces the phytoextraction of P from the biosolids. These results on N mineralization have important implications on the management of moisture during phytoextraction of contaminants from biosolids in end-of-life municipal lagoons.

2.2 Introduction

Biosolids are a rich source of N and P and contain 10–80 g N kg^{-1} and 5–50 g P kg^{-1} dry biosolids (LeBlanc et al., 2008). However, most of this N is in the organic form (Smith and Tibbett, 2004) while the primary form of P depends on the phosphorus removal process during wastewater treatment (Kyle and McClintock, 1995). The primary form of P generated with the use of soluble salts of Fe, Al or Ca to remove P from wastewater is inorganic P (Maguire et al., 2000; Medeiros et al., 2005) while organic P is dominant from biosolids generated without the use of salts during wastewater treatment (Haynes et al., 2009). Plant uptake requires the mineralization of organic forms of N and P and the dissolution of P from precipitates. Many N and P mineralization studies have been conducted on biosolids- or sewage sludge-amended soils (Gilmour et al., 2003; O'Connor et al., 2004; Er et al., 2005; Smith et al., 2006; Gil et al., 2011). However, in cases where plants are grown directly in biosolids, such as during in situ phytoremediation of end-of-life lagoons (Hassan, 2014), knowledge of availability of N and P in the biosolids is important. In biosolids-amended soils, N release from biosolids

is typically estimated from the difference in N mineralization between biosolids-amended soils and unamended soils (Bernal et al., 1998; Gilmour et al., 2003; Rouch et al., 2011). This assumes the addition of amendment does not interfere with the process of mineralization (e.g., boosting the microbial population or making the soil environment more favorable for mineralization), so that any differences in mineralization can be attributed to the amendment alone. Correa et al. (2012) reported lower mineralization rates ranging from 0 to 2.5% of organic N in biosolids incubated alone compared with 10 to 52% of organic N in biosolids-amended soils. They attributed the greater mineralization rates in the latter to soil providing a more conducive environment for mineralization to take place.

Effective phytoremediation requires plants that produce high biomass yields while accumulating high concentrations of contaminants (Chaney et al., 2007; Paz-Alberto and Sigua, 2013). Plants that can also be used for bioenergy, such as *Typha* spp. and *Panicum virgatum*, are more appealing (Sanderson et al., 2006; Grosshans, 2014). Nitrogen and P availability affects plant growth and is therefore an important prerequisite for phytoextraction of nutrients and trace element contaminants from biosolids. In situ phytoremediation of end-of-life lagoons is a promising biosolids management alternative where traditional methods such as spreading on agricultural land or landfilling are restricted, not feasible, or uneconomical.

Mineralization of organic N and P is a microbial-mediated process and is therefore affected by factors which affect microbial processes, such as temperature and moisture. Most published studies on N and P mineralization in biosolids- or sewage sludge-amended soils were conducted at moisture levels near field capacity, which is considered

optimum for mineralization (Gilmour et al., 2003; O'Connor et al., 2004; Er et al., 2005; Gil et al., 2011; Corrêa et al., 2012). Results from such studies may not reflect N and P mineralization in end-of-life municipal lagoons, where optimal conditions are rarely achieved. Municipal lagoons are constructed at sites underlain by clay material in the absence of which a compacted layer of clay is used to control seepage to groundwater (Spellman and Drinan, 2014). As a result, end-of-life lagoons are poorly drained, leading to ponding or saturation during spring snowmelt or periods of heavy rains. Lagoons can also become drier in periods of drought. Therefore, anaerobic and aerobic conditions are common in end-of-life lagoon environments. An understanding of moisture effects on plant available N and P can help predict N and P availability under fluctuating moisture conditions typical of end-of-life lagoons and provide insight into potential impacts on plant health, biomass yield, and therefore phytoextraction of nutrients and other contaminants.

Water-filled pore space has been widely used as a better index than volumetric water content, water potential, gravimetric water content or water holding capacity to study moisture effects on N mineralization (Doran et al., 1990; De Neve and Hofman, 2002; Sleutel et al., 2008). This is because WFPS closely relates to microbial activity and processes such as respiration, mineralization, and denitrification (Doran et al., 1990). Water-filled pore space indicates the amount of water available for microbial activity as well as its relationship to aeration. Maximum aerobic microbial activity in soils has been observed to occur at about 60% WFPS where a balance in the diffusion of substrates and oxygen supply is attained (Davidson, 1991; Liu et al., 2007).

Microbial activity, hence N mineralization, declines at suboptimal WFPS values because of restricted substrate diffusion and reduced microbial mobility (Stark and Firestone, 1995; Schimel et al., 2007). Moisture levels above the optimum WFPS limit oxygen diffusion, thus limiting aerobic microbial growth and activity (Olness et al., 2001; Skiba et al., 2002). In such anaerobic conditions, N mineralization is limited and anaerobic bacteria utilize nitrate-N (NO_3-N) as an alternative electron acceptor, resulting in N losses as nitrous oxide (N_2O). Denitrification reduces the amount of plant available N.

Microbial activity influences processes such as organic P mineralization, microbial P immobilization, and P fixation, which in turn affect changes in plant available P (Brady and Weil, 2002). Increased microbial activity maintains P in plant available pools by reducing sorption of dissolved P and maintaining inorganic P in soluble pools via solubilisation of inorganic phosphates (Lee et al., 1990). Song et al. (2012) reported an increase in Olsen P when soil moisture content was increased from 50% to 90% field capacity during a 42-d incubation.

Nitrogen and P mineralization in biosolids is influenced by biosolids type and properties, the wastewater treatment method used to generate the biosolids, and the type and length of the stabilization process used (Cogger et al., 2006; Corrêa et al., 2012). Generally, P is more available in raw sludge than in digested sludge (Kyle and McClintock, 1995). Nitrogen mineralization has been found to be greater in aerobically-digested than in anaerobically-digested biosolids (Hseu and Huang, 2005). Wang et al. (2003) reported mineralization rates of 32% of organic N in aerobically digested and 15% of organic N in anaerobically digested municipal biosolids incorporated into soil

and incubated for 26 weeks. Biosolids composting or storage in lagoons can result in a decrease in mineralization potential (Gilmour et al., 2003). Correa et al. (2012) reported the absence of mineralization in composted sewage sludge during a 23-week incubation experiment. Biosolids stored in a lagoon for more than 15 years did not mineralize in laboratory incubation at 25°C and moisture content near field capacity and this was attributed to stabilization by long-term lagoon storage (Gilmour et al., 2003).

There is currently a dearth of published information on the effects of moisture on N mineralization in biosolids that are not mixed with soil. Many of the studies on sewage- or biosolids-amended soils have been conducted at moisture levels near field capacity, which is appropriate for agricultural crop production. However, results from such studies are not directly applicable to end-of-life lagoons, which are characterized by widely-fluctuating moisture conditions. The overall objective of this study was, therefore, to determine the effects of moisture levels (30, 60, and 90% WFPS) on N and P availability in biosolids from an end-of-life municipal lagoon destined for in situ phytoremediation. It was hypothesized that (i) moisture level affects N and P availability and (ii) N and P availability differs between PB and SB.

2.3 Materials and Methods

2.3.1 Biosolids

Biosolids samples were collected from an end-of-life municipal lagoon in Niverville, Manitoba, Canada (49°35'42.7"N, 97°02'50.3"W). The lagoon, which operated for 37 years (1971 to 2008), consisted of a primary cell (4.6 ha) and a secondary cell (8.8 ha), which operated in a series flow. The primary cell was the treatment cell, which received

raw wastewater, while the secondary cell was the holding cell, which received effluent from the primary cell for further treatment and storage. When the lagoon ceased operation, the volume of biosolids in the primary and secondary cells were approximately 20,000 and 28,000 m^3, respectively. Biosolids samples were randomly collected from both cells in the summer of 2011. The biosolids depth in the two cells averaged 20 cm at the time of sampling.

2.3.2 Microcosm Set-up

Biosolids (90 g dry wt.) were packed to bulk densities of 0.66 for PB and 0.77 Mg m^{-3} for SB in plastic containers (6 cm diameter × 7 cm height). Deionized water was added to bring the biosolids to 30, 60, and 90% WFPS, which corresponded to 0.22, 0.44, and 0.67 kg H_2O kg^{-1} for PB and 0.12, 0.32, and 0.48 kg H_2O kg^{-1} for SB. These moisture levels correspond to relatively dry, field capacity, and near-saturation moisture conditions, respectively, in the biosolids. After watering, the plastic containers were capped with lids, which had four, 2-mm diameter holes to allow gaseous exchange.

The experiment was laid out as a completely randomized design with a factorial combination of biosolids (PB and SB) and moisture levels (30, 60, and 90% WFPS). The microcosms were placed randomly in the dark in an incubator set at 25°C. A humidifier (Sunbeam Humidifier Model SUL 496-CN, Boca Raton, FL) was placed in the incubator to reduce evaporation from the biosolids. The microcosms were weighed every 5 d and reverse osmosis water was added to replace any moisture lost via evaporation. Triplicate units from each treatment were removed from the incubator on days 0, 3, 6, 13, 20, 30, 50, 70, 100, and 130, and stored at 4°C in sealed Ziploc bags until analysis.

2.3.3 Biosolids Analysis

Total Kjeldahl N (TKN) was determined using a flow injection analyzer (FIAlab 2500, FIAlab Instruments, Bellevue, WA) following digestion with sulfuric acid. Total carbon (TC) was determined by dry combustion using a CNS2000 analyzer (Leco Corp., St. Joseph, MI). Inorganic N (NO_3-N + NO_2-N + NH_4-N) concentration was determined with a Model AA3 autoanalyzer (Bran+Luebbe, Nordersted, Germany) following extraction of a 5-g sub-sample of moist biosolids with 25 mL of 2 M KCl (Mulvaney, 1996). Olsen P was analyzed following extraction of 1 g of biosolids with 20 mL of 0.5 M $NaHCO_3$ buffered at pH of 8.5 (Olsen, 1954). A subsample from each unit was oven-dried for determination of gravimetric moisture content so that concentrations could be expressed on a dry weight basis.

Biosolids pH and electrical conductivity (EC) were measured in a 1:2 biosolids:water suspension with Accumet AB15 and Accumet AB30 meters respectively (Fisher Scientific, Hampton, NH).

2.3.4 Calculations

Water-filled pore space (%) was calculated as:

$$WFPS = 100 \times (\theta_v \times \rho_b)/f \quad [1]$$

where θ_v = volumetric water content (Mg m^{-3}), ρ_b = bulk density (Mg m^{-3}), f is total porosity [= 1 - (ρ_b/ρ_s)], and ρ_s is particle density (assumed to be 1.3 Mg m^{-3} for the biosolids; (Hillel, 1998).

Net mineralized N (N_{min}) concentration (mg kg^{-1}) was calculated as

$$N_{min} = (N_i)_t - (Ni)_0 \quad [2]$$

where $(N_i)_t$ is inorganic N concentration (mg kg^{-1}) at time t (d) and $(Ni)_0$ is inorganic N concentration (mg kg^{-1}) at time 0.

Change in Olsen P concentration [$(P_i)_t$, mg kg^{-1}] was calculated as

$$\text{Net Olsen P} = (P_i)_t - (P_i)_0 \quad [3]$$

where t (d) is the number of days (time) since the start of incubation and $(P_i)_0$ is Olsen P concentration (mg kg^{-1}) at time 0.

2.3.5 Statistical Analysis

2.3.5.1 Analysis of Variance

All data were analyzed with the GLIMMIX procedure for repeated measures in SAS version 9.3 (SAS Institute, 2014), with biosolids and moisture level as fixed effects and time as the repeated measure. Various covariance structures were compared using the Akaike Information Criteria (AIC) and the compound symmetry structure, which had the lowest AIC, was chosen as the best fit for the repeated measures analysis. Treatment differences were deemed significant if $P < 0.05$ using the Tukey−Kramer adjustment for multiple comparisons.

2.3.5.2 Kinetic Model Fitting

Five kinetic models [first-order, combined first- and zero-order, three-half-order (linear and exponential) and a first-order double compartment model] were fitted to the N_{min} data and compared using PROC NLIN in conjunction with the Marquardt algorithm in SAS (SAS Institute, 2014). The model with the lowest Akaike Information Criterion was chosen as the best fit. In PB at 30 and 60% WFPS, the models were fit when N_{min} started to increase on day 6. Models were not tested at 90% WFPS where negative

mineralization was observed, since the models were proposed for positive net mineralization.

Nitrogen mineralization in PB was best described by a linear form of the three-half-order kinetic model:

$$N_{min} = N_0 [1 - \exp (- k_1 t - k_2 t^2/2)] + k_0 t \quad [4]$$

where N_0 (mg N kg^{-1}) is potentially mineralizable N in the easily decomposable pool at the start of incubation, k_1 is a first-order rate constant (d^{-1}), k_2 is a linear second-order microbial biomass growth rate term (d^{-2}), and k_0 is a zero-order rate constant (mg kg^{-1} d^{-1}).

Nitrogen mineralization in SB was best described by a first-order kinetic model:

$$N_{min} = N_0 [1 - \exp (-k_0 t)] \quad [5]$$

where k_0 is the first-order rate constant (d^{-1}). Parameters for different treatments were considered significantly different if their 95% confidence intervals did not overlap.

2.4 Results and Discussion

2.4.1 Biosolids Properties

Total N and total P concentration were significantly greater in PB than in SB (Table 2.1). The difference in N and P concentrations were likely due to the different wastewater treatment processes occurring in the primary and secondary lagoon cells. Primary wastewater treatment, which occurs in the primary cell, involves the removal of heavy solids from the wastewater by sedimentation, resulting in the accumulation of concentrated primary sludge, which is essentially fecal (Research, 1997; Haynes et al., 2009). Effluent containing lighter solids soluble organic matter, reduced nutrients and trace elements overflows to the secondary cell where most of the microbial

biodegradation takes place. Secondary sludge is mainly composed of bacterial biomass (Research, 1997; Haynes et al., 2009). Therefore, PB, which was essentially composed of fecal matter, had greater N, P and C concentrations than SB, which was primarily composed of bacterial biomass.

Both PB and SB had lower total N concentrations (7.7 and 1.8 g kg^{-1}, respectively) compared with total N concentrations reported in other studies. For example, Cooger et al. (2004) reported 23 g kg^{-1} total N in biosolids stored for more than 15 years. About 8% of total N in PB and 10% of total N in SB in the present study was in the inorganic form. Low NH_4-N concentration (< 1% of total N) in both PB and SB biosolids likely reflects nitrification during long term storage of biosolids in the lagoon.

Table 2.1 Selected initial chemical properties of biosolids used in the study.†

Property	PB	SB
Total N, (g kg^{-1})	7.7	1.8
Organic N (g kg^{-1})	7.1	1.6
Total P, (g kg^{-1})	2.2	1.1
Total C, (g kg^{-1})	151	72
C/N ratio	20	40
NO_3^--N (mg kg^{-1})	532	183
NH_4^+-N (mg kg^{-1})	69	3.5
Olsen P (mg kg^{-1})	251	143
pH	7.1	7.6
EC (dS cm^{-1})	4.2	2.8

†PB: biosolids from the primary cell; SB: biosolids from the secondary cell; EC: electrical conductivity.

2.4.2 Inorganic Nitrogen Concentration

There was a significant biosolids × moisture × time interaction ($P < 0.001$) for NO_3-N concentration (Table 2). In PB, NO_3-N concentration differed significantly among moisture levels at all sampling times except Days 3, 13 and 20 (Fig. 1a). Initial NO_3-N concentration flushes were observed in PB from Day 0 to Day 3 at all moisture contents, indicating rapid mineralization of labile organic N following rewetting of biosolids, which stimulated microbial activity (Cabrera, 1993; Smith et al., 1998). The biosolids used in the study were kept at room temperature (21±2°C) until they were used. This probably maintained a high population of nitrifying bacteria (Agehara and Warncke, 2005), which proliferated upon rewetting.

Table 2.2 Biosolids, moisture, and incubation time effects on cumulative nitrate, ammonium, and net mineralized nitrogen concentrations.

Effect†	NO_3-N	NH_4-N	N_{min}‡	Olsen P	pH	EC
	mg kg^{-1}					dS cm^{-1}
Biosolids (B)						
Primary Biosolids	723	15	101	-43	7.2	4.3
Secondary Biosolids	173	3.6	25	-25	7.7	2.5
Moisture (M)						
30% WFPS	463	8.7	77	-45	7.5	3.8
60% WFPS	514	7.8	128	-30	7.5	3.4
90% WFPS	367	11.4	-16	-28	7.5	3.1
	P value					
Biosolids	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
Moisture	<0.001	<0.001	<0.001	0.01	0.52	<0.001
Time (T)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
B × M	<0.001	<0.001	<0.001	0.18	0.13	0.008
B × T	<0.001	<0.001	0.002	<0.001	0.002	0.013
M × T	<0.001	0.006	<0.001	0.14	0.64	0.67
B × M × T	<0.001	0.004	<0.001	0.7	0.04	0.22

†Time main effects are not presented for the ten multiple sampling times. The interactions are presented in figures.

‡ Net mineralized N, $N_{min} = (N_i)_t - (Ni)_0$, where $(N_i)_t$ is the inorganic N at time t (d) and $(Ni)_0$ is the inorganic N concentration (mg kg^{-1}) at Day 0.

The initial (Days 0–3) NO_3-N flushes in PB at 30% and 60% WFPS were followed by a period of temporary net immobilization from Day 3 to 6 (Fig. 1a). The proliferation of microorganisms that rapidly decomposed substrate during the initial NO_3-N flush might have created a huge demand for available N. As a result, available N was assimilated into the microbial biomass, thus temporarily decreasing available N. The brief N immobilization observed in our study is unlikely to adversely affect plant growth.

Following the initial NO_3-N immobilization, NO_3-N concentration in PB at 60% WFPS increased by 268 mg kg^{-1} (37%) from Day 6 to Day 50 and remained relatively constant thereafter, while NO_3-N concentration increased only slightly at 30% WFPS from Day 6 onwards (Fig. 1a). Nitrate-N concentrations were significantly greater in PB at 60% WFPS than at 30% WFPS at Day ≥50. The lower NO_3-N concentrations at 30% WFPS were likely due to inhibition of microbial activity as a result of reduced diffusion of substrates (Schjønning et al., 2003), reduced microbial mobility (Killham et al., 1993), and reduced microbial growth. Manzoni et al. (2012) suggested that aqueous substrate diffusion may be the major factor limiting microbial activity in dry soils.

In PB, NO_3-N concentration at 90% WFPS decreased continuously relative to 30 and 60% WFPS during the entire incubation period after the initial NO_3-N flush (Fig. 1a). This was likely due to low N mineralization under near-saturated conditions and N loss via denitrification. Previous studies have shown increases in N_2O emission with increasing soil water content, with emissions increasing most rapidly above 60% WFPS (Doran et al., 1990; Abbasi and Adams, 2000) and in biosolids-amended soil (Mendoza et al., 2006; Pu et al., 2010). In our study, NO_3-N concentration in PB at 90% WFPS decreased fairly slowly from Day 6 to Day 70 but more sharply thereafter, likely due to

increased anaerobicity as microbial activity depleted oxygen. Therefore, restricted N mineralization or loss of N through denitrification in end-of-life lagoons is expected during long periods of saturation, such as during spring snowmelt or periods of heavy rain. Although from a remediation perspective, N is lost from end-of-life lagoons during long periods of saturation, low N availability under such conditions reduces plant productivity, hence phytoextraction of other contaminants.

Nitrate N concentrations did not differ significantly among moisture levels in SB at all sampling times (Fig. 1b), suggesting that the organic N pool in SB was more resistant to mineralization than that in PB. Similarly, NO_3-N concentration in SB did not vary significantly with sampling time, regardless of moisture level. The labile organic pool in SB might have been biodegraded during long term storage, leaving a recalcitrant organic N fraction, possibly composed of stable bacterial cell walls (Haynes et al., 2009). Mineralization becomes less sensitive to water content as the substrate becomes less decomposable (Paul et al., 2003).

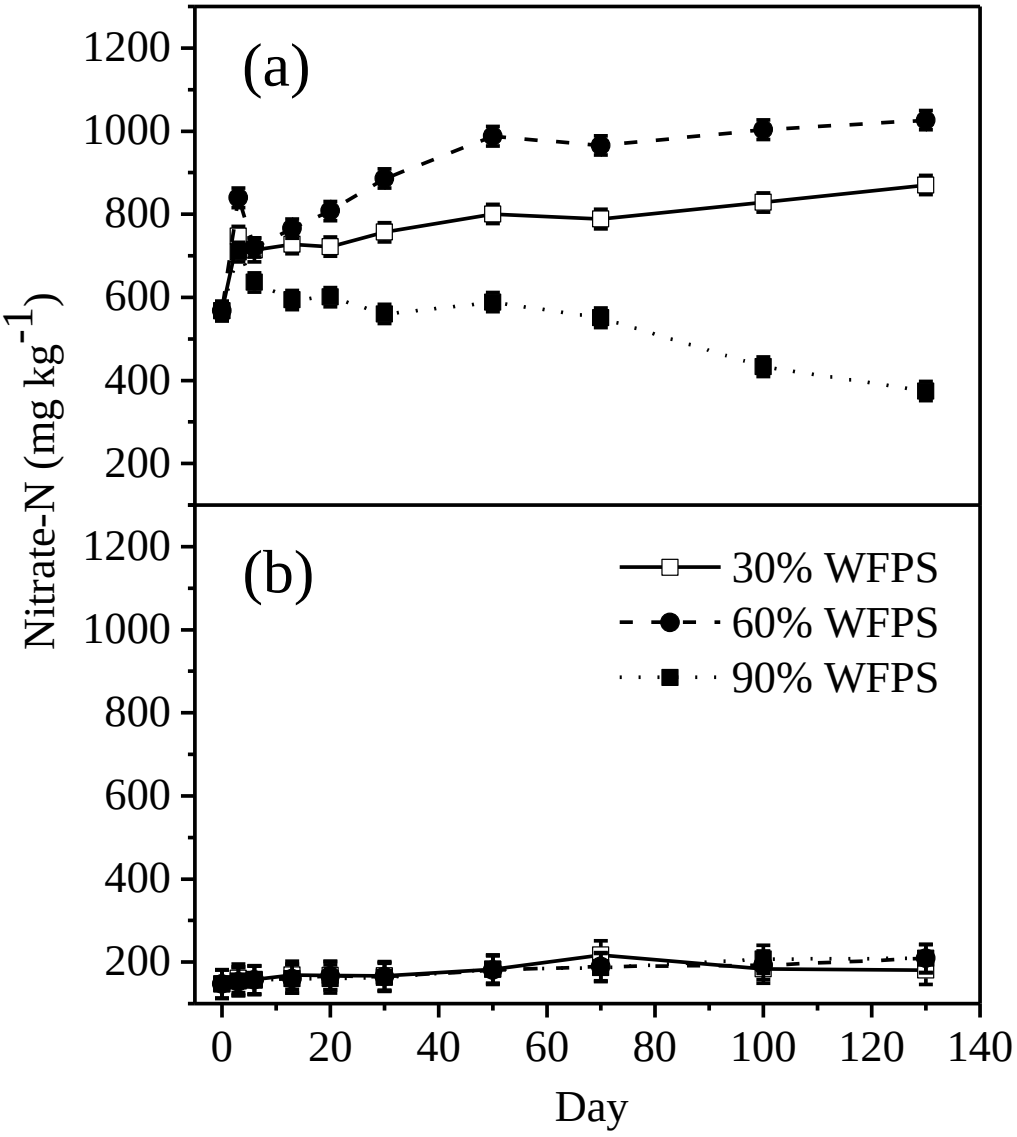

Figure 2.1 Cumulative nitrate-N concentrations in (a) primary cell biosolids (PB) and (b) secondary cell biosolids (SB) as affected by moisture content. Vertical bars represent standard errors of the mean.

