PHYTOREMEDIATION OF MUNICIPAL BIOSOLIDS: TERRESTRIAL AND WETLAND APPROACHES

by

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ABSTRACT

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Growth room experiments were conducted to examine terrestrial and wetland-based phytoremediation approaches as alternatives to biosolids management. Results from both experiments show that biosolids do not need to be amended with soil to encourage plant growth and optimize biomass yields. In the terrestrial phytoremediation approach, two harvests per growth cycle produced greater switchgrass biomass yield than a single harvest but had no significant effect on cattail biomass yield during the first cycle. Repeated harvesting also significantly increased mean nutrient uptake in Cycle 1, reflecting the greater biomass yield from two harvests compared with a single harvest. In the wetland experiment, nutrient phytoextraction under two harvests was 4.25% of initial N content and 2.28% of initial P content compared with 2.9% and 1.58%, respectively, under a single harvest. Terrestrial phytoremediation could be beneficial to small communities that cannot afford the costly excavation, trucking, and eventual spreading of biosolids on agricultural land.

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1. INTRODUCTION

1.1 Municipal Lagoons

Municipal lagoons are engineered structures that store and treat domestic, commercial and, in some cases, industrial waste (Cameron et al., 2003). These systems have a life span of about 20-30 years (Ross, et al., 2003). During their active lives, losses by seepage and other discharges are regulated, but no such controls are in place when the lagoon operation ceases (Miner, et al., 2000). After their life span, municipal lagoons are decommissioned based on certain guidelines and procedures (EPB, 2008).

Municipal lagoons contain organic and inorganic contaminants due to the nature of the waste discharged into them. Although most of these wastes are from biological sources, they also contain trace element (metal, metalloid, and non-metal) contaminants, pathogens, and organic compounds of environmental concern, including pharmaceuticals and personal care products (Díaz-Cruz et al., 2009). The contaminants are typically characterized in terms of biological oxygen demand (BOD), suspended solids content, microbial pathogens, and nutrient (N and P) concentrations (Kadlec and Knight, 1996).

1.2 Challenges of Municipal Lagoons

One of the challenges associated with the decommissioning of municipal lagoons is that of the remediation and restoration of the sites. Spreading of biosolids on agricultural land has been an acceptable method of biosolids disposal in many jurisdictions for many years. In fact, this approach has been used by the City of Winnipeg, Manitoba, Canada since 1937 as it was found to be an efficient way of managing biosolids (Ross, et al., 2003).

Although agricultural land-spreading has been traditionally used to dispose of biosolids because of its benefits as a soil amendment and a source of nutrients, however, this method has a number of limitations. Firstly, field application may result in the introduction of pathogens in the environment (McCoy et al., 2001). Secondly, land spreading is expensive because of the costs associated with excavation and transportation. Thirdly, in some areas, there may not be enough suitable agricultural land to accommodate the biosolids. Lastly, upon removal of biosolids from the lagoon, oxygen diffuses into the underlying soil, allowing conversion of NH_4-N to highly mobile NO_3-N, which can result in ground water contamination (Douglas-Mankin et al., 2010). Therefore, to combat these limitations and still ensure effective remediation, there is a need to employ appropriate, less expensive, in situ remediation approaches (Cameron et al., 2003).

1.4 Terrestrial Phytoremediation

This approach is cost-effective and environmentally-friendly. Phytoremediation utilizes the ability of certain tree, shrub, and grass species to degrade, immobilize, or remove harmful chemicals from contaminated soils, sludges, sediments, and ground water (Zavoda et al., 2001). Many studies have demonstrated that plants can be used successfully to remediate (phytoremediate) soils in livestock manure lagoons after removal of manure at decommissioning (Douglas-Mankin et al., 2010; Kirkham and Madrid, 2002; Liphadzi et al., 2002; Zhu and Kirkham, 2003). An ideal plant for phytoremediation must be able to produce a large biomass yield and accumulate large amounts of contaminants in the aboveground biomass (Alkorta et al., 2004).

The use of hyperaccummulating plants is one of the strategies employed in phytoremediation. Hyperaccumulators are plants that accumulate high amounts of trace elements without showing signs of toxicity (Li et al., 2008). Hyperaccumulators, such as *Thlaspi caerulescens,* which can accumulate as much as 10,000 mg kg^{-1} Zn and 100 mg kg^{-1} Cd (Escarré et al., 2000), and *Vetveria zizanioide* and *Sedium alfredii*, which can hyperaccumulate Cd, Pb and Zn (Zhuang et al., 2007), have attracted a great deal of interest. However, many of the hyperaccumulators cannot be used for large scale remediation because of their low biomass yields. Additionally, they pose harvesting challenges because of their small size (Li et al., 2008).

Another strategy is the use of plants with high biomass, even if they contain relatively low contaminant concentrations. The low concentrations are compensated for by the high plant biomass, leading to reasonable phytoremediation effectiveness (Keller et al., 2003). Non-hyperaccumulator, high biomass plants that can be easily cultivated have been demonstrated to have the potential for phytoremediation. Some common agricultural crops such as corn (*Zea mays*) (Li et al., 2008), sunflower (*Helianthus annuus*) (Meers et al., 2005), and sorghum (*Sorghum bicolor* (L.) Moench) (Zhuang et al., 2009) have been demonstrated to be effective in cleaning up contaminated sites, depending on the types and concentrations of the contaminants. Presently, due to the economic importance of crops and the tendency for bioaccumulation in humans and grazing animals, increasing attention is being shifted to high biomass, non-edible plants and grasses, such as switchgrass (*Panicum vigratum* L.) (Chen et al., 2011), willows (*Salix reichardtii.)* (Laidlaw et al., 2012), and miscanthus (*Miscanthus sinensis* L.) (Arduini et al., 2006).

1.4.1 Switchgrass

Switchgrass is a perennial C_4 plant commonly grown in North America and has been recommended in the last two decades as a potential biomass energy crop (Parrish and Fike, 2005). It also has the ability to thrive on various soil types and in extreme weather conditions (Chen et al., 2011). In eastern Canada and the United States, switchgrass has been widely used as a feedstock in the production of bioenergy (Casler and Boe, 2003). Agronomic properties of switchgrass and its management for production of biomass and biofuel have been extensively studied (Muir et al., 2001; Parrish and Fike, 2005; Cassida et al., 2005; Adler et al., 2006). The established agronomic characteristics (e.g., harvest frequency, plant density) of switchgrass make it a promising candidate for phytoremediation (Chen et al., 2011). Studies have shown that switchgrass has the ability to immobilize trace elements such as Cd (Reed et al., 2002; Chen et al., 2011), Cu (Juang et al., 2011), Cr (Shahandeh and Hossner, 2000), and radionuclides such as ^{90}Sr and ^{137}Cs (Entry and Watrud, 1998). Switchgrass has also been shown to degrade atrazine (Murphy and Coats, 2011) and polychlorinated biphenyls (PCB) (Chekol et al., 2004) in contaminated soils.

Switchgrass produces a large amount of biomass (average yield of 9.1 – 13.5 Mg ha^{-1} in three years) (Grosshans et al., 2011) and does not require replanting. Thus, it requires relatively low energy and economic inputs, which make it a good candidate for phytoremediation (Chen et al., 2011). High biomass yielding plants are relatively easy to harvest and can accumulate high amounts of contaminants in their aboveground biomass (Alkorta et al., 2004). Switchgrass is not considered a food crop, hence using switchgrass for biofuel does not pose any threat to food security (Liu, 2012).

1.5 Wetland-based Phytoremediation

Wetlands are regarded as the most common and productive ecosystems on earth (Reddy and DeLaune, 2008). In comparison with other ecosystems, wetlands have the tendency to accumulate organic matter more rapidly (Mitsch and Gosselink, 2007). Nutrient biogeochemical processes occurring in wetland soils often lead to improvement in environmental quality. Since the 1990s, the use of constructed wetlands for the improvement of water quality has undergone rapid development (Saeed and Sun, 2011; Gu et al., 2006). Several studies have been conducted on the utilization of constructed wetlands for wastewater and stormwater treatment (Birch et al., 2004; Guardo et al., 1995). One ecological benefits of constructed wetlands is that they improve water quality via plant uptake of contaminants (Schulz and Peall, 2001; Wood and Shelley, 1999). They also improve the physical and chemical characteristics of the ecosystem.

The wetland system creates anaerobic conditions in the soil/biosolids which affect the pH and redox potential of the system. This in the long run influences the solubility and phytoavailability of nutrients and other elements (Racz, 2006). Wetland plants play a major role in nutrient cycling and they also help to oxygenate the aerobic-anaerobic interface through their root system (Reddy and DeLaune, 2008).

1.5.1 Cattail

Cattail (*Typha latifolia*) has attributes that make it suitable for use in wetland-based phytoremediation. Cattail is commonly found in a range of soil moisture conditions but mainly in wetlands across North America (Li et al., 2004). It has the ability to produce a large amount of biomass in a single growing season (Grosshans et al., 2014). Cattail also has the ability to absorb nutrients in sediments, does not require replanting, and has low

economic inputs. Maddison et al. (2009) reported the removal of trace elements such as Cd, Zn, Cu, and Pb by cattail in a wastewater treatment wetland. A number of studies have reported the effective uptake of N and P from sediments (Grosshans et al., 2011; Miao, 2004; Martin et al., 2003; Nichols, 1983).

Despite their demonstrated effectiveness at removing contaminants from wastewater and stormwater, there are currently no published studies that have examined the use of constructed wetlands to clean up end-of-life municipal lagoons in situ.

The overall objective of this research was to examine the effectiveness of constructed wetland-based and terrestrial phytoremediation approaches as alternatives to cropland application of biosolids during the decommissioning of municipal lagoons. The second chapter of this thesis covers research on the use of cattail and switchgrass in the terrestrial phytoremediation of an end-of-life municipal lagoon while Chapter 3 examines the use of a constructed wetland system in the phytoremediation of the lagoon using cattail as the wetland plant. Chapter 4 gives the overall synthesis of the thesis.

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2. TERRESTRIAL PHYTOREMEDIATION OF BIOSOLIDS FROM AN END-OF-LIFE MUNICIPAL LAGOON USING CATTAIL AND SWITCHGRASS

2.1 Abstract

Land spreading is a common method of disposal of biosolids in many jurisdictions, including Manitoba, Canada. Biosolids are applied on cropland to improve soil productivity, stimulate plant growth, and establish sustainable vegetation. However, land spreading is expensive and presents a risk of pathogen and contaminant transfer to the environment during transportation and spreading. This growth room study examined the effectiveness of terrestrial phytoremediation using switchgrass (*Panicum vigratum*) and cattail (*Typha latifolia*) as an alternative to land spreading during the decommissioning of municipal lagoons. Switchgrass and cattail seedlings were transplanted into pots containing 3.9 kg of biosolids (dry wt.) from a primary (PB) and a secondary (SB) municipal lagoon cell, and a 1:1 blend (wt./wt.) of PB and soil (PBS). Aboveground biomass was harvested either once at the end of each 90-d growth period or twice during this period. Results from this experiment indicate that biosolids can support a healthy plant population and produce high biomass yields without amending with soil. Trace element accumulation in the aboveground biomass of both plants was < 0.8 mg pot^{-1}, with the exception of Zn, which averaged 1.40 mg pot^{-1}. Switchgrass aboveground biomass yield was greater with two harvests than with one harvest during the first 90-d growth period, whereas cattail yield was not significantly affected by harvest frequency. In the second growth cycle, harvesting frequency had no significant effect on the yield of either plant species. However, repeated harvesting significantly improved nitrogen (N)

and phosphorus (P) uptake in Cycle 1, averaged across the two plants, reflecting the greater biomass yield from two harvests compared with a single harvest. Phytoextraction of P was greater for switchgrass (4.5%) than for cattail (3.0%). Nitrogen and P removal from biosolids decreased in the order PB > PBS > SB, reflecting initial concentrations in these biosolids. Phytoextraction rates attained in this study, particularly for P, suggest that phytoremediation can be an effective approach for removing this environmentally important nutrient from biosolids and offers a potentially viable alternative to the costly disposal of biosolids on agricultural land.

2.2 Introduction

Municipal lagoons, also known as stabilization ponds, are widely used by small municipalities and rural communities across the globe as an affordable, relatively simple technology for treating municipal waste before discharge into the environment. Biosolids are a semi-solid or solid material generated from municipal wastewater facilities upon removal of liquid effluent (CCME, 2010). Biosolids contain organic and inorganic contaminants due to the nature of the waste going into municipal lagoons. At the end of their design life expectancy of 20-30 years, municipal lagoons are decommissioned (that is, closed and remediated) after removal and disposal of biosolids (Ross et al., 2003).

Ex-situ approaches, which involve excavation and transportation of biosolids for off-site disposal, have commonly been used in the decommissioning of municipal lagoons. In Manitoba, Canada, land spreading of biosolids has been the most commonly used disposal method since 1937 (Ross, et al., 2003). In this approach, biosolids serve as soil amendments, which help improve soil physical properties and provide nutrients for

plants. However, land spreading is an expensive and unsustainable approach. It can result in the transfer of pathogens and contaminants to the environment during transportation (McCoy et al., 2001). Canada's more than 3,500 wastewater facilities generate more than 660,000 dry tonnes (2.5 million wet tonnes) of biosolids and sludge per year (CCME, 2012). In 2012, the City of Winnipeg alone produced 13,500 dry tonnes of biosolids, with the annual output expected to increase by 50% in 2037 (City of Winnipeg, 2014). Based on a typical allowable application rate of 15 dry tonnes ha^{-1}, spreading this volume of biosolids on agricultural land would require 900 ha of land annually, with this increasing to 1,273 ha by 2037 (Keam and Whetter, 2008).

Manitoba's Environment Act requires issuance of a license (usually for one cropping season) before land application of biosolids. Additionally, a Nutrient Management Plan must be prepared and submitted in accordance with the Nutrient Management Regulation. Manitoba regulations generally require that cereals, forages, oil seeds, field peas or lentils are grown no less than three years following land application of biosolids (CCME, 2010). Cattle pasturing is also not permitted within three years of biosolids application.

Various studies have demonstrated that plants are effective in cleaning up contaminated soils (e.g., Wenzel et al., 1999), a process known as phytoremediation. Phytoremediation has been widely used in recent years as an in situ, cost-effective strategy for the clean-up of contaminated sites impacted by inorganic and organic contaminants (Salt et al., 1995; Zhuang et al., 2009; Zavoda et al., 2001). Phytoremediation presents great potential for the in situ remediation of municipal lagoons where biosolids are rich in plant nutrients

and contain trace elements in concentrations that are not restrictive to plant growth. There is growing interest in the enhancement and promotion of phytoremediation because of its relatively low cost and minimal environmental side effects (Cui et al., 2004).

Plant species are considered suitable for phytoremediation based on several criteria, including wide distribution, high above-ground biomass yield, high bioaccumulation factors (hyper accumulators), short life cycles, and high propagating rates (Zhuang et al., 2007). One example is switchgrass, a high-biomass perennial grass widely used as a bioenergy crop. Several studies have demonstrated that switchgrass is capable of the phytoextraction of chromium (Cr) (e.g., Shahandeh and Hossner, 2000), cadmium (Cd) (Reed et al., 2002), and the radioisotopes ^{137}Cs and ^{90}Sr (Entry and Watrud, 1998) from contaminated soils. The effective remediative ability of switchgrass has been attributed to its extensive root system (Entry and Watrud, 1998), high biomass yield, rapid growth rate, and ability to tolerate and accumulate high concentrations of trace elements (Murphy and Coats, 2011). Switchgrass can also successfully thrive in different soil types and conditions, even with minimal management (Sladden et al., 1991). It is noteworthy, however, that switchgrass tends to accumulate greater amounts of some trace elements, such as Cd, Cr, Cu, and Zn in its roots rather than in the aboveground biomass (Reed et al., 2002; Jeke et al.,unpublished data, 2014). Higher biomass yields have been reported when switchgrass was harvested twice per season compared with a single harvest (Reynolds et al., 2000).

Cattail (*Typha* spp.), a perennial aquatic plant with a fibrous root system and high biomass yields, has also been demonstrated to be effective at phytoextracting contaminants. Although it can be found in a wide range of moisture conditions, cattail is

usually found in wetlands and ditches across North America. Cattail possesses a number of characteristics that make it suitable for phytoremediation. For example, it can colonise an area within a short period, produces high above-ground biomass yields, and regenerates after each season or harvest. It has also been shown to effectively absorb nutrients from sediments and wastewater (Maddison et al., 2009). Repeated harvesting of cattail in a growing season has been observed to increase its biomass yield compared to one harvest per season (Martin et al., 2003). Harvested cattail biomass can be used as a bioenergy feedstock (Grosshans et al., 2011).

The objectives of this experiment were to (i) compare the effectiveness of cattail and switchgrass in the phytoextraction of contaminants from end-of-life municipal biosolids; (ii) compare phytoextraction under a single harvest vs. multiple annual harvests of the plants; (iii) compare the effectiveness of phytoremediation in the clean-up of biosolids from a primary vs. a secondary lagoon; and (iv) determine if mixing of biosolids with soil will enhance plant growth and phytoremediation.

2.2.1 Hypotheses

The following hypotheses were tested:

i. Terrestrial phytoremediation will effectively remediate a municipal lagoon;
ii. Repeated harvesting will enhance phytoremediation relative to a single annual harvest;
iii. The rate of biosolids clean-up using phytoremediation will be the same for the secondary and the primary cells; and
iv. Amending biosolids with soil will enhance the effectiveness of phytoremediation.

2.3 Materials and Methods

2.3.1 Treatments

2.3.1.1 Biosolids

Three biosolids treatments were tested in this experiment: (i) biosolids from a primary lagoon cell (PB); (ii) biosolids from a secondary lagoon cell (SB); and (iii) a 1:1 mixture (dry wt. basis) of PB and soil (PBS). The biosolids were collected from an end-of-life municipal lagoon in Niverville, Manitoba, Canada, while the soil (0- to 15-cm layer) used for the PBS treatment was a clayey, Black Chernozem (Udic boroll) sampled ~500 m from the municipal lagoon. The PBS treatment was included because, at the start of the experiment, there was concern that plants may not be able to establish because of the high contaminant concentrations in the primary cell. If this turned out to be the case, diluting with soil would reduce the potential for harm to plants and therefore, enhance phytoremediation. The biosolids and soil were analyzed for chemical properties, as described below, before the start of the experiment.

2.3.1.2 Plant Species

The two plant species tested were switchgrass (var. Alamo) and common cattail (*T. latifolia*). Both species produce high biomass yields and are known to be tolerant to relatively high concentrations of trace elements . Cattail seeds were extracted from cattail heads collected from ditches near Winnipeg. The cattail heads were blended for 30 seconds in a Contrad detergent solution using a household blender (Model 54227C, Hamilton Beach, CA, USA), according to the method described by McNaughton (1968).

This was followed by repeated washing of the seeds settled at the bottom of the blender with tap water followed by reverse osmosis (RO) water. Switchgrass seeds were obtained from Native Plant Solutions, a branch of Ducks Unlimited Canada (Winnipeg, Manitoba).

2.3.2 Experimental Design and Setup

The experiment was laid out as a completely randomized design with a 3 × 2 × 2 factorial treatment layout consisting of three biosolids types (PB, SB, and PBS), two plant species (cattail and switchgrass), and two harvest frequencies [one harvest (at the end of the 90-day growth cycle) vs. two harvests (on Day 60 and Day 90) per growth cycle]. Three controls (biosolids with no plants) were included for comparison. All treatments were replicated three times and tested over two plant growth cycles (equivalent to two growing seasons).

The three biosolids were weighed (3.93 kg dry wt.) into each of 12 plastic pots (24 cm diameter × 26 cm height) to give a bulk density of approximately 0.58 g cm^{-3}. Four weeks after germination, seedlings produced as described above were transplanted into each pot. Based on seeding rates, five switchgrass seedlings were transplanted into each pot in a circular pattern, maintaining an equal distance between seedlings and the walls of the pot. For the cattail treatment, three seedlings were transplanted in a triangular pattern, with equal distance from the walls of the container to each transplant.

The pots were placed in a growth room maintained at day/night temperatures of 22/15ºC, a relative humidity of 65%, and a light intensity of 270 µmole m^{-2} s^{-1} during the 12-hr photoperiod. Each pot was weighed once every 2 days and moisture replenished as

needed. After 47 days, moisture targets were increased by 10% to account for increased evapotranspiration.

2.3.3. Plant and Biosolids Sampling

At the end of each cycle, plants in each pot were harvested by cutting the stems using a knife at a height of 5 cm to allow for regrowth. The harvested biomass was dried for 72 h at 65°C in an oven. The samples were then weighed to determine dry matter yield and ground (< 0.2 mm) using a SPEX 8000D ball mill (Metuchen, NJ, USA).

Immediately after each harvest, biosolids samples were taken from each pot using a 2-cm diameter auger to avoid damaging the roots. Samples were immediately stored in a refrigerator at 4°C.

2.3.4. Laboratory Analysis

2.3.4.1. Biosolids and Soil

Total P concentration in biosolids and soil samples was determined colorimetrically at a wavelength of 880 nm (ascorbic acid method, Murphy and Riley, 1962) with an Ultrospec 2100 Pro spectrophotometer (Biochrom Ltd, Cambridge, UK) following wet oxidation (Akinremi et al., 2003). Olsen P concentration was similarly measured following extraction of 1.0 g soil (dry wt.) with 20 mL of 0.5 M $NaHCO_3$ (Olsen et al., 1954). For TKN determination, samples were digested with 30% H_2O_2 in a block digester, followed by TKN measurement in digests with an autoanalyzer (Technicon AA II, Technicon Instrument Corp., Tarrytown, New York, USA) using the colorimetric automated phenate method (APHA et al., 2005). Inorganic (NO_3^-, NO_2^- and NH_4^+) N concentrations were determined using the autoanalyzer described above following

extraction of 5 g biosolids or soil samples with 25 mL of 2 M KCl (Keeney and Nelson, 1982). Biosolids and soil pH and electrical conductivity (EC) were measured in a 1:2 (wt./vol.) sludge or soil/water suspension using a pH/conductivity meter (Accumet AP85, Fisher Scientific, Singapore).

2.3.4.2. Plant Tissue

Ground plant tissue samples were digested with aqua regia (concentrated HNO_3/HCl) in a microwave oven (MARS 5, CEM Corp., Matthews, NC, USA) and analyzed for trace element concentrations using an Elan 6000 inductively coupled plasma (ICP) mass spectrometer (Perkin Elmer Sciex Instruments, Concord, ON, Canada). Total P was determined by ICP optical emission spectroscopy (Vista-MPX, Variance Analytical Instruments, Mulgrave, Victoria, Australia) following Kjeldahl digestion. Nitrogen was analysed using the Kjeldahl method with samples digested in a block digester (APHA et al., 2005). Total N was measured spectrophotometrically using a FIAlab 2500 flow injection analyser (FIAlab Instruments, Bellevue, WA, USA).

2.3.5 Calculations

Total uptake of each nutrient or trace element was calculated by multiplying dry matter yield by the concentration of the analyte prior to analysis of variance. Phytoextraction was calculated by dividing the amount removed by the initial contaminant amount in the biosolids.

2.3.5 Statistical Analysis

Data were analysed using PROC MIXED in SAS 9.3 (SAS Institute, 2014). Data for N, Cd, Zn and Cr uptake, Cd phytoextraction (%), and available N and TKN concentrations

in the biosolids were not normally distributed based on the Shapiro-Wilk test from PROC UNIVARIATE and therefore required natural log-transformation. Treatment means were compared using the Tukey multiple comparison procedure. Treatment effects were considered significant if $P < 0.05$.

2.4 Results

2.4.1. Biosolids Characterization

Concentrations of the three most abundant trace elements in the biosolids decreased in the order Zn > Cu > Cr (Table 2.1). Trace element, N, and P concentrations were higher in the PB than in the SB. Approximately 98% of available N in the biosolids was in the NO_3 form. Interestingly, trace element concentrations were higher in the soil than in the SB (Table 2.1).

Biosolids pH was higher for SB than for PB (Table 2.1). The soil used in the PBS blend had a pH of 8.2, while PBS had a pH higher than that of PB. Electrical conductivity ranged from 2.76 for SB to 4.84 for PB.

Table 2.1 Initial nutrient and trace element concentrations (mg kg^{-1}) in the biosolids and soil.

Medium†	TKN	NH_4-N	NO_3-N	TP	OP‡	Cd	Cu	Zn	Cr	Pb	pH	EC
					mg kg^{1}							dS m^{-1}
Soil	2565	0.9	32.9	605	10.7	0.43	37.1	115	52.9	15.2	8.2	4.75
PB	6000	29.4	551	2730	139	1.16	119	356	44.7	25.5	7.36	4.84
PBS	3700	26.4	329	1590	78	0.75	70.1	212	45.3	20.3	7.57	3.29
SB	1706	28.4	264	1530	86	0.36	32.5	95.1	43.2	13.3	7.81	2.76
SQG¶	550	-	-	600	-	0.6	35.7	123	37.3	35	-	-

† PB, biosolids from the primary cell; PBS, 1:1 mixture of PB and soil; SB, biosolids from the secondary cell.

‡ OP, Olsen P.

¶ SQG, Sediment Quality Guideline (CCME 1998)

Table 2.2 Aboveground biomass yield and nutrient and trace element phytoextraction as affected by harvest frequency, plant species, biosolids treatment and growth cycle.

Effect	Biomass	N	P	Ca	Mg	Cd	Cu	Cr	Zn	Pb
	g DM pot^{-1}				mg pot^{-1}					
Cycle										
1	23.3	387	61.5	152	88.9	0.003b†	0.15	0.06	1.85	0.01
2	21.0	284	45.5	66.3	72.7	0.02a	0.09	0.06	0.96	0.04
‡Biosolids treatment										
PB	34.3	584	78.4	149	142	0.03	0.19	0.11	2.88a	0.05
SB	7.55	84.7	29.9	40.9	20.7	0.004	0.04	0.22	0.26c	0.002
PBS	24.6	309	51.9	136	79.3	0.005	0.14	0.05b	1.07b	0.02
Harvests										
Single	17.6b	227	33.9	90.8b	58.1	0.0002	0.10	0.03b	0.92b	0.004
Two	26.7a	425	73.1	127a	103	0.02	0.14	0.09	1.89a	0.04
Plant species										
Cattail	19.5	312	45.0	139	77.7	0.01	0.10	0.05	0.89	0.03
Switchgrass	24.8	340	61.8	78.9	83.9	0.02	0.14	0.07	1.91	0.02
					P value					
Cycle (C)	0.20	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	0.001
Biosolids (B)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Harvest (H)	<0.001	0.003	<0.001	0.01	0.001	<0.001	0.007	<0.001	0.04	<0.001
Plant species (P)	0.004	0.67	0.001	0.01	0.98	0.35	0.001	0.22	0.04	0.06
C × B	<0.001	0.001	<0.001	0.02	<0.001	0.11	0.001	<0.001	0.11	0.07
C × P	<0.001	0.001	0.001	0.01	<0.001	0.04	<0.001	0.001	<0.001	0.71
C ×H	0.88	0.01	<.001	0.30	0.15	<0.001	<0.001	<0.001	0.21	<0.001
B × P	<0.001	0.66	0.01	0.05	0.25	0.06	0.002	0.002	0.09	0.07
B × H	<0.001	0.004	0.02	0.20	0.04	0.006	0.05	0.05	0.30	0.003

P × H	0.004	0.26	0.02	0.16	0.50	0.10	0.33	0.01	0.07	0.97
C × B × P	0.004	0.49	0.03	0.99	0.03	0.30	0.002	0.26	0.74	0.02
C × B × H	0.07	0.37	0.02	0.27	0.001	<0.001	0.32	0.003	0.05	0.001
C × P × H	0.06	0.87	0.41	0.99	<0.001	0.02	0.97	0.003	0.04	0.01
B × P × H	0.08	0.42	0.25	0.18	0.95	0.02	0.11	0.81	0.57	0.62
C × B × P × H	0.15	0.38	0.84	0.56	0.13	0.19	0.06	0.94	0.99	0.48

†Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

‡PB = biosolids from the primary cell; SB = biosolids from the secondary cell; PBS = 1:1 mixture of PB and soil.

2.4.2 Biomass Yield

The effect of growth cycle on biomass yield varied with plant species and biosolids treatment, as indicated by the significant ($P < 0.001$) cycle × biosolids × plant species interaction (Table 2.2). The biomass yield of cattail in PBS was 171% greater at the end of Cycle 1 than at the end of Cycle 2. Cattail biomass yields in PB and SB did not differ significantly between Cycle 1 and Cycle 2. Switchgrass biomass yield in PB was significantly greater at the end of Cycle 2 (57 g DM pot^{-1}) than at the end of Cycle 1 (28 g DM pot^{-1}). For switchgrass grown in PB and SB, there was no significant difference in biomass yield between growth cycles.

2.4.3 Nitrogen Uptake

There was a significant ($P < 0.001$) cycle × biosolids interaction for N uptake averaged over plant species and harvest frequencies (Table 2.2). Nitrogen uptake (mean of the two plant species) by plants grown in PBS and SB was greater in Cycle 1 than in Cycle 2 but did not differ significantly between cycles in PB (Fig. 2.2). Nitrogen uptake decreased in the order PB ≈ PBS > SB in Cycle 1 and PB > PBS > SB in Cycle 2.

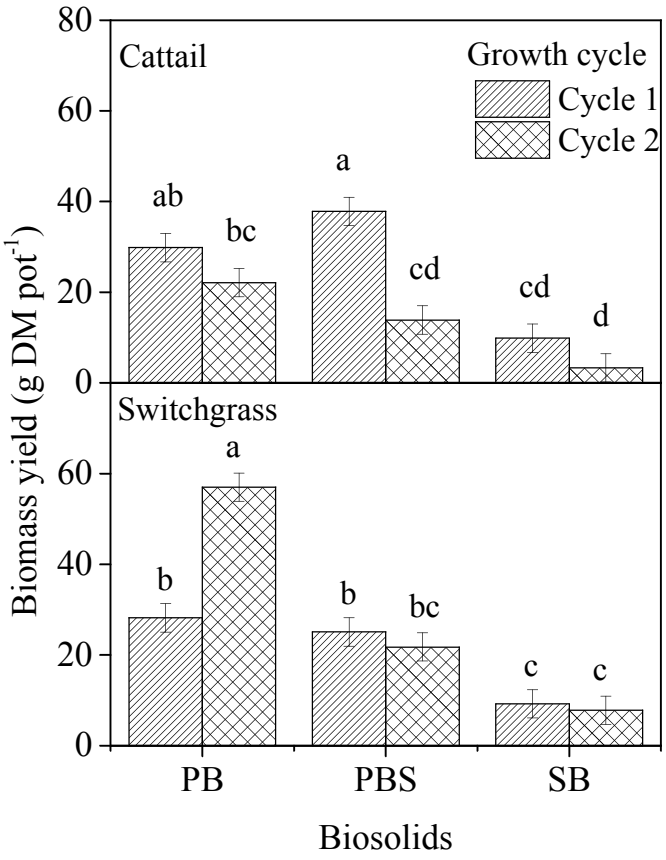


Figure 2.1 Aboveground biomass yield of cattail and switchgrass, averaged across harvest frequencies, as affected by biosolids type and growth cycle. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

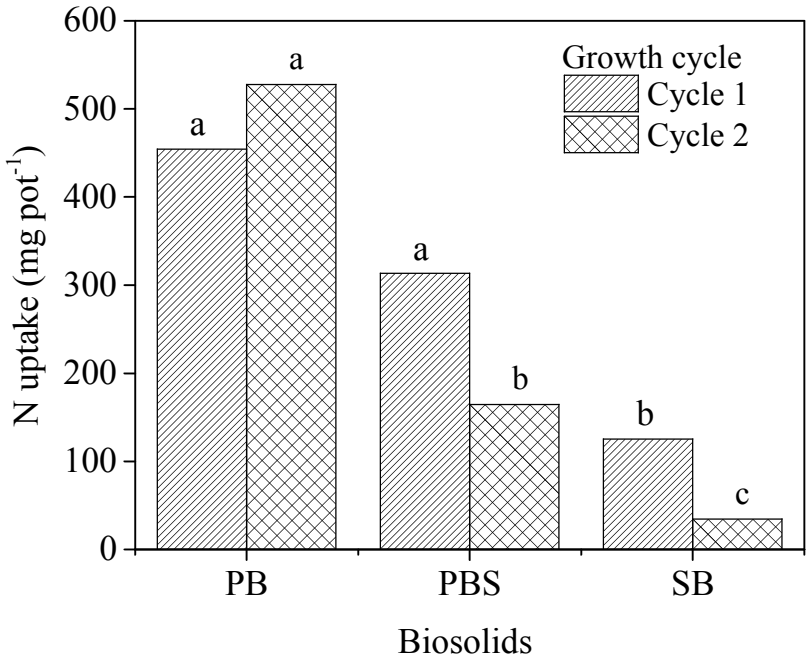


Figure 2.2 Geometric mean N uptake, averaged across plant species and harvest frequencies, as affected by biosolids and growth cycle. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

The plant species × growth cycle interaction was significant for N uptake (Table 2.2). Nitrogen uptake by cattail, averaged over biosolids treatments and harvest frequencies, was significantly greater in Cycle 1 (334 mg pot^{-1}) than in Cycle 2 (107 mg pot^{-1}) (Fig. 2.3). By comparison, N uptake by switchgrass did not differ significantly between growth cycles. Nitrogen uptake was significantly greater for cattail than for switchgrass in Cycle 1 but significantly greater for switchgrass than for cattail in Cycle 2 (Fig. 2.3).

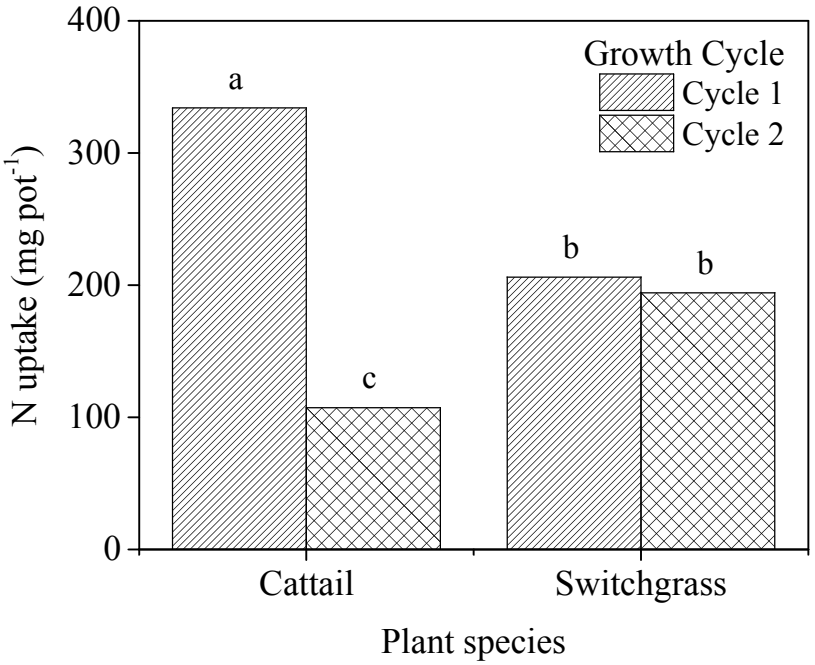


Figure 2.3 Geometric mean N uptake, averaged over different biosolids and harvest frequencies, as affected by plant species and growth cycle. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

The cycle × harvest frequency interaction was significant for N uptake (Table 2.2). Nitrogen uptake, averaged across plant species, was significantly greater for two harvests than for a single harvest in Cycle 1, whereas harvest frequency had no significant effect on N uptake in Cycle 2 (Fig. 2.4). While N uptake did not differ significantly between cycles under a single harvest, it decreased significantly in Cycle 2 compared with Cycle 1 for the two-harvest treatment.

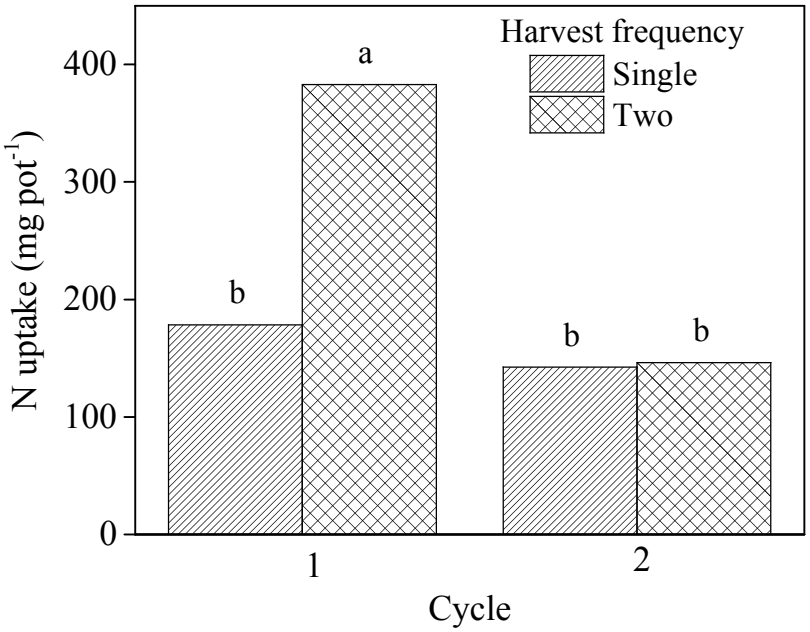


Figure 2.4 Geometric mean N uptake, averaged across biosolids and plant species, as affected by harvest frequency and growth cycle. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

There was a significant ($P < 0.001$) biosolids × harvest frequency interaction for N uptake averaged across plant species (Table 2.2). Harvesting plants twice in PB and PBS resulted in a significantly greater N uptake compared with a single harvest. However, for plants grown in SB, there was no significant difference in N uptake between harvest frequencies (Fig 2.5). Across plant species, N uptake decreased in the order PB > SB ≈ PBS under a single harvest and PB > PBS > SB when plants were harvested twice per growth cycle.

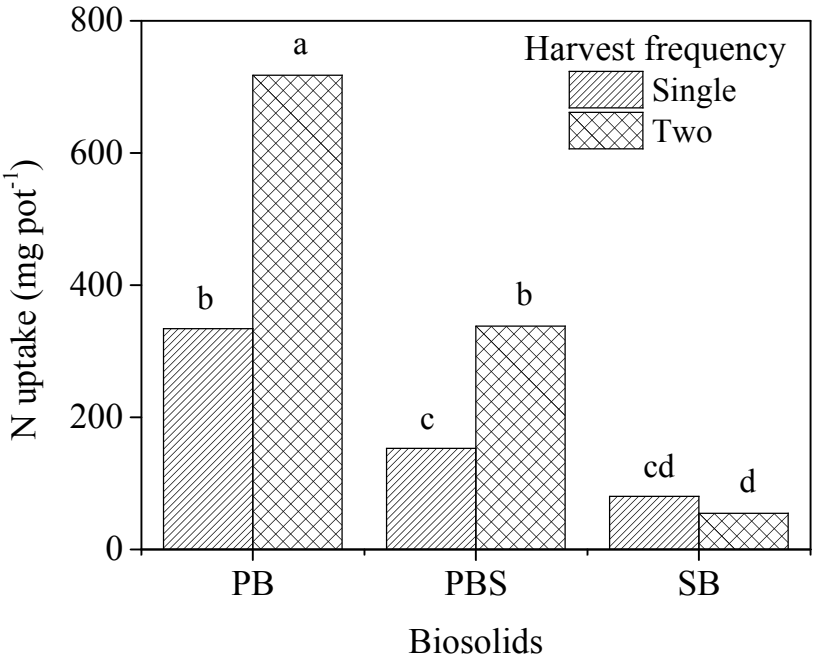


Figure 2.5 Geometric mean N uptake, averaged over two plant species, as affected by biosolids treatment and harvest frequency. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure

2.4.4 Phosphorus Uptake

There was a significant ($P = 0.03$) biosolids × plant species × growth cycle interaction for plant P uptake averaged across harvest frequencies. In PB, P uptake by switchgrass was significantly greater in Cycle 2 than in Cycle 1 (Fig. 2.6). By comparison, P uptake by cattail in PBS and SB was significantly greater in Cycle 1 than in Cycle 2 whereas growth cycle had no significant effect on P uptake by cattail in PB. In Cycle 2, P uptake by cattail did not differ significantly between PB and PBS and was lowest for SB, whereas P uptake by switchgrass was significantly greater for PB than PBS and SB, which did not differ significantly.

The cycle × biosolids × harvest frequency interaction was significant (P = 0.02) for plant P uptake averaged across plant species (Table 2.3). In Cycle 1, P uptake, averaged over the two plant species, was significantly greater when plants were harvested twice compared with a single harvest regardless of biosolids type (Fig. 2.7). In fact, P uptake from each of the biosolids in Cycle 1 was significantly greater with two harvests compared with a single harvest (Fig 2.7). By comparison, in Cycle 2, harvesting twice resulted in significantly greater P uptake than a single harvest only in the PB, whereas harvest frequency had no significant effect on P uptake in the SB and PBS treatments. Regardless of harvest frequency, P uptake did not vary significantly between the two growth cycles for PB but decreased significantly in Cycle 2 when compared to Cycle 1 for SB and PBS.

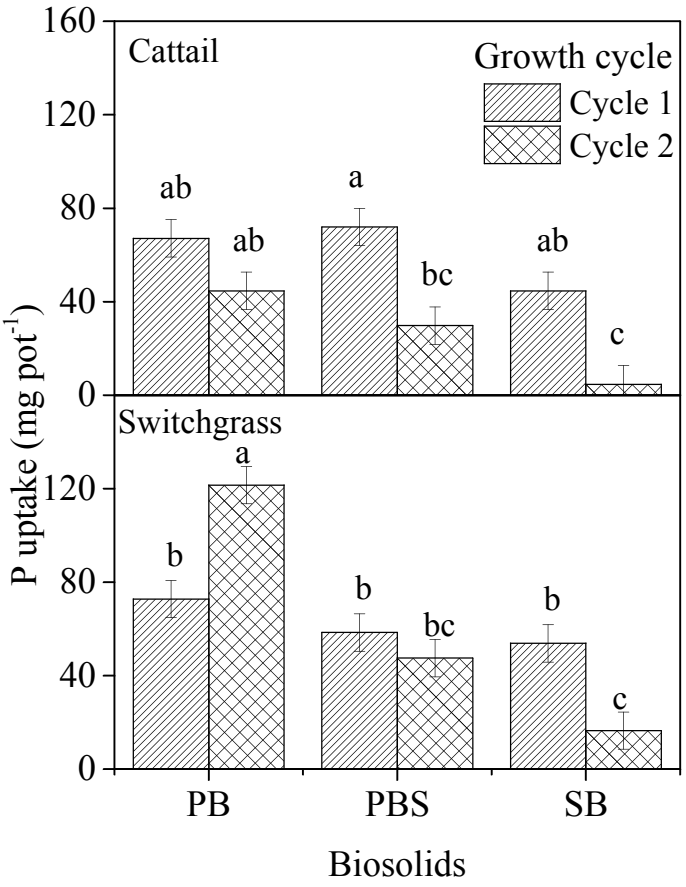


Figure 2.6 Phosphorus uptake, averaged over harvest frequencies, as affected by growth cycle, biosolids and plant species. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

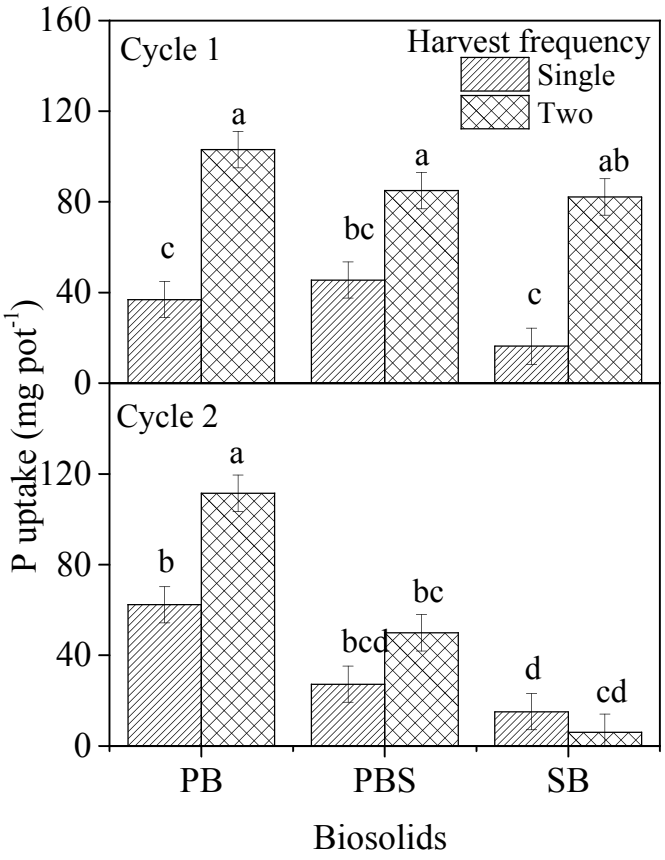


Figure 2.7 Growth cycle, harvest frequency and biosolids effects on P uptake averaged over plant species. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparism procedure.

2.4.5 Cadmium Uptake

The effect of harvest frequency on Cd uptake varied with biosolids treatment and growth cycle, as indicated by the significant ($P = 0.02$) cycle × biosolids × harvest interaction (Table 2.3). Cadmium uptake from SB, averaged across plant species, was significantly greater with two harvests (0.044 mg pot^{-1}) than with a single harvest in Cycle 1 (below detection limit) (Fig. 2.8). On the other hand, no significant difference in plant Cd uptake

was observed in PB and PBS in Cycle 1. In Cycle 2, two harvests resulted in a significant increase in Cd uptake across biosolids compared with a single harvest (Fig. 2.8).