On average, NH_4-N accounted for less than 3% of total inorganic N in the biosolids during the incubation. Nonetheless, there was a significant moisture × biosolids × time interaction ($P < 0.004$) for NH_4-N concentration (Table 2). A distinct, rapid decline in NH_4-N concentration occurred in PB at all moisture levels during the first 3 d of incubation (Fig. 2a). This rapid decline in NH_4-N concentration coincided with the initial NO_3-N flush reported above (Fig. 1a) and was likely due to rapid nitrification, hence rapid consumption of NH_4-N. The rapid decline in NH_4-N concentration was followed by a slight increase in NH4-N concentration, after which the NH4-N concentration decreased to less than 4 mg kg^{-1} on Day 100 at all moisture contents (Fig. 2a). Trace amounts of NH_4-N (<5.5 mg kg^{-1}) were measured in SB throughout the sampling period, with no significant temporal changes.

The NH_4-N/NO_3-N ratio observed in PB at 90% WFPS was lower than expected, considering that ammonification is mediated by heterotrophic microorganisms that function in both aerobic and anaerobic conditions (Nugroho and Kuwatsuka, 1990; Paul and Clark, 1996; Vymazal, 2007). In contrast, nitrifying bacteria are obligate aerobes and their activities are restricted in near-saturated conditions, resulting in limited nitrification (Sierra et al., 2001). The low NH_4-N concentrations measured at 90% WFPS in our study suggest that biosolids were aerobic enough under the near-saturated conditions to allow nitrification to occur to the extent that it caused measurable decreases in NH_4-N concentration. The subsequent decrease in NO_3-N concentrations can be attributed to denitrification. Alternatively, our results may suggest that ammonium loss occurred due to NH_3 volatilization or in the form of N_2O through nitrifier denitrification by autotrophic NH_3-oxidizers (Webster and Hopkins, 1996; Wrage et al., 2001).

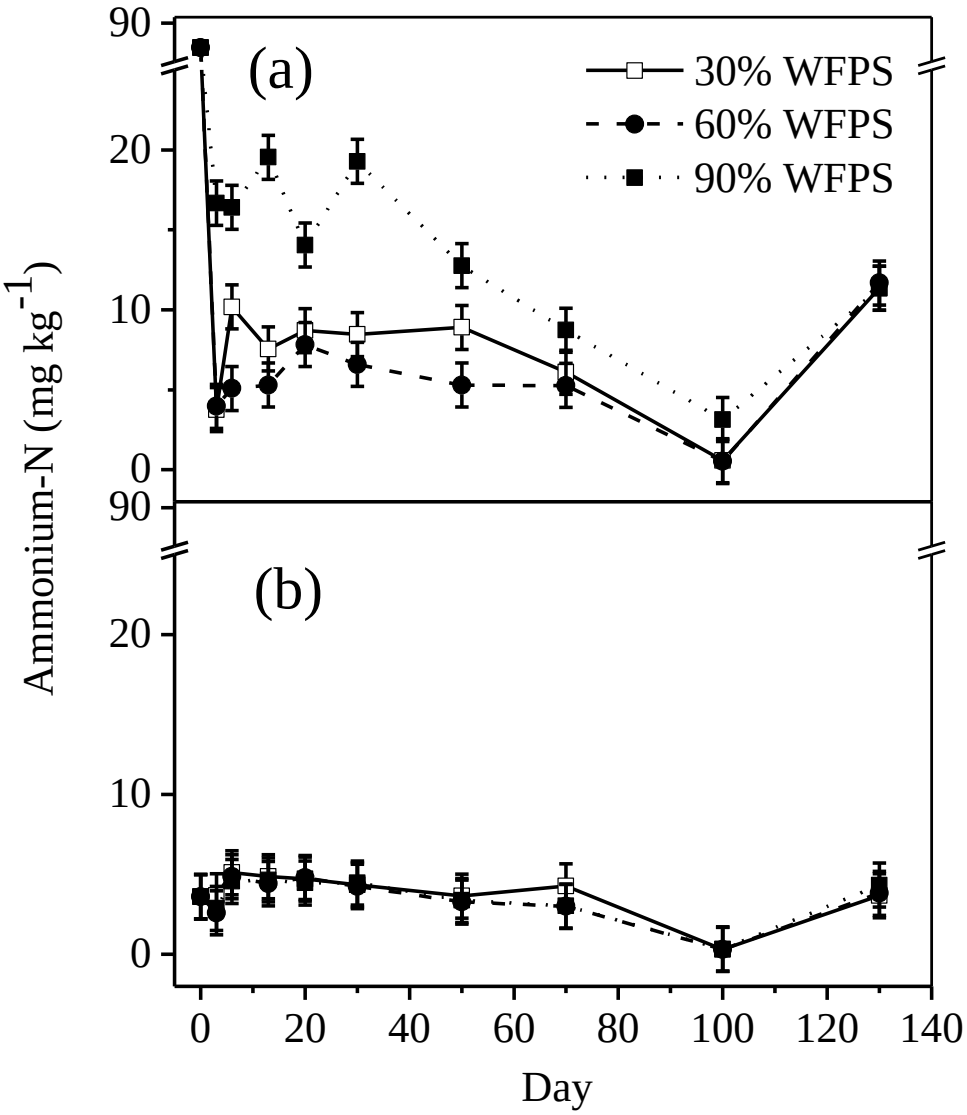

Figure 2.2 Cumulative ammonium concentrations in (a) primary cell biosolids and (b) secondary cell biosolids as affected by moisture content. Vertical bars represent standard errors of the mean.

2.4.3 Mineralized Nitrogen Concentration

The effects of moisture level on net mineralized N concentration (N_{min}) varied with biosolids type and sampling time, as indicated by the significant biosolids × moisture level × sampling time interaction (Table 2). In PB, N_{min} was positive for 30 and 60% WFPS at all sampling times, while N concentration decreased at 90% WFPS (Fig. 3a). Net mineralized N concentration in PB was significantly greater at 60% WFPS than at 30% WFPS on Day ≥ 50. The low N_{min} at 30% WFPS suggests that N availability in end-of-life lagoons during long periods of drought may not sustain healthy plant growth of high N demanding plants. Low plant available N reduces the phytoextraction of N and other contaminants due to reduced biomass yields. The apparent negative N_{min} at 90% WFPS in PB, which was probably due to denitrification, suggests that N deficiency may adversely affect the development and biomass yields of high N demanding plants in near-saturated biosolids, thus reducing the effectiveness of phytoextraction in cases where the plants are used for phytoremediation. Despite the low N_{min} at 30% WFPS and decreasing N_{min} at 90% WFPS in PB, total available N concentrations at the end of the experiment (880 mg kg^{-1} and 390 mg kg^{-1}, respectively), were still sufficient for plant growth.

Moisture level had no significant effect on N_{min} in SB, regardless of sampling time (Fig. 3b). Additionally, N_{min} did not differ significantly among sampling times, regardless of moisture level. The lack of treatment effects in SB can be attributed to a non-labile pool of organic N. Secondary biosolids will likely provide inadequate available N to support high biomass yields during periods of rapid growth, regardless of moisture level. Low plant productivity reduces the phytoextraction of N and other contaminants. Harvesting of plants may be limited to a single cut per season instead of

multiple harvesting because low N mineralization in SB may not replenish available N fast enough to support a robust regrowth during the growing season.

Plants such as switchgrass that can produce high biomass yields in N limiting conditions would likely successfully remediate N and other contaminants in SB. Switchgrass was selected by the U.S Department of Energy's Bioenergy Feedstock Development Program as a model crop because of its high biomass yield and low water and nutrient requirements (McLaughlin, 1992; McLaughlin and Walsh, 1998). Giannoulis and Danalatos (2014) studied nutrient use efficiency and uptake characteristics of switchgrass and reported that switchgrass has low N requirements and can be grown in less fertile soils and still produce high biomass yields.

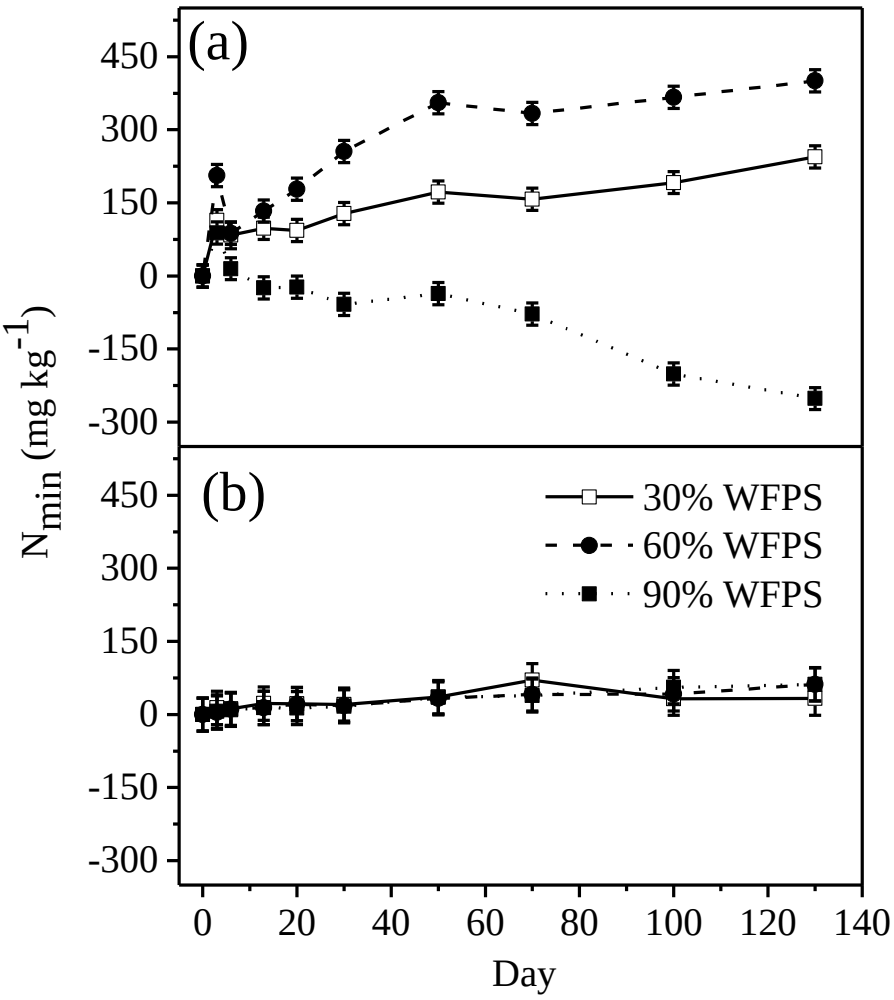

Figure 2.3 Net mineralized nitrogen (N_{min}) concentration in (a) primary cell biosolids (PB) and (b) secondary cell biosolids (SB) as affected by moisture content. Vertical bars represent standard errors of the mean.

2.4.4 Nitrogen Mineralization Kinetics

2.4.4.1 Primary Cell Biosolids

Nitrogen mineralization in PB incubated at 30 and 60% WFPS followed three-half-order kinetics (Table 3; Fig 4). This suggests that PB contained two N pools with

different degrees of biodegradability. The first-order kinetic component of the three-half-order kinetic model describes mineralization of the labile organic N fraction and takes into account microbial growth (Trefry and Franzmann, 2003), while the zero-order component describes mineralization of the less labile N fraction. The slow N mineralization described by the zero-order component may also be caused by depletion of essential nutrients or cessation of microbial growth (Trefry and Franzmann, 2003). Several laboratory incubation studies have shown that N mineralization in biosolids is characterized by at least two N pools: a rapid- and a slow-release pool (Lerch et al., 1992; Smith et al., 1998; Gil et al., 2011).

Potentially-mineralizable N (N_0) concentration in PB was greater at 60% WFPS (222 mg kg^{-1}) than at 30% WFPS (30 mg kg^{-1}) (Table 3), indicating that the full mineralization potential was not attained at 30% WFPS. The lower N_0 at 30% WFPS was probably due to a smaller microbial population under the low moisture conditions. Schimmel et al. (1999) and Bottner (1985) reported low microbial diversity under dry soil conditions, with microbial communities better adapted to the drier conditions thriving. Agehara and Warncke (2005) attributed the increase in N_0 with increasing moisture content to a shift in microbial populations, with those microbes that thrive at higher moisture content metabolizing substrates not utilized at lower moisture contents. Therefore, at 30% WFPS, limited microbial diversity likely resulted in a lower fraction of the total mineralizable N being mineralized while a greater microbial diversity at 60% WFPS produced a greater N_0 and total mineralized N concentration. Rate constants (k_0, k_1, and k_2) did not differ significantly between 30 (0.995 mg kg d^{-1}, 0.024 d^{-1} and 0.004 d^{-2}) and 60% (0.672 mg kg d^{-1}, 0.012 d^{-1} and 0.003 d^{-2}) WFPS in PB (Table 3), which is

likely a result of the large standard errors associated with rate constant estimates (Benbi and Richter, 2002).

The better fit of N_{min} data to a linear rather than exponential growth component in the three-half-order kinetic model was unexpected since microbial growth typically follows exponential growth (Brunner and Focht, 1984). Brunner and Focht (1984) suggested that linear rather than exponential microbial growth can be attributed to restricted diffusion of substrate or nutrients to microbes. Diffusion of substrate to microbes can be limited because of the medium matrix or more prominently by the distribution of microbes on the medium surface (Zobell, 1943; Marshall, 1971). When microbial cell density exceeds the surface that the microbes can occupy due to a large microbial community, multilayers of cells are formed on the surface and diffusion of substrate to the inner cells is limited (Brunner and Focht, 1984). Comparable results from published literature are currently nonexistent since the three–half–order kinetic model has not been widely used to describe N mineralization data. The model has instead been widely fitted to C mineralization and organic compound biodegradation data (Trefry and Franzmann, 2003).

Table 2.3 Three-half-order kinetic model parameter estimates for net mineralized N in primary cell biosolids incubated at 30 and 60% WFPS. †

WFPS (%)	N_0	k_0	k_1	k_2
	mg kg^{-1}	mg kg d^{-1}	d^{-1}	d^{-2}
30	30b	0.995a	0.024a	0.004a
60	222a	0.672a	0.012a	0.003a

† $N_{min} = N_0 [1 - \exp (- k_1 t - k_2 t^2/2)] + k_0 t$, where N_0 is the potentially mineralizable N pool at the start of incubation, k_1 is a first-order rate constant, k_2 is a linear second-order microbial biomass growth rate term, and k_0 is the zero-order rate constant.

Parameter estimates followed by the same letter are not significantly different. Parameters were considered significant if 95% confidence intervals did not overlap.

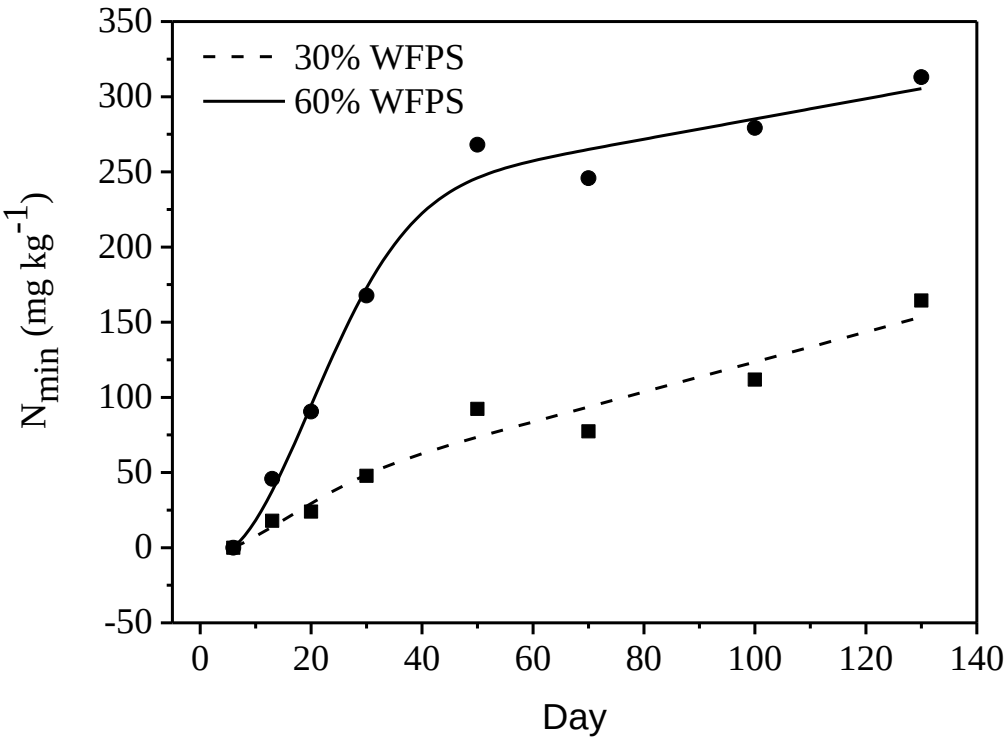

Figure 2.4 Net mineralized N (N_{min}) concentration in primary cell biosolids (PB) at 30 and 60% WFPS as described by a three-half-order kinetic model.

2.4.4.2 Secondary Cell Biosolids

Nitrogen mineralization in SB was adequately described by the first-order kinetic model at all moisture levels (Fig. 5), suggesting the existence of a single pool of potentially mineralizable N that mineralized at a rate proportional to its concentration (Stanford and Smith, 1972). The three-half-order kinetic model failed to converge for SB. First-order kinetics are fitting when the half–life is independent of time and concentration, indicating that the population of degrader microorganisms does not change with time (Johnsen et al., 2013). At 60% and 90% WFPS, N_{min} in SB increased from 0 to 60 mg kg^{-1} during the entire incubation period while a rapid N release was observed at 30% WFPS during the first 30 d, after which it plateaued at about 30 mg kg^{-1} (Fig. 5). Potentially mineralizable N in SB was significantly greater at 60% (68.4 mg kg^{-1}) and 90% WFPS (94.1 mg kg^{-1}) than at 30% WFPS (32 mg kg^{-1}) (Table 4). The lower microbial activity as a result of limited substrate diffusion and microbial mobility in SB at 30% WFPS (Stark and Firestone, 1995; Schimel et al., 2007) likely resulted in the N_{min} plateau and low N_0.

The first-order rate constant for N mineralization in SB was significantly greater at 30% WFPS (0.069 d^{-1}) than at 60% (0.013 d^{-1}) and 90% WFPS (0.008 d^{-1}) (Table 4). Rapid N mineralization at 30% WFPS during the first 20 d followed by a plateau thereafter (Fig 5), most likely resulted in the higher k_0 value compared with those at 60% and 90% WFPS. Similarly, the half-life of N_{min} in SB at 30% WFPS (10 d) was lower than the half-life of N_{min} in SB at 60% (53 d) and 90% WFPS (83 d) because of the low potentially mineralizable N (32 mg kg^{-1}) that plateaued at about Day 20.

Table 2.4 First-order kinetic model parameter estimates for net mineralized N in secondary biosolids incubated at 30, 60 and 90% WFPS. †

WFPS (%)	N_0	k_0	$t_{1/2}$
	mg kg^{-1}	d^{-1}	d
30	32.0b	0.069a‡	10
60	68.4a	0.013b	53
90	94.1a	0.008b	83

†$N_{min} = N_0 [1 - exp (-k_0 t)]$ where N_{min} = Net mineralized N at time t, N_0 is the potentially mineralizable N pool, k_0 is the first-order rate constant and $t_{1/2} = 0.693/k$, is the time required to mineralize one half of the organic nitrogen pool (half-life).

‡Means followed by the same letter are not significantly different. Parameters were considered significant if 95% confidence intervals did not overlap.

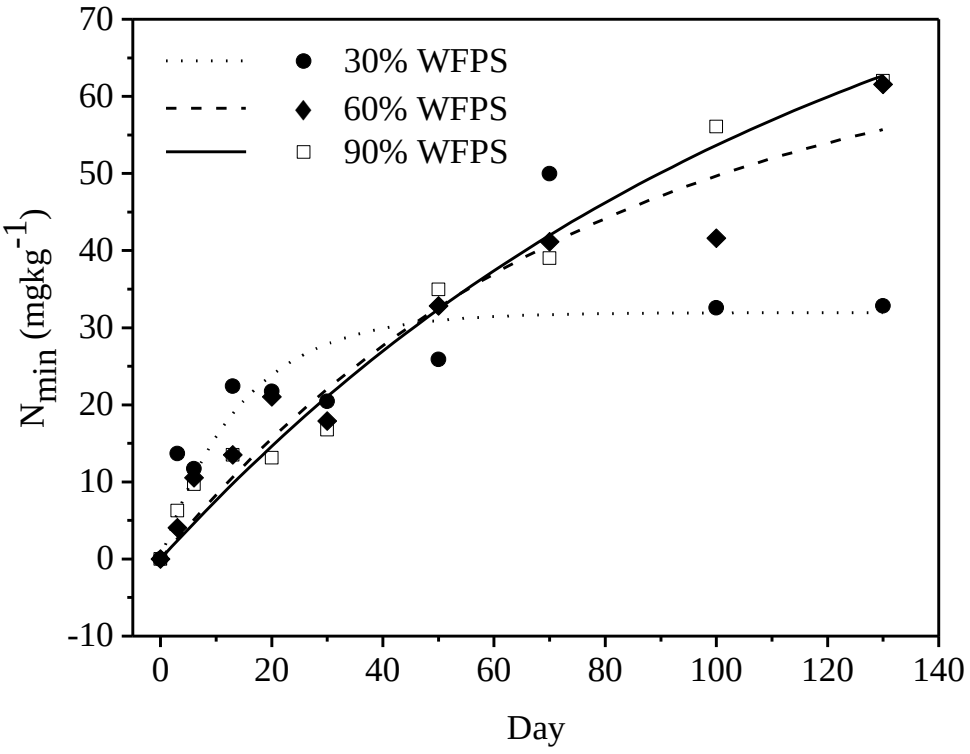

Figure 2.5 Net mineralized N (N_{min}) concentration in secondary cell biosolids (SB) at 30, 60 and 90% WFPS as described by a first-order kinetic model.

2.4.5 Biosolids pH

Moisture level had no significant effect on pH in both PB and SB, regardless of sampling time (Fig 2.6). Biosolids pH was significantly affected by biosolids type and time of sampling ($P = 0.002$). Biosolids pH increased by 0.4 units in both PB (7.1-7.5) and SB (7.6-8.0) from the start to the end of the incubation (Fig 2.7). Biosolids pH was significantly greater in SB than in PB across the entire incubation period. The increase in pH in both biosolids with sampling time may be attributed to aerobic decomposition, which has an alkalinizing effect (Bernal and Kirchmann, 1992). Significantly greater pH in SB might have been due to greater alkalization effect caused by greater decomposition of SB in the secondary cell compared with decomposition of PB in the primary cell. Greater microbial biodegradation takes place during secondary wastewater treatment than during primary wastewater treatment (Haynes et al., 2009). Alkaline pH in both biosolids favors precipitation of P with Ca. Formation of calcium phosphates is more favored in PB, which are more alkaline than SB.