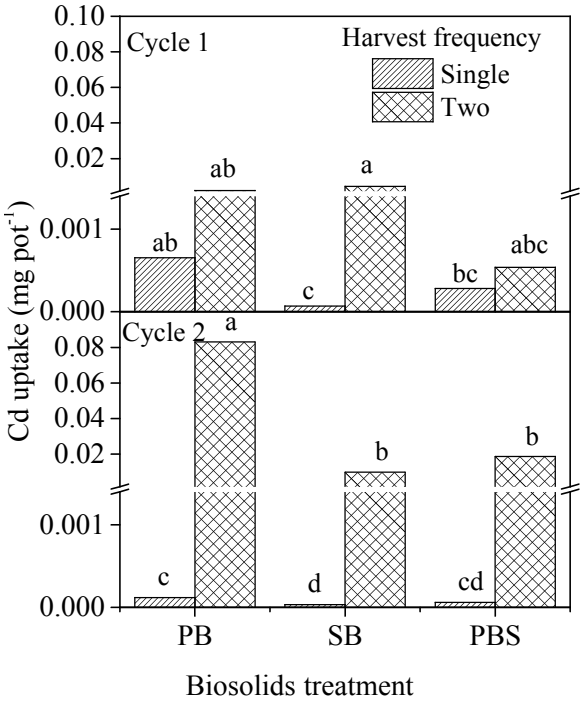


Figure 2.8 Geometric mean plant Cd uptake, averaged over plant species, as affected by growth cycle, biosolids treatment, and harvest frequency. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

The effect of harvest frequency on Cd uptake varied with biosolids treatment and plant species, as indicated by the significant ($P = 0.02$) biosolids × plant × harvest interaction (Table 2.3). For all biosolids treatments, Cd uptake was significantly greater when cattail and switchgrass were harvested twice compared with one harvest per cycle (Fig 2.9). When switchgrass was harvested once per cycle, Cd uptake was significantly greater in

PB than in SB. Under the two harvest system, Cd uptake by switchgrass was significantly greater in PB than in PBS.

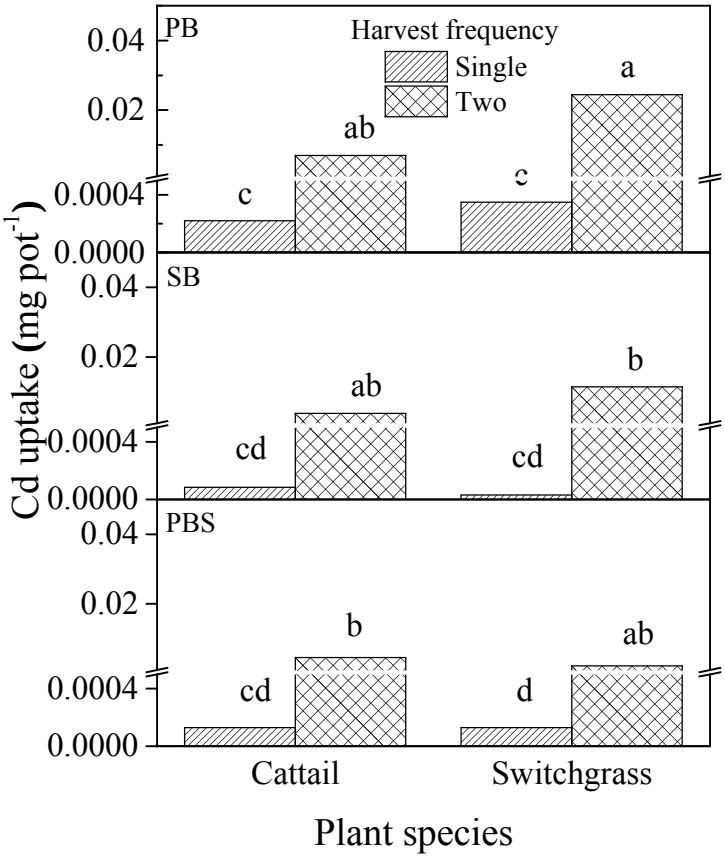


Figure 2.9 Biosolids, plant species and harvest frequency effects on Cd uptake averaged across cycles. Bars with the same letters represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

2.4.6 Copper Uptake

Of all trace elements tested, Cu was taken up by plants in the second largest amount after Zn. The biosolids × plant species × growth cycle interaction was significant ($P = 0.002$) for Cu uptake (Table 2.3). Copper uptake by cattail was significantly greater in Cycle 1

than in Cycle 2 for all biosolids treatments (Fig 2.10). By comparison, the growth cycle effect on Cu uptake by switchgrass was significant only for PBS (Cycle 1 > Cycle 2) but not for PB and SB.

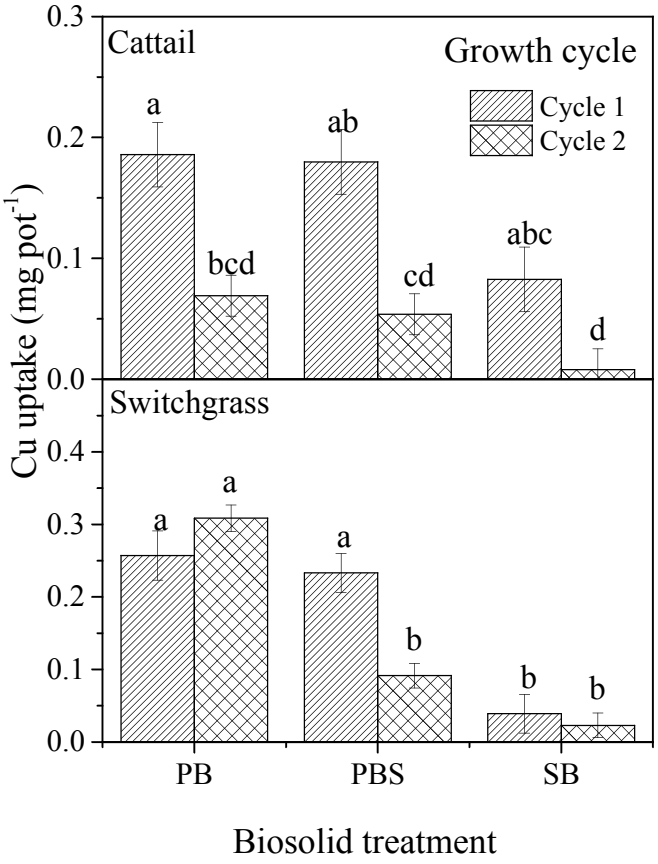


Figure 2.10 Plant species, growth cycle and biosolids effects on Cu uptake (averaged over harvest frequency). Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

2.4.7 Zinc Uptake

The effect of harvest frequency on Zn uptake varied with plant species and growth cycle, as indicated by the significant ($P = 0.04$) cycle × plant × harvest interaction (Table 2.3).

Zinc uptake by cattail was significantly greater in Cycle 1 than in Cycle 2 regardless of harvest frequency (Fig 2.11). On the other hand, there was no significant harvest frequency or cycle effect on Zn uptake by switchgrass. In both growth cycles and for both harvest frequencies, there was no significant difference in Zn uptake between cattail and switchgrass.

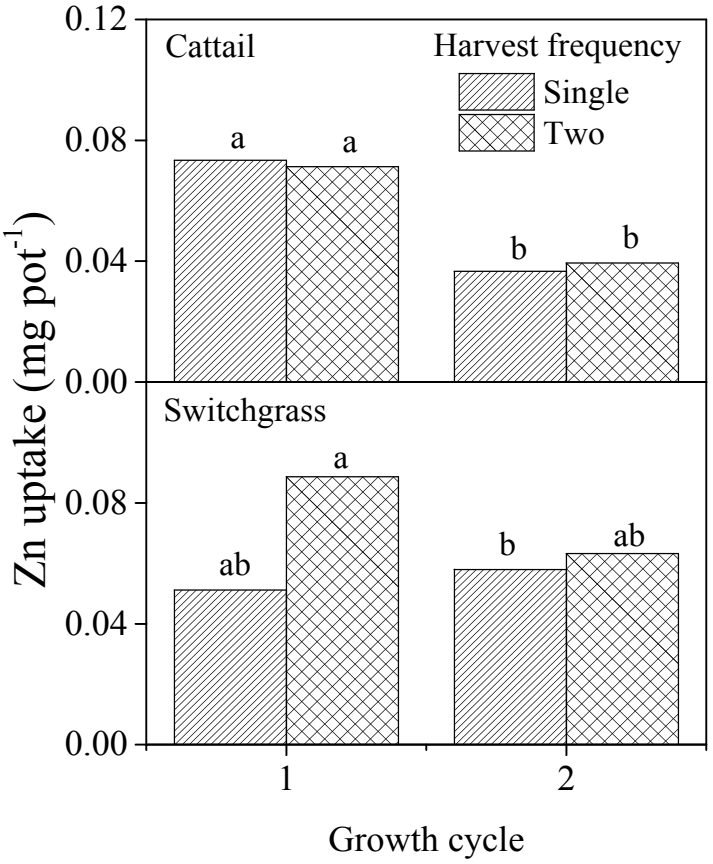


Figure 2.11 Geometric mean Zn uptake as affected by plant species, harvest frequency and growth cycles. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

2.4.8 Chromium Uptake

The plant species × harvest frequency × growth cycle interaction was significant for plant Cr uptake averaged across biosolids types ($P = 0.003$). In Cycle 1, Cr uptake by switchgrass was 87% greater for two harvests than for a single harvest, whereas Cr uptake by cattail did not differ significantly between harvest frequencies (Fig 2.12). In Cycle 2, Cr uptake by both plant species increased significantly with two harvests compared with one harvest. Cattail accumulated significantly more Cr than switchgrass with a single harvest in Cycle 1, but Cr uptake did not differ significantly between the two plant species when harvested twice in this cycle. By comparison, in Cycle 2, Cr uptake did not differ significantly between plant species with a single harvest but was significantly greater for switchgrass than cattail when harvested twice per growth cycle.

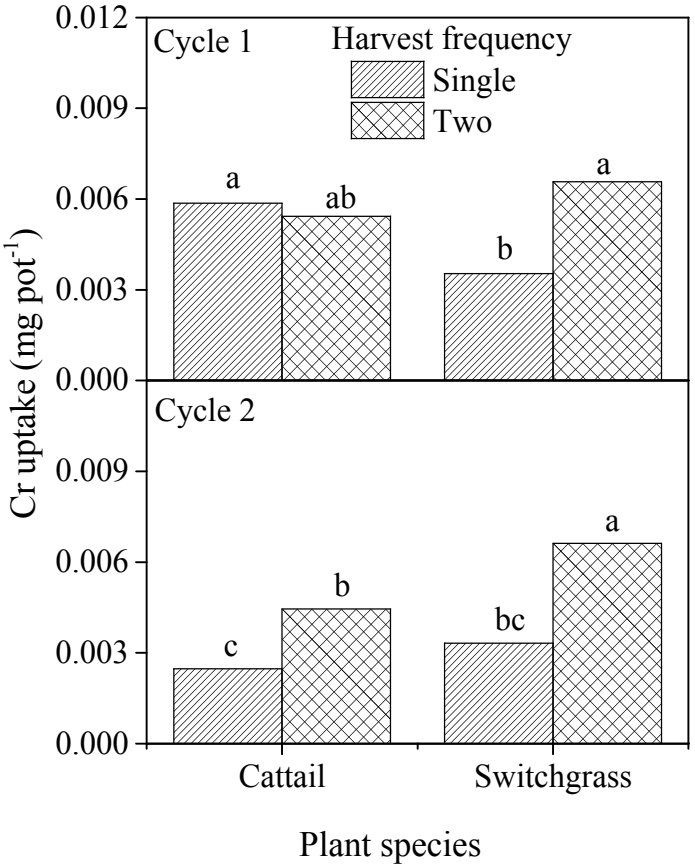


Figure 2.12 Geometric mean Cr uptake, averaged across biosolids, as affected by plant species, harvest frequency, and growth cycle. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

There was a significant cycle × biosolids × harvest frequency interaction ($P = 0.003$) for Cr uptake averaged across plant species (Fig. 2.13). In Cycle 1, there was no significant difference in Cr uptake between harvest frequencies regardless of biosolids type. On the other hand, in Cycle 2, Cr uptake by plants grown in PB and PBS was significantly greater for two harvests than for one harvest, while harvest frequency had no significant effect on Cr uptake by plants grown in SB. Chromium uptake decreased significantly in Cycle 2 compared with Cycle 1 for all biosolids under a single harvest and for SB and PBS under two harvests.

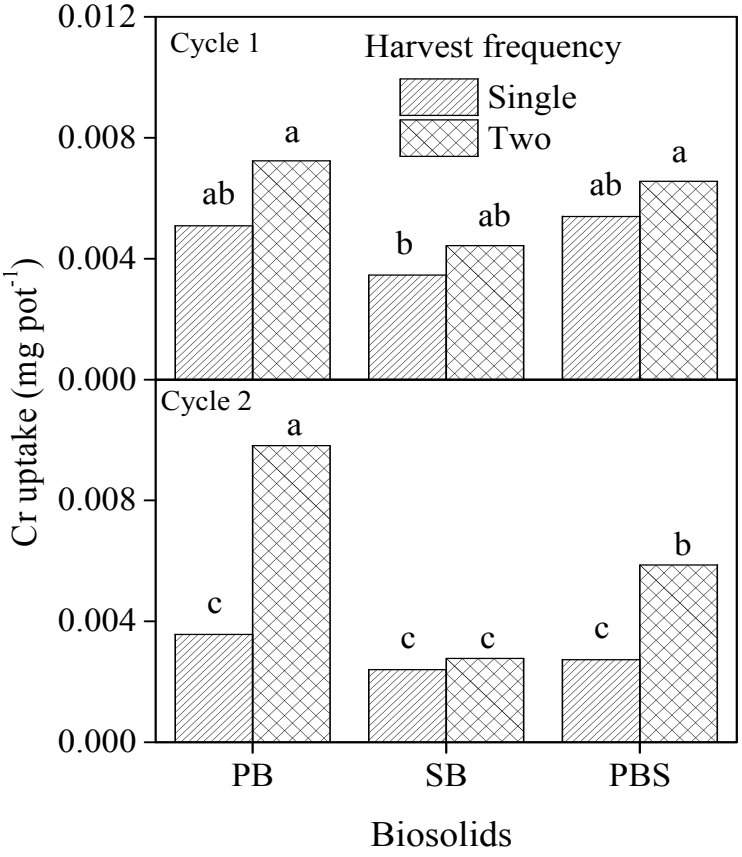


Figure 2.13 Geometric mean Cr uptake, averaged across plant species, as affected by biosolids and harvest frequency. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

Table 2.3 Concentrations of N and P species and trace elements in biosolids as affected by harvest frequency, plant species and growth cycle.

Effect	TKN	NO_3-N	NH_4-N	AN	TN	Total P	Olsen P	Cd	Cu	Cr	Zn	Pb
	mg kg^{-1}											
Cycle												
1	3604	184	1.62a†	185	4088	1486	53.4	0.73	73.5	45.5b	209	18.7
2	3507	17.9	6.46b	24.8	3752	7081	118	0.69	77.3	50.2a	202	17.9
Biosolids treatment												
PB	5225a	229	4.97	234	5684a	4875a	121	1.11a	119	47.7	322a	23.5a
SB	2160c	19.0	4.76	22.9	2311c	3789b	63.1	0.34c	36.2	47.2	96.9c	12.7c
PBS	3836b	55.8	2.23	58.6	3766b	4186ab	72.4	0.71b	71.3	48.6	196b	18.7b
Harvest												
Single	3587	103	6.0	109	3888	4262	90.8	0.75a	80.1	47.9	219	18.8
Two-harvest	3523	99.3	2.07	101	3951	4304	79.9	0.67b	70.8	47. 7	191	17.8
Plant species												
Cattail	3484	58.7	4.48	63.1	3986	4120	84.3	0.69	73.8	47.9	195	17.8
Switchgrass	3628	143	3.36	147	3855	4446	86.4	0.73	77.0	47.7	215	18.6
							P value					
Cycle (C)	0.56	<0.001	<0.001	<0.001	0.25	<0.001	<0.001	0.25	0.38	<0.001	0.52	0.17
Biosolids treatment (B)‡	<0.001	<0.001	0.09	<0.001	0.001	0.02	<0.001	<0.001	<0.001	0.53	<0.001	<0.001
harvest (H)	0.78	0.88	0.39	0.76	0.84	0. 88	0.004	0.03	0.05	0.79	0.06	0.11

Plant species (P)	0.53	0.001	0.55	0.002	0.77	0.30	0.64	0.21	0.37	0.86	0.37	0.08
C × B	0.04	<0.001	0.16	<0.001	0.26	0.33	0.001	0.92	0.43	0.04	0.06	0.28
C × P	0.87	<0.001	0.82	<0.001	0.09	0.19	0.13	0.68	0.58	0.26	0.52	0.67
C × H	<0.001	0.44	0.35	0.35	0.001	0.24	0.01	0.56	0.46	0.38	0.28	0.56
B × P	0.13	0.004	0.12	0.008	0.59	0.98	0.19	0.81	0.85	0.73	0.57	0.89
B × H	0.41	0.61	0.19	0.64	0.26	0.34	0.04	0.17	0.02	0.38	0.31	0.31
P × H	0.08	0.28	0.37	0.29	0.19	0.50	0.04	0.14	0.17	0.76	0.33	0.20

†Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

‡PB = biosolids from the primary cell; SB = biosolids from the secondary cell; PBS = biosolids from the primary cell + soil

AN = available N; TKN= total Kjedahl N**.**

2.4.9 Concentrations in Biosolids

2.4.9.1 Total Kjedahl nitrogen and nitrate nitrogen

There was a significant cycle × harvest frequency interaction (P = 0.04) for TKN concentration in the biosolids. At the end of Cycle 1, TKN concentration, averaged across biosolids and plant species, was significantly greater under two harvests than under a single harvest (Fig. 2.14). On the other hand, TKN concentration in Cycle 2 was significantly greater under a single harvest compared with two harvests (Fig 2.14).

The cycle × biosolids × plant species interaction was significant (P = 0.002) for NO_3-N concentration in the biosolids (Table 2.3). At the end of Cycle 1, NO_3-N concentration was significantly greater in PB and PBS planted to switchgrass than in PB and PBS planted to cattail (Fig. 2.15). By comparison, there was no significant difference in NO_3-N concentration between the two plant species in SB in Cycle 1. In all biosolids, NO_3-N concentrations at the end of Cycle 1 were greater in the non-vegetated controls than in the biosolids planted to cattail, while differences between the control and the switchgrass treatments were not significant. At the end of Cycle 2, NO_3-N concentration was significantly greater in PB planted with cattail than in PB planted with switchgrass. Nitrate-N concentrations in the biosolids in Cycle 2 were significantly greater in the non-vegetated controls than in PB and PBS vegetated with switchgrass. In the SB, NO_3-N concentrations did not differ significantly between the control and the vegetated biosolids. Nitrate-N concentration was also greater at the end of Cycle 1 than at the end of Cycle 2 (Fig 2.15).

Ammonium N concentration in biosolids, averaged across biosolids types, plant species, and harvest frequencies, was significantly greater at the end of Cycle 2 than at the end of Cycle 1 (Table 2.3). The concentrations of NH_4-N, averaged across biosolids, however, were 96% lower than NO_3-N concentrations.

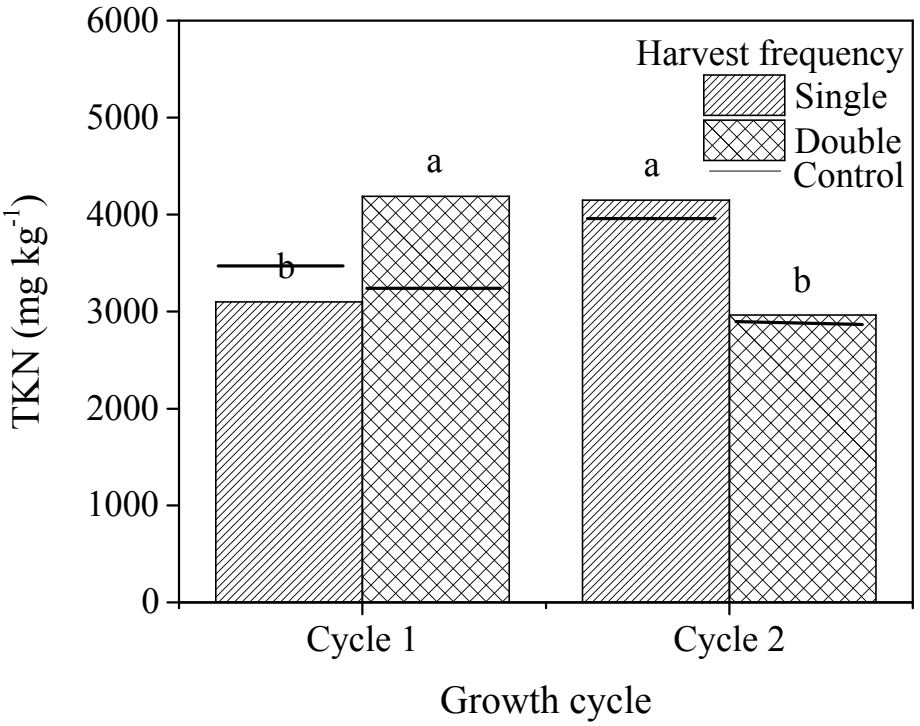


Figure 2.14 Geometric mean total N concentration, averaged across biosolids and plant species, as affected by growth cycle and harvest frequency. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

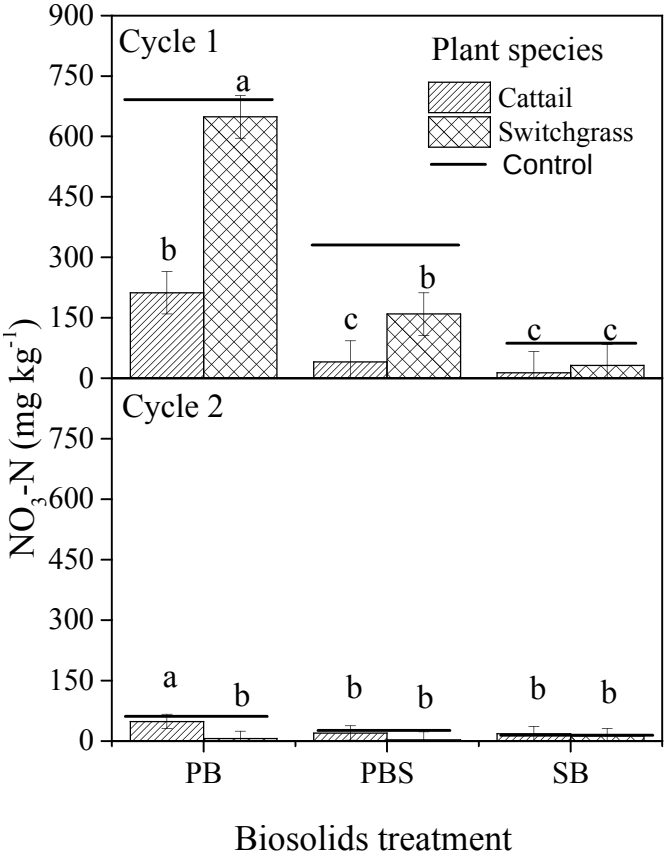


Figure 2.15 Effects of biosolids, plant species, and growth cycle on NO3-N concentration averaged across harvest frequencies. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

2.4.9.2 Available Phosphorus

The growth cycle × biosolids × plant species × harvest frequency interaction was significant ($P = 0.03$) for available (Olsen) P concentration in the biosolids. At the end of both growth cycles, available P concentration was significantly greater in PB than in PBS and SB, regardless of harvest frequency (Fig. 2.17). Available P concentration did not

differ significantly between plant species, regardless of biosolids type and harvest frequency (Fig 2.17A, B, C), with the exception of PB in Cycle 2 in which available P concentration was significantly greater when vegetated with switchgrass than when vegetated with cattail (Fig 2.17D). Available P concentration was significantly greater at the end of Cycle 2 compared with Cycle 1, irrespective of biosolids type or harvest frequency. There was no significant difference in available P between the control and the vegetated biosolids except in the PB planted with cattail and harvested twice in Cycle 1 and Cycle 2 (Fig 2.17C).

2.4.9.3 Copper

There was a significant biosolids × harvest interaction (P = 0.02) for Cu concentration in the biosolids (Table 2.3). Copper concentration, averaged over plant species and growth cycles, was significantly greater under a single harvest than under two harvests in PB, whereas harvest frequency had no significant effect on Cu concentration in PBS and SB (Fig 2.18). For both harvest frequencies, Cu concentration in the biosolids decreased in the order PB > PBS > SB.

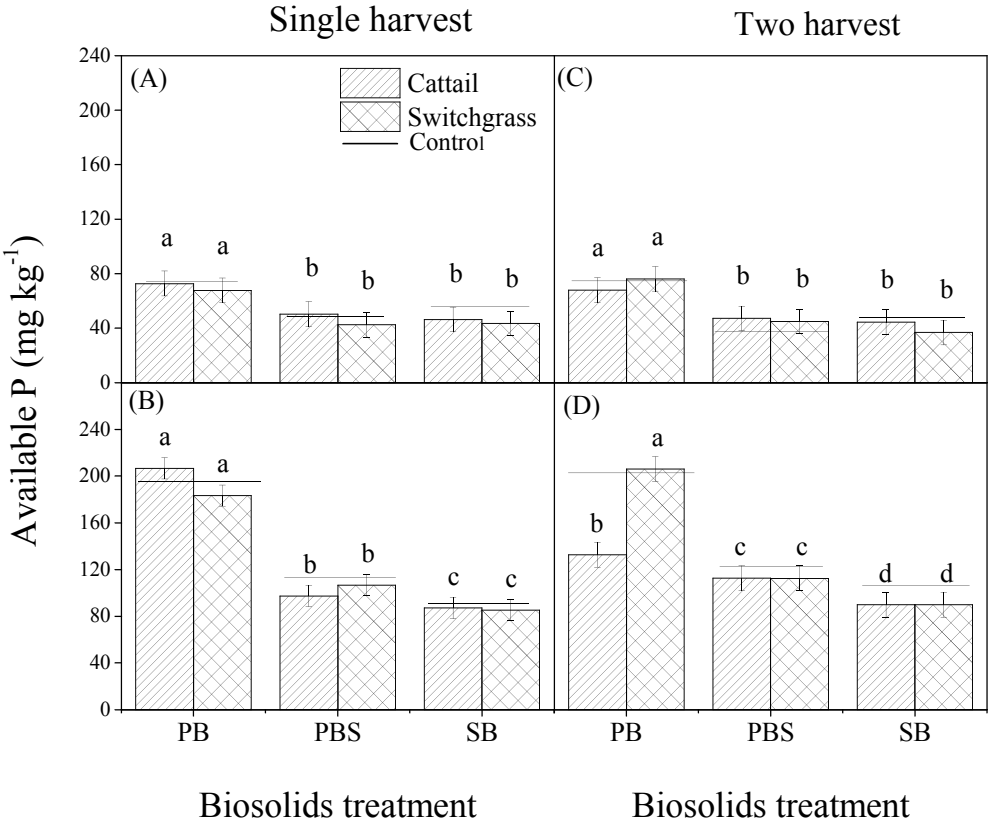


Figure 2.16 Available (Olsen) P concentration at the end of Cycle 1 (A and C) and Cycle 2 (B and D) as affected by plant species, growth cycle, biosolids treatment and harvest frequency. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

2.4.9.4 Chromium

There was a significant biosolids × plant species interaction ($P = 0.02$) for Cr concentration in the biosolids (Table 2.3). Nonvegetated biosolids had significantly greater Cr concentration than vegetated biosolids in Cycle 2. However, in Cycle 1, there was no significant difference between the nonvegetated and the vegetated biosolids (Fig

2.19). Chromium concentration in PB and PBS was significantly greater in Cycle 1 than in Cycle 2, but there was no significant difference in Cr concentration between the two cycles when plants were grown in SB (Fig 2.19).

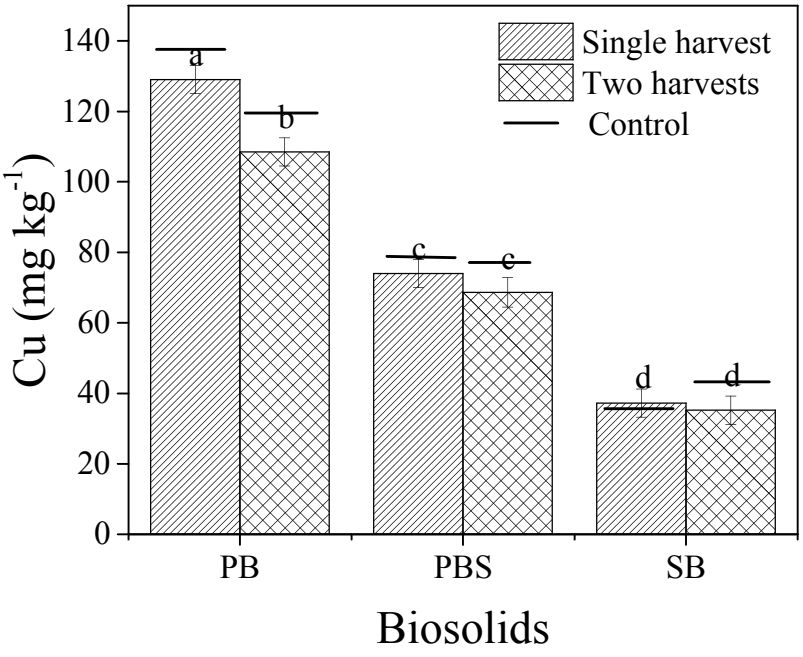
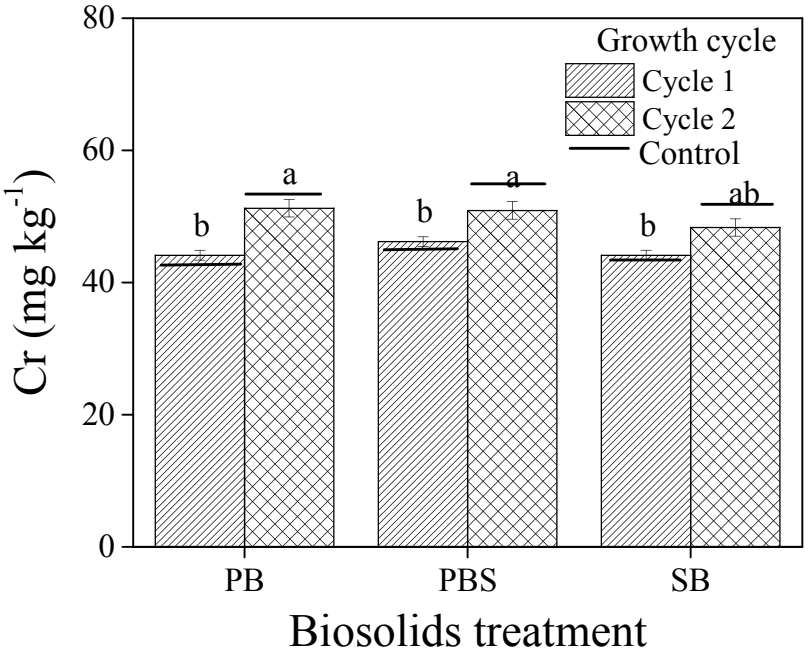


Figure 2.17 Effect of biosolids treatment and harvest frequency on Cu concentration in biosolids at harvest. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.



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. Figure 2.18 Biosolids treatment and growth cycle effects on biosolids Cr concentration, averaged across plant species and harvest frequencies. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

Table 2.4 Decrease in nitrogen, phosphorus, and trace element concentrations in biosolids after two growth cycles as affected by biosolids type, plant species, and harvest frequency.

Effect	ΔN†	ΔP	ΔCd	ΔCu	ΔZn	ΔCr
	mg pot^{-1}					
‡Biosolids						
PB	1042	663	0.05	0.34a¶	4.37	0.04
PBS	619	248	0.01	0.28b	1.96	0.02
SB	169	94.5	0.01	0.08c	0.27	0.02
Harvest						
Single	455	268	0.001	0.19b	1.72	0.06
Two-harvest	765	400	0.05	0.26a	2.68	0.20
Plant species						
Cattail	625a	249	0.02	0.19a	1.78	0.10
Switchgrass	595a	419	0.03	0.26a	2.61	0.17
			P value			
Biosolids	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Harvest	<0.001	<0.001	<0.001	0.04	<0.001	<0.001
Plant species	0.64	<0.001	0.87	0.05	<0.001	<0.001
B × H	0.01	0.01	0.15	0.26	0.001	<0.001
B × P	0.28	<0.001	0.17	0.11	<0.001	<0.001
H × P	0.96	0.78	0.003	0.95	0.001	<0.001
B × H × P	0.39	0.64	0.01	0.38	0.06	<0.001

† ΔN = decrease in N relative to initial N content; ΔP = decrease in P relative to initial P content; ΔCd = decrease in Cd relative to initial Cd content; ΔCu = decrease in Cu relative to initial Cu content; ΔZn = decrease in Zn relative to initial Zn content.

‡ PB = biosolids from the primary cell; SB = biosolids from the secondary cell;PBS = 1:1 mixture of PB and soil

¶ Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

2.4.10 Cumulative phytoextraction of nutrients and trace elements after two growth cycles

2.4.10.1 Nitrogen

The effect of harvest frequency on cumulative N removal during the two growth cycles varied with biosolids type, as indicated by the significant (P = 0.01) biosolids × harvest frequency interaction (Table 2.4). Averaged over plant species, cumulative N removal from PB after two growth cycles was significantly greater with two harvests than with a single harvest (Fig 2.19). By comparison, there was no significant difference in the phytoextraction of N from PBS and SB between the two harvest frequencies.

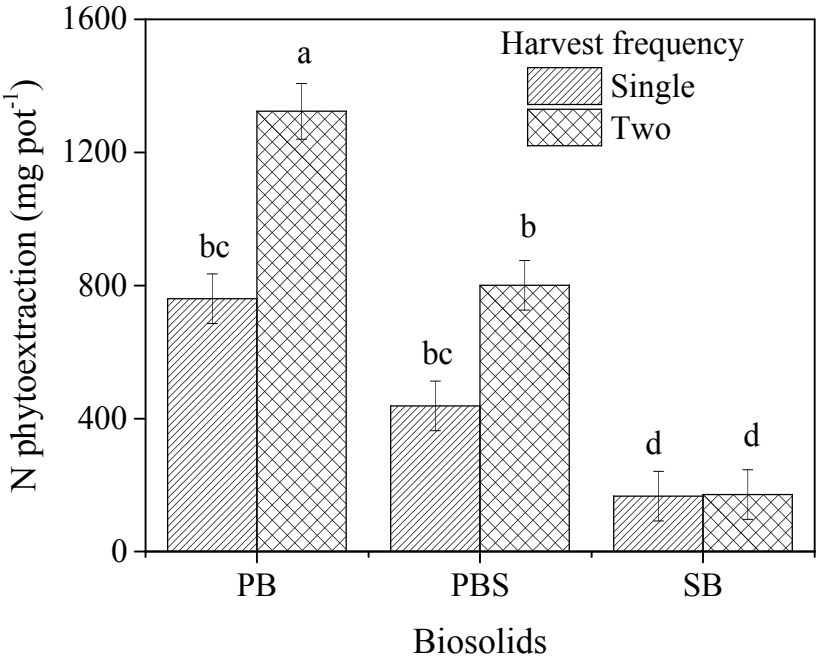

Figure 2.19 Effect of harvest frequency and biosolids treatment on the amount of N removed in the harvested plant biomass. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different (P ≥ 0.05) according to the Tukey multiple comparison procedure.

2.4.10.2 Phosphorus

The effect of plant species on cumulative P phytoextraction varied with biosolids treatment, as indicated by the significant ($P < 0.001$) biosolids × plant species interaction (Table 2.4). Averaged over harvest frequencies, cumulative P phytoextraction from PB after two growth cycles was twice greater for switchgrass than for cattail (Fig 2.20). On the other hand, there was no significant difference in cumulative P phytoextraction from PBS and SB between the two plant species. For both plant species, cumulative P phytoextraction decreased in the order PB > PBS > SB.

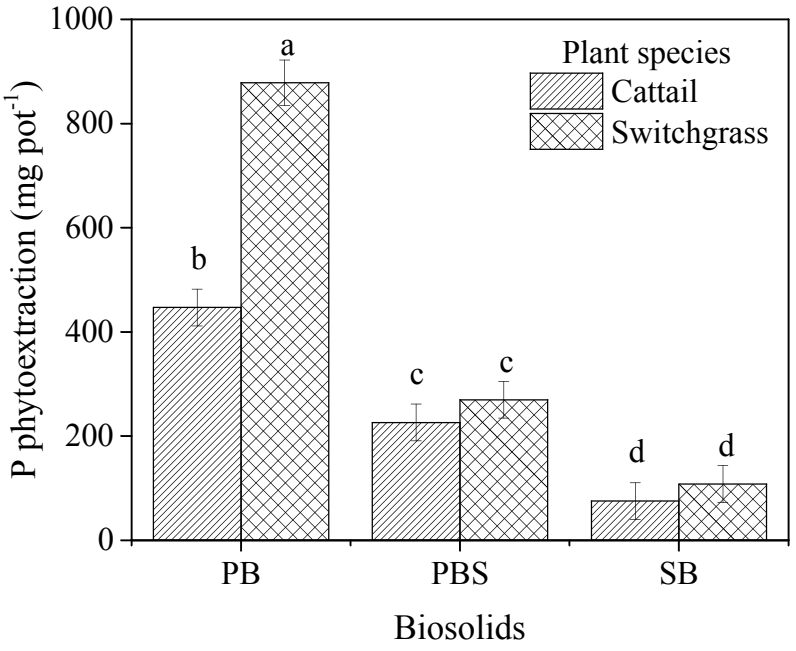

Figure 2.20 Cumulative P phytoextraction, averaged across harvest frequencies and growth cycles, as affected by biosolids and plant species. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

2.4.10.3 Cadmium

There was a significant (P = 0.01) harvest frequency × plant species × harvest frequency interaction for cumulative Cd phytoextraction from biosolids (Table 2.4). For both plant species, cumulative Cd phytoextraction was greater for two harvests than for a single harvest (Fig. 2.21). Cumulative Cd phytoextraction by two harvests of cattail was significantly greater for PB than SB but did not differ significantly between PB and PBS or between PBS and SB. Phytoextraction by a single harvest of cattail did not differ significantly among biosolids. By comparison, cumulative Cd phytoextraction by switchgrass was significantly greater for PB than PBS and SB with two harvests and significantly greater for PB and PBS than SB with a single harvest.

2.4.10.4 Zinc

The biosolids × harvest frequency interaction was significant (P = 0.001) for cumulative Zn phytoextraction from the biosolids (Table 2.4). The amount of Zn removed by plants after two growth cycles (mean of the two plant species) was significantly greater for two harvests than a single harvest for PB but did not differ significantly between the two harvest frequencies for PBS and SB (Fig 2.22). For both harvest frequencies, cumulative Zn phytoextraction decreased in the order: PB > PBS > SB.

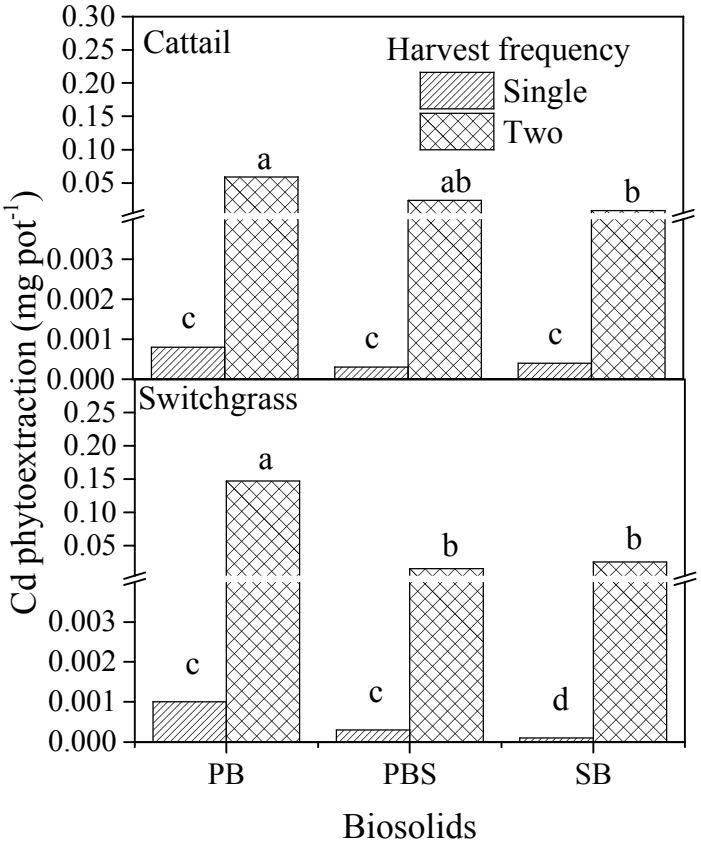

Figure 2.21 Geometric mean Cd phytoextraction as affected by plant species, biosolids and harvest frequency. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

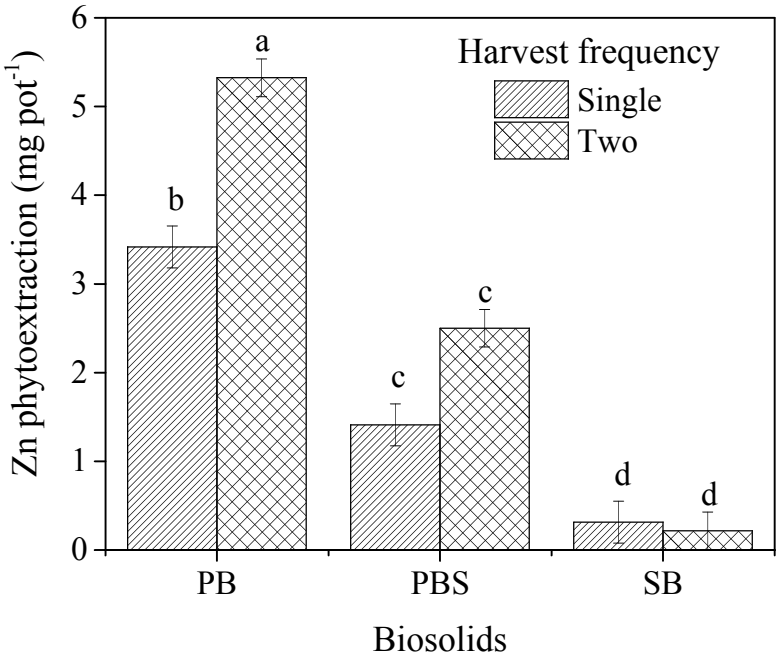

Figure 2.22 Cumulative phytoextraction of Zn, averaged over plant species, as affected by biosolids treatment and harvest frequency. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

The effect of plant species on cumulative Zn phytoextraction also differed with biosolids type, as indicated by the significant ($P < 0.001$) biosolids × plant species interaction (Table 2.4). Cumulative Zn phytoextraction at the end of the two growth cycles (mean of the two harvest frequencies) was significantly greater for switchgrass than for cattail in PB but did not differ significantly between the two plant species in PBS and SB (Fig 2.23). Cumulative Zn phytoextraction decreased in the order PB ≈ PBS > SB for cattail and PB > PBS > SB for switchgrass.

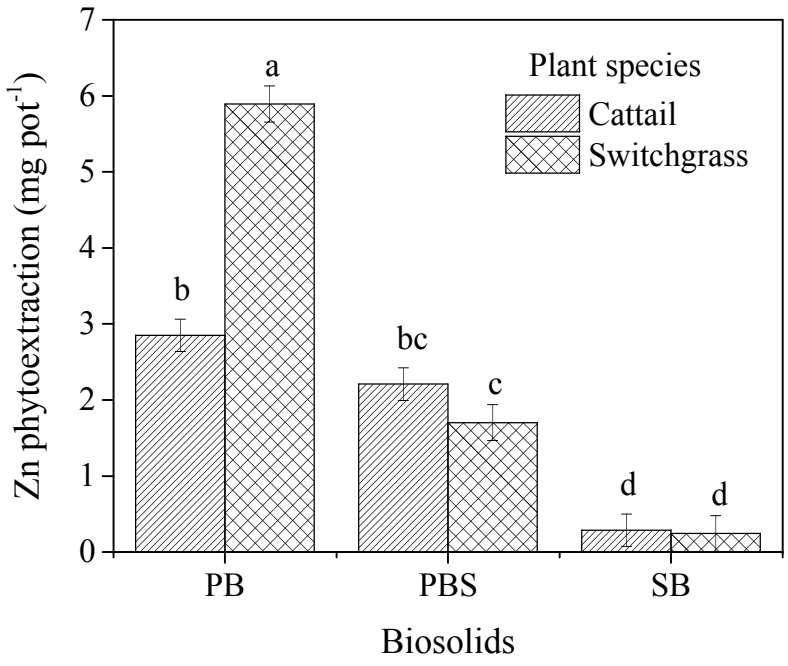

Figure 2.23 Effect of biosolids treatment and plant species on cumulative Zn phytoextraction averaged over harvest frequencies. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

There was a significant plant species × harvest frequency interaction on cumulative Zn phytoextraction after two growth cycles ($P = 0.001$). Harvesting switchgrass twice resulted in a significantly greater phytoextraction of Zn from the biosolids compared to a single harvest (Fig 2.24). On the other hand, there was no significant difference in Zn phytoextraction by cattail between the two harvest frequencies.

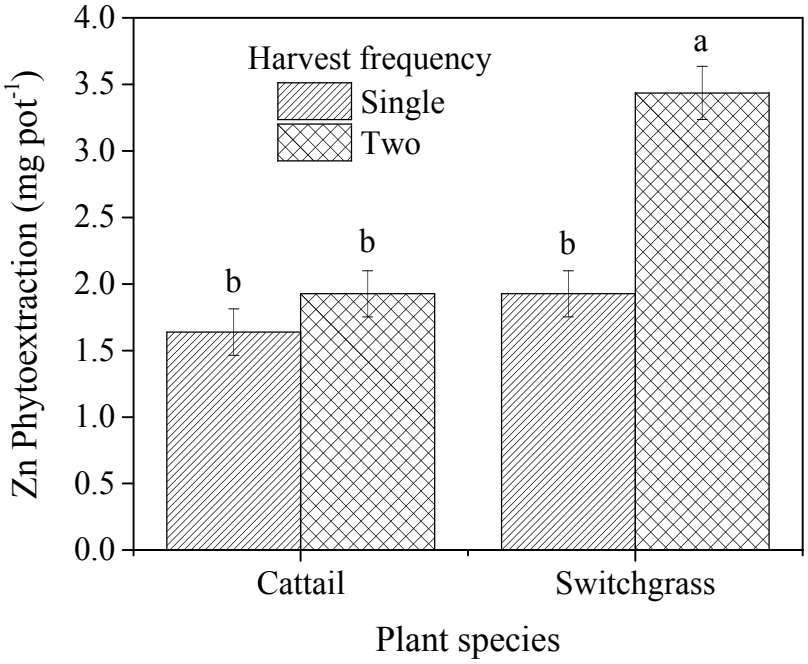

Figure 2.24 Cumulative Zn phytoextraction, averaged over biosolids, as affected by plant species and harvest frequency. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

2.4.10.5 Chromium

The effect of harvest frequency on cumulative Cr phytoextraction differed with plant species and biosolids type, as indicated by the significant ($P < 0.001$) biosolids × plant species × harvest frequency interaction (Table 2.4). Cumulative Cr phytoextraction at the end of the two growth cycles was significantly greater for cattail and switchgrass when harvested twice compared with a single harvest in PB but did not differ significantly between harvest frequencies in PBS and SB regardless of plant species (Fig 2.25).