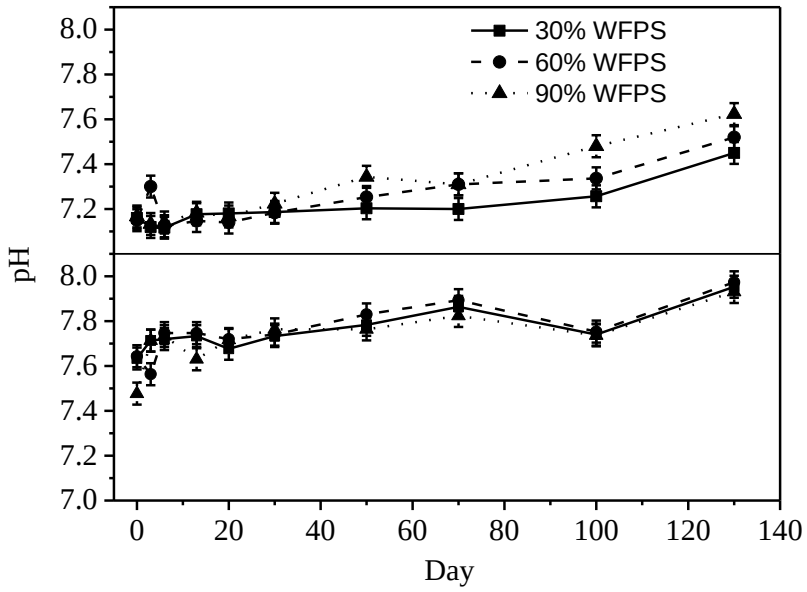

Figure 2.6 Change in pH of (a) primary cell biosolids and (b) secondary cell biosolids as affected by moisture content.

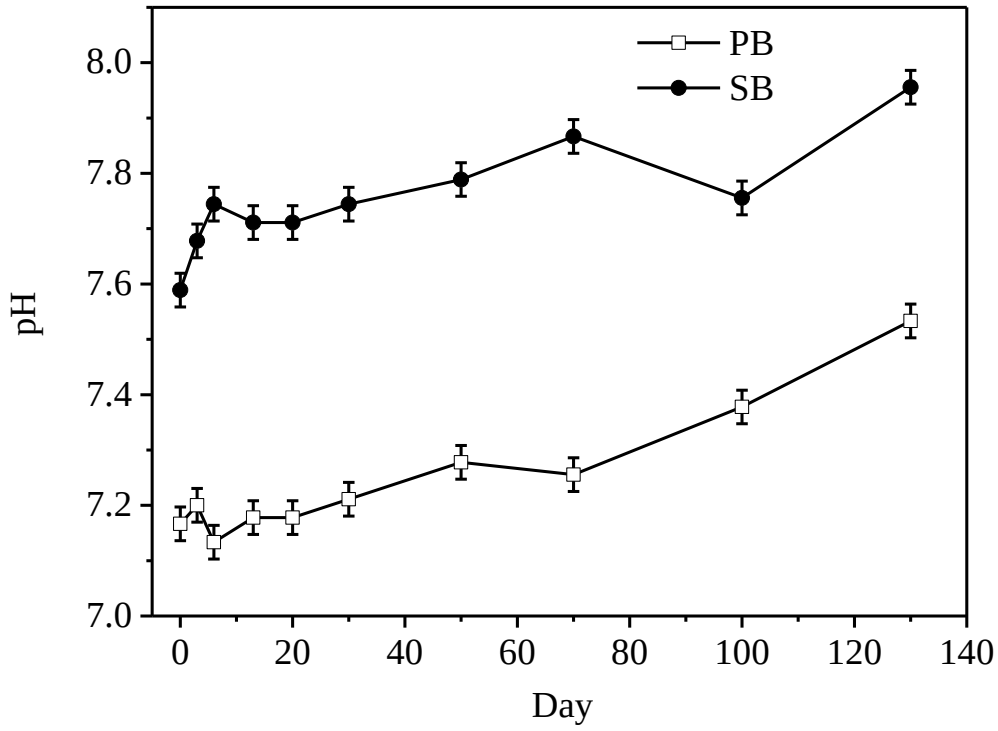

Figure 2.7 Change in pH of (a) primary cell biosolids and (b) secondary cell biosolids as affected by sampling time.

2.4.6 Electrical Conductivity

There was a significant biosolids × time interaction for EC (P = 0.01) (Table 2.2). In PB, EC decreased from Day 0 to 3 and then increased from Day 3 to 30, after which it remained fairly constant up to Day 70 (Fig 2.8). Electrical conductivity increased from Day 70 onwards. In SB, changes in EC followed the same pattern as observed in PB. Electrical conductivity decreased from Day 0 to 6 and remained fairly constant until Day 70, after which it increased from Day 70 onwards. Overall, EC was significantly greater in PB than in SB throughout the incubation. Electrical conductivity in PB (3.8 – 5.1 dS

cm^{-1}) was in the saline range (EC > 4 dS cm^{-1}) and therefore potentially injurious to plants (Brady and Ray, 2008). By comparison, EC in SB (2.3–2.9 dS cm^{-1}) was in the range for non-saline soil (0–4 dS cm^{-1}) (Bateman and Baggs, 2005). High EC in biosolids is attributed to high concentrations of ions such as Ca^{2+}, Mg^{2+}, Cl^{-}, and CO_3^{2-} and accumulation of NO_3-N (Pascual et al., 2007). The increase in EC with incubation time was likely due to release of ions during mineralization of organic matter (Moreno et al., 1999).

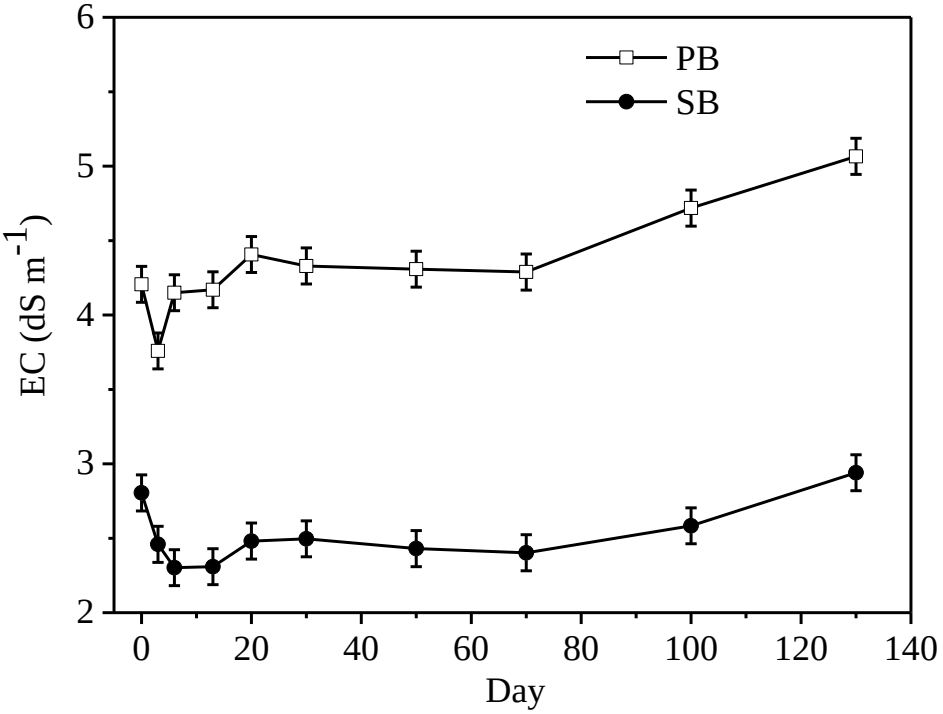

Figure 2.8 Change in EC of (a) primary cell biosolids and (b) secondary cell biosolids as affected by sampling time.

In PB, EC significantly decreased with increasing moisture content (Fig 2.9). In SB, EC was significantly greater at 30% WFPS than at 60% and 90% WFPS. The decrease in

EC with increasing moisture content was probably a dilution effect as moisture content increased. The results suggest that higher EC in biosolids, specifically PB, may be an additional factor limiting microbial activity under dry conditions compared to near field capacity or near-saturated moisture conditions

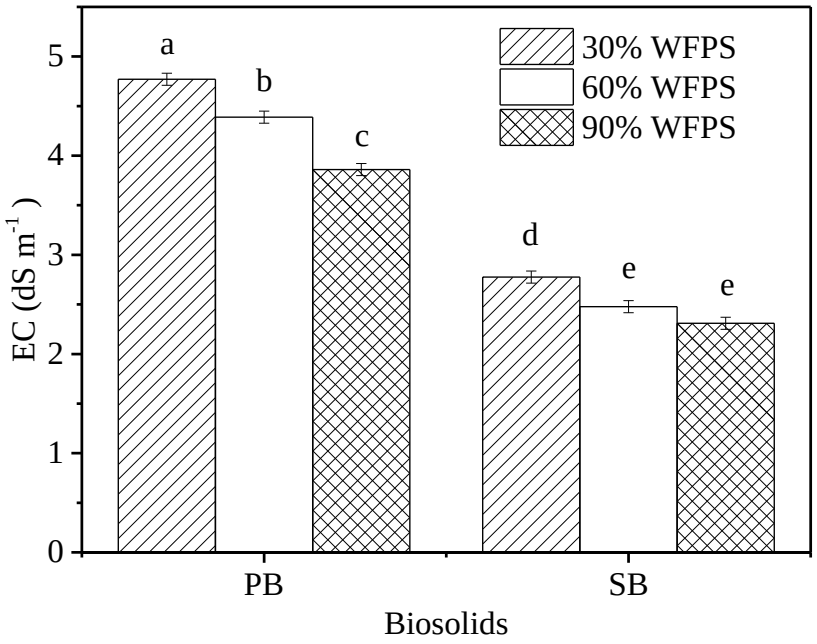

Figure 2.9 Change in EC of (a) primary cell biosolids and (b) secondary cell biosolids as affected by moisture content.

2.4.7 Olsen Phosphorus Concentration

An initial flush of Olsen P (Day 0 to 3) was observed followed by decreasing Olsen P concentrations in both biosolids at all moisture contents over the entire incubation period (Fig 2.10).The lack of moisture effects on P mineralization in both biosolids suggests that organic P mineralization by microbes was not the dominant process determining P availability. Some other immobilization process, probably adsorption and/or precipitation of P with iron (Fe) , aluminium (Al), or Ca^{2+} salts in the biosolids or

microbial P immobilization decreased available P (Hosseinpur and Pashamokhtari, 2013). Several authors reported P microbial immobilization in sewage sludge-amended soils (Goshal and Jansson, 1975; Akhtar et al., 2002). Iron and Al react with P in acidic conditions while reactions of P with Ca are more dominant in alkaline conditions (Brady and Ray, 2008). The pH ranges for PB (7.1–7.5) and SB (7.6–8.0) suggest that formation of Ca–P compounds may be the dominant process removing available P from solution. To a lesser extent, increase in microbial activity due to high C availability in biosolids can result in microbial P uptake (Hosseinpur and Pashamokhtari, 2013), thus reducing available P concentration. Adsorption and precipitation are the main mechanisms that remove P from solution (Braschi et al., 2003). Song et al. (2012) reported an increase in Olsen P concentration as soil moisture increased from 50 to 90% of field capacity. However, their study did not indicate Al, Fe or Ca concentrations in the soil.

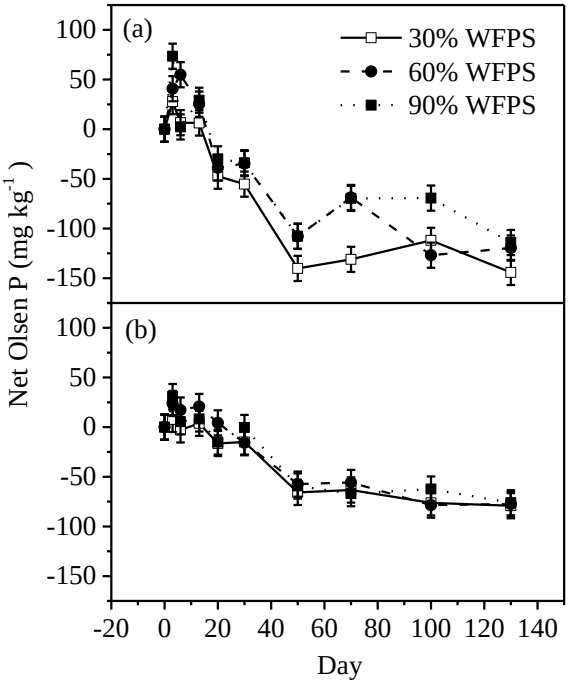

Figure 2.10 Net phosphorus concentration in (a) primary cell biosolids (PB) and (b) secondary cell biosolids (SB) as affected by moisture content. Vertical bars represent standard errors of the mean.

Biosolids type effects on net change in Olsen P concentration varied with incubation time, as indicated by the significant biosolids × time interaction ($P < 0.001$) (Table 2.2). In PB, an initial flush of Olsen P concentration (Day 0 to 3) was followed by a decrease of 166 mg P kg^{-1} (350% of peak Olsen P concentration) from Day 3 to Day 50 (Fig 2.11). The initial flush of P during the first 3 d coincided with the N flush observed in PB (Fig 2.1) and is attributed to proliferation of microbial population when the biosolids were rewetted. In SB, the initial flush of Olsen P concentration (Day 0 to 3) was followed by a decrease in Olsen P concentration to 82 mg P kg^{-1} (392% of peak Olsen P). The decrease

in Olsen P concentration in both PB and SB is likely a result of precipitation of P with Ca salts in the biosolids.

The decrease in Olsen P concentration reduces the phytoextraction of P from the biosolids and has potential to limit plant growth, thereby reducing the phytoextraction of other contaminants because of reduced biomass and poor health of plants. Olsen and Sommers (1982) reported that Olsen P concentration greater than 0.01 g kg^{-1} is sufficient for plant productivity in soil and addition of P fertilizers will likely produce no response. By the end of the incubation study, PB and SB had available P concentrations of 0.124 g kg^{-1} and 0.065 mg kg^{-1}, respectively, which are 12 and 7 times greater than the critical concentration reported by Olsen and Sommers (1982). Therefore, using whole biosolids alone as a medium for plant growth will likely provide sufficient P for plant growth.

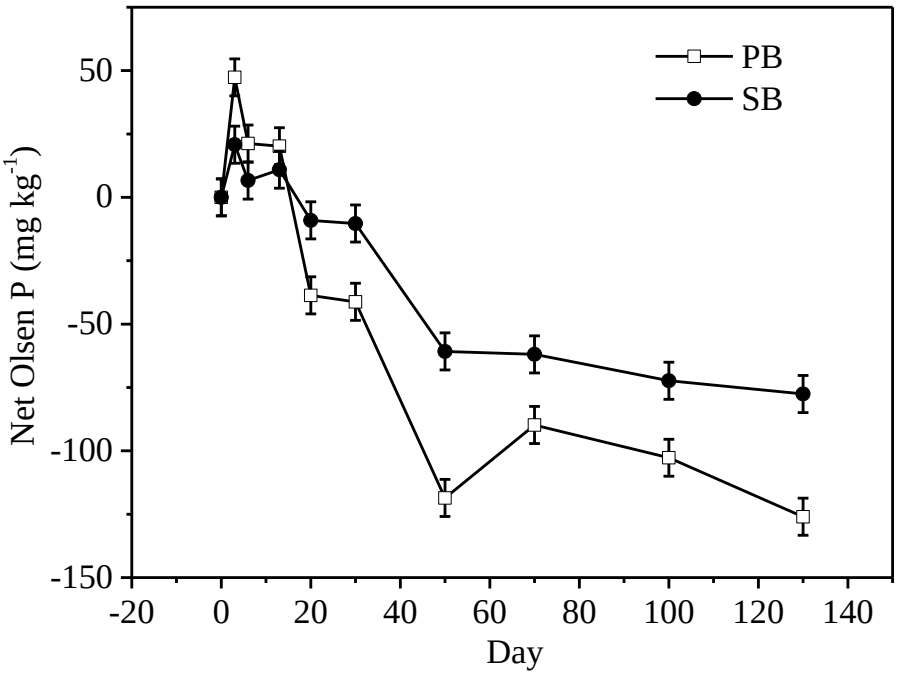

Figure 2.11 Net inorganic phosphorus concentration in (a) primary cell biosolids (PB) and (b) secondary cell biosolids. Vertical bars represent standard errors of the mean

2.5 Conclusions

Moisture content influenced N mineralization differently in PB and SB. Our results indicate that N mineralization potential in PB was significantly greater at 60% WFPS than at 30% WFPS. Plant available N supply from PB under relatively dry conditions in end-of-life lagoons may fail to support a healthy plant population that produces high biomass yields, thus reducing the effectiveness of phytoextraction as a strategy for nutrient and trace element removal from the biosolids. The decrease in N concentration with time observed in PB at 90% WFPS, probably as a result of denitrification, suggests a potential risk of N deficiency and therefore low biomass yields

of plants growing in the biosolids, which would reduce phytoremediation potential. However, unlike in the near-saturated microcosms of our study, which had no plants growing, in the presence of plant roots, higher dissolved oxygen concentrations are expected due to the presence of plant roots in the rhizosphere, which should enhance mineralization. Moreover, at the end of the experiment, total available N concentrations in PB were still adequate to support high biomass growth.

Moisture level had no significant effect on N mineralization in SB. The low N mineralization in SB suggests that high N demanding plants would have limited effectiveness to remediate SB. Phytoextraction in SB may be enhanced by using plant species such as switchgrass that can produce appreciable biomass yields in low N conditions. High biomass plants that have high N requirements can be grown in PB to phytoextract high concentrations of mineralized N and other contaminants. Multiple harvesting of plants during the growing season to increase total biomass yield and hence contaminant uptake is likely feasible in PB because of the high mineralization rates. Low mineralization rates in SB may not support sufficient available N concentrations to support plant regrowth, restricting harvesting to a single cut per season.

Moisture level did not have a significant effect on Olsen P concentration in both biosolids. Olsen P concentrations decreased with time, likely due to P fixation. Phosphorus fixation reduces the phytoextraction potential of P in biosolids. Despite the decrease in Olsen P concentrations with time, Olsen P concentrations still remained above the critical concentrations required for plant growth in both biosolids. In addition, unlike in the microcosms of our study, which had no plants growing, in the presence of

plant roots, root exudates such as organic acids can solubilize inorganic P precipitates, thus increasing P availability.

Given the lower mineralization in SB, it may be prudent to supplement inorganic N in SB, for example, by mixing SB with PB to increase plant available N to support high biomass growth. Low mineralization potential in PB in dry conditions may necessitate irrigation of PB during long periods of drought to increase mineralization.

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3. BIOMASS, NUTRIENT AND TRACE ELEMENT ACCUMULATION AND PARTITIONING IN CATTAIL DURING WETLAND PHYTOREMEDIATION OF MUNICIPAL BIOSOLIDS

3.1 Abstract

Biomass and contaminant accumulation and partitioning in plants determine the harvest stage for optimum contaminant uptake during phytoremediation of municipal biosolids. This wetland microcosm bioassay characterized the accumulation and partitioning (between aboveground and belowground plant tissue) of biomass, nutrients [nitrogen (N), phosphorus (P)] and trace elements [zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd)] in cattail (*Typha latifolia*) in a growth room. Four cattail seedlings were transplanted into each 20–L plastic pail containing 3.9 kg (dry wt.) biosolids from an end–of–life municipal lagoon previously used for treatment of municipal wastewater. A 10–cm deep water column was maintained above the 12–cm thick biosolids layer in the microcosm. Plants were harvested every 14 d over a period of 126 d for determination of aboveground biomass (AGB) and belowground biomass (BGB) yields, along with contaminant concentrations in these plant tissues. Logistic model fits to biomass yield data indicated no significant difference in asymptotic yield between aboveground and belowground biomass. Aboveground biomass accumulated significantly greater amounts of N and P and lower amounts of trace elements than BGB. Maximum N accumulation in the AGB occurred 83 d after transplanting (DAT) while peak P uptake occurred 86 DAT. At maximum accumulation of each element, the percentage of total accumulation partitioned to AGB was 72% for N, 56% for P, 47% for Zn, 34% for Cu, 44% for Cr, and 7% for Cd. Harvesting at maximum aboveground

accumulation removed (percent of the initial element concentration in the biosolids) 4% N, 3% P, 0.05% Zn, 0.6% Cu, 0.1% Cd, and 0.2% Cr. Therefore under the conditions of this study, phytoremediation would be most effective if cattail is harvested 86 DAT. These results will contribute towards identification of the harvest stage that will optimize contaminant uptake and enhance in situ phytoremediation of biosolids using cattail.

3.2 Introduction

Many small communities in Canada and the United States use lagoons or stabilisation ponds for wastewater treatment. In the United States, over 7,000 facultative lagoons are in use (USEPA, 2002). The disposal or management of biosolids in an economical and environmentally sustainable manner after operation of these lagoons cease is a challenge for many municipalities. In the United States and Canada, about 60% of biosolids produced are spread on agricultural lands (Cogger et al., 2006).

Where spreading of biosolids on agricultural land is limited or not feasible, wetland–based phytoremediation offers a promising alternative. Several studies have demonstrated the effectiveness of wetland systems as an efficient and cost–effective treatment approach for removal of nutrients and trace elements from wastewaters (Cameron et al., 2003; Maine et al., 2007; Vymazal, 2007). However, most of the published studies have focused primarily on wastewater treatment with soil or gravel based sediments. In such cases, wastewater is retained in a wetland system for a period of time, taking advantage of the wetland's purification system, before discharge of the treated wastewater into receiving water bodies.

Using constructed wetlands for in situ remediation of end–of–life lagoons contributes to a wider effort to limit potential nutrient losses, especially P, which has

caused serious eutrophication of fresh water bodies such as Lake Winnipeg in Manitoba, Canada. Erosion and surface runoff from nutrient rich agricultural soils, including those receiving biosolids application, are important contributors to high N and P in river systems in western Canada (Manitoba Conservation, 2002). With in situ wetland phytoremediation, contaminants are sequestered within the wetland, reducing contamination of surrounding environments, while harvesting plants permanently removes contaminants from the biosolids. Harvesting high biomass plants such as cattail removes contaminants from the wetland system and can provide feedstock for bioenergy production (Cicek et al., 2006; Grosshans, 2014), thereby addressing public concerns with human and environmental health issues arising from contaminants when biosolids are applied to agricultural land.

Harvesting of wetland plants is an important step in the phytoremediation of contaminated sediments. Harvesting removes contaminants taken up by the plants and prevents contaminant recycling in the wetland ecosystem from decaying plant tissues (Martin and Fernandez, 1992; Vymazal, 2007). It is therefore important to harvest plants when contaminant accumulation is optimal to effectively remove contaminants in the harvested aboveground plant tissues. The uptake of contaminants, hence removal by harvesting, depends on biomass yield and contaminant concentration in the harvested biomass (Vymazal, 2007; Kadlec and Bevis, 2009). As plants mature, biomass and nutrient accumulation diminish and nutrients and photoassimilates are retranslocated from aboveground to belowground tissues where they are stored for use during spring regrowth (Mitsch and Gosselink, 2007). Martin and Fernandez (1992) reported 40–45% of N and P initially present in secondary effluent was removed when cattail growing in

the effluent was harvested in November; by comparison, a late September to early October harvest, that is, before nutrient retranslocation to the root biomass, could have potentially removed 70% of these nutrients. Retranslocation can reduce the concentration of nutrients accumulated in the aboveground biomass by more than 50% (Vymazal, 1995; Reddy and DeLaune, 2008; Grosshans, 2014). Therefore, harvesting before the onset of retranslocation is important to ensure optimum contaminant removal through harvesting AGB. Harvesting plants at maximum nutrient accumulation also increases rhizome uptake of nutrients such as P so that they can be stored for use the following season (Kim and Geary, 2001; Headley et al., 2003).

Removal of nutrients through harvesting macrophytes in wetlands has been reported to be efficient in wetlands with low inflow contaminant concentrations (Cicek et al., 2006). Vymazal (2007) reported <10% N removal through harvesting plants from various constructed wetlands for secondary wastewater treatment systems while harvesting plants was reported to be more important in N removal when inflow N concentrations are low, such as in constructed wetlands for tertiary wastewater treatment. Other authors have reported higher removal of N and P through harvesting of plants. For example, Martin and Fernandez reported 70% of N and P removal from secondary effluent when cattail was harvested in the fall. Kadlec and Bevis (2009) reported that 20% of added N and 14% of added P were sequestered by *Typha* spp. growing in a natural peatland receiving treated wastewater over a 30–yr period. Weng et al. (2006) reported 40% and 45% P removal by cattail grown in microcosms treating primary and secondary effluents, respectively.