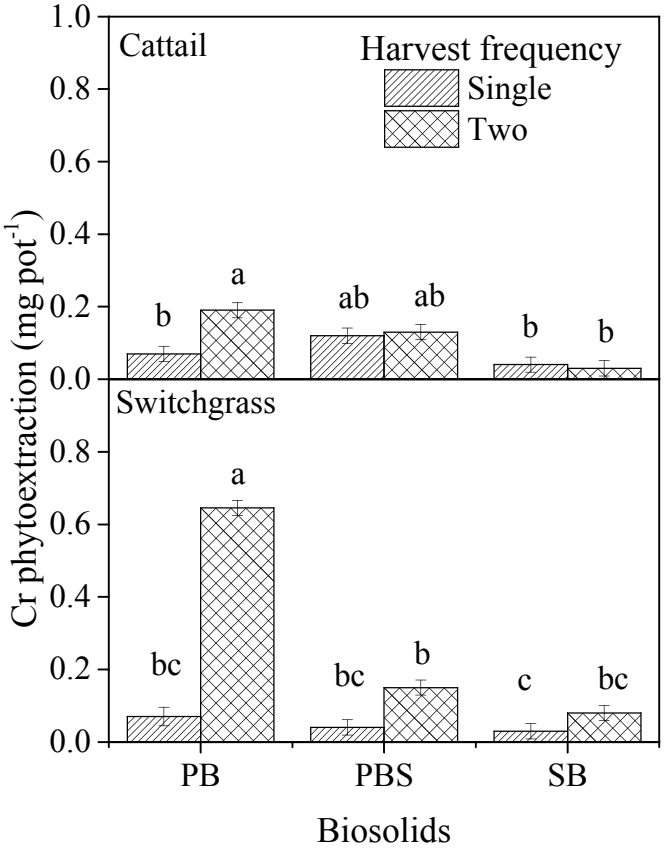

Figure 2.25 Cumulative Cr phytoextraction as affected by plant species, biosolids, and harvest frequency. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

Table 2.5 Percent decrease in nitrogen, phosphorus, and trace element concentrations in biosolids after two growth cycles as affected by biosolids type, plant species, and harvest frequency.

Effect	ΔN†	ΔP	ΔCd	ΔCu	ΔZn	ΔCr
			%			
Biosolids (B)‡						
PB	4.96	5.54	1.20	0.07b¶	0.31	0.12
PBS	4.25	4.24	0.35	0.10a	0.26	0.06
SB	2.53	1.52	0.62	0.06b	0.07	0.03
Harvests (H)						
Single	2.91	3.08	0.02	0.06a	0.18	0.04
Two	4.92	4.33	1.43	0.09a	0.25	0.10
Plant species (P)						
Cattail	3.95	3.01	0.49	0.07a	0.18	0.05
Switchgrass	3.88	4.48	0.95	0.08a	0.24	0.08
			P value			
Biosolids	0.003	<0.001	0.01	0.03	<0.001	<0.001
Harvest	0.001	0.001	<0.001	0.14	0.01	<0.001
Plant	0.90	<0.001	0.87	0.29	0.01	<0.001
B × P	0.12	0.01	0.17	0.14	<0.001	<0.001
B ×H	0.04	0.004	0.15	0.39	0.02	<0.001
P ×H	0.55	0.99	0.003	0.46	0.03	<0.001
B × P × H	0.23	0.06	0.01	0.09	0.39	<0.001

† ΔN = percent decrease in biosolids N concentration; ΔP = percent decrease in biosolids P concentration; ΔCd = percent decrease in biosolids Cd concentration; ΔCu = percent decrease in biosolids Zn concentration; ΔZn = percent decrease in biosolids Zn concentration.

‡ PB = biosolids from the primary cell; SB = biosolids from the secondary cell; PBS = 1:1 mixture of PB and soil.

¶ Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

2.4.11 Percentage Removal (Phytoextraction) of Nutrients and Trace Elements

2.4.11.1 Nitrogen

The biosolids × harvest frequency interaction was significant (P = 0.04) for the percentage of N (initially present in the biosolids) that was removed by plants (phytoextracted) (Table 2.5). Nitrogen phytoextraction from PB was significantly greater when plants were harvested twice compared with a single harvest (Fig 2.26). In contrast, N phytoextraction did not differ significantly between harvest frequencies in PBS and SB.

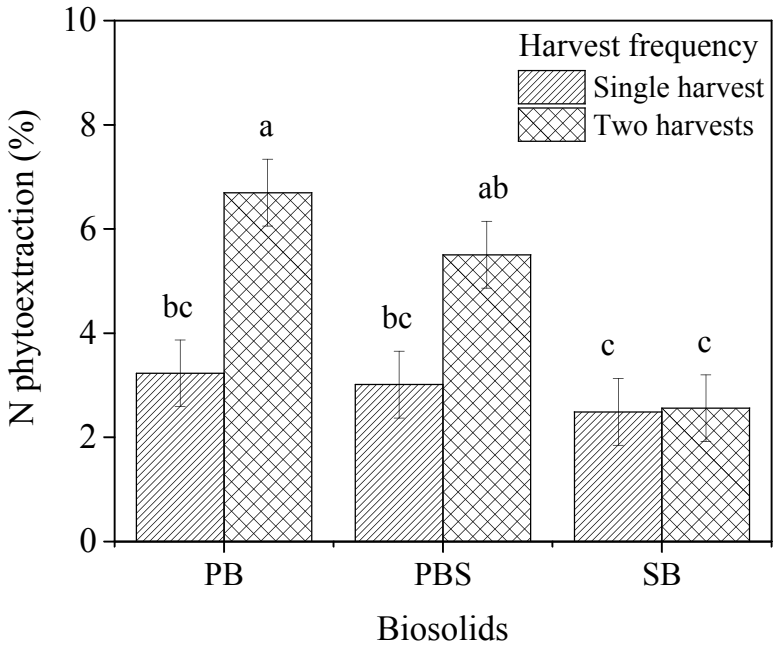

Figure 2.26. Effect of biosolids treatment and harvest frequency on N phytoextraction (% of initial total N concentration in biosolids) averaged over plant species. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different (P ≥ 0.05) according to the Tukey multiple comparison procedure.

2.4.11.2 Phosphorus

The effect of plant species on the phytoextraction of P from biosolids varied with biosolids type, as indicated by the significant (P = 0.01) biosolids × plant species interaction (Table 2.5). The percentage of initial biosolids P taken up by plants from PB was significantly greater for switchgrass than for cattail (Fig. 2.27). In contrast, P phytoextraction from PBS and SB did not differ significantly between the two plant species. Phosphorus phytoextraction decreased in the order PB ≈ PBS > SB for a single harvest and PB > PBS > SB for two harvests per cycle.

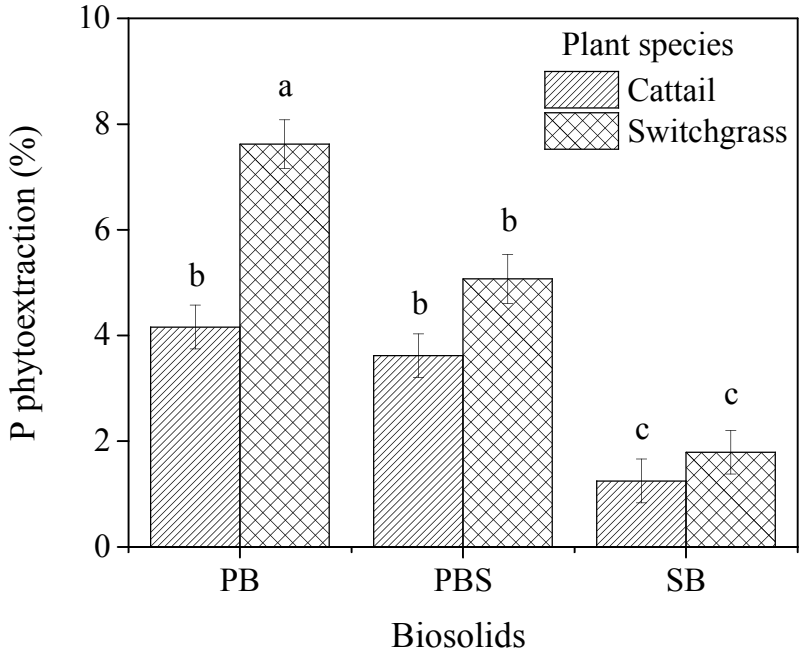

Figure 2.27 Effect of biosolids treatment and plant species on P phytoextraction (% of initial P concentration in biosolids) averaged across growth cycles and harvest frequencies. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different (P ≥ 0.05) according to the Tukey multiple comparison procedure.

There was a significant (P = 0.004) biosolids × harvest frequency interaction for percent P phytoextraction from biosolids, averaged across plant species and growth cycles. The percentage of initial P removed from PB was significantly greater for two harvests than for a single harvest (Fig. 2.28). By comparison, percent P removal from PBS and SB did not differ significantly between harvest frequencies. Phosphorus phytoextraction decreased in the order PB ≈ PBS > SB under a single harvest compared with PB > PBS > SB when the plants were harvested twice per cycle.

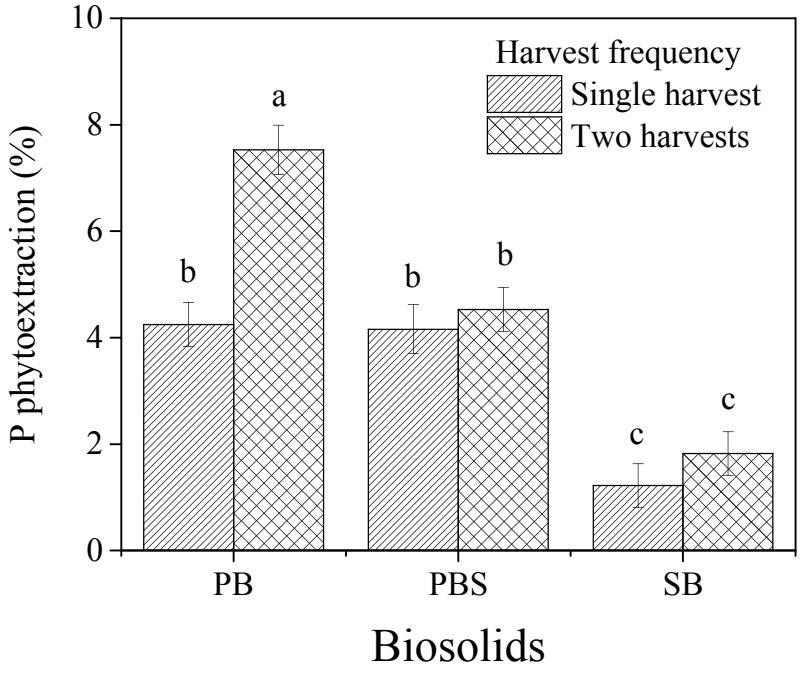

Figure 2.28 Effect of biosolids treatment and harvest frequency on P phytoextraction (% of initial P concentration in biosolids) averaged over plant species and growth cycles. Error bars represent standard errors of the mean. Bars with the same letter represent means that are not significantly different (P ≥ 0.05) according to the Tukey multiple comparison procedure.

2.4.11.3 Cadmium

The plant species × biosolids × harvest frequency interaction was significant (P = 0.001) for percent Cd phytoextraction (Table 2.5). In all three biosolids and for both plant species, the percentage of initial Cd removed was significantly greater with two harvests than with a single harvest (Fig 2.29). The percentage of initial Cd removed by cattail did not differ significantly among biosolids regardless of harvest frequency. By comparison, while Cd phytoextraction by switchgrass did not differ significantly among biosolids under a single harvest, with two harvests, it was significantly greater in PB than in PBS and SB, whereas the latter two did not differ significantly.

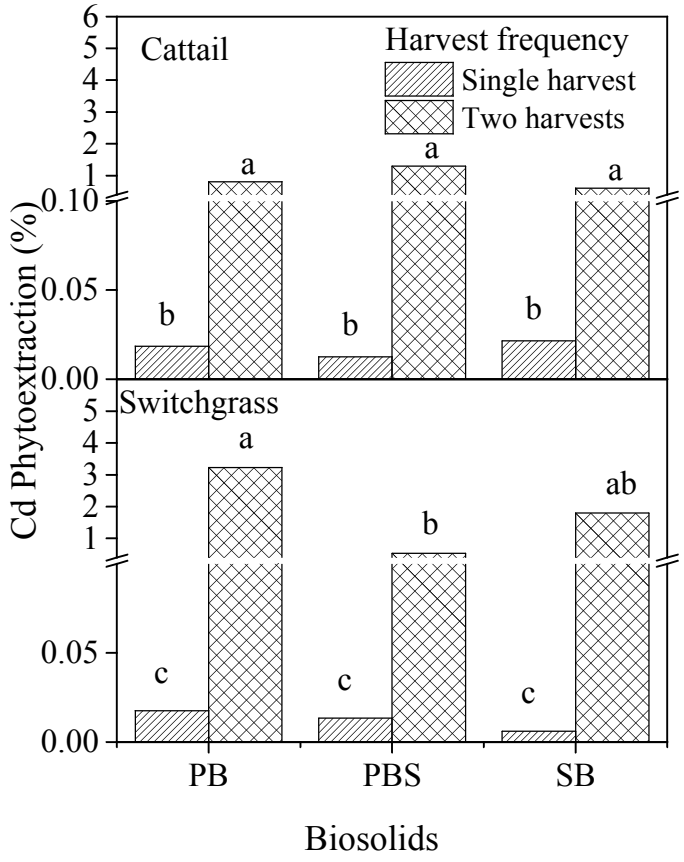

Figure 2.29 Percentage of initial biosolids Cd removed by plants (geometric means) as affected by plant species, harvest frequency, and biosolids treatment. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey comparison procedure.

2.4.11.4 Zinc

There was a significant ($P < 0.001$) biosolids × plant species interaction for the percentage of initial Zn content removed from the biosolids (Table 2.5). In PB, percent Zn removal was significantly greater for switchgrass than for cattail, whereas plant species differences were not significant in PBS and SB (Fig. 2.30). Zinc removal by

cattail decreased in the order PB ≈ PBS > SB while removal by switchgrass decreased in the order PB > PBS > SB.

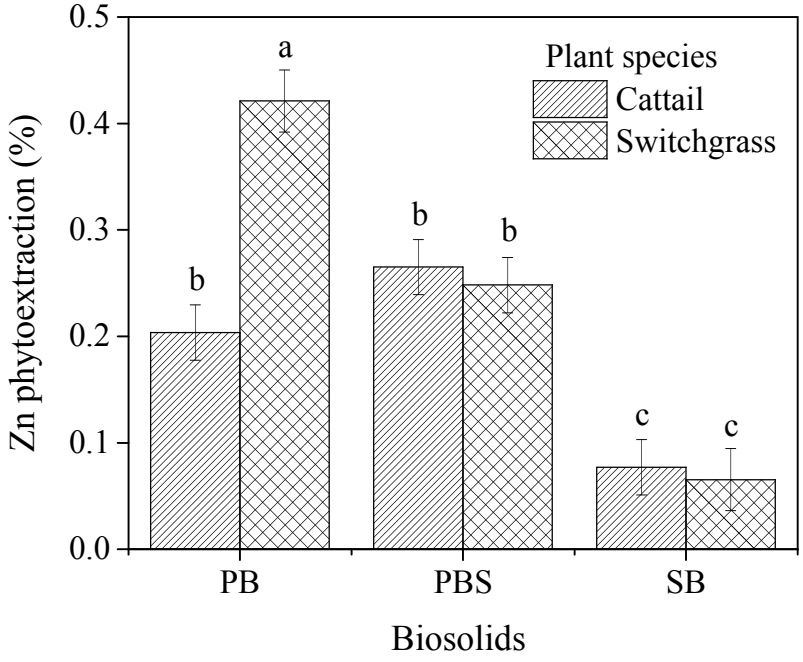

Figure 2.30 Effect of biosolids and plant species on the percentage of initial biosolids Zn removed in the harvested plant biomass, averaged across growth cycles and harvest frequencies. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different (P ≥ 0.05) according to the Tukey multiple comparison procedure.

The plant × harvest frequency interaction was also significant (P = 0.001) for percent Zn removal. Switchgrass removed a significantly greater percentage of Zn (mean of all biosolids) from the biosolids when harvested twice compared to a single harvest (Fig 2.31). On the other hand, no significant difference in percent Zn phytoextraction was observed in biosolids planted to cattail irrespective of harvest frequency.

The effect of harvest frequency on percent Zn phytoextraction varied with biosolids, as indicated by the significant (P = 0.001) biosolids × harvest frequency interaction (Table 2.5). Across plant species, two harvests in PB resulted in a significantly greater percent Zn phytoextraction than a single harvest. By comparison, there was no significant difference in percent Zn removal from PBS and SB between the two harvest frequencies (Fig 2.32).

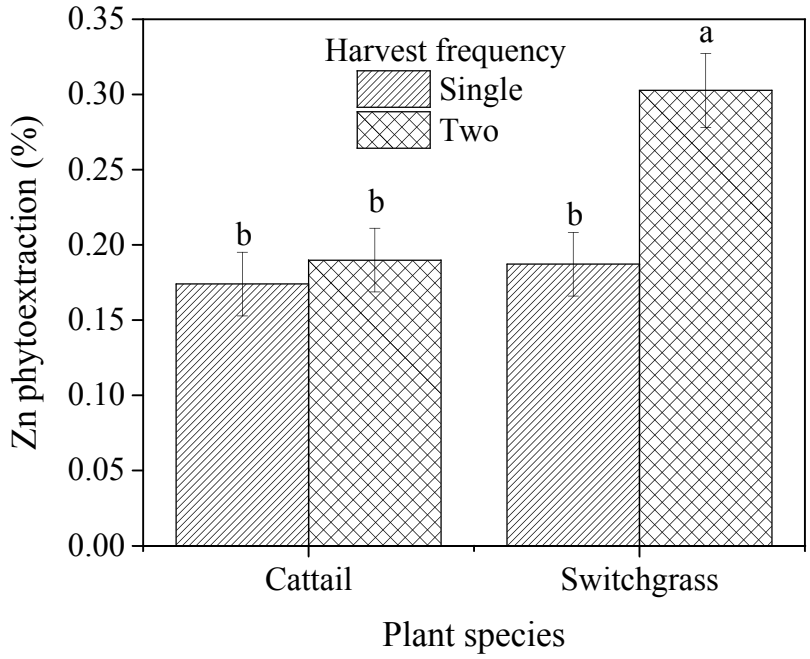

Figure 2.31 Effect of harvest frequency and plant species on the percentage of initial biosolids Zn removed in the harvested plant biomass, averaged across biosolids and growth cycles. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different (P ≥ 0.05) according to the Tukey multiple comparison procedure.

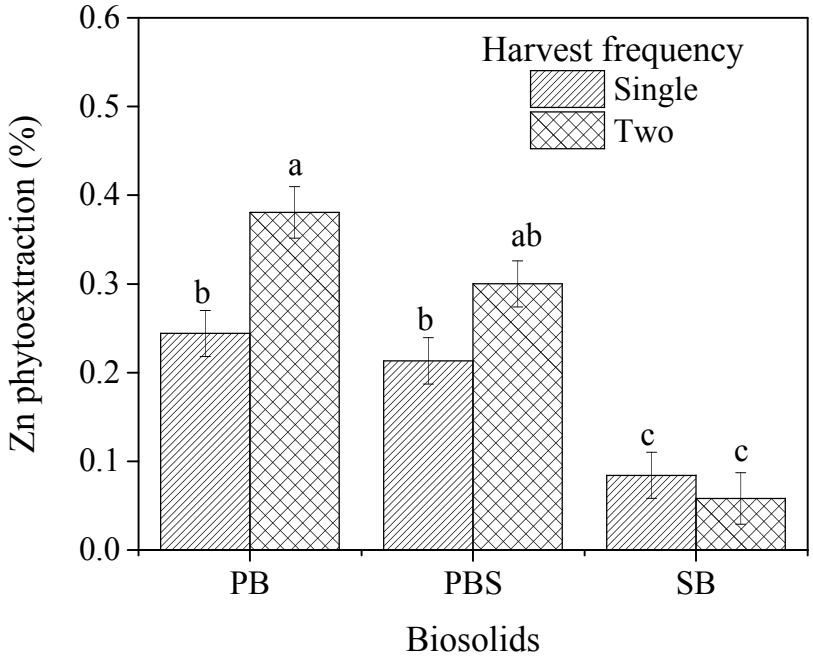

Figure 2.32 Effect of biosolids treatment and harvest frequency on the percentage of initial biosolids Zn removed in the harvested plant biomass, averaged across plant species and growth cycles. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

2.4.11.5 Chromium

The effect of harvest frequency on Cr phytoextraction varied with biosolids and plant species, as indicated by the significant ($P < 0.001$) biosolids × plant species × harvest frequency interaction (Table 2.5). Cattail and switchgrass removed a significantly greater percentage of Cr from PB when harvested twice compared with a single harvest (Fig 2.33). There was no significant difference in Cr phytoextraction by cattail and switchgrass from PBS and SB irrespective of harvest frequency.

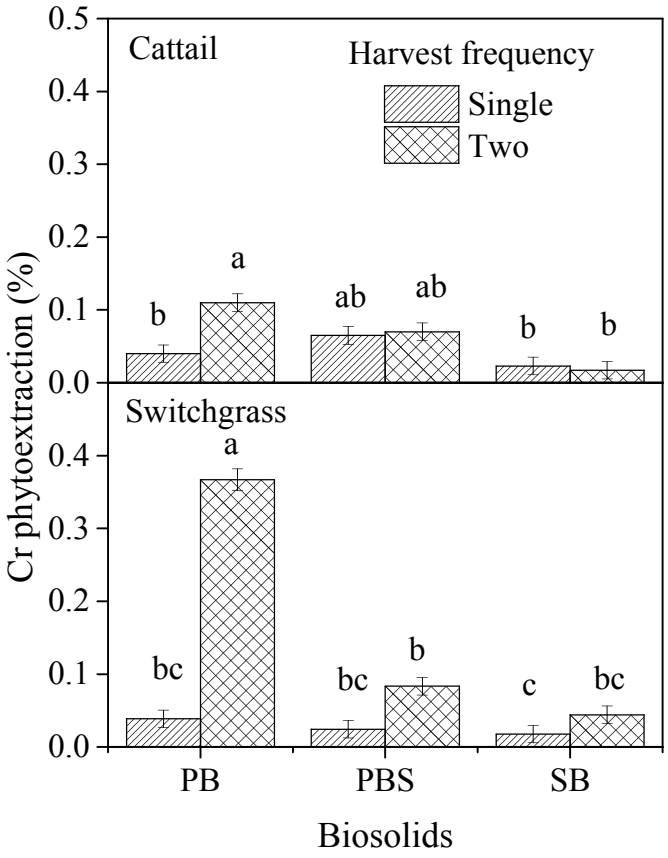

Figure 2.33 Effect of biosolids treatment, plant species and harvest frequency on the percentage of initial biosolids Cr removed in the harvested plant biomass, averaged across growth cycles. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

2.5 Discussion

2.5.1 Biosolids and Soil Characterization

The greater trace element, N and P concentrations observed in PB than in SB could be because most of the elements in the influent sludge are removed in the primary cell before the effluent is transferred to the secondary cell. Interestingly, the trace element

(Cr, Zn, Cu and Cd) concentrations were greater in the soil than in the SB, perhaps reflecting historical amendment and/or fertilizer applications to the soil. The low trace element concentrations measured in the present study are expected of this type of biosolids, which originates primarily from domestic wastewater. The concentrations are consistent with those reported for other municipal lagoons. For example, Nielsen and Hrudey (1983) reported Cr and Cd concentrations of 0.254 and 0.002 mg kg^{-1}, respectively, in biosolids from a municipal treatment plant in Edmonton, AB, Canada.

The concentrations of Cd, Zn, Cu, and Cr in PB were below CCME sediment quality guidelines for aquatic life, while only Cr was above the guidelines in the SB (CCME, 1998). The sources of trace elements in the biosolids likely include pharmaceutical and personal care products, plumbing, food products, pesticides, and health supplements (Díaz-Cruz et al., 2009; Monteith et al., 2010). The relative abundance of trace elements in the biosolids in the present study (Zn > Cu > Cr > Cd) is similar to that observed in biosolids from a municipal lagoon in Steinbach, MB (Sahulka and Keam, 2013) and in biosolids from Kansas, USA (Madrid et al., 2003), indicating the common anthropogenic sources of these trace elements.

Nitrogen, particularly in the NO_3 form, is a big concern in decommissioned lagoons because it can contaminate ground water, which is a source of drinking water for some communities. More than 98% of available N in the biosolids in the present study was in the NO_3 form, with much of the remainder in the NH_4 form. This is consistent with Liphadzi et al. (2002), who observed lower NH_4 concentrations in an end-of-life animal

waste lagoon compared with the operational phase of the lagoon, as NH_4-N was converted to NO_3-N during drying (hence aeration) of the lagoon.

The pH of the biosolids and soil from the present study ranged from 7.4 to 8.2. The PB and soil were weakly saline (EC 4-5 dS m^{-1}) while the PBS was slightly saline (3-4 dS m^{-1}) and the SB was non-saline (EC < 2 dS m^{-1}). This range of salinity did not seem to pose a threat to the growth of plants tested in this experiment and is not expected to pose a threat to crops if spread on agricultural land. In any case, perennial grasses such as switchgrass (Alexopoulou et al., 2008) and cattail (Beare and Zedler, 1987) have the ability to thrive well in saline soils.

2.5.2 Plant Biomass

As expected, cattail established faster after transplanting compared with switchgrass, which established much more slowly. Overall, aboveground biomass yields of both plant species in all biosolids were modest, despite the limited volume of the pots used. However, the yields were much lower than those expected under field conditions. For example, in a study near Lake Winnipeg, MB, Grosshans et al. (2011) reported cattail biomass yields of 1.5 kg m^{-2} compared with a mean of ~0.4 kg m^{-2} in the present study. Similarly, Reynolds et al. (2000) obtained switchgrass biomass yields of 2 kg m^{-2} compared with a mean of 0.5 kg m^{-2} in this growth room study. This difference is probably due to the controlled conditions in the growth room, including the small volume of biosolids in the pots and the absence of an 'off-season' break between growth cycles.

The decline in biomass yields of cattail in growth cycle two relative to cycle one in the present study likely reflects the restrictions imposed by the volume of the potted biosolids, which could no longer supply enough nutrients to sustain continued aboveground and belowground biomass growth. Contrary to the results from this study, greater biomass yield of cattail was observed in the second season compared to the first season in field studies (Grosshans et al., 2011; Dickerman et al., 1986; Martin et al., 2003). These authors also reported increased yield after repeated harvesting annually.

The high plant biomass observed in PB compared with PBS and SB was likely due to the higher nutrient concentration of PB. The much reduced biomass yield of cattail in SB relative to PB might also be due to autotoxicity. Unlike in the primary cell where there had been no previous cattail growth prior to biosolids collection for the present bioassay, the secondary cell had already been colonized by a healthy crop of cattail in the preceding growing season. Cattail has been reported to exhibit autotoxicity feedback, which restricts seed germination and the growth of successive plant populations in media where cattail has previously been established (McNaughton, 1968).

Little difference in switchgrass biomass was observed between growth cycles in PBS and SB. The greater switchgrass biomass observed in the second growth cycle compared to the first cycle in PB might be due to the inherently slow establishment of switchgrass (Muir et al., 2001). Resources during plant establishment are largely used to develop an extensive root system. By the second cycle in the present study, the roots would have been more fully developed, enabling a concomitant increase in tiller density, hence

greater biomass accumulation (Muir et al., 2001). The biomass yields of 0.56 kg m^{-2} (28 g DM pot^{-1}) in Cycle 1 and 1.16 kg m^{-2} (58 g DM pot^{-1}) in Cycle 2 obtained in the present study are consistent with those from a study by Liu (2012), who reported a successive increase in switchgrass biomass yield from 0.6 kg m^{-2} in the first year through 1 kg m^{-2} in the second year to 1.2 kg m^{-2} in the third year. These results confirm the general observation that switchgrass stands that appear poor in the seeding year often produce high yields in subsequent years (Samson, 2007). Nonetheless, due to the plant's slow establishment, it is often recommended that switchgrass should not be grazed or cut during the seeding year, except when growth is exceptional or weeds are problematic (Renz et al., 2009).

2.5.3 Nitrogen and Phosphorus Uptake

In Cycle 1, N uptake was greater for cattail than for switchgrass while in Cycle 2 the N uptake was greater for switchgrass than for cattail. These results reflect the corresponding differences in biomass yield between the two plant species, with cattail outyielding switchgrass in Cycle 1, while switchgrass outyielded cattail in Cycle 2.

Averaged across plant species, N uptake was greater under two harvests compared with a single harvest in PB and PBS. This is consistent with Reynolds et al. (2000), who studied the effect of harvest frequency on N uptake by switchgrass grown in field soils. They attributed the higher N uptake in the two harvest system to the greater biomass yield under this system. The order (PB > PBS > SB) in which N was taken up by plants in the

present study reflects N and P concentrations in these biosolids, which followed the same order.

In the present study, nutrient uptake was greater when plants were harvested twice compared to a single harvest per cycle in the three biosolids. This corroborates previous studies (e.g., Thomason et al., 2005; Liu, 2012; Fike et al., 2006), which showed higher nutrient uptake from multiple harvests than from single harvests per season.

On average, cattail and switchgrass phytoextracted 5% of the N initially present in the PB. A similar percentage (5.5%, mean of the two plant species) of P was also removed from PB. When PB was mixed with soil (PBS treatment), phytoextraction rates were lower (4.3% for N and 4.2% for P). By comparison, only 2.5% of N and 1.5% of P initially present in SB were removed in the aboveground biomass after two growth cycles. These results indicate that phytoextraction of N and P was most effective in PB, which had the highest concentrations of these nutrients. As indicated previously, the higher nutrient concentrations in PB resulted in greater biomass yields, which in turn resulted in greater N and P uptake, hence greater phytoextraction of these nutrients.

The P removal rates of 3% by cattail and 4.5% by switchgrass achieved for PB in this study indicate a great potential for phytoremediation of biosolids. Based on Ontario's sediment quality guidelines, it would take 52 years when phytoremediating with cattail and 32 years with switchgrass to bring the level of P in PB to the lowest effect level (LEL) of 600 mg kg^{-1} (Persaud et al., 1993), assuming these phytoextraction rates did not change in subsequent growing seasons. Similarly for N, it would take 46 years to bring N

concentrations to the LEL of 550 mg kg^{-1} (Persaud et al., 1993) using cattail and 47 years using switchgrass species, based on N removal rates of 3.95% for cattail and 3.88% for switchgrass.

Averaged over the two growth cycles in this study, the removal rate of N by cattail was 4% and that of P was 3% (of initial biosolids), which are lower than cattail removal rates of 6-12% N reported by Martin et al. (2003). The removal rate of nutrients from biosolids by switchgrass has not been fully explored. However, in this study, switchgrass removed 6.8 g m^{-2} N (340 mg pot^{-1}), and 1.2 g m^{-2} P (61.8 mg pot^{-1}). By comparison, Guretzky et al. (2011) reported removals of 18.6 g m^{-2} N and 4.9 g m^{-2} P. Reynolds et al. (2000) reported a mean removal of 10 g $m^{-2.}$ N while Lemus et al. (2008) reported a removal of 13.5 g m^{-2} N. The higher removals of N and P from these studies reflects the higher biomass yields in the field study compared to the present study.

2.5.4 Trace elements

Zinc was taken up by plants in the greatest amount of all trace elements tested, reflecting its high concentration in the biosolids relative to the other trace elements. Overall, however, the phytoextraction of Zn (mean of all biosolids = 0.21% of initial concentration) was low. Copper was taken up in the second greatest amounts, which account for just 0.07% of the Cu initially in the biosolids. Phytoextraction rates were similarly low for Cr (0.07%) and Cd (0.74%). The low uptake of these trace elements reflects their low bioavailability in the biosolids. The biosolids used in the present study had high pH values (7.6-8.2), which may have reduced the bioavailability of trace

elements, which decreases with increasing pH. High P concentrations in the biosolids may also have contributed to low bioavailability of these trace elements. For example, Deng et al. (2004) reported that high P concentration may reduce the bioavailability of Zn, Cu and Cd. Concentration of trace elements in this study (0.01-79.7 mg kg^{-1}) (Table A.1) tend to fall within the normal range as identified by Zhu and Kirkham (2003), who measured Zn uptake of 0.01-200 mg kg^{-1} DM in sorghum, barley, sunflower and wheat, despite the high N and P concentrations in the old animal lagoon that was being remediated.

Although Cu was taken up in the second highest amounts after Zn, its phytoextraction was low (0.04-0.19 mg pot^{-1}) relative to concentrations in the biosolids (36.2-119 mg kg^{-1}). In addition to its precipitation as hydroxides or sulfides at the high pH of the biosolids, the high organic matter content of biosolids likely reduced the bioavailability of Cu, hence plant uptake. Studies have shown that Cu has a strong affinity for organic matter (e.g., Laidlaw et al., 2012; Meers et al., 2007). Huynh et al. (2008) reported a linear increase in Cu concentration with increasing dissolved organic carbon concentration. Copper phytoextraction by the aboveground cattail biomass in the present study was greater than that of Zn. This is consistent with the assertion by Deng et al. (2004) that Cu concentration in plant shoots is usually higher than that of Zn.

Chromium concentration in aboveground switchgrass biomass was near or below detection limit (0.05 mg kg^{-1}), indicating that Cr phytoextraction made little impact on the initial Cr concentration of 43.2-45.7 mg kg^{-1} in the biosolids. While Cr in the root system

was not measured, biosolids analysis in conjunction with plant shoot analysis indicated that 0.08% of Cr removed from the biosolids was translocated to the aboveground biomass. This, in turn, suggests that virtually all (99.9%) of the Cr taken up by plant roots were sequestered in the belowground biomass. This is consistent with the findings of Barceló et al. (1985), who reported preferential accumulation of Cr in the roots of bush beans. Similarly, Shahandeh and Hossner (2000) screened 36 plants for Cr phytoextraction and found that plants growing in media with high levels of Cr rarely accumulated Cr in the shoots but rather accumulated it in their roots, with an average of 5.5% of Cr removed in the shoots and 34.5% of Cr removed in the roots. The authors reported that, in as much as switchgrass could tolerate Cr-contaminated media, its uptake of Cr was very low (1.4% of the initial concentration of Cr), although higher than the mean uptake rate from the present study (0.08%). They concluded that Cr was precipitated at high pH as Cr (III) hydroxide and is unavailable to plants. In a more recent bioassay, Jeke (unpublished data) 2014, reported that 61% of absorbed Cr was in cattail roots, while a mere 39% accumulated in the aboveground biomass. Chromium partitioning in the roots in our study, nonetheless, was inexplicably much higher than in these other studies.

Cadmium phytoextraction by aboveground plant biomass after two growth cycles was low (0.004 to 0.08 mg kg^{-1}) relative to Cd concentrations in the biosolids (0.34-1.11 mg kg^{-1}). This may have been due to low bioavailability of Cd at the high pH values of the biosolids. In a study on Cd accumulation by switchgrass, Reed et al. (2002) reported that Cd concentration in the plant tissue increased as the pH of the sludge amended soil

decreased. They also observed that switchgrass could thrive in Cd-contaminated media to produce acceptable biomass yields and that Cd tended to preferentially accumulate in the roots instead of the shoots. This corroborates the findings of Jeke et al. (unpublished data, 2014), who studied the partitioning of trace elements in cattail using the same biosolids as used in this study and reported higher Cd concentrations in the roots (98% of absorbed Cd) than in the shoots (2.3% of absorbed Cd). Cadmium, Zn, Cu, and Cr concentrations in PB exceeded CCME sediment quality guidelines by 93%, 189%, 233%, and 19.8%, respectively. Their low phytoextraction rates suggest that reducing concentrations of these trace elements to compliant levels will likely take a much longer timeframe compared with nutrients.

2.6 Conclusion

Results from this study show that biosolids from both the primary and the secondary end-of-life municipal lagoon cells can support a healthy vegetation growth and high biomass yields, which are critical for the attainment of successful remediation of the lagoons. There was a significant benefit of harvesting cattail and switchgrass, grown in PB and PBS, twice per growth cycle, as this resulted in a greater percentage removal (phytoextraction) of nutrients and trace elements. Results from the study further demonstrated that there was no significant benefit of adding soil to the biosolids (thus, essentially diluting the nutrients and trace elements by 50%), since biomass yields and nutrient and trace element uptake values were greater in PB than in PBS. This indicates that phytoremediation of municipal lagoons does not require the costly trucking and mixing-in of soil into the biosolids layer prior to plant establishment. Both cattail and

switchgrass were effective in N and P phytoextraction from the biosolids. Although, on average, switchgrass was able to take up more nutrients and trace elements than cattail, both plants were capable of phytoextracting only small amounts of trace elements. Nonetheless, it is well-established that trace elements such as Cd, Zn, Cu, and Cr accumulate preferentially in the belowground biomass, which is also a mechanism of phytoremediation. Our results show that switchgrass is a better option for terrestrial phytoremediation than cattail. This would also be expected under field conditions where drier conditions would tend to favour switchgrass ahead of cattail. Although contaminant concentrations were lower in SB than in PB, phytoremediation of SB would be expected to take longer due to lower biomass yields, hence low contaminant uptake. It is noteworthy that biomass yields obtained under controlled environmental conditions in this pot experiment were much lower than those expected under field conditions. Thus, the phytoextraction rates measured in this study underestimate those under field conditions, suggesting satisfactory remediation, particularly with switchgrass. Thus, overall, our results indicate that phytoremediation is a promising strategy for the remediation of municipal lagoons during decommissioning. If proven to work under field conditions, this option could greatly benefit small communities that either cannot afford the costly excavation, trucking, and spreading of biosolids on agricultural land or where disposal in landfills or incineration are unacceptable practices. More importantly, phytoremediation may prove to be the only viable option for communities where agricultural land is not available for spreading of biosolids.

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3. WETLAND-BASED PHYTOREMEDIATION OF BIOSOLIDS FROM AN END-OF-LIFE MUNICIPAL LAGOON: A MICROCOSM STUDY

3.1 Abstract

This growth room study examined the effectiveness of a wetland system for the in situ remediation of biosolids from an end-of-life municipal lagoon. The wetland microcosm experiment tested factorial combinations of biosolids type [primary cell (PB) and primary cell mixed (50/50 by dry wt.) with soil (PBS)] and harvest frequency (one harvest vs. two harvests per growing cycle) in a completely randomized design. Two controls, which consisted of the biosolids types with no plants, were included for comparison. Cattail seedlings were transplanted into pots containing 4.54 kg (dry wt.) of biosolids, above which a 10-cm deep water column was maintained. Measurements were taken weekly to determine biogeochemical parameters in the sediment and in the water column. Plants were harvested for biomass yield and phosphorus (P), nitrogen (N), and trace element determination. Results showed that cattail plants phytoextracted more nutrients and trace elements from PB than from PBS, reflecting the higher concentrations in the PB. Two harvests per cycle significantly increased N, P, and trace element phytoextraction relative to a single harvest. Overall, the three cycles of cattail, equivalent to three growing seasons, removed about 2.1% of total P originally present in the biosolids and 3.8% of initial N content. These phytoextraction rates are expected to be higher under field conditions where biomass yields are much higher than obtained under growth room conditions in this study. These results indicate that wetland-based phytoremediation can effectively clean up nutrients from biosolids, and therefore presents a potential alternative

to the spreading of biosolids on agricultural land, which may not be readily available in some communities. Phytoextraction rates of trace elements, however, were much lower and ranged from 0.01% to 0.51% for Zn, Cu, Cd, and Cr. The rest of the environmentally important trace elements were below concentrations considered harmful to aquatic life.

3.2 Introduction

Municipal lagoons are constructed structures that store and treat domestic, commercial, and, in some cases, industrial waste (Cameron et al., 2003). These structures are widely used by small municipalities and rural communities across the world because of their low cost and ease of operation. The life expectancy of municipal lagoons ranges from 20-30 years (Ross, et al., 2003), after which they are decommissioned. Decommissioning of municipal lagoons requires removal and disposal of biosolids. Biosolids are the organic, stabilized material produced following treatment of domestic sewage sludge. While they are a good soil-conditioner and a source of plant nutrients (Cui et al., 2008), biosolids also contain both organic and inorganic contaminants from various domestic and, in some cases, commercial sources.

During lagoon decommissioning, biosolids are often spread on agricultural land to provide nutrients to crops. However, the requisite dewatering, stabilization, and trucking of biosolids to agricultural land makes land spreading an expensive approach that can also lead to secondary contamination of the environment during transportation. Recently, there has been growing interest in the use of constructed wetlands for the removal of trace elements from sediments and soils. The use of constructed wetlands for biosolids

treatment, a phenomenon that has not been tested to date, may be a promising, less expensive, in situ alternative to the land spreading of biosolids. This is because it involves lower operating and maintenance costs and presents no risk of secondary contamination (Cui et al., 2008).

Constructed wetlands are a common and productive ecosystem that serves as a sink, source and transformer of nutrients (Reddy and DeLaune, 2008). They are a complex ecosystem which functions with a number of physical, chemical and biological processes. Unlike other ecosystems, wetlands have the tendency to accumulate organic matter more rapidly (Mitsch and Gosselink, 2007). They are unique in their ability to treat large volumes of water at relatively low cost. Constructed wetlands can be designed for specific purposes, such as wildlife habitat and wastewater treatment and storage (Reddy and DeLaune, 2008). They are relatively easy to construct, environmentally friendly, and cost- and energy-effective in removing nutrients, making them a preferred alternative to the spreading of sludges on agricultural land (Nichols, 1983). Constructed wetlands possess certain properties that make them suitable for treating wastewaters, such as high plant productivity; large adsorptive surface on sediments, plants and roots; aerobic and anaerobic interfaces; and various active microbial populations (Maehlum et al., 1995).

There is growing interest in the development of techniques with minimal environmental side effects, such as wetland-based phytoremediation, for remediation of contaminated sites (Cui et al., 2004). Phytoremediation utilizes the ability of certain plant species to remove, degrade, or immobilize harmful chemicals in contaminated soils, sludges,

sediments, and ground water (Zavoda et al., 2001). Phytoremediation presents great potential for the in situ remediation of municipal lagoons where biosolids are rich in nutrients such as N and P and contain trace elements in concentrations that are not restrictive to plant growth. The increased biodiversity in constructed wetlands gives them aesthetic value. Ensuring a good combination of higher plants, lower plants, and bacteria allows for detoxification and, to an extent, helps to control eutrophication (Horne et al., 2000). Wetlands with a range of plants (rooted and unrooted) tend to remove more nitrate (NO_3) N than wetlands with just one plant type (Reddy and DeLaune, 2008). For the effective removal of nutrients by wetland plants, there is a need to harvest at the end of each growing season to prevent a drawback of nutrients into the sediments (Ciria et al., 2005).

Nutrient and trace element removal in a constructed wetland system can be enhanced by judiciously selecting the wetland plant species to be utilized (Deng et al., 2004). Cattail is a large emergent plant found in wetlands and drainage ditches across North America, with the ability to produce high biomass yields within a growing season. Cattail sequesters carbon from the atmosphere and takes up nutrients from the sediments as it grows, incorporating these components into its plant biomass (Grosshan et al., 2011). Cattail has been effectively utilized for extraction of trace elements and environmentally important nutrients such as P. It has been used for phytoextraction of metals from waste water (Ye et al., 2001a) and metalliferous waters (Manios et al., 2003). Cattail absorbs significant amounts of P, which causes eutrophication issues in aquatic systems (Nichols,

1983). Repeated harvesting of cattail has been observed to enhance its growth (Martin et al., 2003; Grosshan, 2014).

The process of nitrification/denitrification removes N from constructed wetlands while P is removed by adsorption (Reddy and DeLaune, 2008). Nitrification is a microorganism-mediated redox reaction that occurs at the water-sediment interface. The resulting NO_3 diffuses into the anaerobic zone where denitrification takes place. The presence of lower and higher plants in wetlands enhances N removal (Reddy and DeLaune, 2008). One major benefit of denitrification is its ability to control the intensity of eutrophication. Plant biomass, growth rate, and plant type are factors that determine how effectively vegetation will affect denitrification (Bachand and Horne, 2000). Old decaying plant parts can increase the rate of denitrification. On the contrary, the presence of submerged vegetation limits the rate of denitrification. This is because oxygen produced by plants during photosynthesis will create an aerobic environment which is unfavourable to denitrification (Bastviken et al., 2005).

The main mechanisms of P retention in a wetland system are adsorption and precipitation reactions (Nichols, 1983). Phosphorus sorption processes and the solubility of P are governed by pH. In acidic conditions, the solubility of iron and aluminum P compounds determines the bioavailability of P (Racz, 2006). In alkaline conditions, the solubility of calcium phosphates controls phosphate bioavailability (Racz, 2006). Phosphorus transformation processes are affected indirectly by redox conditions through the reduction of iron in anaerobic conditions (Racz, 2006). Microorganisms, periphyton, and

vegetation play an important role in the short term recycling of P (Reddy and DeLaune, 2008). Vegetated constructed wetland structures have been observed to be more efficient in nutrient removal than unvegetated structures (Henderson et al., 2007).

Trace element removal is enhanced by plants through filtration, adsorption, and cation exchange (Du Laing et al., 2009). The anoxic soil in constructed wetlands creates conditions that enable the mobilization of trace elements in a highly reduced form (Deng et al., 2004). In addition to ecological benefits, constructed wetlands improve water quality by reducing pollutant loads (Schulz and Peall, 2000; Wood and Shelley, 1999). Despite these benefits, to our knowledge, no study has examined the potential of constructed wetlands for in situ treatment of biosolids during the decommissioning of municipal lagoons.

This growth room experiment was carried out to mimic a wetland system for the remediation of an end-of-life primary municipal lagoon. Specific objectives were to:

(i) Characterize contaminant dynamics in a wetland microcosm system under controlled environment conditions;
(ii) Quantify changes in contaminant concentrations in biosolids under a single harvest of wetland plants vs. two harvests per growth cycle (season); and
(iii) Compare contaminant dynamics in 100% biosolids vs. biosolids mixed with soil (50/50 by dry wt.).