Trace element translocation from roots to aboveground biomass is restricted in wetland plants (Deng et al., 2004; Maddison et al., 2005). Consequently, aboveground biomass harvesting is mostly ineffective in trace element removal (Batty and Younger, 2004; Vymazal et al., 2009). Immobilisation in the root system (rhizostabilization) is the dominant phytoremediation mechanism for trace elements. During wastewater treatment, rhizostabilization and adsorption to plant roots (rhizofiltration) and sediments are the major mechanisms of trace element removal (Marchand et al., 2010). However, remediation of trace element-contaminated sediments is challenging. Immobilised trace elements in plant roots still exist in the contaminated media and may be cycled back to the sediments when belowground biomass dies and decomposes.

Several published studies on nutrient removal by the harvesting of wetland plants have been conducted in wastewater treatment systems, which receive readily available nutrients and whose sediments contribute to the removal of contaminants such as P from wastewater. This study examined the phytoremediation of biosolids sediment in a closed wetland system. The overall objective was to characterize the accumulation and partitioning of biomass, N, P and trace elements in cattail plants using wetland microcosms. Such information is important to determine the harvest stage that will optimize contaminant uptake and enhance in situ phytoremediation of biosolids using cattail. There is potential for contamination of the overlying water column from nutrients and trace elements diffusing from the sediment, thus posing risk to aquatic or aquatic-dependent organisms. Therefore, the study also examined nutrient and trace element concentrations in the wetland water column. It was hypothesized that (i) biomass, N, P and trace element partitioning differs between AGB and BGB in cattail; (ii) harvesting

AGB at peak accumulation of biomass, N, P and trace elements enhances the effectiveness of phytoremediation; and (iii) N, P and trace element concentrations in the wetland water column differs between vegetated and unvegetated treatments.

3.3 Materials and Methods

3.3.1 Biosolids

Biosolids samples were collected from a primary cell of an end–of–life municipal lagoon in Niverville, MB, Canada (49°35'42.7"N, 97°02'50.3"W). The municipality used a wastewater stabilization treatment system that included a primary cell (4.6 ha) as the treatment cell, which received raw effluent, and a secondary cell (8.8 ha) into which effluent was transferred for further treatment and storage. The volumes of biosolids at the time of lagoon closure were approximately 20,000 m^3 for the primary cell and 28,000 m^3 for the secondary cell. The lagoon operated for 37 years (1971 to 2008). The lagoon treated predominantly domestic effluent and served a population of approximately 2500 in 2006 (Statistics Canada, 2006). Biosolids for this study were collected in the summer of 2011 from random sampling points in the primary cell. The biosolids had an average depth of 20 cm at the time of sampling.

3.3.2 Wetland Microcosm Setup

Seeds were extracted by blending cattail fruits in a Contrad detergent for 30 s in a blender (Model 54227C, Hamilton Beach, CA, USA), according to the method described by (McNaughton, 1968). Seeds which settled at the bottom of the blender were repeatedly washed with tap water and then rinsed with reverse osmosis (RO) water. Seeds were sowed in plastic trays placed in seed trays which were filled with RO water

to supply water from the bottom. After 28 d, four uniform cattail seedlings were transplanted into each plastic pail (27.5 cm diameter × 32 cm height) to give a plant density of ~70 plants m^{-2} (Kadlec and Bevis, 2009). Each pail contained 3.94 kg (dry wt.) of biosolids approximately 12 cm deep. The experiment was arranged in a completely randomized design consisting of 24 planted pails (3 replicates × 8 sampling times) and three pails containing no plants (controls).

The pails were placed in a controlled environment growth room under a 16-h photoperiod and day/night temperatures of 22/15°C. Relative humidity during the experiment was set at 65% while daytime light intensity was 270 µmole photons m^{-2} s^{-1}. Microcosms were weighed every other day and watered to replace water lost during evapotranspiration. Moisture content was initially maintained at ~60% water filled pore space and then increased by 20% seven days after transplanting (DAT). The microcosms were flooded to a depth of 10 cm above the biosolids surface 14 DAT to provide wetland conditions.

3.3.3 Sampling and Laboratory Analysis

Plants were harvested from three pails 28 DAT and every 14 d thereafter. Aboveground biomass was harvested by clipping plants at the surface of the biosolids after decanting the 'wetland' water from each pail. Belowground (roots) biomass was recovered by washing the roots with tap water to remove biosolids and then rinsing with RO water. Harvested biomass samples were placed in paper bags and dried for 72 h in an oven set at 60°C. The dry biomass samples were weighed for biomass yield determination and then ground (< 0.2 mm) with a mixer/mill grinder (Model 8000D, Spex Sample Prep, Metuchen, NJ). Total Kjeldahl N (TKN) concentrations were

determined by an autoanalyzer (FIAlab 2500, FIAlab Instruments, Bellevue, WA) following digestion with sulfuric acid in a block digester. For determination of P and trace element concentrations, a 0.5 g sample was digested in aqua regia for 2 h at 90^{o}C using a microprocessor–controlled digestion block. Phosphorous concentration was then measured using a Varian 735 ES inductively coupled plasma mass spectrometer (ICP–MS) (Varian Inc., Palo Alto, CA) while trace element concentrations were measured with a Perkin Elmer SCIEX ELAN 6000 ICP–MS (Perkin–Elmer SCIEX Instruments, Concord, Ontario).

Electrical conductivity (EC), pH and dissolved oxygen (DO) in the water column were measured just before harvesting of biomass. Electrical conductivity and pH were measured using an Accumet AP85 pH/conductivity meter (Fisher Scientific, Ottawa, ON). Dissolved oxygen was measured using an optical ProODO oxygen meter (YSI Inc, Yellow Springs, OH).

3.3.4 Statistical Analysis

Five growth models – a three-parameter and a four-parameter logistic function, the Gompertz function, the Richards model, and a modified Richards model (Archontoulis and Miguez, 2013) – were fitted to biomass data using PROC NLIN in conjunction with the Marquardt algorithm in SAS version 9.3 (SAS Institute., 2014). Model comparison using the Akaike Information Criterion (AIC) indicated that the three-parameter logistic model provided the best fit for AGB and BGB yields. The three-parameter logistic model is given by the equation:

$$y = \frac{y_{asymp}}{1 + e^{-k(x-x_m)}} \quad [1]$$

where y_{asymp} is the maximum attainable biomass yield (g pail^{-1}), x is the time elapsed since transplanting (d), x_m is the inflection point at which growth rate is maximized (d), and k controls the steepness of the curve.

Nutrient and trace element uptake values were calculated by multiplying concentrations by the corresponding biomass yields. Segmented regression models were fitted using PROC NLIN in SAS to predict the time at which peak nutrient and trace element accumulation occurred.

3.4 Results and Discussion

3.4.1 Biosolids Properties

Selected chemical properties of the biosolids are presented in Table 3.1 along with applicable sediment quality guidelines. High N and P concentrations were the major concern in the biosolids due to potential losses to aquatic systems where biosolids are applied to agricultural lands, leading to potential contribution to eutrophication especially P. Although a wide range of trace elements were measured in the biosolids, only Zn, Cu, Cr, and Cd are reported because they were above the threshold effect level (TEL) set by the Canadian Sediment Quality Guidelines (CCME, 2001). The low concentrations of the rest of the trace elements, including nickel, mercury, lead, selenium, and arsenic, were expected since the biosolids were produced from domestic wastewater.

Table 3.1 Selected initial chemical properties of biosolids used in the microcosm study, along with applicable sediment quality guidelines

Property	Biosolids	†Ontario SQG		‡CCME SQG
		LEL	SEL	
TKN, (g kg^{-1})	7.8	0.55	4.8	–
Total P, (g kg^{-1})	3.4	0.6	2	–
NO_3–N, (mg kg^{-1})	119	–	–	–
NH_4^+–N,(mg kg^{-1})	63	–	–	–
Olsen P, (mg kg^{-1})	155	–	–	–
Cu, (mg kg^{-1})	143	–	–	35.7
Zn, (mg kg^{-1})	396	–	–	123
Cd, (mg kg^{-1})	1.4	–	–	0.6
Cr, (mg kg^{-1})	42.4	–	–	37.3
pH	7.3	–	–	–

†Guidelines for the protection and management of aquatic sediment quality in Ontario (1993). The lowest effect level (LEL) describes clean to marginally polluted sediments, indicating contamination levels that can be tolerated by most sediment–dwelling organisms. The severe effect level (SEL) describes significantly polluted sediments, indicating contamination levels that can be detrimental to the majority of sediment-dwelling organisms.

‡Canadian Council of Ministers of the Environment Sediment Quality Guidelines for the protection of aquatic life (2001). The threshold effect level (TEL) represents concentrations below which adverse biological effects are expected to occur rarely.

3.4.2 Biomass Yield

The three-parameter logistic model fitted AGB and BGB yield data better than all the other models tested (Fig 3.1). Periods of slow growth lasting for up to ~50 DAT for AGB (Fig. 3.1a) and ~60 DAT for BGB (Fig. 3.1b) were observed. These were followed by periods of rapid growth lasting approximately 30 d for AGB and 40 d for BGB in the near-linear portions of the curves. There was a significant difference in the time to maximum growth rate between AGB and BGB. Maximum AGB accumulation rate occurred on Day 70, corresponding to a cumulative AGB yield of 82 g pot^{-1} (1.38 kg m^{-2}) or 63% of the total biomass (aboveground plus belowground) yield at that time point. Peak cumulative AGB yield accounted for ~50% of the total AGB produced during the entire study period. By comparison, maximum BGB accumulation rate occurred on Day 82, corresponding to a cumulative BGB yield of 81 g $pail^{-1}$ (1.36 kg m^{-2}) or 41% of the total biomass accumulation at that time point. Thus, growth rate was maximized 12 d later for BGB, likely due to translocation of absorbed nutrients from BGB to aboveground photosynthetic tissues to support growth.

The rate of AGB accumulation began to decrease at about 80 DAT when cumulative biomass yield reached 112 g $pail^{-1}$ (1.88 kg m^{-2}) or 70% of the total AGB produced during the 126–d bioassay. At that stage, cumulative AGB yield accounted for 60% of total biomass accumulation. Belowground growth continued rapidly until diminishing growth began at about 100 DAT, with biomass accounting for 82% of the total final BGB yield and 47% of the total biomass yield. The 20-d delay in onset of diminishing growth of BGB indicates that cattail continued to rapidly accumulate BGB

even after AGB growth had slowed down, with some of the biomass increase probably a result of retranslocation of photoassimilates from AGB.

Asymptotic biomass yields did not differ significantly between aboveground (161 g $pail^{-1}$ or 2.71 kg m^{-2}) and belowground plant tissues (165 g $pail^{-1}$ or 2.78 kg m^{-2}). In contrast, Maddison et al., 2009 reported greater BGB (0.61–1.31 kg DW m^{-2}) than AGB (0.37 – 1.76 kg DW m^{-2}) yields for *Typha spp.* at peak AGB accumulation under field conditions. Similarly, (Gagnon et al., 2012) reported 1.1 kg DW m^{-2} for *Typha* spp. AGB compared with 2.8 kg DW m^{-2} for BGB, while Grosshans (2014) reported 1.5 kg DW m^{-2} of AGB vs. 2.0 kg DW m^{-2} of BGB. The reason for the difference between results in these studies and the present study was that, in the former, cattail had long been established and therefore had accumulated a large BGB over several years, whereas, in our study, cattail was established from seeds and, therefore, belowground biomass was still developing and expanding.

The *k* parameter from the three–parameter logistic model did not differ significantly between AGB and BGB, indicating similar growth rates for the two yield components. Aboveground biomass yield was greater than BGB yield until the end of the study period when a more even biomass partitioning occurred. If the experiment had continued for a longer period, it appears belowground biomass (Fig 3.1b) would have continued increasing, albeit at a slower rate.

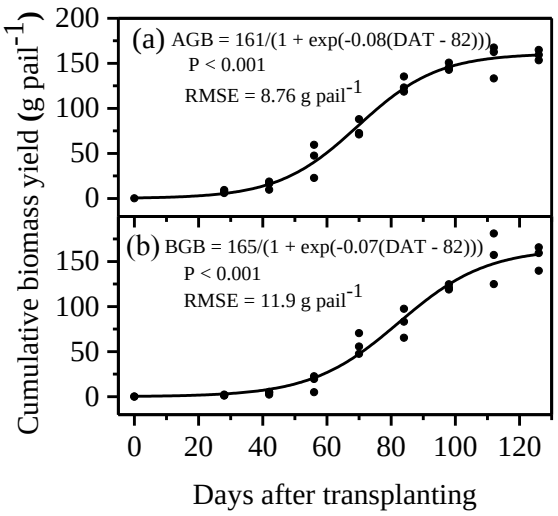

Figure 3.1 Aboveground (a) and belowground (b) cattail biomass accumulation as described by a three–parameter logistic model.

3.4.3 Nitrogen Uptake

A linear-linear segmented model provided the best description for temporal changes in cumulative N uptake by AGB (Fig. 2a). Cumulative N uptake (CNU) increased linearly (CNU = –386 + 17.4DAT) with time for $28 \leq DAT \leq 86$, peaking at 1114 mg $pail^{-1}$ (18.8 g N m^{-2}) on 86, after which it decreased linearly (CNU = 1644 – 6.2DAT for DAT > 86) until the end of the experiment (Fig 3.2).The decrease in N uptake was likely due to retranslocation of the nutrient to BGB. The peak cumulative N uptake by AGB accounted for 72% of the total N uptake by the plant, indicating that phytoextraction in conjunction with aboveground biomass harvesting at peak N accumulation can effectively remove a large fraction of N absorbed by the plant. Unlike AGB, cumulative N uptake by BGB increased linearly with time (CNU = –178 + 7.1DAT) over the entire study period. This was likely due in part to retranslocation of N from AGB to BGB and to absorption of N by plant roots, although BGB was senescing.

However, a plateau in N uptake is expected at sampling times beyond the sampling time used in this study. Cumulative N uptake was generally greater in AGB than in BGB throughout the study period. The rate of increase in CNU in AGB (17.4 mg $pail^{-1}$ d^{-1}) was 2.5 times the rate of CNU increase in BGB (7.1 mg $pail^{-1}$ day^{-1}) (Table 3.2), indicating that cattail readily translocated N to aerial parts to support shoot growth. Photosynthetic tissues require more N and other macronutrients than non-photosynthetic roots and rhizome tissues (Baldantoni et al., 2004; Sharma et al., 2006).

Harvesting AGB at peak N accumulation on Day 86 resulted in the removal of 3.7% of TN initially present in the biosolids. Based on the Ontario sediment quality guidelines for the protection and management of aquatic sediment (Persaud et al., 1993) twenty-five harvest cycles, corresponding to twenty-five growing seasons, would be required to remediate the sediment to the lowest effect level (LEL, 550 mg kg^{-1}), which defines clean to marginally polluted sediment. Eleven harvest cycles would be required to remediate the sediment to the severe effect level (SEL, 4800 mg kg^{-1}), which defines marginally to significantly polluted sediments. The estimates disregarded other N removal pathways, such as denitrification, and are based on the assumption that biomass production and uptake of N and other contaminants are relatively constant in each harvest cycle. However, contaminant uptake may differ with time as a result of repeated harvesting or changes in plant growth and development. Moreover, available nutrient concentrations in the biosolids decrease with time as they are taken up by plants, resulting in a decrease in uptake with time.

The timeframe required to remediate the biosolids to the LEL is satisfactory, if it can be realized under field conditions, considering that attainment of remediation goals

within 30 years using phytoremediation is generally considered satisfactory (USEPA, 2012). The actual timeframe to attain the N remediation goal will be less than estimated by harvesting AGB because denitrification is an important pathway for N loss in wetlands (Vymazal, 2007) and can remove up to 50% of total nitrate-N (Hanson et al., 1994). Harvesting at the end of the study period, that is, after retranslocation had occurred, removed 2.8% of the TN initially present in the biosolids, which translates to a 33 harvest cycle (growth seasons) requirement to meet the LEL remediation goal, which is eight cycles longer than that achieved by harvesting at peak N accumulation. Retranslocation of N to BGB may occur to a greater extent under field conditions where plants are left in the field well past the peak N uptake period.

The Ontario sediment quality guidelines for the protection and management of aquatic sediment used to estimate phytoremediation timeframes in this study are meant to protect freshwater life and may be stringent for remediation of biosolids as sediments. Therefore, there is a need to develop site-specific guidelines that can be used to define remediation goals when remediating biosolids as sediments in wetland systems. This could take into account typical nutrient concentrations in local natural wetlands or the intended use of the site after remediation. The remediation targets or concentrations are expected to be higher for biosolids than those intended for freshwater life.

The percentage of initial biosolids N content removed through harvesting at peak N accumulation in our study is lower than the 20% (Kadlec and Bevis, 2009), 20-26% (Tanner, 1996), and 40-45% (Martin and Fernandez, 1992) reported in other studies for wastewater treatment wetlands. Higher plant N uptake in wastewater studies may be attributed to the readily bioavailable ammonia and ammonium forms of N, which are the

predominant inorganic N forms in wastewater (Cronk and Fennessy, 2001). On the contrary, biosolids contain predominantly organic N, which must be mineralized prior to plant uptake. Moreover, wastewater treatment wetlands receive fresh supplies of nutrients, whereas, in our closed wetland microcosms, plants obtained a continuous supply of nutrients from the biosolids.

Table 3.2 Segmented and linear models for nitrogen, phosphorus, and trace element uptake (mg kg^{-1} $pail^{-1}$) in aboveground and belowground biomass of cattail grown in biosolids for 126 days in a wetland microcosm. †

Element	Aboveground uptake					Belowground uptake				
mg kg^{-1} $pail^{-1}$	a	b_1	b_2	X_0	r_2	a	b_1	b_2	X_0	r_2
N	–386	17.4	–6.2	86	0.67	–178	7.1	–‡	–	0.87
P	–115	6.1	–2.4	83	0.83	–193	5.9	–	–	0.92
Cu	0.034	0.04	–0.006	82	0.85	–0.429	0.026	–	–	0.75
Zn	–2	0.104	–0.124	99	0.78	–1.318	0.11	–	–	0.71
Cd	–0.002	9×10^{-5}	-2×10^{-4}	106	0.67	–0.044	0.002	6×10^{-5}	76	0.76
Cr	0.277	0.049	0.001	80	0.95	–0.226	0.007	–	–	0.78

† *a*, intercept (mg kg^{-1} $pail^{-1}$); b_1, rate of change of response variable when $x_i \leq x_0$ (mg kg^{-1} $pail^{-1}$ day^{-1}); b_2, rate of change of response variable when $x_i > x_0$ (mg kg^{-1} $pail^{-1}$ day^{-1}); x_i, number of days after transplanting (DAT); x_0, critical point at maximum nutrient and trace element uptake (d).

‡ Parameters did not apply to the model and simple linear regression analysis was conducted.

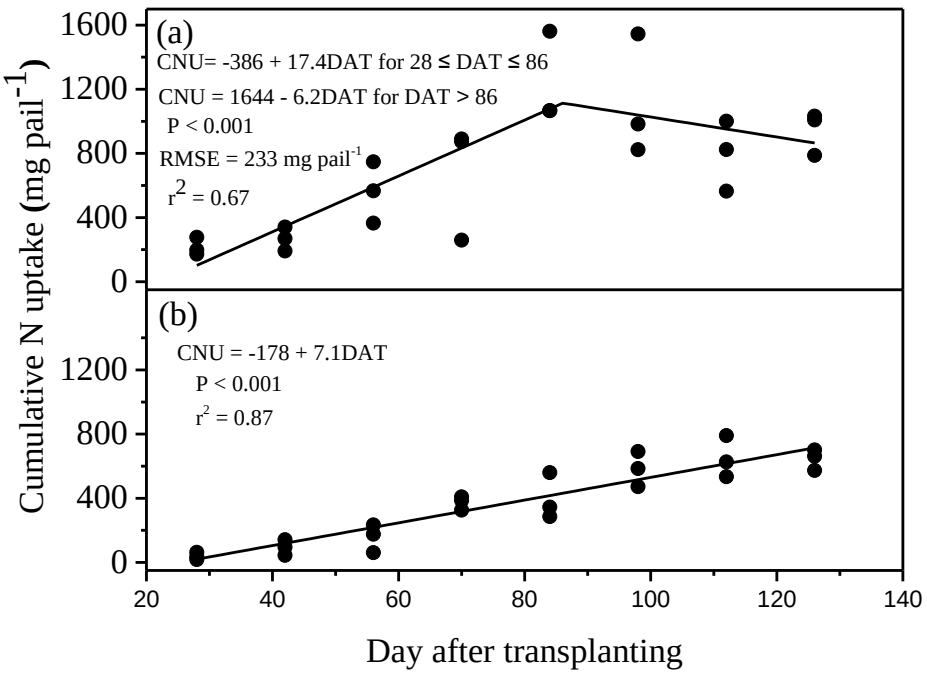

Figure 3.2 Cumulative nitrogen uptake as a function of time in (a) aboveground and (b) belowground cattail biomass.

3.4.4 Phosphorus Uptake

A linear–linear segmented model provided the best description for temporal changes in cumulative P uptake (CPU) by AGB (Fig. 3a). Cumulative P uptake increased linearly (CPU = –115 + 6.1DAT) with time for 28 ≤ DAT ≤ 83 peaking at 385 mg pail^{-1} (6.5 g P m^{-2}) on Day 86, after which it decreased linearly (CPU = 591 – 2.4DAT for DAT > 83) until the end of the experiment. The peak CPU by AGB accounted for 56% of total P uptake by the plant, indicating the effectiveness of phytoextraction, in conjunction with AGB harvesting at peak CPU. Cumulative P uptake by BGB increased linearly with time (CPU = –193 + 5.6DAT) over the entire study period.

Cumulative P uptake was greater in AGB than in BGB until peak CPU, after which the CPU was greater in BGB. Cumulative P uptake in BGB was 33% greater than that in AGB by the end of the study period. The rate of P accumulation in AGB during the linear increase phase (5.9 mg pail $^{-1}$ d^{-1} for DAT $\leq$ 83) was approximately equal to that in BGB (6.1 mg $pail^{-1}$ day^{-1}), indicating that BGB accumulated P as fast as aboveground tissues. The increase in cumulative P uptake in belowground biomass after peak CPU in AGB was likely a result of retranslocation of nutrient reserves to BGB and to absorption of P by plant roots although BGB growth rate was decreasing.

Harvesting AGB at peak CPU on Day 83 resulted in the removal of 2.9% of TP initially present in the biosolids. Based on the Ontario sediment quality guidelines for the protection and management of aquatic sediment (Persaud et al., 1993), 28 harvest cycles, corresponding to 28 growing seasons, would be required to remediate the sediment to the LEL (600 mg P kg^{-1}), while 14 harvest cycles would be required to remediate to SEL (2000 mg kg^{-1}). Higher P phytoextraction has been reported in other studies. For example, using synthetic wastewater in a laboratory constructed treatment wetland microcosm study, Weng et al. (2006) reported 40–45% P removal by cattail plants growing in gravel substrate. Using model simulation, Mitsch and Wang (2000) estimated that 74% of P flowing into four constructed riparian wetlands along the Des Plaines River in Illinois, USA, was taken up by macrophytes. However, much of the P was expected to cycle back into the sediments since the macrophytes were not harvested. Other studies have shown no significant effect of wetland plant harvesting on P removal from wastewater (Comeau et al., 2001; Stottmeister et al., 2003). This was attributed to the much greater contribution of sediments to P removal relative to the proportion of P

removed by harvesting. By comparison, harvesting was the only method that permanently removed P from biosolids in the present study.