3.3 Hypotheses

i. A wetland-based phytoremediation system will reduce nutrient and trace element concentrations in the biosolids;

ii. Two harvests per growth cycle will improve phytoremediation of biosolids compared with a single harvest of wetland plants per growth cycle.

iii. A combination of soil and biosolids will enhance the effectiveness of phytoremediation.

3.4 Materials and Methods

3.4.1 Biosolids

The biosolids used in this experiment were collected from the primary cell of an end-of-life municipal lagoon in Niverville, MB, Canada; while the Black Chernozemic clay soil (Udic boroll; 0 to 15-cm) used to prepare the biosolids/soil mixture (PBS treatment described below) was sampled from a site 0.5 km from the lagoon. The lagoon was constructed in 1971 as a combined sewer system (two-cell) (Bodnar, 2006) and the decommissioning process was initiated in 2008 (Keam and Whetter, 2008). At the start of decommissioning, the volume of biosolids in the primary cell was ~20,000 m^3. The biosolids were analyzed for physical and chemical properties, as described below, before the start of the experiment.

3.4.2 Cattail Seeds and Germination

Cattail seeds were extracted from seed fruits according to the method described by McNaughton (1968). Briefly, cattail fruits were blended for 30 s in a Contrad 70

detergent solution (Fisher Scientific, Devon, PA, USA) in a blender (Model 54227C, Hamilton Beach, CA, USA). This was followed by repeated washing of the seeds settled at the bottom of the blender with tap water, followed by reverse osmosis water. Seeds were germinated in a growth room set at a temperature of 25ºC and a relative humidity of 65%. Ten seeds per cell were sown into triplicate 12-cell seedling trays containing one of the biosolids treatments tested. The seedling trays were placed in plastic trays filled with water to provide watering from the bottom of the cells.

3.4.3 Experimental Design and Setup

The experiment was laid out in a completely randomized design with a 2 × 2 factorial treatment structure and three replications. The factors tested were biosolids type and harvest frequency. The biosolids treatments were (i) biosolids from the primary cell (PB) and (ii) a 1:1 mixture (dry wt. basis) of PB and soil (PBS). Two harvest systems were compared: (i) one harvest per growth cycle and (ii) two harvests per growth cycle. Two controls (checks), which consisted of the biosolids with no plants, were included for comparison.

Five weeks after germination, three cattail (*Typha latifolia*) seedlings were transplanted into each of the 18 plastic containers (microcosms, 27.5 cm diameter × 32 cm height) containing 4.54 kg (dry wt.) of PB or PBS (approximately 15 cm deep). The microcosms were placed in a growth room maintained at 22°C during the 12-h photoperiod and 15°C during the 8-h dark period. Humidity was set at 65% and daytime light intensity at 270 μmole photons m^{-2} s^{-1}. The containers were weighed every other day to determine and

replace any moisture lost via evapotranspiration. Starting 9 days after transplanting, the target moisture content was gradually increased, initially by 20% and subsequently by progressively larger amounts, in order to gradually introduce wetland conditions. The final water level in each wetland microcosm was maintained at 10 cm above the biosolids surface. The experiment ran for an equivalent of three growing seasons, with each season (growth cycle) lasting 90 d. At the end of each growth cycle, biosolids samples were collected from each microcosm and immediately stored at 4°C.

3.4.4 Plant, Biosolids, and Water Analysis

At the end of each cycle, aboveground biomass was harvested from each microcosm by cutting the stems 5 cm above the water/biosolids interface using a knife. Root samples were taken at the end of Cycle 3. Harvested biomass was dried in the oven at 65°C for 72 h, weighed for dry matter yield, and finely ground (< 0.2 mm) using a SPEX 8000D ball mill (SPEX SamplePrep LLC, Metuchen, NJ, USA). Ground plant tissue samples were digested with aqua regia (concentrated HNO_3/HCl) in a microwave oven (MARS 5, CEM Corp., Matthews, NC, USA) and analysed for trace element concentrations by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Sciex ELAN 6000, MA, USA). Total P in digests was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES; Vista-MPX, Variance Analytical Instruments, Mulgrave, Victoria, Australia).

For determination of total Kjeldahl N (TKN) and total P (TP) concentrations, biosolids samples were digested with 30% H_2O_2 (APHA et al., 2005). Total Kjeldahl N

concentration in the digestate was measured using a Technicon autoanalyzer (Technicon AA II, Technicon Instrument Corporation, Tarrytown, NY, USA), while TP was measured using the molybdate method at a wavelength of 882 nm with a spectrophotometer (Ultrospec 2100 pro, Biochrom Ltd, Cambridge, UK). Available N (NO_3-N + NH_4-N) concentration was determined with the autoanalyzer described above following extraction with 2 M KCl (Keeney and Nelson, 1982). Biosolids pH and electrical conductivity (EC) were measured in a 1:2 (wt./vol.) biosolids:water suspension using an Accumet pH/conductivity meter (AP85, Fisher Scientific, Singapore). Redox potential of the biosolids in the microcosms was measured weekly using platinum and reference electrodes connected to a digital meter (Model 52-0060-2, Mastercraft Inc., Mississauga, ON, Canada).

Water samples were analyzed in situ weekly for dissolved oxygen (DO), redox potential (Eh), pH, and EC. Redox and pH were measured using a YSI Professional Plus probe (YSI Inc., Ohio, USA) while DO was determined using a YSI Pro ODO probe. Electrical conductivity of the water column was determined using a conductivity meter (Accumet AP85, Fisher Scientific, Singapore).

3.4.5 Statistical Analysis

Data were analysed using PROC MIXED for repeated measures in SAS 9.3 (SAS Institute, 2014), with growth cycle as the repeated factor. The heterogeneous compound symmetry (CSH) covariance structure was used to model the repeated measures effect for P, Cu, and Zn uptake, while the first-order autoregressive [AR(1)] structure was used for

Cr uptake, the toeplitz (TOEP) for N uptake and biomass yield, and the unstructured [UN(1)] for all variables measured in the biosolids. For water chemistry data, sampling time was the repeated factor, and the compound symmetry (CS) covariance structure was used for all aquatic chemistry parameters. Data for P, Cd, Cu, and Zn uptake by cattail, and NO_3-N and NH_4-N concentrations in the biosolids were not normally distributed, according to the Shapiro-Wilk test from PROC UNIVARIATE, and required natural log-transformation to approximate a normal distribution. Treatment means were compared using the Tukey multiple comparison procedure. Treatment effects were considered significant if $P < 0.05$.

3.5 Results

3.5.1. Biosolids and Soil Properties

Approximately 98% of inorganic N in the biosolids was in the NO_3 form (Table 3.1)

The three most abundant trace elements in the biosolids were, in order of increasing concentration, cadmium (Cd), copper (Cu) and zinc (Zn). Trace element and nutrient concentrations were higher in the PB than in the PBS. The biosolids and the soil used in the experiment had pH values ranging from 7.4-8.2, with the soil having the highest pH and the PB having the lowest (Table 2.2). Primary biosolids had the highest mean EC (4.84 dS m^{-1}), followed by soil (4.75 dS m^{-1}) and PBS (3.29 dS m^{-1}).

Table 3.1 Initial nutrient and trace element concentrations (mg kg^{-1}) in the biosolids and soil.

Medium†	TKN	NH_4-N	NO_3-N	TP	OP‡	Cd	Cu	Zn	Cr	Pb	pH	EC
	mg kg^{1}											dS m^{-1}
Soil	2565	0.9	32.9	605	10.7	0.43	37.1	115	52.9	15.2	8.2	4.75
PB	6000	29.4	551	2730	139	1.16	119	356	44.7	25.5	7.36	4.84
PBS	3700	26.4	329	1590	78	0.75	70.1	212	45.3	20.3	7.57	3.29
SQG¶	550	-	-	600	-	0.6	35.7	123	37.3	35	-	-

† PB, biosolids from the primary cell; PBS, 1:1 mixture of PB and soil; SB, biosolids from the secondary cell.
‡ OP, Olsen P.
¶ SQG, CCME Sediment Quality Guideline (CCME, 1998)

Table 3.2 Aboveground cattail biomass yield (dry wt.), nutrient uptake and trace element uptake as affected by growth cycle, biosolids treatment and harvest frequency in the wetland microcosms.

Effect	Biomass	N	P	Ca	Mg	Cd	Cu	Cr	Zn	Pb
	g DM pot^{-1}				mg pot^{-1}					
Cycle										
1	42.3	313	98.1	358	85.8	0.0005	0.26	0.05b	1.15	0.01a
2	27.9	272	46.5	213	66.1	0.0002	0.16	0.08 a	0.48	0.004a
3	19.7	234	48.4	170	59.2	0.0001	0.11	0.05b	0.33	0.003b
†Biosolids treatment										
PB	38.2	347a‡	85.4	320a	86.9	0.0003	0.25	0.04b	0.90a	0.003b
PBS	21.7	199b	43.3	174b	53.8	0.0002	0.11	0.08a	0.41b	0.01a
Harvest										
Single	28.0	221	53.3	245	64.2	0.0003	0.17	0.17a	0.62	0.003a
Two-harvest	31.9	325	75.3	249	76.5	0.0002	0.18	0.18a	0.69	0.01b
					P value					
Cycle(C)	<0.001	0.05	<0.001	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	<0.001
Biosolids (B)	<0.001	<0.001	0.001	<0.001	0.004	0.46	<0.001	0.001	<0.002	<0.002
Harvest(H)	0.72	0.001	0.01	0.44	0.19	0.26	0.31	0.25	0.16	<0.001
C × B	0.001	0.08	0.03	0.09	0.01	0.29	0.02	0.13	0.06	0.78
C ×H	0.003	<0.001	0.003	0.04	0.01	0.07	0.01	0.08	0.001	0.15
B × H	0.88	0.14	0.88	0.61	0.72	0.96	0.76	0.78	0.89	0.67
C ×B × H	0.22	0.28	0.09	0.23	0.73	0.08	0.18	0.19	0.37	0.59

†PB = biosolids from the primary cell; PBS = 1:1 mixture of PB and soil.

‡Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

3.5.2 Cattail Biomass

The effect of growth cycle on biomass yield varied with biosolids treatment, as indicated by the significant cycle × harvest interaction (P = 0.003) (Table 3.2). Aboveground biomass yield in PB decreased significantly in the order Cycle 1 > Cycle 2 > Cycle 3. Cattail biomass yield in PB exceeded that in PBS by > 100% in Cycle 1 and by 59% in Cycle 2, while yield difference between the two biosolids treatments was not significant in Cycle 3. Biomass yield of cattail grown in PBS was significantly greater in Cycle 1 than in Cycle 3, but there was no significant difference between Cycles 1 and 2 and between Cycles 2 and 3 (Fig 3.1).

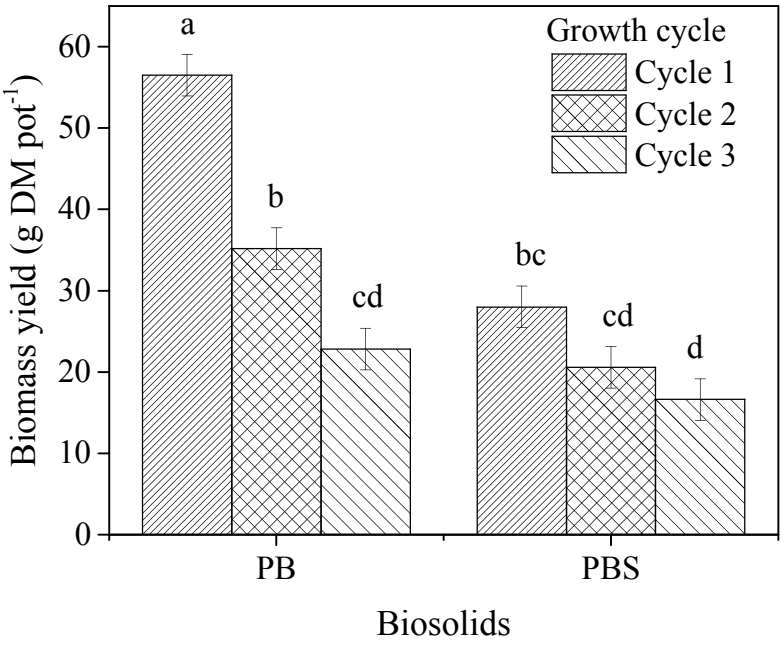

Figure 3.1 Cattail aboveground biomass yield, averaged across harvest frequencies, as affected by growth cycle and biosolids. Error bars represent standard errors of the means. Bars with the same letter are not significantly different (P ≥ 0.05) according to the Tukey multiple comparison procedure.

The effect of harvest frequency on biomass yield varied with growth cycle, as indicated by the significant cycle × harvest interaction (P = 0.003) (Table 3.2). Two harvests per growth cycle produced significantly greater yields than a single harvest in Cycle 1 and Cycle 3, whereas no significant difference was observed between harvest frequencies in Cycle 2 (Fig 3.2). For the single harvest treatment, there was no significant change in biomass yield from Cycle 1 to Cycle 2, but there was a significant decrease in the yield in Cycle 3 compared to the previous two cycles. By comparison, for the two-harvest treatment, biomass yield decreased significantly from Cycle 1 to Cycle 2 but did not change significantly in Cycle 3 relative to Cycle 2.

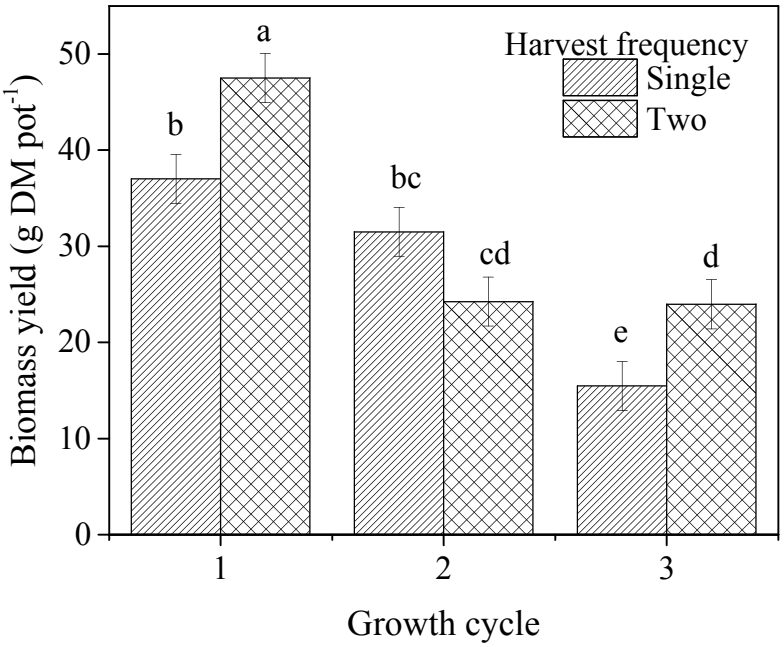

Figure 3.2 Cattail aboveground biomass yield as affected by growth cycle and harvest frequency (averaged across biosolids treatments). Error bars represent standard errors of the means. Bars with the same letter are not significantly different (P ≥ 0.05) according to Tukey multiple comparison procedure.

3.5.3 Nitrogen Uptake

There was a significant cycle × harvest interaction ($P < 0.001$) for N uptake (Table 3.2). Nitrogen uptake by cattail in Cycles 1 and 3 was significantly greater for two harvests than for a single harvest (Fig. 3.3). In Cycle 2, there was no significant difference in N uptake between the two harvest frequencies. Under a single harvest, N uptake did not change significantly in Cycle 2 relative to Cycle 1, but decreased significantly in Cycle 3 compared with Cycle 2. By comparison, when cattail was harvested twice per growth cycle, N uptake decreased significantly in Cycle 2 relative to Cycle 1 but did not change significantly in Cycle 3 relative to Cycle 2.

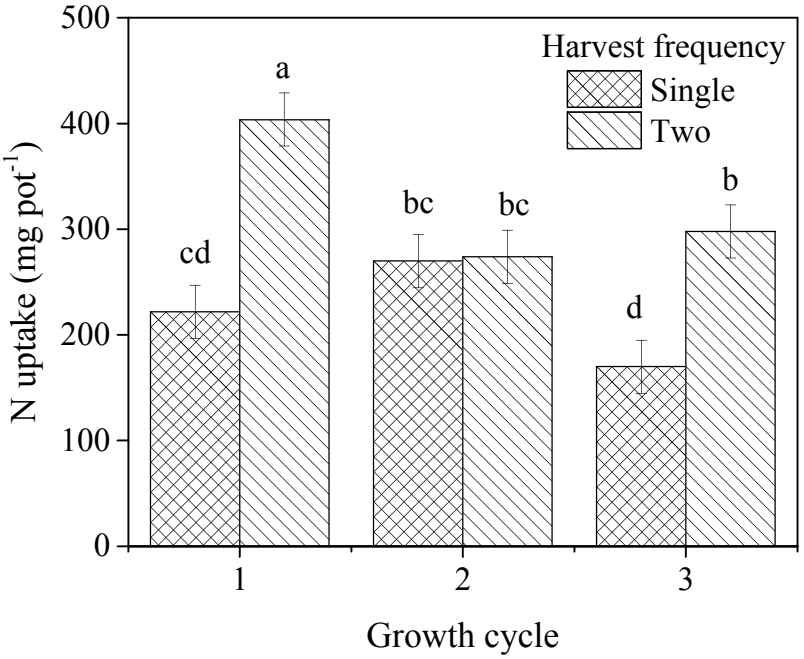

Figure 3.3 Harvest frequency and growth cycle effects on N uptake by cattail, averaged across biosolids. Error bars represent standard errors of the means. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

3.5.4 Phosphorus Uptake

The effect of growth cycle on P uptake varied with biosolids, as indicated by the significant (P = 0.03) cycle × biosolids interaction (Table 3.2). Phosphorus uptake from PB was significantly greater in Cycle 1 than in Cycles 2 and 3 while P uptake from PBS was significantly greater in Cycle 1 than in Cycle 2 (Fig. 3.4).

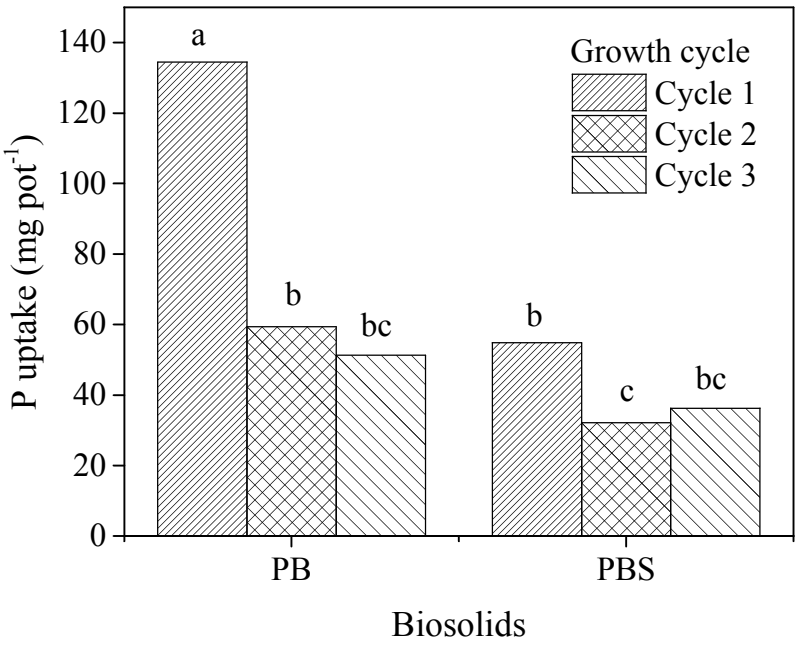

Figure 3.4 Geometric mean P uptake (averaged across harvest frequencies) as affected by biosolids and growth cycle. Bars with the same letter are not significantly different (P ≥ 0.05) according to the Tukey multiple comparison procedure.

There was a significant (P = 0.003) cycle × harvest frequency interaction for P uptake (Table 3.2). Harvesting cattail twice resulted in a significantly greater P uptake than a single harvest in Cycles 1 and 3 (Fig. 3.5). In Cycle 2, there was no significant difference in P uptake between the harvest frequencies. Harvesting cattail once resulted in

significantly greater P uptake in Cycle 1 than in Cycles 2 and 3, whereas there was no significant difference between Cycles 2 and 3.

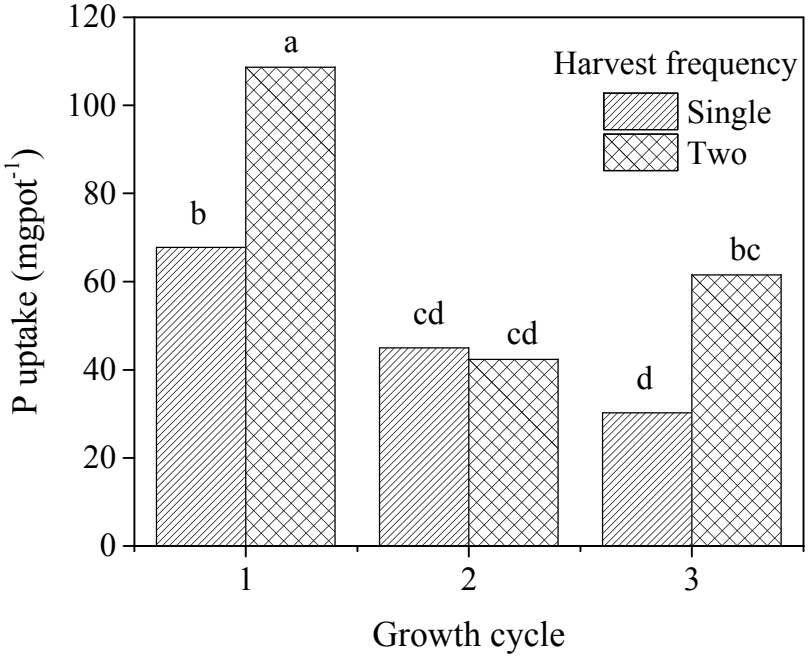

Figure 3.5 Harvest frequency and growth cycle effects on geometric mean P uptake (averaged over plant species and harvest frequencies). Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

3.5.5 Copper Uptake

Growth cycle effect on Cu uptake varied with biosolids treatment, as indicated by the significant ($P = 0.023$) cycle × biosolids interaction (Table 3.2). Copper uptake from PB decreased in the order Cycle 1 > Cycle 2 > Cycle 3 (Fig 3.6). In PBS, the uptake of Cu by cattail was significantly greater in Cycle 1 than in Cycles 2 and 3 but did not differ significantly between the latter two cycles. Copper uptake from PB exceeded that from

PBS by 153% in Cycle 1 and by 83% in Cycle 2 but did not differ significantly between biosolids in Cycle 3.