Harvesting at the end of the study period, that is, after retranslocation had occurred, removed 2.1% of the TP initially present in the biosolids, which translates to a 39 harvest cycle (growth season) requirement to remediate the sediment to the LEL (600 mg P kg^{-1}), which is 11 cycles longer than that achieved by harvesting at peak P accumulation. Phosphorus retranslocation may be more pronounced under field conditions than under the controlled environment conditions of the present study. For example, Sharma et al. (2006) and Maddison (2009) reported P retranslocation of about 37−45% when cattail was harvested in the winter compare with 77–83% when it was harvested in the fall. Grosshans (2014) reported an 80% decrease in P uptake from a spring harvest (5 kg P ha^{-1}) of cattail growing in a coastal wetland compared with a summer harvest (25 kg P ha^{-1}). Although winter harvesting facilitates easier operation of harvesting equipment and minimizes ecological impacts (Cicek et al., 2006), retranslocation of nutrients to BGB might reduce nutrient phytoextraction efficiency.

Overall, our results indicate that P (and N) phytoextraction can be maximized if cattail harvest coincides with peak P accumulation. However, Vyzamal (2004) and Asaeda et al. (2006) suggested that harvesting plants before retranslocation of nutrients and assimilates to belowground tissues may cause serious damage to plant productivity. On the contrary, Kim and Geary (2001) and Headly et al. (2003) reported that harvesting plants at the time of peak nutrient accumulation increases nutrient uptake and storage for rapid spring growth. In a field study examining cattail biomass production, Pratt et al. (1988) showed that annual harvesting of cattail over three seasons did not have any

adverse effect on plant productivity while harvesting during peak nutrient uptake resulted in significant nutrient removal from rhizomes, thus necessitating fertilizer application to maintain biomass yield. Grosshans (2014) reported no significant change in belowground P reserves during a 4-year field study, ostensibly due to readily available P from the sediment and litter.

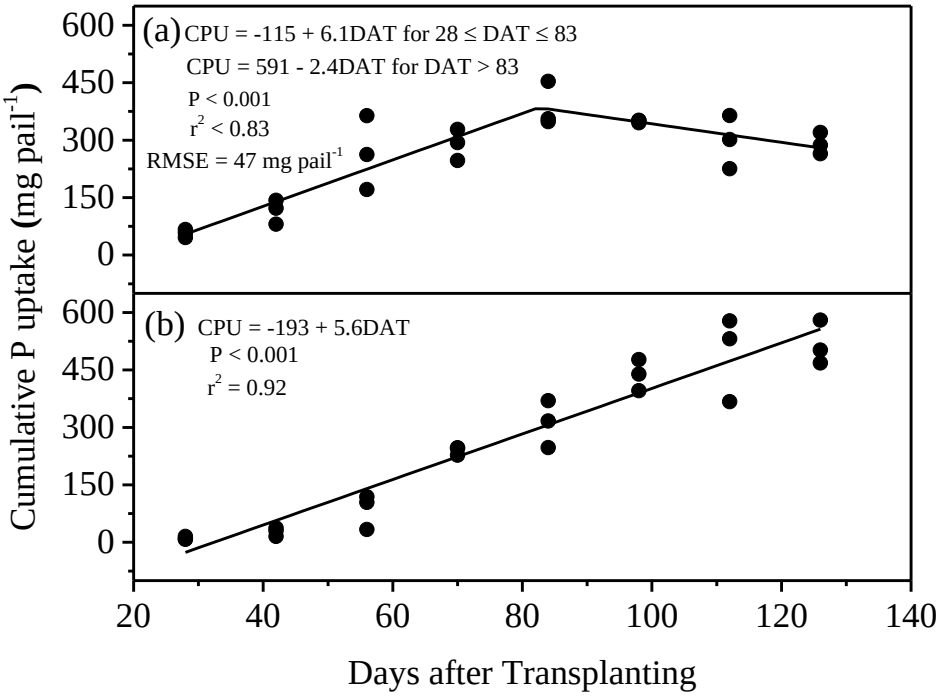

Figure 3.3 Cumulative phosphorus uptake as a function of time in (a) aboveground and (b) belowground cattail biomass.

3.4.5 Zinc Uptake

Cumulative Zn uptake (CZU) by the aboveground biomass increased as a linear function of time (CZU = –2 + 0.104DAT) for 28 ≤ DAT ≤ 99 and decreased linearly with time (CZU = 21 – 0.124DAT) for DAT > 99 (Fig. 3.4a). Peak cumulative Zn uptake on Day 99 was 8.3 mg $pail^{-1}$ (0.14 g Zn m^{-2}), accounting for 47% of total Zn uptake by the plant. Although Zn uptake was low, it was readily translocated to aboveground tissues, with about 50% of total plant Zn uptake partitioned to the AGB. Throughout the experiment, Zn accumulation in the BGB increased linearly (CZU = – 1.32 + 0.1DAT) with time. The rate of Zn accumulation was similar for AGB (0.10 mg $pail^{-1}$ d^{-1}) and BGB (0.11 mg $pail^{-1}$ d^{-1}) until 99 DAT, after which Zn accumulation in the AGB decreased by 3.3 mg $pail^{-1}$ d^{-1} during the rest of the experiment while that in the BGB continued to increase linearly. When harvesting coincided with peak cumulative Zn uptake, 0.05% of the Zn initially present in the biosolids was removed in the harvested biomass. This translates to 127 harvest cycles required to remediate to the TEL (123 mg kg^{-1}) of the CCME sediment quality guidelines for protection of aquatic life (CCME, 2001) under conditions of the present experiment, assuming that phytoextraction rate remains constant season to season. Although this estimate cannot be directly translated to field conditions, it nonetheless suggests that Zn phytoextraction may not be efficient enough to remove the contaminant from biosolids.

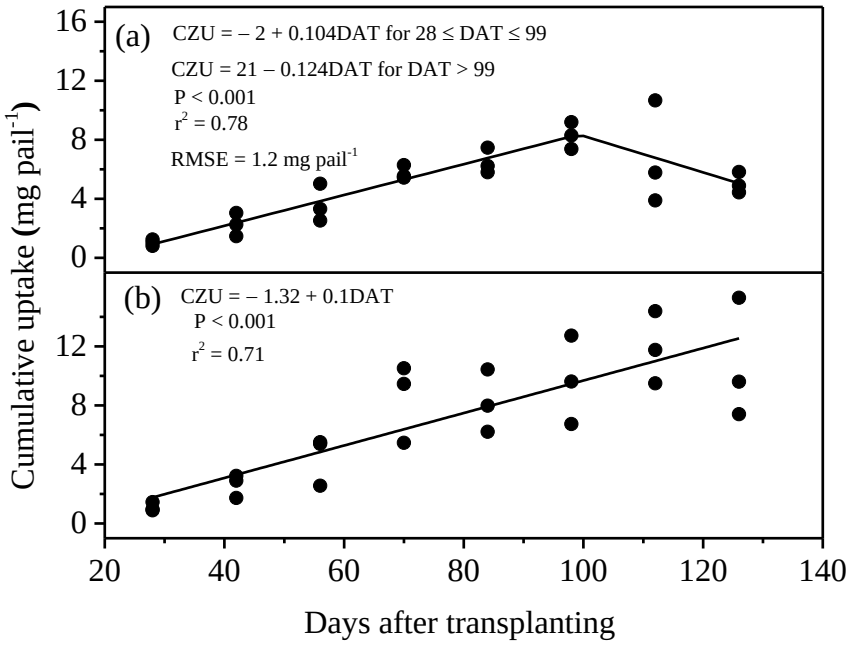

Figure 3.4 Cumulative zinc uptake as a function of time in (a) aboveground and (b) belowground cattail biomass.

3.4.6 Copper Uptake

Small amounts of Cu were taken up by cattail plants, most of which were sequestered in the belowground plant tissues, with very little translocation to AGB. Temporal changes in cumulative Cu uptake (CCU) in the aboveground biomass were best described by an exponential–linear function. For 28 ≤ DAT ≤ 82, Cu accumulation in the aboveground tissues increased as an exponential function of time (CCU = $0.034e^{0.04DAT}$), after which it decreased linearly (CCU = 1.4 – 0.006DAT) for DAT > 82. The peak Cu accumulation of 0.87 mg $pail^{-1}$ (0.01 g Cu m^{-2}) on Day 82 represented 34% of total plant Cu uptake at that stage (Fig 3.5). Retranslocation of Cu to BGB after peak uptake was not evident given the low concentrations translocated to aboveground plant

tissues in the first place. Total Cu accumulation in the BGB was 2–3 times greater than that in the AGB prior to peak aboveground Cu accumulation, and was five times greater by the end of the study period, indicating that roots continued to absorb Cu with no significant translocation to the shoots. Low Cu uptake by the plant was likely due to strong adsorption of the metal by organic matter (Laidlaw et al., 2012). When plants were harvested at the time of peak Cu accumulation in the AGB, 0.6% of Cu initially present in the biosolids was removed in the harvested biomass.

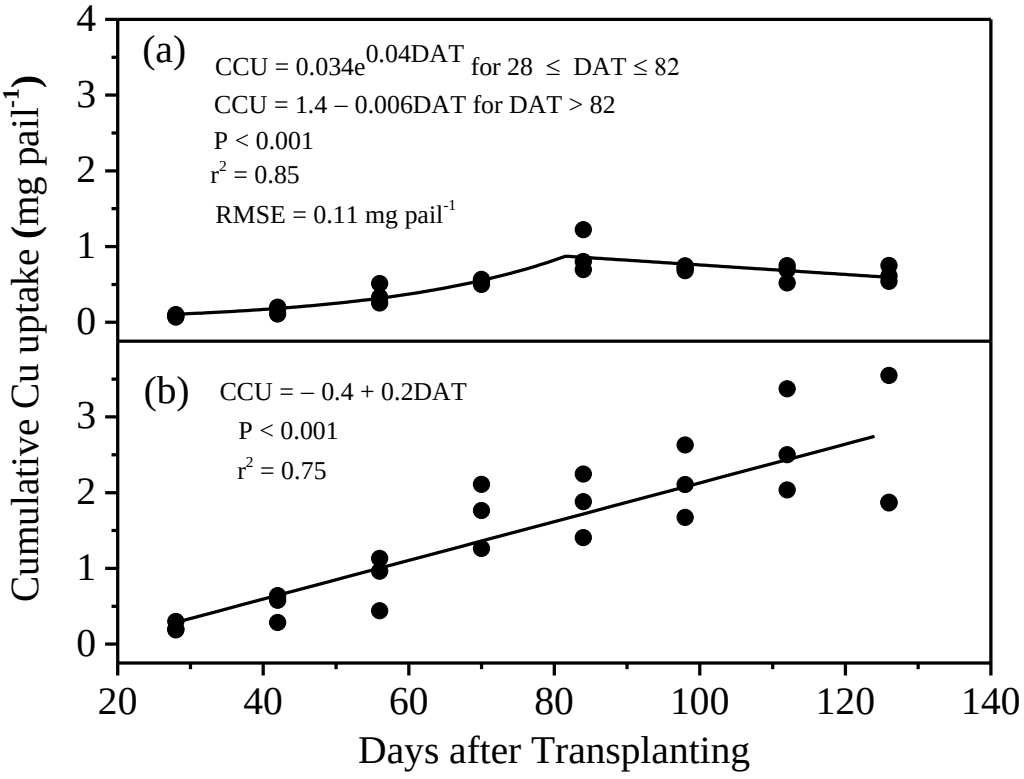

Figure 3.5 Cumulative copper uptake as a function of time in (a) aboveground and (b) belowground cattail biomass.

3.4.7 Chromium Uptake

Cumulative Cr uptake (CCrU) by the aboveground biomass increased as an exponential function ($CCrU = 0.277e^{0.049DAT}$) of time for $28 \leq DAT \leq 80$, after which it increased by 0.06 mg pot^{-1} ($CCrU = 0.2 + 0.001DAT$) for $DAT > 80$ (Fig 3.6a). Peak Cr accumulation on Day 80 was 0.28 mg $pail^{-1}$ (0.005 g Cr m^{-2}), which represented 44% of total plant accumulation by that day. Cumulative Cr uptake at all sampling times was greater in the BGB than in the AGB, and was about twice greater in the BGB by the end of the experiment. Greater accumulation of Cr by BGB could be a result of Cr immobilization in the vacuoles of root cells as a mechanism to reduce toxicity to AGB (USEPA, 2012). Harvesting AGB at peak cumulative Cr uptake removed 0.2% of the Cr initially present in the biosolids.

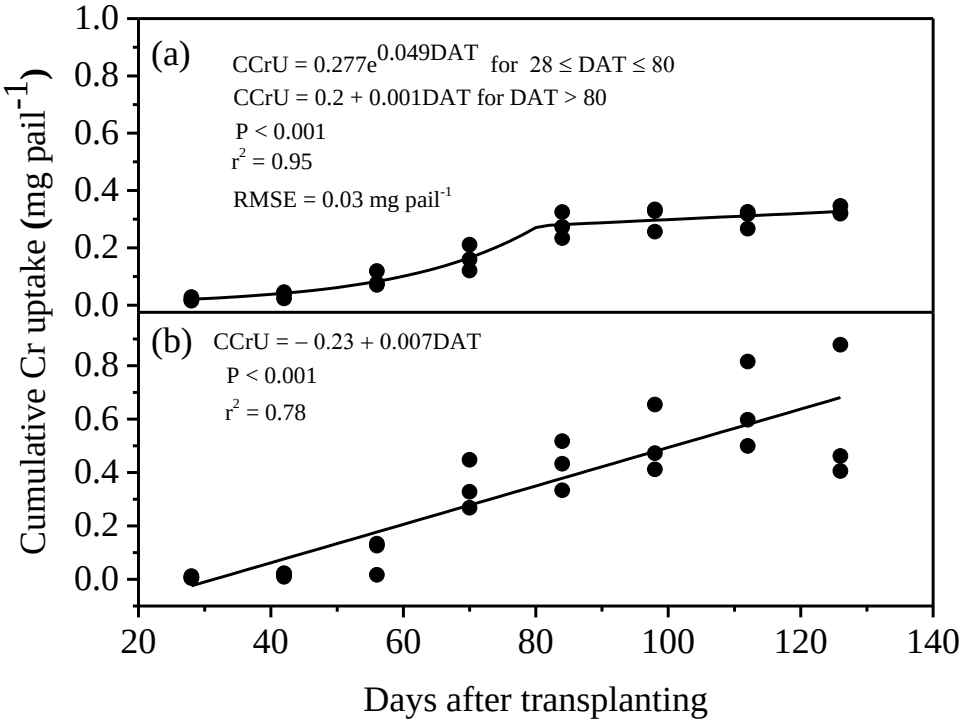

Figure 3.6 Cumulative chromium uptake as a function of time in (a) aboveground and (b) belowground cattail biomass

3.4.8 Cadmium Uptake

Cadmium accumulation in the aboveground biomass was low, peaking at just 0.0075 mg pail^{-1} (0.0001 g Cd m^{-2}), or 7% of total plant uptake on Day 107 (Fig 3.7a). Almost all of the absorbed Cd was in the BGB. Harvesting at peak Cd accumulation removed 0.1% of the Cd initially present in the biosolids. Cattail plants may immobilize Cd in the cell wall of roots as an exclusion mechanism to protect aerial plant tissues from Cd toxicity (Stoltz and Greger, 2002; Bah et al., 2011; Salem et al., 2014). Trace elements such as Cd and Cr, generate free radicals and toxic reactive oxygen species (ROS) in the cytoplasm, inducing oxidative stress to plants (Cho and Seo, 2005) that can

damage proteins, lipids and DNA in plant cells (Fu and Huang, 2001). Bah et al. (2011) demonstrated the activation of enzymatic antioxidants superoxidase dismutase and peroxidase in *Typha angustifolia* to detoxify ROS as a defense mechanism to protect the plant from oxidative stress induced by Cd and Cr.

Cadmium is a non-essential element and is toxic to plants. Most plants do not have mechanisms to absorb non-essential elements such as Cd, and uptake is through Ca^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} transporters when plants absorb essential trace elements (Verbruggen et al., 2009; Marchand et al., 2010). Translocation of absorbed Cd to aboveground tissues was restricted probably to reduce Cd toxicity to photosynthetic aerial plant parts. Roots of many plant species have been reported to release chelating compounds into the rhizosphere that mobilize trace elements, thus reducing their uptake and toxicity (McGrath et al., 2001).

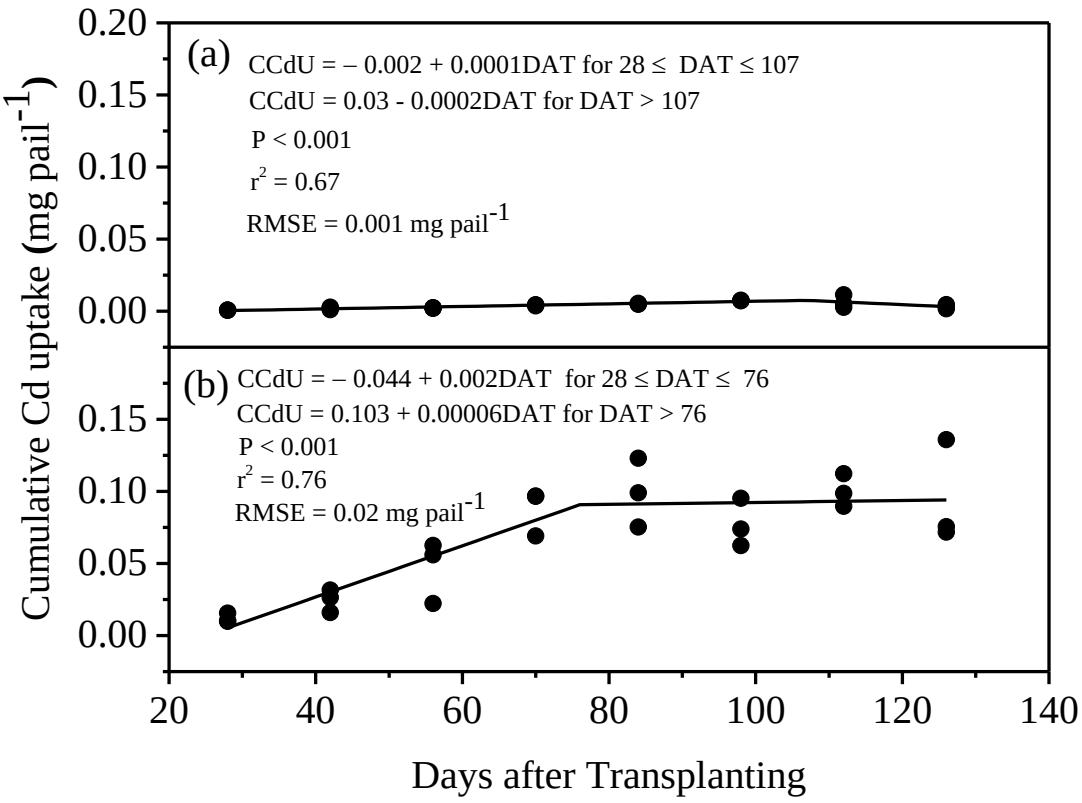

Figure 3.7 Cumulative cadmium uptake as a function of time in (a) aboveground and (b) belowground cattail biomass.

The low trace element uptake in the present study may be due to low trace element concentrations or bioavailability. Bioavailability of trace elements in wetlands is affected by environmental conditions, such as redox potential, pH, and salinity of the sediment and overlying water column (Reddy and DeLaune, 2008). As a result of low trace element uptake, the timeframe required to achieve trace element remediation goals by phytoextraction would be undesirably long. Phytostabilization is the dominant mechanism of phytoremediation for the trace elements. The trace elements are

immobilized in the roots and are therefore not available to affect sediment-dwelling organisms. Although the trace element concentrations in the biosolids used in this study were above TEL values of the CCME sediment quality guidelines for protection of aquatic life, the concentrations were nonetheless below threshold values of all the four land uses in the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (1999) and would therefore not pose a threat to humans and the environment under any of the land uses after remediating for N and P.

3.4.9 Aquatic Chemistry

3.4.9.1 Copper

There was a significant treatment × day interaction ($P < 0.001$) for Cu concentration in the overlying water column (Table 3.3). In vegetated microcosms, Cu concentration in the overlying water column decreased by 20 µg L^{-1} (82%) from Day 42 (25 µg L^{-1}) to Day 84 (4.6 µg L^{-1}) followed by a slow increase thereafter (Fig 3.8). By comparison, Cu concentration increased in the overlying water column of the unvegetated (control) microcosms to a peak of 43 µg L^{-1} on Day 84, followed by a decrease of 11 µg L^{-1} (26%) in Cu concentration until Day 112.

In the vegetated sediment, the exchangeable Cu was likely absorbed by plants or immobilized in the sediment due to plant effects such as release of organic compounds which may decrease trace element mobility through chelation or complexation or radial oxygen loss from plant roots, which causes iron-oxidation and coprecipitation of Cu (Jacob and Otte, 2003). In the unvegetated control, the absence of plant effects likely resulted in an influx of exchangeable Cu to the overlying water column, hence greater Cu concentration measured in the control microcosms during

the entire experiment compared with Cu concentration in the overlying water column of the vegetated sediments.

Ye et al. (2009) reported weak Cu adsorption in the inorganically bound fraction in the sediment of unvegetated wetland controls and acknowledged that this could be a source of pollution to the overlying water column. This is in agreement with our results, which suggest that in vegetated sediments, Cu toxicity to aquatic life is reduced since Cu concentration in the overlying water column decreased to concentrations slightly greater than the Canadian water quality guidelines for the protection of aquatic life (CCME, 1999) (Fig 3.8). Copper concentration in the control was about two orders of magnitude higher than the CCME water quality guidelines throughout the entire study period.

Table 3.3 Effect of presence or absence of cattail on copper, zinc, and cadmium concentration, and on electrical conductivity, dissolved oxygen concentration, and pH of the overlying water column.

Effect†	Cu	Zn	Cd	EC	DO	pH
	———	$\mu g\ L^{-1}$	———	$\mu S\ cm^{-1}$	$mg\ L^{-1}$	
Treatment`						
Vegetated	13.2	25.8	0.13	776	8.5	7.8
Control	34.7	22.4	0.35	2846	1.5	8.9
			P − value			
Treatment (T)	0.01	0.39	0.006	0.01	0.01	0.002
Time (D)	0.65	0.11	0.86	<0.001	0.02	0.003
T × D	<0.001	0.08	<0.001	<0.001	0.06	0.001

†Time main effects are not presented for the eight multiple sampling times. The interactions are presented in figures.

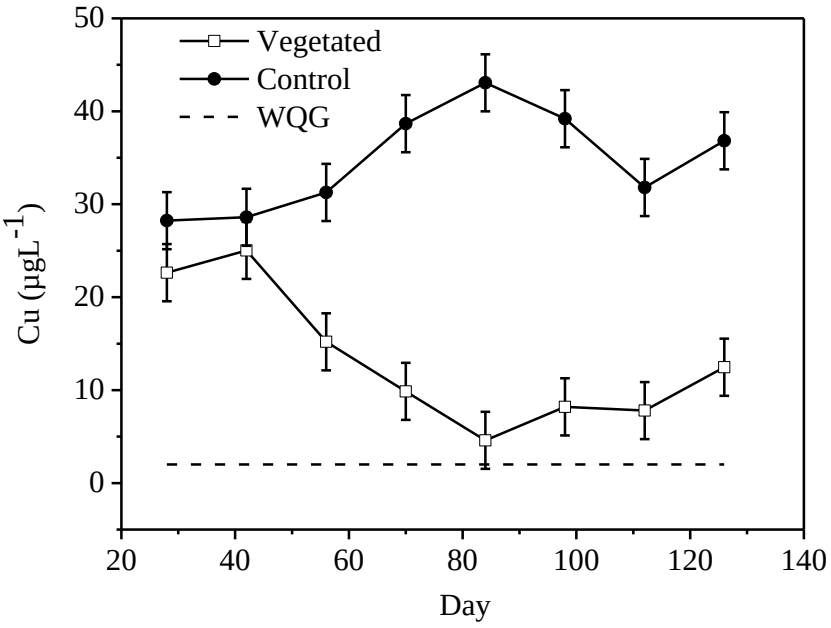

Figure 3.8 Effect of vegetated and unvegetated microcosm on the copper concentration of the overlying water column. WQG, Canadian water quality guideline for the protection of aquatic life (CCME, 1999).

3.4.9.2 Zinc

The presence or absence of cattail did not have a significant effect on Zn concentration in the overlying water column (P = 0.08) (Table 3. 3). Doyle and Otte (1997) and Yeh et al. (2009) did not find any significant difference in Zn removal from wastewater between vegetated and non-vegetated sediments. However, in their wastewater study, deposition of trace elements in the sediment was the main mechanism for Zn removal from wastewater and plants did not have any effect considering that plants absorb trace elements from the sediment pore water and not the overlying water column. In our study, we expected cattail to have an effect since

Zn was diffusing from the sediment pore water, which was in direct contact with the roots, into the overlying water column.

Goulet et al. (2001) reported that Zn was predominantly associated with the persistent organic fraction in the sediment of their constructed wetland while Cu was associated mainly with the reactive fraction associated with oxides and monosulfides or adsorbed to organic matter and was readily available. Copper in biosolids is more firmly bound to organic matter than Zn (Laidlaw et al., 2012). In the present study, trace element fractions in the biosolids sediment were not determined and would be important for future studies.

It is unclear why the Zn in the water column of the vegetated sediment was higher than that in the control during the first 60 d of sampling. Our water sampling began on Day 28, synchronizing harvesting of biomass with water sampling. However, sampling a few hours or days after flooding the microcosms would have given better information on the trend of Zn concentrations from the onset of the experiment.

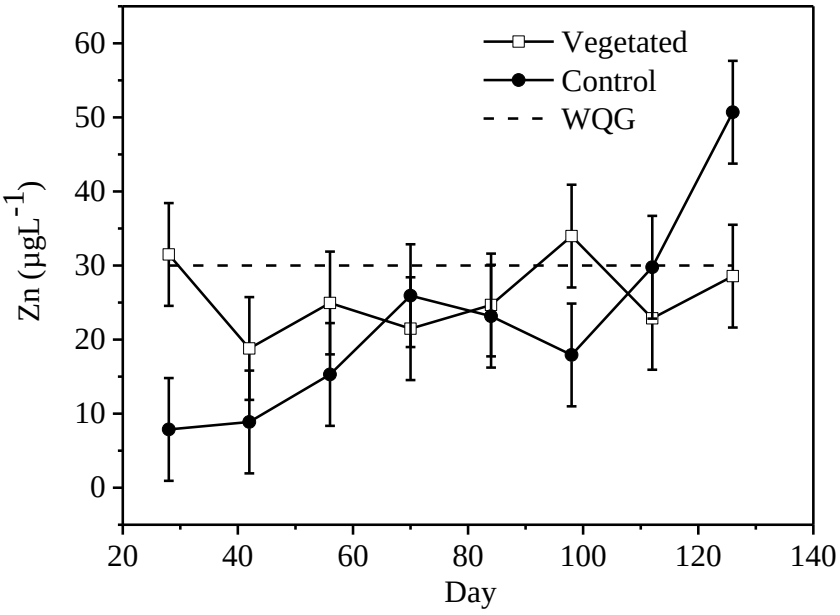

Figure 3.9 Effect of vegetated and unvegetated microcosm on the zinc concentration of the overlying water column. WQG is the Canadian water quality guideline for the protection of aquatic life.

3.4.9.3 Cadmium

There was a significant treatment × Day interaction ($P < 0.001$) for Cd concentration (Table 3.3). In the vegetated sediments, Cd concentration in the overlying water column decreased from 0.33 µg L^{-1} (Day 42) to a constant Cd concentration of 0.05 µg L^{-1} on Day 84 and thereafter. The decrease in Cd concentration is probably a result of Cd uptake by cattail from the sediment pore water. In the control, Cd concentration generally increased in the water column, probably as a result of flux of bioavailable Cd from the sediments. Cadmium concentration in the water column of the vegetated sediments was below the CCME water quality guidelines for protection of aquatic life (0.09 µg L^{-1}) from Day 72 and

onwards, while in the control, Cd concentration was above the CCME water quality guideline thoroughout the entire experiment.

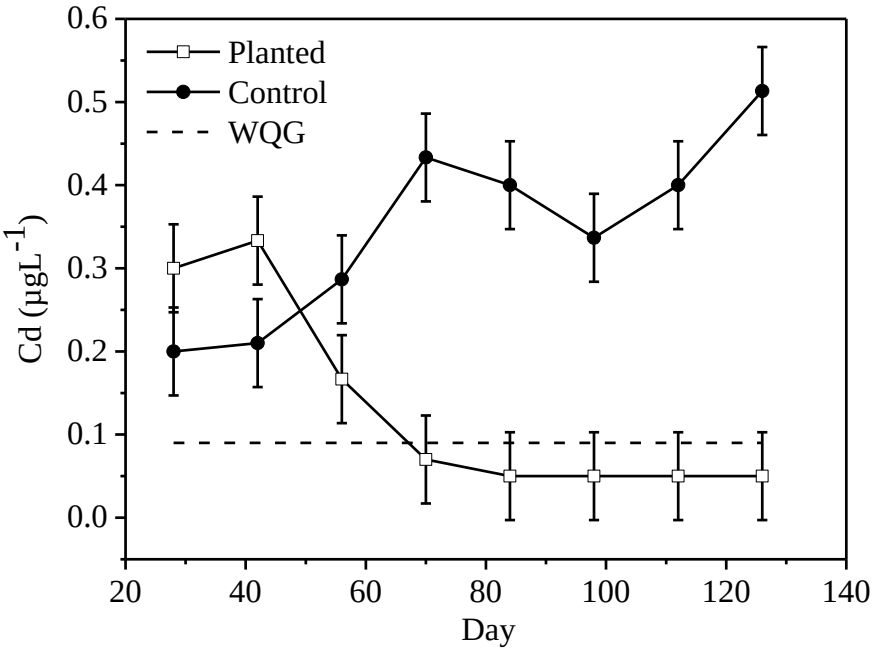

Figure 3.10 Effect of vegetated and unvegetated microcosm on the cadmium concentration of the overlying water column. WQG is the Canadian water quality guideline for the protection of aquatic life.

Total nitrogen, total P, and Cr concentrations were below the detection limits of 20 000 µg L^{-1}, 500 µg L^{-1} P, and 7.5 µg L^{-1}, respectively. The Canadian guidance framework for P considers 35 -100 µg L^{-1} to trigger eutrophic conditions in aquatic systems (CCME, 2004). Our study could not give conclusion on P in the water column since the detection limit of our analysis was greater than the range of P

concentration indicated by the CCME guidance framework. The CCME guidance framework does not provide guidance on N.

3.4.9.4 Electrical Conductivity

Presence or absence of cattail had a significant effect on electrical conductivity (EC) in the water column (Table 3.3). In the vegetated sediments, EC in the overlying water column decreased from 2.2 dS cm^{-1} (Day 28) to 0.2 dS cm^{-1} (Day 84) followed by a fairly constant EC thereafter (Fig 3.11). In the control, EC did not significantly differ at all sampling dates. Electrical conductivity decreased in the overlying water column of vegetated sediments due to uptake of ions by cattail plants in the sediment. Several studies have shown that plants in wetlands are able to remove ions such as sodium (Na^{+}) and chlorine (Cl^{-1}) ions through plant uptake. Nilratnisakorn et al. (2007) and (2009) reported a 44% decrease in Na^{+} ions to plant uptake by *Typha angustifolia* in a constructed wetland treating synthetic dye wastewater. Morteau et al. (2009) reported *Typha Latifolia* accumulating high concentration of Cl^{-1} ions (63 mg g^{-1} DW) in its tissues and they concluded that *Typha Latifolia* has great potential to remove salts in wastewater.

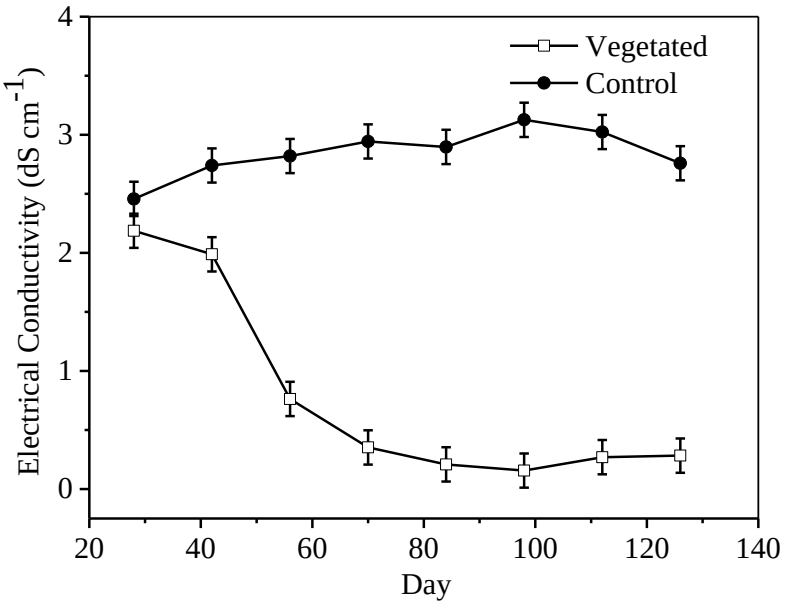

Figure 3.11 Effect of vegetated and unvegetated microcosm on the electrical conductivity in the overlying water column.

3.4.9.5 Dissolved Oxygen

Dissolved oxygen (DO) in the overlying water column did not differ significantly between vegetated and unvegetated sediments (P = 0.06) (Table 3.3). Dissolved oxygen in the water column of vegetated microcosms deceased from 11 mg L^{-1} (Day 28) to 7 mg L^{-1} (Day 56) and remained fairly constant thereafter (Fig 3.12). The presence of cattail lowered DO while greater DO was measured in the unvegetated microcosms. The lower DO in the water column of vegetated sediments may be a result of oxygen consumption by roots or roots exudates such as organic compounds and microbial activity associated with roots (Zhai et al., 2013). Greater DO in the unvegetated control microcosms than in the vegetated microcosms might be

a result of high production of DO by photosynthesizing algae that developed in the control. Borne et al. (2014) attributed high DO and pH >8.5 in their control wetland cell to abundant algal growth.

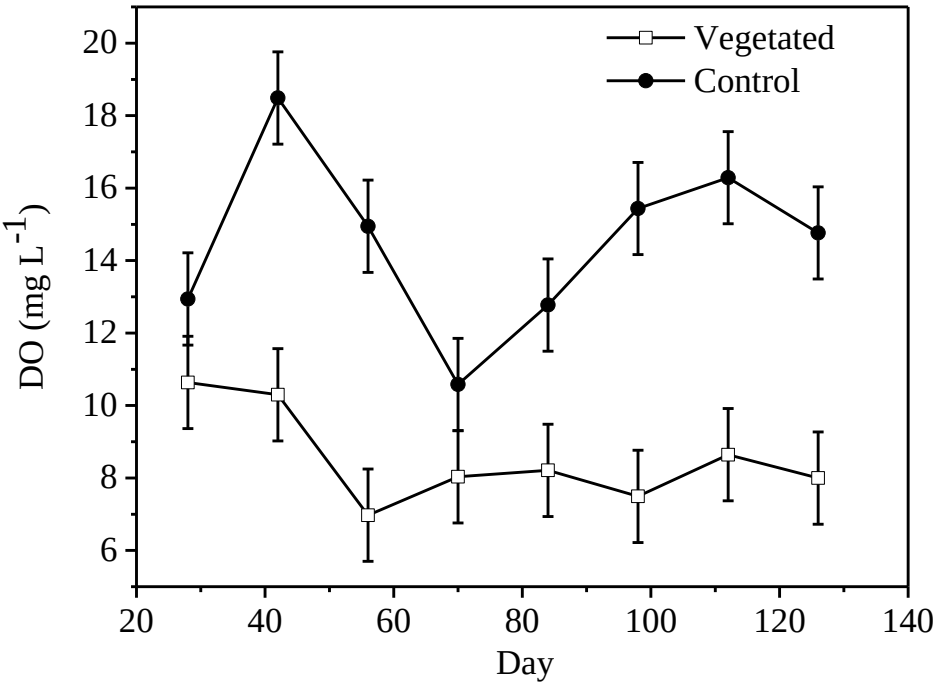

Figure 3.12 Effect of vegetated and unvegetated microcosm on the cadmium concentration of the overlying water column.

3.4.9.6 pH

The presence or absence of plants had a significant effect on the pH in the overlying water column (P = 0.001) (Table 3.4.3). In the vegetated sediments, the pH in the overlying column decreased from 8.2 (Day 28) to 7.6 (Day 56) and remained fairly constant thereafter, except for the peak on Day 112. In the control, pH was

significantly greater than pH in the vegetated microcosm through the entire experiment, except on Days 28, 70 and 84. The presence of cattail may have promoted nitrification through the release of oxygen and the protons produced during nitrification were not completely neutralized, resulting in lower pH in the vegetated sediments than the unvegetated sediments (Lee and Scholz, 2007). The pH in the overlying water column in vegetated sediments (7.5-8.2) was within the range (6.5 to 9.0) at which adverse biological effects are unlikely (CCME, 1999).

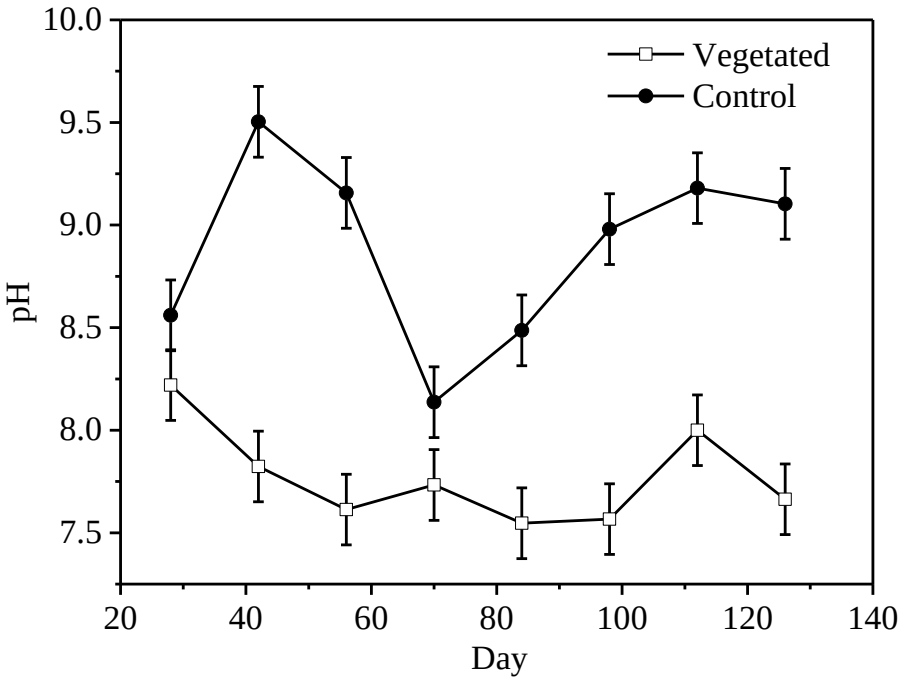

Figure 3.13 Effect of vegetated and unvegetated microcosm on the pH in the overlying water column.

3.5 Conclusions

Maximum biomass yields were similar between AGB and BGB. At peak N and P accumulation in AGB, greater proportions of N and P were partitioned to AGB than to BGB. We suggest that optimum removal of these nutrients from biosolids would occur if harvesting of cattail plants coincides with peak AGB nutrient accumulation. Phytoextraction was not effective in removing trace elements from biosolids as small amounts of trace elements were absorbed, most of which were partitioned to the belowground biomass. Several decades would be required to reduce trace elements to the CCME TEL. These results indicate that harvesting cattail AGB removes a greater portion of N and P taken up by the plants. Findings from this study will inform policy makers and landowners on the timing of harvesting for maximum phytoextraction and removal of nutrients from biosolids. However, long-term studies are needed to determine the nutrient and biomass dynamics and hence timing of harvesting under field conditions. Results on concentration of trace elements in the overlying water column indicate reduced risk to aquatic life if the wetland is vegetated with cattail.

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4. BIOMASS, NUTRIENT AND TRACE ELEMENT ACCUMULATION AND PARTITIONING IN SWITCHGRASS DURING TERRESTRIAL PHYTOREMEDIATION OF MUNICIPAL LAGOONS

4.1 Abstract

In situ phytoremediation of municipal biosolids is a promising biosolids management option to the spreading of biosolids on land and landfilling of biosolids from end-of-life municipal lagoons. Accumulation and partitioning of dry matter, nitrogen (N), phosphorus (P) and trace elements [zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd)] were determined in aboveground biomass (AGB) and belowground biomass (BGB) of switchgrass (*Panicum virgatum*) to determine the harvest stage that maximizes phytoextraction of contaminants from municipal biosolids. Five switchgrass seedlings were transplanted into each 15-L plastic pail containing 3.9 kg (dry wt.) biosolids from the secondary cell of a municipal lagoon. Aboveground biomass and BGB yields and contaminant concentrations were determined by harvesting switchgrass every 14 d for 161 d. Logistic model fits to biomass yield indicated no significant differences in asymptotic yield between AGB (58 g pail^{-1}) and BGB (56 g pail^{-1}). Switchgrass partitioned significantly greater quantities of N and P to the AGB than to the BGB. Maximum uptake occurred 86 d after transplanting (DAT) for N and 102 DAT for P. Belowground biomass accumulated greater proportions of trace elements than AGB, except for Cr, which was allocated more to AGB. The percentage of total accumulation partitioned to AGB at peak accumulation of each element was 68% for N, 65% for P, 46% for Zn, 38% for Cu, 57% for Cr, and 9% for Cd. Harvesting at peak aboveground accumulation removed (percent of initial concentration in the biosolids) 5% N, 1.6% P,

0.2% Zn, 0.1% Cu, 0.05% Cd, and 0.1% Cr. The large portion of N and P taken up by switchgrass can be removed by harvesting AGB while low trace element uptake limits the effectiveness of phytoextraction as a remedial approach for trace elements. Low phytoextraction, particularly of P, could be due to the root system not being fully established in the first season. These results will contribute towards identification of the harvest stage that will optimize contaminant uptake and enhance in situ phytoremediation of biosolids using switchgrass.

4.2 Introduction

Phytoremediation has gained wide attention as a cost effective and environmentally-friendly approach for removal of soil contaminants in situ (Sadowsky, 1999; Adams et al., 2000; Pilon-Smits, 2005). Phytoremediation takes the advantage of plants' ability to remove, immobilize, or render pollutants harmless (Garbisu et al., 2002; McIntyre, 2003) and is practical for a wide range of contaminants, such as nutrients (N and P) (Sanderson et al., 2001; Liphadzi et al., 2002; Ashworth, 2010; Silveira et al., 2013), trace elements (Alkorta et al., 2004; Chen et al., 2012), and organic contaminants (Dzantor et al., 2000; Euliss et al., 2008; Nedunuri et al., 2009).

In situ phytoremediation of municipal biosolids in end-of-life lagoons is a promising alternative to the traditional agricultural land spreading or landfilling of biosolids. Plants can utilize biosolids N and P for biomass production, and harvesting the aboveground biomass removes these nutrients and other contaminants from the biosolids, thus cleaning up end-of-life lagoons. Harvested biomass can be used as a feedstock for bioenergy production (McKendry, 2002; Ashworth, 2010).

Plants that tolerate and accumulate high contaminant concentrations while producing high biomass yields are attractive species of choice for phytoremediation (Delorme et al., 2000; Sanderson et al., 2001; Garbisu et al., 2002), particularly if they have a post-harvest use, such as feedstock for bioenergy production. Switchgrass is a drought-tolerant, perennial grass with high biomass yield potential, and is adapted to a wide range of soil conditions (Parrish and Fike, 2005). These qualities make switchgrass a notable candidate for in situ phytoremediation of biosolids in end-of-life lagoons. Switchgrass has been widely adopted as a feedstock for bioenergy production in Canada and the United States (Casler and Boe, 2003), and is often grown on marginal agricultural lands, producing high biomass yields with minimal inputs (Chen et al., 2012). Due to its high nutrient efficiency and high yield potential (Heaton et al., 2004; Adler et al., 2006), which is typical of C4 plants (Brown et al., 2000; Dohleman et al., 2012), switchgrass has potential for phytoremediation of contaminated media with low plant-available N concentrations or those in which N mineralization rates are low, such as biosolids used in this study (Chapter 2).

Switchgrass has been widely used for phytoextraction of N and P (Sanderson et al., 2001; Ryan et al., 2006; Ashworth, 2010; Silveira et al., 2013) and trace elements (Shahandeh and Hossner, 2000; Alkorta et al., 2004; Chen et al., 2012) in soil. Switchgrass reduced total molybdate-reactive P in surface runoff from dairy manure by 47–76% (Sanderson et al., 2001), indicating its potential to effectively remove excess P from soil. Missasoui et al. (2005) reported high accumulation of P in switchgrass grown in high-P soils under both greenhouse and field conditions. High biomass yields and P-removal potential of forage crops, including switchgrass, has led to a growing interest in

their use for nutrient uptake in land-applied manure (Woodard et al., 2002; Mikhailova et al., 2003; Newton et al., 2003; Rowe and Fairbrother, 2003). Several studies have shown that N fertilization of switchgrass increased aboveground N uptake (Brejda et al., 1996; Vogel et al., 2002; Heggenstaller et al., 2009) and therefore N removal can be achieved by harvesting aboveground biomass. Although switchgrass is considered an accumulator rather than a hyperaccumulator of trace elements such as Cd, Cr, and Zn, its high biomass yields enable its practical use in phytoextraction of trace elements (Chen et al., 2012).

Nutrient concentrations in aboveground switchgrass biomass decreases as the growing season progresses because of retranslocation of the nutrients to belowground biomass, leaching of soluble nutrients from aboveground biomass, and shedding of senescing leaves (Beale and Long, 1997; Vogel, 2004; Dell and Rice, 2005; Parrish and Fike, 2005). Harvesting switchgrass for bioenergy production is often delayed past peak nutrient accumulation to target low nutrient concentrations in aboveground biomass after retranslocation has occurred (McKendry, 2002; Lewandowski and Heinz, 2003; Parrish and Fike, 2005; Adler et al., 2006). Switchgrass biomass as a bioenergy feedstock requires low N concentrations in the biomass because N reduces the efficiency of converting biomass to biofuel (McKendry, 2002; Boateng et al., 2006; Heaton et al., 2009). In contrast, for maximum phytoextraction of nutrients and contaminants, harvesting at peak aboveground accumulation is ideal to optimize the contaminant removal in the aboveground biomass. Harvesting biomass past peak accumulation results in reduced biomass yields and lower nutrient removal (Beale and Long, 1997; Parrish and Fike, 2005; Gouzaye et al., 2014). Heaton et al. (2009) reported greater removal potential

of N (187 kg N ha^{-1}) when switchgrass was harvested during the peak accumulation period compared with a late winter harvest (5 kg N ha^{-1}).

Several studies have examined phytoextraction of contaminants from soils (Delorme et al., 2000; Dzantor et al., 2000; Alkorta et al., 2004; Chen et al., 2012) or biosolids-amended soils (Liphadzi, 2002; Liphadzi et al., 2006), but there are currently no published studies on direct phytoextraction from biosolids alone. In the case of switchgrass, the studies have typically been conducted in soils fertilized with nutrients to optimize biomass production (Adler et al., 2006; Fike et al., 2006; Garten Jr et al., 2010). Results from such studies are not directly transferable to cases where phytoremediation is used to remove contaminants directly from biosolids. There is, therefore, a need to investigate the effectiveness of phytoremediation when plants are grown directly in biosolids, an organic medium with different physiochemical conditions to soil. The objective of this study was, therefore, to determine the accumulation and partitioning of biomass, nutrients, and trace elements in the aboveground and belowground biomass of switchgrass grown in biosolids from the secondary cell of a municipal lagoon system. Such information is needed to determine the timing of harvesting that will optimize contaminant removal. It was hypothesized that (i) biomass, N, P and trace element partitioning differs between AGB and BGB in switchgrass and (ii) harvesting AGB at peak accumulation of biomass, N, P and trace elements enhances the effectiveness of phytoremediation

4.3 Materials and methods

4.3.1 Biosolids

Biosolids samples were collected from a secondary cell of an end-of-life lagoon in Niverville, MB, Canada (49°35'42.7"N, 97°02'50.3"W). The 8.8 ha secondary cell received effluent from a 4.6 ha primary cell for further treatment and storage. The effluent was primarily domestic effluent and the municipality used a wastewater stabilization treatment system. The volume of biosolids (20-cm thick layer) in the secondary cell at the time of lagoon closure was 28,000 m^3. The biosolids (0- to 20-cm layer) used in this study were collected in the summer of 2011 from random points covering the entire lagoon cell.

4.3.2 Microcosm Set-up

Switchgrass seeds were sowed into plastic trays containing the biosolids described above. After 35 d, five uniform switchgrass seedlings were transplanted into each plastic pail (24 cm diameter × 26 cm height) containing a 15-cm thick layer of biosolids (3.9 kg dry wt.). The experiment was arranged in a completely randomized design consisting of 30 planted pails (3 replicates × 10 sampling times).

The pails were placed in a controlled environment growth room maintained at a 16–h photoperiod and day/night temperatures of 22/15°C. Relative humidity during the experiment was set at 85% while daytime light intensity was 270 µmole photons m^{-2} s^{-1}. Microcosms were weighed every other day and watered to replace water lost via evapotranspiration. Moisture content was initially maintained at 60% water filled pore space and then increased by 10% seven days after transplanting (DAT).

4.3.3 Sampling and Laboratory Analysis

Plants were harvested from three pails 35 DAT and every 14 d thereafter. Aboveground biomass was harvested by clipping plants at the surface of the biosolids layer using a knife. Plant roots (belowground biomass) were washed with tap water to remove the bulk of the biosolids and then rinsed with reverse osmosis water. Harvested biomass was oven-dried for 72 h followed by weighing for determination of biomass yield. The dry biomass was ground (<0.2 mm) using a SPEX 8000D ball mill (Metuchen, NJ, USA). Total Kjeldahl N (TKN) concentration was determined using a FIAlab 2500 flow injection analyzer (FIAlab Instruments, Bellevue, WA, USA) following digestion with sulfuric acid in a block digester. For trace element and P determination, 0.5 g samples were digested in aqua regia (concentrated HNO_3/HCl) for 2 h at 90°C in a microprocessor–controlled digestion block. Trace element concentrations were measured in the digest with a Perkin Elmer SCIEX ELAN 6000 ICP–MS (Perkin–Elmer SCIEX Instruments, Concord, Ontario) while P concentration was measured using a Varian 735 ES inductively coupled plasma mass spectrometer (ICP-MS) (Varian Inc., Palo Alto, CA).

4.3.4 Statistical Analysis

Six growth models – a three–parameter logistic, a four–parameter logistic, Gompertz, Beta, Richards, and the modified Richards model – were fit to the biomass data (Archontoulis and Miguez, 2013) using PROC NLIN in conjunction with the Marquardt algorithm in SAS version 9.3 (SAS Institute, 2014). The three–parameter logistic model had the lowest Akaike information Criterion (AIC) and was therefore chosen as the best fit. The three-parameter logistic model is given by the equation:

$$y = \frac{y_{asymp}}{1 + e^{-k(x-x_m)}} \quad [1]$$

where, y_{asymp} is the asymptotic biomass yield (g pail^{-1}), x is the time elapsed since transplanting (d), x_m is the inflection point at which growth rate is maximized (d), and k controls the steepness of the curve. Parameter estimates were considered significant if 95% confidence intervals did not overlap.

Nutrient and trace element uptake values were calculated by multiplying concentrations and biomass yield. Segmented regression models were fitted using PROC NLIN to predict the critical time when cumulative uptake of nutrients and trace elements peaked.

4.4 Results and Discussion

4.4.1 Biosolids Properties

Selected initial chemical properties of the biosolids are presented in Table 4.1. High N and P concentrations were the major concern with the biosolids, especially for P which causes eutrophication in aquatic systems. About 11% of the total N content was in the plant available form. Nitrate-N represented 84% of the plant-available N while the rest was ammonium-N. Low ammonium concentrations were likely due to nitrification during drying of the biosolids after operation of the lagoon ceased (Liphadzi et al., 2002). The low trace element concentrations observed were expected since the biosolids were produced from domestic wastewater treatment.

Table 4.1 Initial nutrient and trace element concentrations (mg kg^{-1}) in the biosolids

TKN	NH_4-N	NO_3-N	TP	Olsen P	Cu	Zn	Cd	Cr
mg kg^{-1}								
1800	32	165	1210	84	37.8	85.3	0.39	50

4.4.2 Biomass Yield

The three-parameter logistic model fitted AGB and BGB yield data better than the other models tested. A 40-d lag phase in biomass accumulation occurred in the aboveground plant partition (Fig. 4.1a) while a 60-d lag was indicated in the belowground tissues (Fig. 4.1b). Rapid biomass accumulation occurred for approximately 80 d in aboveground tissues and 60 d in belowground tissues. Maximum growth rate for belowground biomass occurred at a significantly latter time (96 DAT) than for aboveground biomass (77 DAT). Growth rate was maximized 19 d later for belowground biomass, likely due to translocation of absorbed nutrients from the belowground biomass to the aboveground photosynthetic tissues to support growth. At the maximum AGB accumulation rate, cumulative AGB yield was 29 g $pail^{-1}$ (0.64 kg m^{-2}) or 74% of the total (aboveground plus belowground) biomass yield.

The *k* parameter which indicates the steepness of the curves from the three–parameter logistic model did not differ significantly between AGB (0.084) and BGB (0.078) (Table 2), showing similar growth rates for the two plant partitions. Asymptotic (maximum) biomass yields (y_{asymp}) did not differ significantly between aboveground (58 g $pail^{-1}$ or 1.28 kg m^{-2}) and belowground biomass (56 g $pail^{-1}$ or 1.24 kg m^{-2}) (Table 2). The results contradict those from several other studies, which showed greater aboveground than belowground biomass yields for switchgrass under field conditions.

For example, Dohleman et al. (2012) reported greater aboveground (21.1 t ha^{-1}) than belowground (13.1 t ha-1) biomass yield across 3 growing seasons. Frank et al. (2004) reported belowground biomass of switchgrass averaging 27% of the total biomass yield in a 3-year field study. Garten et al. (2010) reported an aboveground-to-belowground biomass ratio of 0.77.

Table 4.2 Three-parameter logistic model parameter estimates for aboveground and belowground biomass accumulation. †

	y_{asymp}	x_m	k
	g $pail^{-1}$	d	
Aboveground biomass	58a	77b	0.084a
Belowground biomass	56a	96a	0.078a

†y_{asymp} is the maximum attainable biomass yield (g $pail^{-1}$), x is the time elapsed since transplanting (DAT), x_m is the inflection point at which growth rate is maximized (DAT), and k controls the steepness of the curve.

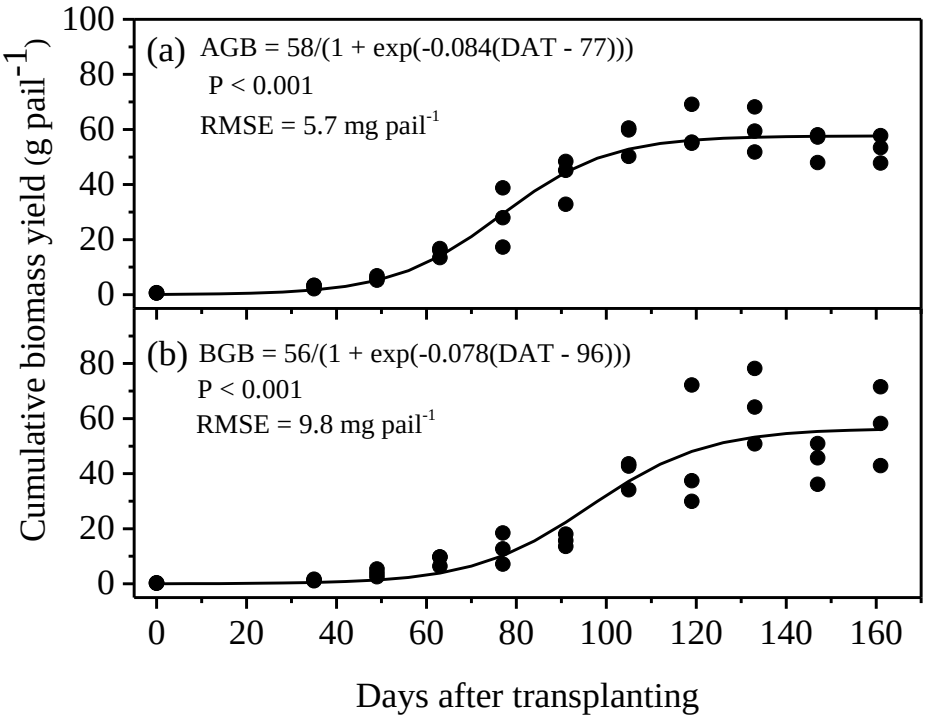

Figure 4.1 Aboveground (a) and belowground (b) switchgrass biomass accumulation as described by a three-parameter logistic model.

4.4.3 Nitrogen Uptake

Temporal changes in cumulative N uptake (CNU) by aboveground biomass were described by a linear-linear segmented regression (Fig. 4.2). Cumulative N uptake increased linearly (CNU = –204 + 6.9DAT) with time for 35 ≤ DAT ≤ 86, peaking at 391 mg pail^{-1} (8.6 g N m^{-2}) on Day 86, after which it decreased linearly (CNU = 699 – 3.6DAT) until the end of the experiment. The linear decrease in N uptake after peak N accumulation was primarily due to retranslocation of N to belowground biomass. Retranslocation of N in switchgrass has been reported by many other authors (Beale and Long, 1997; Lewandowski et al., 2003; Heggenstaller et al., 2009; Wilson, 2012).

Similarly, Madakadze et al. (1999) reported N content in switchgrass decreasing from 25 g kg^{-1} in spring to 5 g kg^{-1} at the end of the growing season in eastern Canada.

Peak cumulative N uptake by aboveground biomass accounted for 68% of total N uptake by switchgrass. The greater partitioning of N to aboveground biomass indicates that a large fraction of the N absorbed by switchgrass can be effectively removed by harvesting aboveground biomass. Nitrogen removal at peak accumulation in the present study (8.6 g N m^{-2}) was within the range reported across the eastern United States (4 to 13 g N m^{-2}) for N phytoextraction when switchgrass was harvested once per season (Fike et al., 2006).

In contrast to aboveground biomass, cumulative N accumulation in belowground biomass increased linearly with time (CNU = -144 + 3.5DAT) over the entire study. The rate of increase of cumulative N uptake during the linear increase phase in aboveground biomass (6.9 mg $pail^{-1}$ d^{-1}) was nearly double the rate of increase in belowground cumulative N uptake (3.6 mg $pail^{-1}$ day^{-1}) (Table 3), indicating that switchgrass readily translocated N to aerial parts to support photosynthesizing tissues (Dohleman et al., 2009).

Harvesting aboveground biomass at peak N accumulation (86 DAT) resulted in the removal of 5% of total N initially present in the biosolids. Fourteen harvesting cycles, corresponding to fourteen growing seasons would be required to remediate the biosolids to the lowest effect level (LEL= 550 mg N kg^{-1}) (Persaud et al., 1993) if switchgrass was harvested at peak N accumulation under conditions of the present experiment, assuming that phytoextraction rate remains constant from one growing season to the next. The LEL defines clean-marginally polluted sediments. Harvesting at the end of the study period,

that is, after retranslocation had occurred, removed only 1.6% of the total N initially present in the biosolids, which translates to 45 growth cycles to meet the LEL, which is 31 cycles longer than that achieved by harvesting at peak N accumulation. Evidently, harvesting switchgrass well past peak N uptake reduces the effectiveness of N removal by phytoextraction and harvesting of aboveground biomass. Vogel et al. (2002) and Wilson et al. (2013) reported two times and 1.5 times greater N removal, respectively, at peak N uptake compared with N removal from fall harvest.

The guidelines used to estimate phytoremediation timeframes are meant to protect freshwater life and are not appropriate for terrestrial life. In the absence of appropriate guidelines, there is need to develop site specific guidelines based on local natural wetlands and the planned post-remediation land-use of the site.

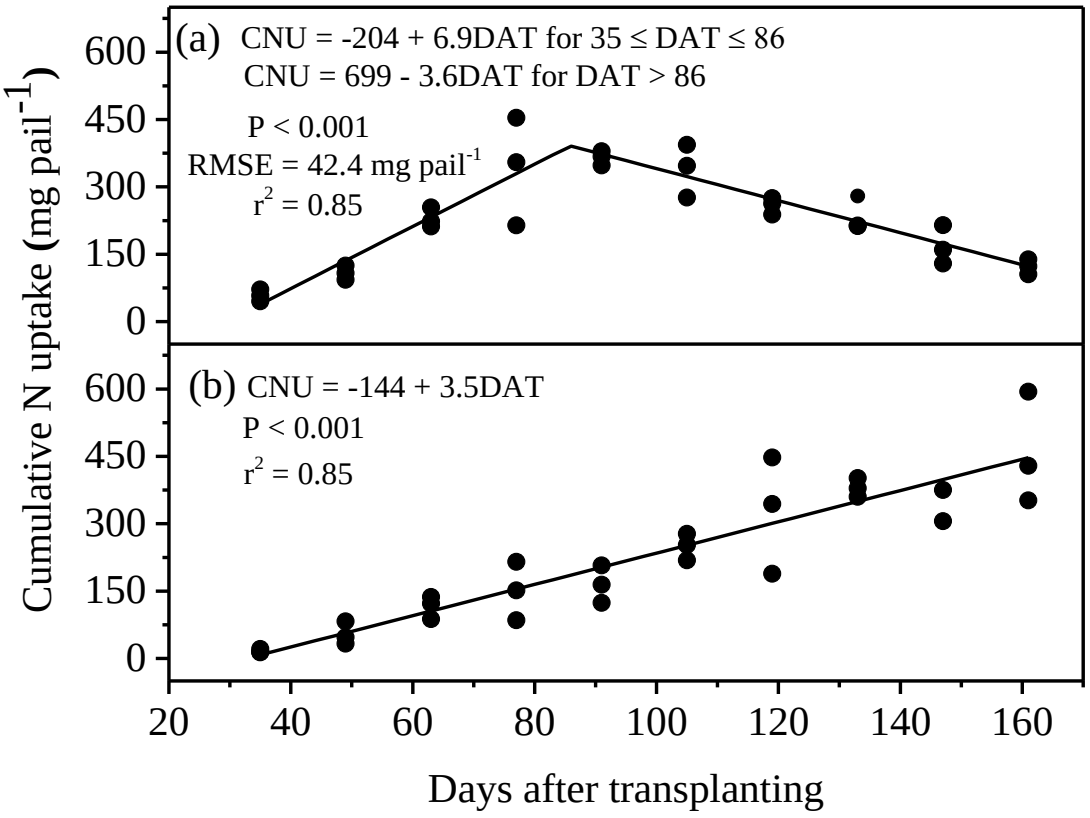

Figure 4.2 Cumulative nitrogen uptake as a function of time in (a) aboveground and (b) belowground switchgrass biomass.

Table 4.3 Segmented and linear models for nitrogen, phosphorus, and trace element uptake (mg kg^{-1} $pail^{-1}$) in aboveground and belowground biomass of switchgrass grown in secondary biosolids for 126 days. †

Element	Aboveground					Belowground		
	a	b_1	b_2	x_0	r_2	a	b_1	r_2
N	–204	6.9	–3.6	86	0.85	–114	3.5	0.85
P	–36	1.1	–0.2	102	0.73	–30	0.7	0.71
Cu	-0.086	0.003	–	82	0.75	–0.289	0.007	0.6
Zn	–0.34	0.011	–0.006	108	0.79	–0.623	0.015	0.74
Cd	6×10^{-5}	7×10^{-6}	–	102	0.27	–0.004	0.0001	0.73
Cr	–0.082	0.002	–0.002	128	0.78	–0.074	0.002	0.69

† *a*, intercept (mg kg^{-1} $pail^{-1}$); b_1, rate of change of response variable when $x_i < x_0$ (mg kg^{-1} $pail^{-1}$ day^{-1}); b_2, rate of change of response variable when $x_i > x_0$ (mg kg^{-1} $pail^{-1}$ day^{-1}); x_i, number of days after transplanting; x_0, critical point at maximum nutrient and trace element uptake (DAT).

4.4.4 Phosphorous Uptake

A linear–linear segmented model provided the best description for temporal changes in cumulative P uptake (CPU) by aboveground biomass (Fig. 4.3a). Cumulative P accumulation in the aboveground biomass increased linearly with time (CPU = –36 + 1.1DAT) for $35 \leq DAT \leq 102$, peaking at 74.6 mg $pail^{-1}$ (1.6 g P m^{-2}) on Day 102, after which it decreased linearly (CPU = 97 – 0.2DAT for $x > 83$) until the end of the experiment. Retranslocation of P in switchgrass was minimal, with P decreasing by only 12.5 mg $pail^{-1}$ from peak P accumulation (74.6 mg $pail^{-1}$) to 62.1 mg $pail^{-1}$ at the end of the study period. Heggenstaller et al. (2009) reported marginal P retranslocation to belowground biomass relative to N retranslocation in perennial warm-season grasses, including switchgrass, and they suggested that retranslocation of P is less efficient in the

grasses. Peak cumulative P uptake by aboveground biomass accounted for 65% of the total P uptake by the plant, indicating that phytoextraction in conjunction with aboveground biomass harvesting at peak N accumulation can remove a large fraction of P absorbed by the plant. Cumulative P accumulation in the belowground biomass increased linearly with time (CPU = –30 + 0.7DAT) over the entire study period. Since retranslocation of P to BGB was minimal, the linear increase in BGB P uptake suggests that BGB continued to absorb P. However, P uptake is expected to reach an uptake plateau with a sampling period greater than 160 d. Cumulative P uptake was greater in aboveground biomass until Day 140. Switchgrass actively removed P from a P-enriched soil (573 mg P kg^{-1}) in a greenhouse experiment, attaining a shoot to root ratio of 1.8 and maximum potential P removal of 1.1 g m^{-2} (Delorme et al., 2000) which is comparable to peak P removal in this study (1.6 g m^{-2}).

Harvesting aboveground biomass at peak P accumulation on Day 102 removed 1.6% of P initially present in the biosolids. Based on the guidelines for the protection and management of aquatic sediment quality in Ontatio (Persaud et al., 1993), 32 harvest cycles, corresponding to 32 growing seasons, would be required to remediate the sediment to the LEL (600 mg kg^{-1}), assuming that phytoextraction rate remains constant from one growing season to the next. Harvesting at the end of the study period, that is, after retranslocation had occurred, removed 1.3% of the P initially present in the biosolids, which translates to a 39 harvest cycle (growth season) requirement to meet the remediation goal, which is 7 cycles longer than that achieved by harvesting at peak P accumulation.

Low P uptake by switchgrass suggests that the plant uses low P amounts or is an efficient P user, as suggested by Nathanial et al. (2014). Nathanial et al. (2014) reported that high P rates applied in poultry litter did not enhance yield, suggesting a low P requirement or efficient P use by switchgrass. Low P uptake may also be a result of low P availability in the biosolids.

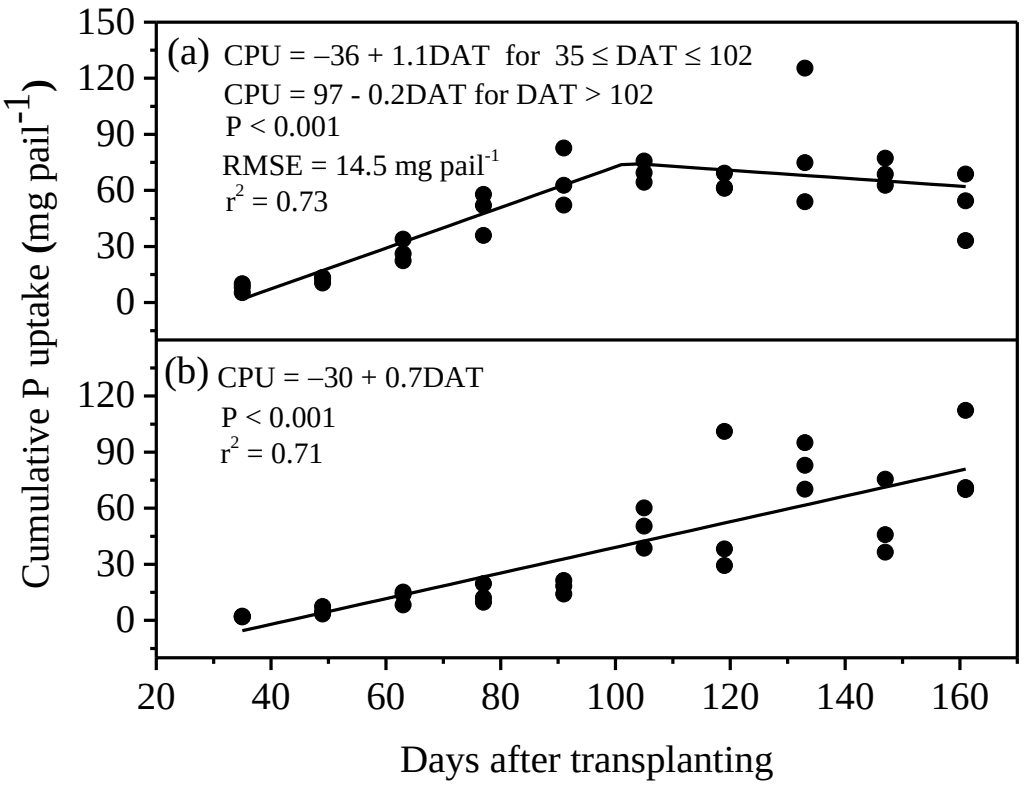

Figure 4.3 Cumulative phosphorus uptake as a function of time in (a) aboveground and (b) belowground switchgrass biomass.

4.4.5 Copper Uptake

Copper accumulation in the aboveground biomass was low and plateaued 82 DAT with a cumulative Cu uptake (CCU) of only 0.16 mg $pail^{-1}$ (0.004 g Cu m^{-2}) or 38% of total Cu uptake (Fig 4.4). A greater fraction of the Cu taken up by the plant was sequestered primarily in the belowground biomass. Cumulative Cu uptake in belowground biomass increased linearly (CCU = -0.289 +0.007DAT) with time throughout the study period and was 5 times greater than aboveground Cu accumulation by the end of the study. This indicates that switchgrass roots continued to absorb Cu since Cu retranslocation did not occur. Although phototoxocity of Cu is low in switchgrass, a limited capacity exists for Cu accumulation in the plant tissues (Juang et al., 2011) and may restrict the amount of Cu taken up by switchgrass.

Harvesting at peak Cu accumulation removed 0.1% of the total Cu initially present in the biosolids. Although the initial Cu concentration in the biosolids (37.8 mg kg^{-1}) was only slightly greater than the CCME threshold effect level (TEL = 35.7 mg kg^{-1}) for protection of aquatic life (CCME, 2001), 51 harvest cycles would be required to meet the TEL, assuming that phytoextraction rate remains constant from one growing season to the next. This is a result of low phytoextraction of Cu from the biosolids. Strong adsorption of Cu to organic matter and precipitation of the metal as sulfides or hydroxides can reduce Cu availability for plant uptake (Donner et al., 2012; Laidlaw et al., 2012; Lu et al., 2012). About 80% of Cu in biosolids is typically bound to organic matter (Haynes et al., 2009) because of the high stability constant of Cu complexes with organic matter (Ashworth and Alloway, 2004).

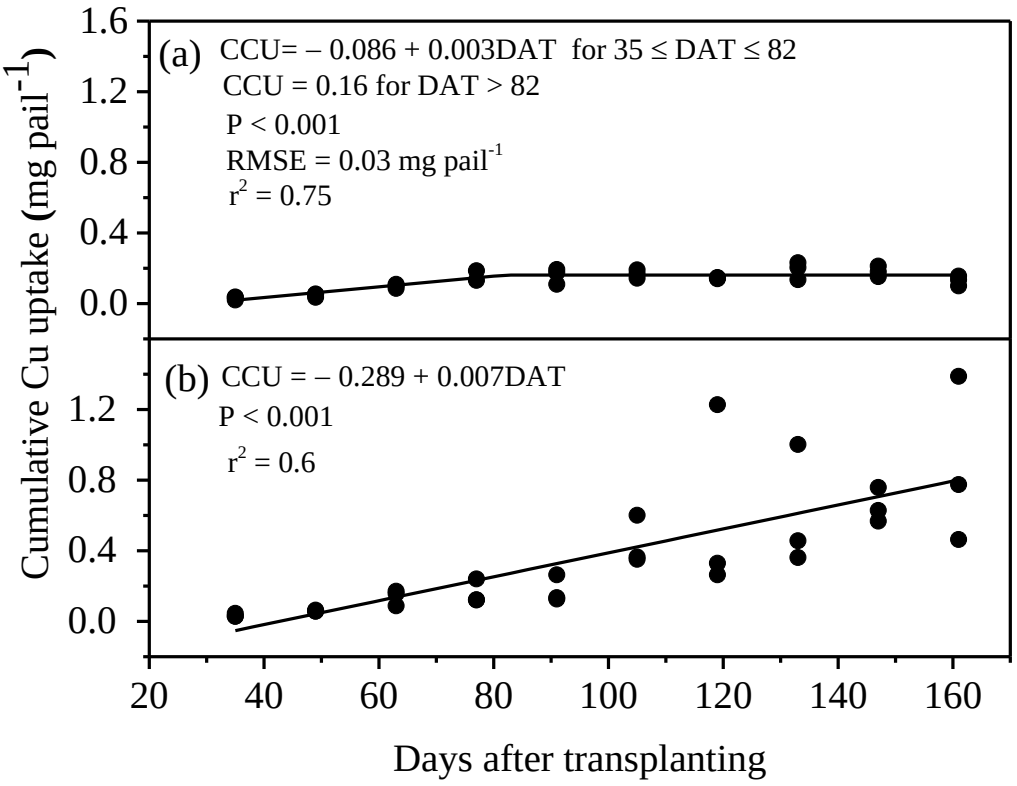

Figure 4.4 Cumulative copper uptake as a function of time in (a) aboveground and (b) belowground switchgrass biomass.

4.4.6 Zinc Uptake

A linear–linear function provided the best description for temporal changes in cumulative Zn uptake (CZU) by aboveground biomass (Fig. 4.5). Cumulative Zn uptake increased linearly with time (CZU = –0.34 + 0.011DAT) for 35 ≤ DAT ≤ 108 , peaking at 0.8 mg pail^{-1} (0.02 g Zn m^{-2}) or 46% of the total Zn uptake on Day 108, after which it decreased linearly (CZU = 1.5 – 0.006DAT) until the end of the experiment. Accumulation of Zn in the belowground biomass increased linearly (CZU = – 0.62 +

0.015DAT) with time throughout the experiment. Zinc accumulation in the aboveground biomass was more than 50% of total Zn uptake until 70 DAT. This is because Zn is easily accumulated in green aboveground plant tissues such as leaves (Stoltz and Greger, 2002). Partitioning of Zn shifted to belowground biomass on and after Day 70 and Zn accumulation in the belowground biomass was about four times greater than uptake in aboveground by the end of the experiment.

Harvesting at peak cumulative Zn uptake removed 0.2% of the Zn initially present in the biosolids. The initial concentration of Zinc in the biosolids (85.3 mg kg^{-1}) was lower than the CCME TEL (123 mg kg^{-1}) (CCME, 2001).

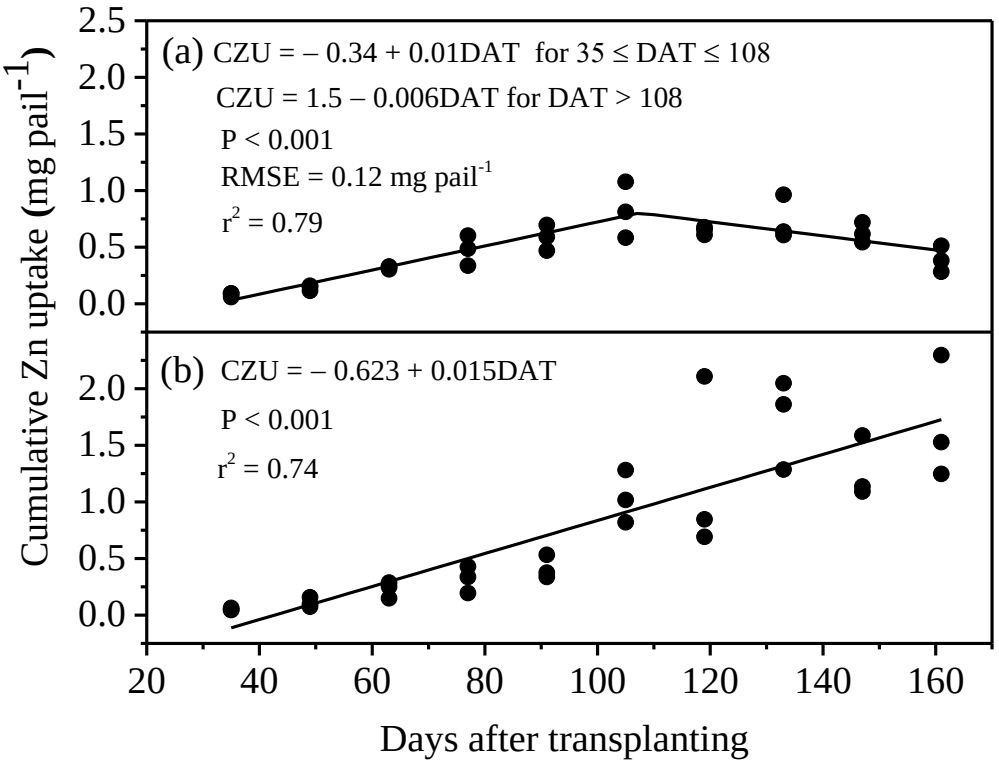

Figure 4.5 Cumulative zinc uptake as a function of time in (a) aboveground and (b) belowground switchgrass biomass.

4.4.7 Cadmium Uptake

Cadmium accumulation in the aboveground biomass was very low, peaking at just 0.0007 mg pail^{-1} (0.00002 g Cd m^{-2}), or 9% of total plant uptake, on Day 102 (Fig 4.6). The plateau in cumulative Cd uptake (CCdU) in aboveground biomass on and after Day 102 was likely due to the relatively constant aboveground biomass during that period (Fig. 4.1). This indicates that practical phytoextraction of the low amounts of Cd taken up by the plant largely depends on the high biomass production since switchgrass is not a Cd hyperaccumulator (Chen et al., 2012). Nearly all of the Cd taken up by the plant was in the belowground biomass. Although Cd is relatively mobile in plants, it is easily bound to exchange sites on cell walls, thus restricting its translocation (Reed, 1997).

When harvesting coincided with peak cumulative Cd uptake, 0.05% of the Cd initially present in the biosolids was removed in the harvested biomass. The concentration of Cd initially present in the biosolids (0.39 mg kg^{-1}) was way below the CCME TEL (37.3 mg kg^{-1}) for protection of aquatic life and therefore does not pose significant risk to aquatic life. Cadmium concentrations in biosolids from residential sources are usually less than 2 mg kg^{-1} while Cd concentration in biosolids from industrial sources can be up to 840 mg kg^{-1} (Reed, 1997).

Switchgrass has been reported to be tolerant of Cd (Reed et al., 2002), and cultivars such as Alamo are better suited to phytoextraction of Cd from contaminated soils due to their high biomass yields (Chen et al., 2012). The low accumulation in aboveground biomass observed in the present study may be due to the low initial Cd concentration in the biosolids, which resulted in low uptake by roots and limited translocation to aboveground biomass.

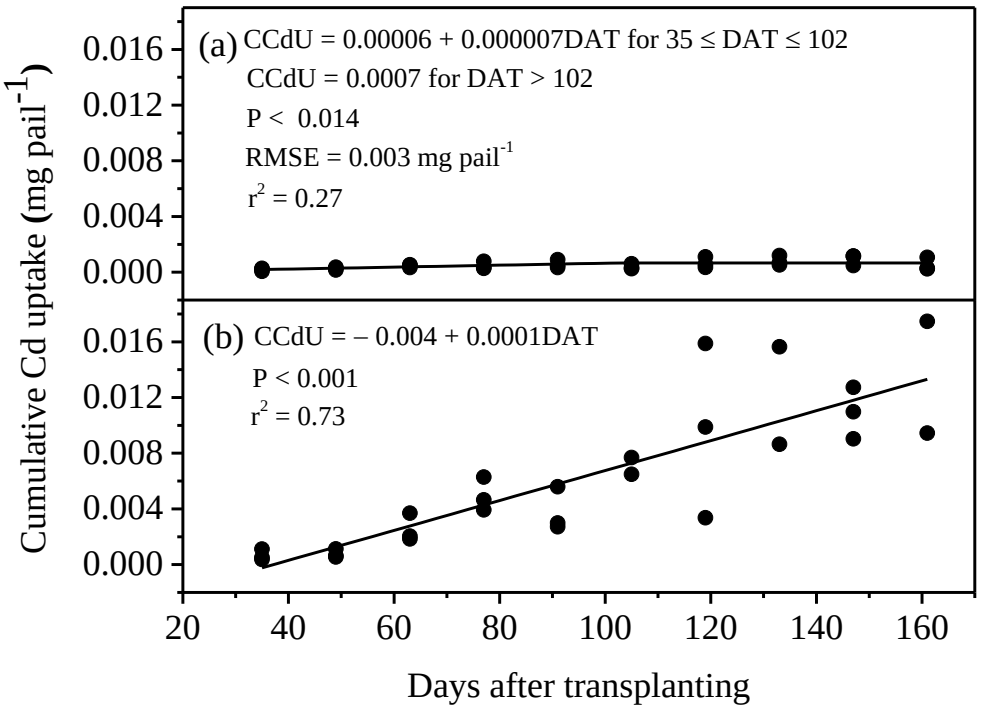

Figure 4.6 Cumulative cadmium uptake as a function of time in (a) aboveground and (b) belowground switchgrass biomass.

4.4.8 Chromium Uptake

Temporal changes in cumulative Cr accumulation in the aboveground biomass were best described by a linear–linear regression (Fig. 4.7). For 35 ≤ DAT ≤ 128, cumulative Cr uptake (CCrU) accumulation in the aboveground tissues increased as a linear function of time (CCrU = -0.082 + 0.002DAT), after which it decreased linearly with time (CCrU = 0.48 – 0.002DAT). Peak Cr accumulation on Day 128 was 0.22 mg pail^{-1} (0.005 g Cr m^{-2}), which represented 57% of total Cr accumulation. Harvesting at peak cumulative Cr uptake removed 0.11% of the Cr initially present in the biosolids, which translates to 226 harvest cycles required to meet the TEL (37.3 mg kg^{-1}) for protection of aquatic life

(CCME, 2001), assuming that phytoextraction rate remains constant from one growing season to the next.

Low Cr uptake in the present study can be attributed to the low initial Cr concentration (50 mg kg^{-1}) in the biosolids or low availability for plant uptake. Li et al. (2011) reported increased Cr accumulation in shoots and roots of switchgrass grown in soil with Cr concentrations ranging from 131 to 600 mg kg^{-1}. The highest accumulation of Cr (49 g m^{-2}) was observed in a heavily polluted soil (600 mg kg^{-1}). This was greater than peak Cr uptake in the present study, (0.22 mg $pail^{-1}$, or 0.004 g m^{-2}). Although Li et al. (2011) observed high Cr accumulation in the heavily polluted soil, biomass of the shoots and roots was significantly decreased while a moderately polluted soil (<350 mg kg^{-1}) did not inhibit biomass production.

More than 90% of the total Cr content of biosolids is typically in unavailable forms and a significant fraction is bound to organic matter (Haynes et al., 2009). Therefore, low Cr availability in the biosolids in the present study may explain the observed low Cr uptake by switchgrass.

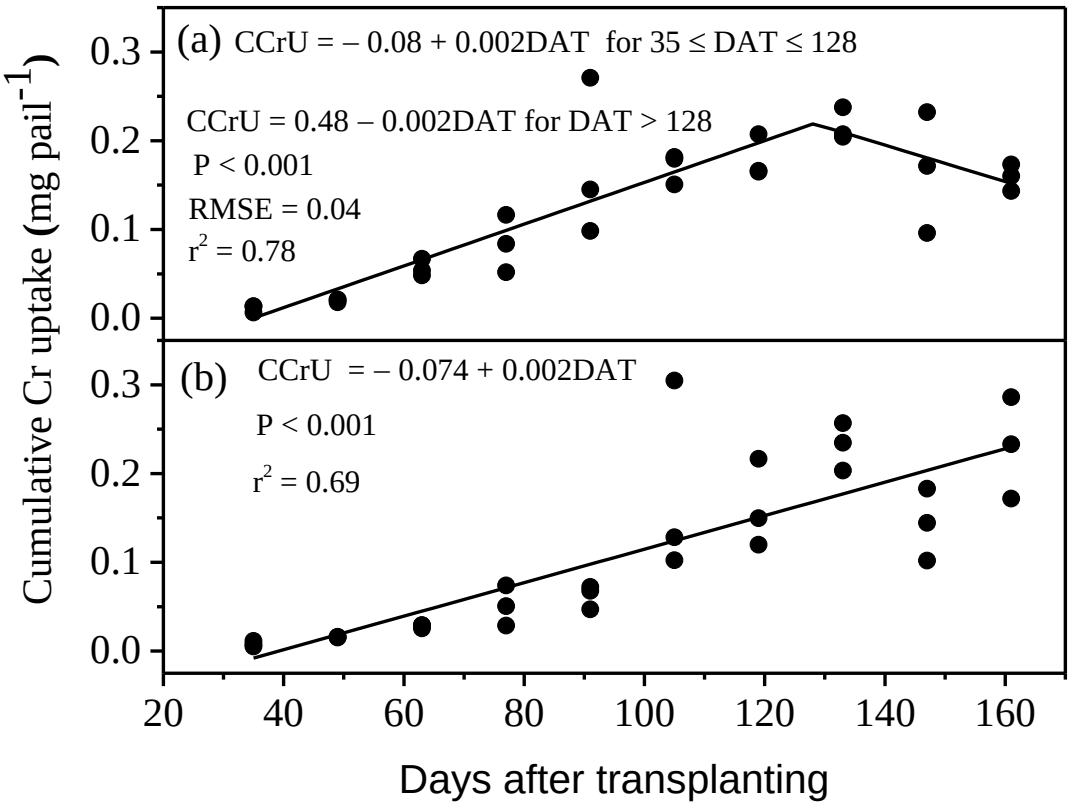

Figure 4.7 Cumulative chromium uptake as a function of time in (a) aboveground and (b) belowground switchgrass biomass.

4.5 Conclusion

Asymptotic biomass yields between shoots and roots were not significantly different. Peak N and P accumulation was greater in the shoots than in the root biomass, indicating that a large fraction of the accumulated N and P can be removed by harvesting aboveground biomass. Our results indicate that maximum nutrient and P removal from the biosolids can be attained if harvesting coincides with peak nutrient accumulation. Maximum N and P accumulation occurred on Days 86 and 102, respectively. We suggest harvesting around the time of peak P accumulation to maximize P removal which is of

more concern for eutrophication of aquatic systems. Our results suggest that phytoextraction is not an effective approach to remove trace elements as trace element accumulation in switchgrass was low and a large portion of the trace elements taken up by the plant were partitioned to the root biomass. Trace elements in this study were, however, below applicable Canadian environmental quality guidelines and posed no threats to humans and the environment. Findings from this experiment will inform policy makers and landowners on the timing of harvesting for maximum phytoextraction and removal of nutrients from biosolids using switchgrass. The microcosm experiment evaluated the phytoextraction potential of switchgrass with greater degree of experimental control and thererefore results from the experiment cannot be directly extrapolated to field conditions. Therefore, long-term field studies are required to evaluate the phytoextraction potential under field conditions.

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5. GENERAL SYNTHESIS

Spreading of biosolids on agricultural lands has been traditionally practiced as a beneficial biosolids management option (Ross et al., 2003). However, shortage of suitable land base within economic distances for transport of biosolids, stricter regulations, contamination of food sources, or issues of emerging contaminants may limit or restrict the use of biosolids on agricultural land. Any restriction on spreading biosolids on agricultural land will be felt more by small municipalities.

This study examined wetland and terrestrial phytoremediation approaches as alternatives to the traditional land application and landfilling of municipal biosolids from end-of-life lagoons. Effective phytoextraction of nutrients and contaminants is greatly affected by the establishment and health of the plant population, which are in turn influenced by the availability of nutrients. Chapter 2, therefore, examined N and P availability in primary and secondary biosolids and identified the plant growth stages at which biomass yield and nutrient accumulation are optimized. To effectively remove nutrients and trace elements in the harvested aboveground biomass, it is important to harvest biomass at peak nutrient uptake. Therefore, Chapter 3 and 4 examined trends in biomass, nutrient and trace element uptake in aboveground and belowground biomass to determine the harvest stage that maximizes contaminant uptake in cattail and switchgrass

grown in biosolids under wetland and terrestrial-based phytoremediation approaches, respectively.

Results from the incubation experiment indicated high N mineralization in primary biosolids incubated at moisture content near field capacity. In near-saturated biosolids, negative mineralization occurred while in the dry moisture content N mineralization did not significantly change with time. Therefore, plant-available N in near-saturated and dry conditions may fail to support a healthy plant population that gives high biomass yields, thus reducing the effectiveness of phytoextraction as a strategy for nutrient and trace element removal from biosolids. In biosolids from the secondary lagoon cell, N mineralization was low and did not significantly change with time regardless of moisture level, indicating that secondary biosolids were stable and therefore resistant to microbial degradation. Limited plant growth and biomass due to low N mineralization may limit phytoextraction potential of plants grown in SB.

Phosphorus availability, as measured by the Olsen method, was not affected by moisture level across the entire sampling period in both biosolids. Available P decreased with time in both biosolids, likely due to P adsorption to Fe or Ca in the biosolids. However, total Olsen P concentrations in both biosolids were still available the critical concentrations required for plant growth.

Peak biomass accumulations and growth rates were similar for aboveground and belowground plant partitions for both cattail and switchgrass. Nitrogen and P accumulations were greater in aboveground biomass than in belowground biomass regardless of plant species, indicating that a major portion of the nutrients taken up by the plants can be removed by harvesting aboveground biomass. Peak aboveground N uptake

occurred on Day 86 in both cattail and switchgrass while maximum P uptake occurred on Days 83 and 102 for cattail and switchgrass, respectively. Trace element uptake was low and trace elements taken up by cattail were restricted to belowground biomass. Phytoextraction was therefore not effective in removing trace elements in harvested aboveground biomass.

5.1 Practical Implications

High N mineralization potential in primary biosolids, as indicated at the moisture content near field capacity (60% WFPS), can support high biomass yields and plants can effectively function to remove contaminants. However, in dry conditions, e.g., during drought, irrigation may be needed to optimize N mineralization. Unlike in our incubation experiment (Chapter 2) that had no plants growing, in the presence of plants, N mineralization is enhanced in near-saturated conditions as a result of oxygen supplied by plant roots. Low mineralization in secondary biosolids at all moisture contents may not support high biomass growth and harvesting may be restricted to one cut per season. Given the lower N mineralization potential in secondary biosolids, it may be beneficial to amend the secondary biosolids with primary biosolids to improve N mineralization rates and optimize biomass yields. Alternatively, high biomass plants with high N requirements may be grown in primary biosolids to phytoextract high concentrations of mineralized N while phytoextraction in the secondary biosolids can be enhanced by use of C_4 plant species such as switchgrass that can produce high biomass yields in low N conditions (Brown et al., 2000; Dohleman et al., 2009).

Harvesting at peak P accumulation removed 2.9% of P from primary biosolids, which translates to 65 kg P ha^{-1} yr^{-1} under the conditions of the experiment. Harvesting at

peak N uptake removed 3.7% in this study, which translate to 191 kg N ha^{-1} yr^{-1}. Although the estimates from this study cannot be directly translated to field conditions, they nonetheless demonstrate how harvesting cattail in an in situ wetland-based phytoremediation system reduces the potential of nutrient loading to surface water bodies that would occur if biosolids were spread on agricultural land. Harvested biomass can be used as a feedstock for bioenergy production. Harvesting at peak uptake of trace elements removed less than 0.6% of trace elements initially present in the biosolids. Therefore, phytoextraction is not effective for trace element phytoextraction.

Harvesting switchgrass at peak P and N accumulation translated to removals of 17 kg P ha^{-1} yr^{-1} and 78 kg N ha^{-1} yr^{-1}. Trace element removal in switchgrass translated to less than 0.2% of trace elements initially present in the biosolids.

5.2 Research Limitations and Future Research

- In Chapter 2, N mineralization in primary biosolids decreased with time in the near-saturated moisture content and this was assumed to be due to denitrification losses. Further studies should determine nitrous oxide emissions from primary biosolids at different moisture continents.
- In this study, plants were established from seeds and dynamics of biomass, nutrients and trace element uptake were examined over a period equivalent to one growing season. Belowground biomass was still developing and expanding and therefore, different patterns of biomass and nutrient partitioning may be more noticeable from the second growing season and thereafter. Further studies on biomass and nutrient dynamics in the aboveground and belowground biomass should consider more than one harvest cycle.

- Plant growth in the small pots may have been limited by small space available for root and rhizome expansion, which limited access to available nutrients. Moreover, the rhizosphere effect on P uptake is greater in pots where roots are crowded compared with conditions in the field and, as a result, P uptake may be increased by changes in P availability induced by altered pH in the rhizosphere (Armstrong and Helyar, 1992; Bolan et al., 1997; Delorme et al., 2000).
- The controlling of environmental factors such as temperature, water level, light intensity and humidity eliminated the effects of these variables and the study provided insights in biomass and nutrient dynamics in cattail and switchgrass under constant environmental conditions. Therefore, this data cannot be directly translated to field conditions and field validation is required to determine biomass and nutrient dynamics under fluctuating seasonal conditions.

5.3 References

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