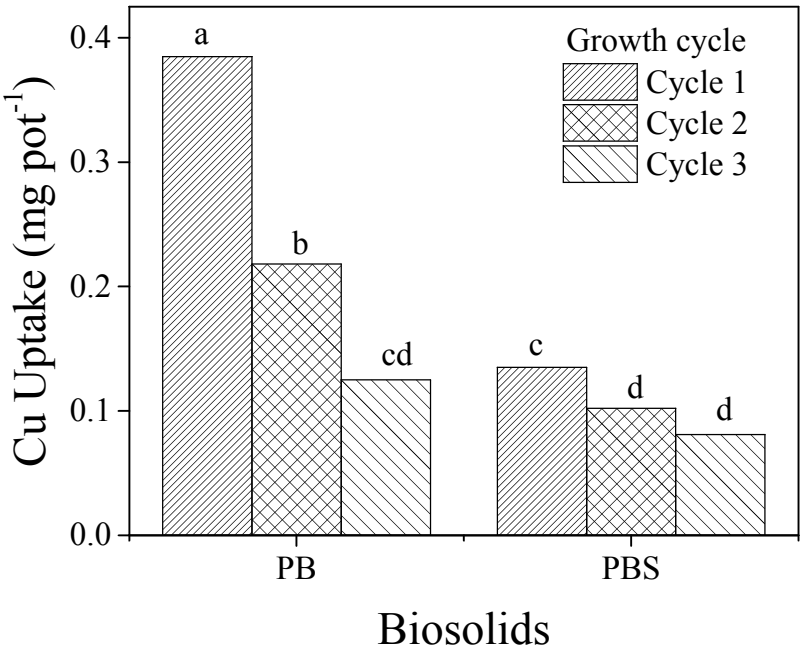

Figure 3.6 Geometric mean Cu uptake, averaged across harvest frequencies, as affected by biosolids and growth cycle. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

Averaged across amendments, Cu uptake generally decreased with growth cycle, but the effect of cycle depended on harvest frequency, as indicated by the significant cycle × harvest interaction ($P = 0.01$) (Table 3.2). Under a single harvest per cycle, there was a significant decrease in Cu uptake with each successive cycle (Fig. 3.7). With two harvests per cycle, however, a significant decrease in Cu uptake occurred in Cycle 2 relative to Cycle 1 but there was no significant change in Cu uptake in Cycle 3 compared with Cycle 2.

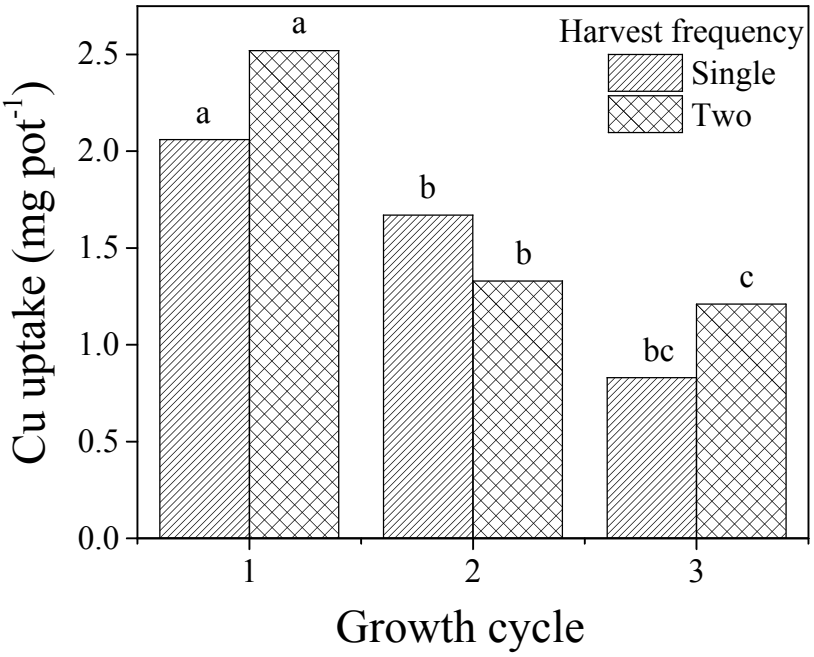

Figure 3.7 Geometric mean uptake of Cu as affected by harvest frequency and growth cycle (averaged over biosolids treatment). Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

3.5.6 Zinc Uptake

The effect of harvest frequency on Zn accumulation in the aboveground biomass differed with growth cycle, as indicated by the significant cycle × harvest frequency interaction ($P = 0.001$) (Table 3.2). In Cycle 1, there was no significant difference in Zn uptake between harvest frequencies (Fig. 3.8). By comparison, Zn uptake was significantly greater under the single harvest in Cycle 2 and under the two-harvest treatment in Cycle 3. For the single harvest treatment, there was a significant decrease in Zn uptake with each successive cycle. In contrast, under the two-harvest treatment Zn uptake decreased

significantly in Cycle 2 relative to Cycle 1 but did not change significantly in Cycle 3 compared with Cycle 2.

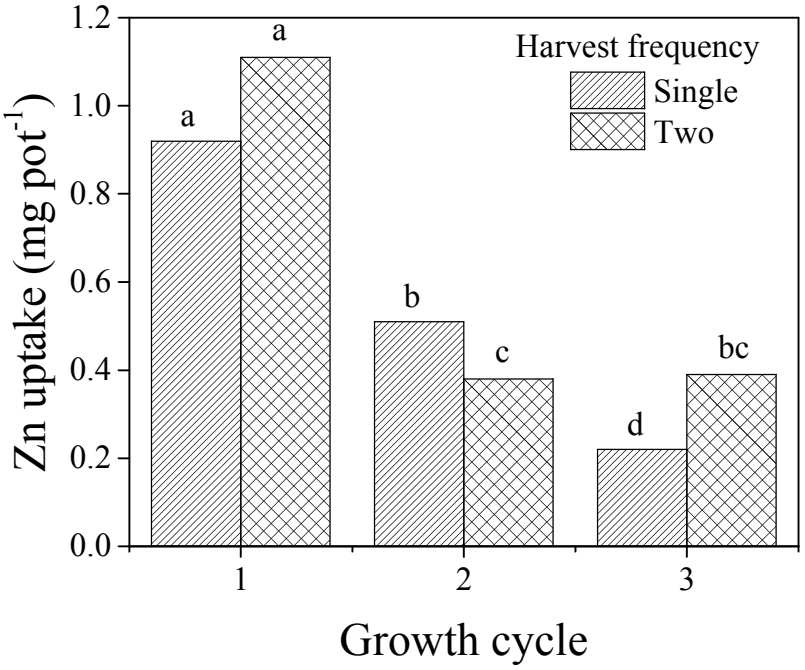

Figure 3.8 Geometric mean Zn uptake, averaged across biosolids, as affected by harvest frequency and growth cycle. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

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Table 3.3 Decrease in nitrogen, phosphorus, and trace element concentrations in biosolids as affected by biosolids type and harvest frequency.

Effect	ΔN†	ΔP	ΔCd	ΔCu	Δ Zn	ΔCr
			mg pot^{-1}			
Biosolids‡ treatment						
PB	1040a¶	256a	0.001a	0.75a	2.69a	0.23a
PBS	596b	130b	0.001a	0.36b	1.22b	0.13b
Harvest						
Single	662b	160b	0.001a	0.55a	1.85a	0.19a
Double	975a	226a	0.001a	0.52a	2.05a	0.16a
			P value			
Biosolids treatment (B)	<0.001	<0.001	0.44	<0.001	<0.001	0.01
Harvest (H)	0.002	0.01	0.74	0.57	0.34	0.24
B × H	0.14	0.74	0.61	0.53	0.85	0.77

† ΔN = percent decrease in biosolids N concentration; ΔP = percent decrease in biosolids P concentration; ΔCd = percent decrease in biosolids Cd concentration; ΔCu = percent decrease in biosolids Cu concentration; ΔZn = percent decrease in biosolids Zn concentration; ΔCr = percent decrease in biosolids Cr concentration.

‡ PB = biosolids from the primary cell; PBS = 1:1 mixture of PB and soil.

¶ Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

3.5.7 Cumulative nitrogen and phosphorus phytoextraction after 3 growth cycles

Cattail plants removed more N and P from PB than from PBS by the end of the three cycles (Table 3.3). Cattail also removed significantly more N and P when harvested twice compared with a single harvest.

3.5.8 Cumulative phytoextraction of trace elements after 3 growth cycles

After three cycles, cattail removed significantly ($P < 0.001$) more Cu and Zn, averaged across harvest frequencies, from PB than from PBS (Table 3.3). Harvest frequency had no significant effect on trace element phytoextraction.

Table 3.4 Percent decrease in nitrogen (ΔN), phosphorus (ΔP), and trace element (ΔCd, ΔCu, ΔZn and ΔCr) concentrations in biosolids as affected by biosolids type and harvest frequency.

Effect	ΔN†	ΔP	Δ Cd	ΔCu	Δ Zn	ΔCr
			%			
Biosolids‡treatment						
PB	3.82	2.07	0.04a¶	0.46a	0.51a	0.23a
PBS	3.33	1.80	0.01b	0.06b	0.08b	0.13b
Harvest						
Single	2.90b	1.58b	0.03	0.25	0.28a	0.19
Two harvest	4.25a	2.28a	0.03	0.26	0.31a	0.16
			P value			
Biosolids treatment (B)	0.15	0.20	0.002	<.0001	<.0001	0.01
Harvest (H)	0.002	0.01	0.77	0.86	0.53	0.24
B × H	0.49	0.60	0.97	0.81	0.82	0.77

† ΔN = percent decrease in biosolids N concentration; ΔP = percent decrease in biosolids P concentration; ΔCd = percent decrease in biosolids Cd concentration; ΔCu = percent decrease in biosolids Cu concentration; ΔZn = percent decrease in biosolids Zn concentration; ΔCr = percent decrease in biosolids Cr concentration

‡ PB = biosolids from the primary cell; PBS = 1:1 mixture of PB and soil.

¶ Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

3.5.9 Phytoextraction of Nitrogen and Phosphorus

Total N and P removal (percent of N and P initially present in the biosolids) during the three growth cycles was significantly greater for two cattail harvests than for a single

harvest. There was no significant difference in N and P phytoextraction between PB and PBS (Table 3.4).

3.5.10 Phytoextraction of trace elements

The percentage of Cd, Cu, Cr and Zn removed from biosolids during the three growth cycles was significantly greater for PB than for PBS but did not vary with harvest frequency (Table 3.4).

3.5.11 Belowground biomass yield and N, P and trace element uptake

After three growth cycles, the cumulative root biomass of plants grown in PB was ~46% greater than that of plants grown in PBS, but the difference between the two biosolids was not significant (Table 3.5). Similarly, there was no significant difference in nutrient and trace element uptake by the below ground biomass between PB and PBS (Table 3.5).

Table 3.5 Biomass, nutrient and trace element uptake in roots after three growth cycles

Biosolids† treatment	Biomass	Mg	N	P	Ca	Cr	Cu	Zn	Cd	Pb
	g DM pot^{-1}				mg pot^{-1}					
PB	72.0	430	436	381	1071	0.29	1.79	8.55	0.02	1.21
PBS	49.3	260	243	186	576	0.18	0.88	2.68	0.01	0.13
					P value					
Biosolids treatment	0.16	0.15	0.17	0.06	0.08	0.23	0.06	0.06	0.11	0.34

†PB = biosolids from the primary cell; PBS = 1:1 mixture of PB and soil.

Table 3.6 Effect of harvest frequency on nitrogen, phosphorus, and trace element concentrations in biosolids after three growth cycles.

Effect	NO_3-N	NH_4-N	AN	TKN	TP	Avail P	Cd	Cu	Cr	Zn	Pb
						mg kg^{-1}					
Cycle											
1	4.62	15.1	16.9	7111	1990	170a†	1.004	112	48.7	291	20.2
2	0.13	10.6	10.8	3711	1280	157b	1.02	129	54.5	314	20.6
3	1.28	126	123	5205	2066	184a	1.04	103	41.4	273	19.1
Biosolids treatment‡											
PB	0.83	48.7	49.6	6130	2343	197a	1.31a	148	46.9	375	23.0a
PBS	0.61	31.6	32.2	4316	1216	132b	0.73b	77.6	48.3	208	17.5b
Harvests											
Single	1.83	23.4	24.0	4651	2068	176	1.02	115	50.9	303	20.0
Two	2.19	77.8	76.0	6034	1524	166	1.02	111	45.7	282	20.0
						P value					
Cycle (C)	<0.001	<0.001	<0.001	<0.001	<0.001	0.01	0.14	<0.001	<0.001	0.002	0.30
Biosolids (B)	0.99	0.16	<0.001	<0.001	<.0001	<0.001	<0.001	<0.001	0.64	<0.001	<0.001
Harvest (H)	0.21	0.63	<0.001	<0.001	<0.001	0.51	0.33	0.01	0.001	0.001	0.22
C × B	0.79	0.56	<0.001	<0.001	0.01	0.66	0.36	0.06	0.004	0.03	0.14
C ×H	<0.002	<0.001	<0.001	<0.001	<0.001	0.08	0.02	0.004	0.005	0.001	0.21
B × H	0.62	0.57	<0.001	0.05	0.001	0.55	0.76	0.65	0.002	0.03	0.14
C × B × H	0.69	0.76	<0.001	0.01	0.002	0.94	0.16	0.02	0.003	0.05	0.48

†Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

‡ PB = biosolids from the primary cell; PBS = 1:1 mixture of PB and soil.

3.5.12 Nutrient and trace element concentrations in biosolids after three growth cycles

3.5.12.1 Available Nitrogen

The effect of harvest frequency on NO_3-N concentration in the biosolids varied with growth cycles, as indicated by the significant growth cycle × harvest frequency interaction ($P < 0.001$). There was no significant difference in NO_3-N concentration between harvest frequencies in Cycle 1. In Cycle 2 and Cycle 3, NO_3-N concentration was significantly greater when cattail was harvested twice compared with a single harvest. The highest NO_3-N concentration was measured at the end of Cycle 3 when cattail was harvested twice.

Averaged over biosolids, the effect of harvest frequency on NH_4-N concentration varied with growth cycle, as indicated by the significant cycle × harvest frequency interaction ($P < 0.001$) (Table 3.5). Ammonium N concentration was significantly greater with a single harvest compared with two harvests per growth cycle (Fig. 3.10). In Cycle 3, NH_4-N concentration in biosolids was significantly greater with two harvests than with a single harvest. Generally, across the three cycles, the highest concentration of NH_4-N was observed at the end of Cycle 3 under two harvests.

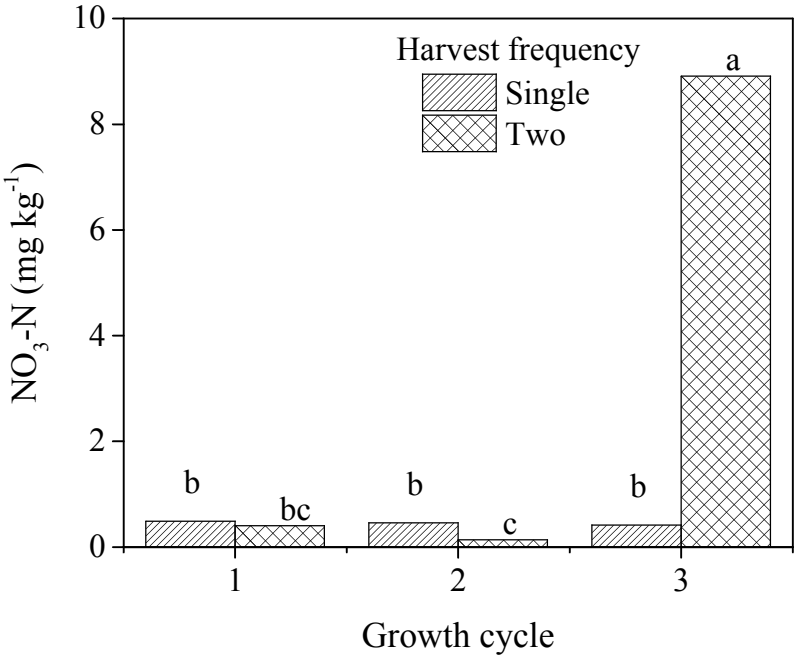

Figure 3.9 Geometric mean NO_3-N concentration as affected by growth cycle and harvest frequency averaged over biosolids. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

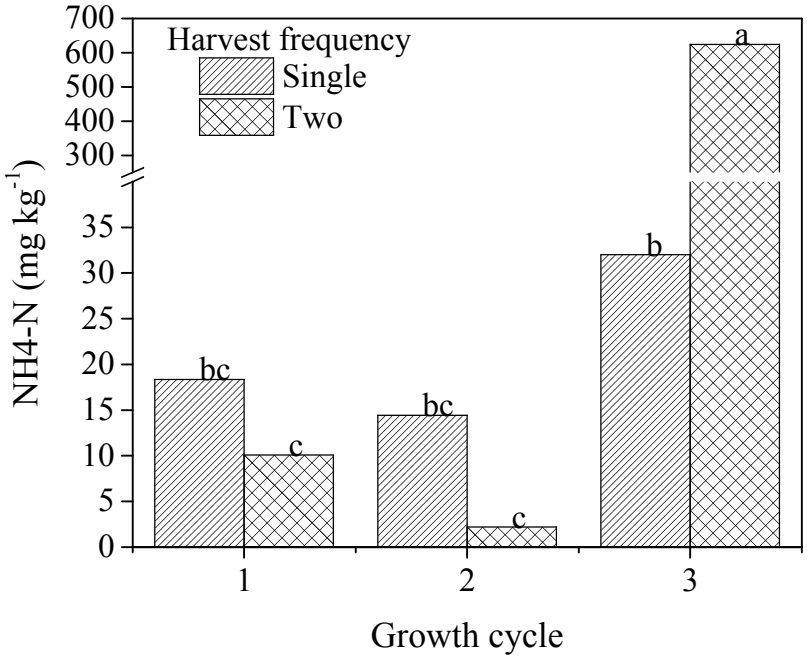

Figure 3.10 Geometric mean concentration levels of NH4-N as affected by growth cycle and harvest frequency averaged over two media. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

3.5.12.2 Total Kjeldahl Nitrogen

The effect of harvest frequency on biosolids TKN concentration varied with growth cycle and biosolids treatment as indicated by the significant ($P = 0.01$) cycle × biosolids × harvest frequency interaction (Table 3.5). In Cycle 1, TKN concentration was greater under a single cattail harvest than with two harvests, whereas the opposite was true for TKN concentration in PBS (Fig. 3.11). In Cycle 2, there was no significant difference in TKN concentration between harvest frequencies irrespective of biosolids treatment, while TKN concentrations were greater in PB than PBS regardless of harvest frequency. In

Cycle 3, harvesting cattail twice resulted in significantly greater TKN concentrations compared with a single harvest, regardless of biosolids treatment.

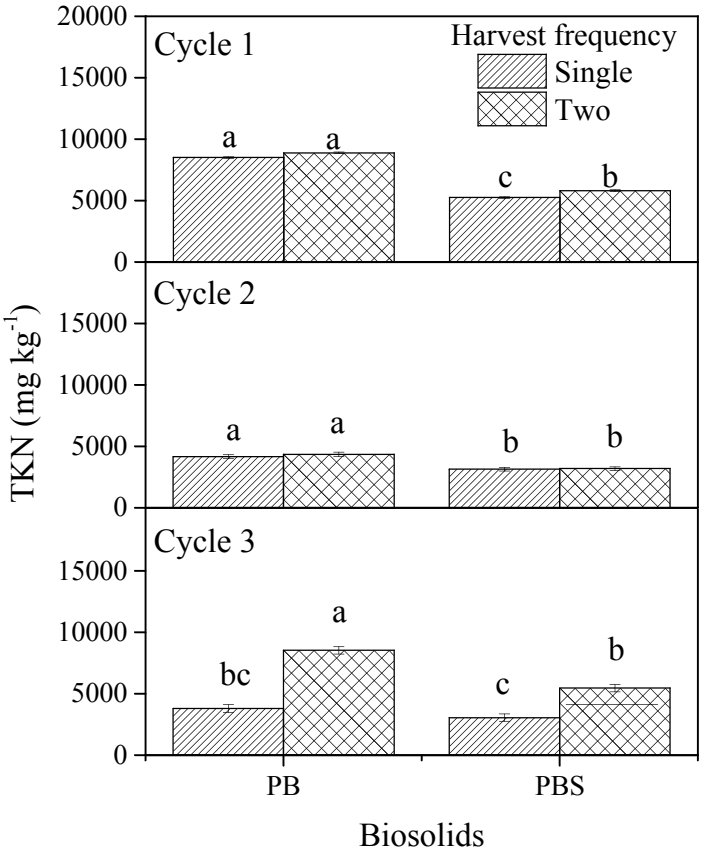

Figure 3.11 Growth cycle, biosolids treatment, and harvest frequency effects on TKN concentration in biosolids. Error bars represent standard errors of the means. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

3.5.12.3 Total P concentration in biosolids

There was a significant ($P = 0.002$) cycle × biosolids × harvest frequency interaction for TP concentration in biosolids (Table 3.5). At the end of Cycles 1 and 3, TP

concentrations were ~50% higher in PB than in PBS irrespective of harvest frequency (Fig 3.12). However, there was no significant difference in TP concentration between harvest frequencies in both biosolids in the two cycles. By comparison, TP concentration at the end of Cycle 2 was significantly greater for two harvests than a single harvest, regardless of biosolids treatment.

3.5.12.4 Copper concentration

There was a significant ($P = 0.02$) growth cycle × harvest frequency × biosolids interaction for Cu concentration in biosolids (Table 3.5). At the end of Cycle 1, Cu concentration was ~50% greater in PB than in PBS, regardless of harvest frequency (Fig. 3.13). Harvest frequency had a significant effect only in PB in Cycle 2 in which Cu concentration was 20% greater with one harvest than with two harvests. Treatment effects in Cycle 3 mirrored those in Cycle 1(Fig 3.13).

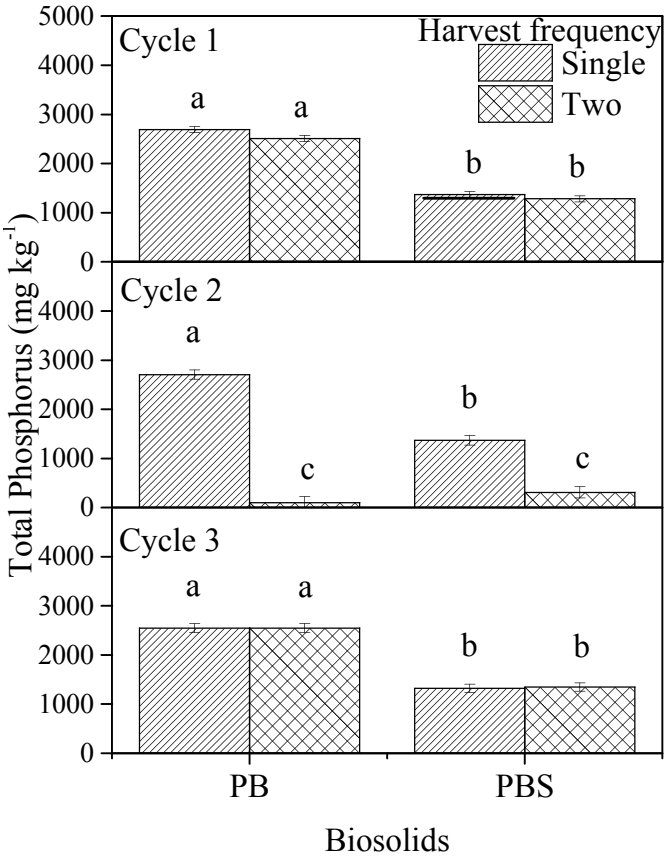

Figure 3.12 Total P concentration as affected by growth cycle, harvest frequency, and growth cycle. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

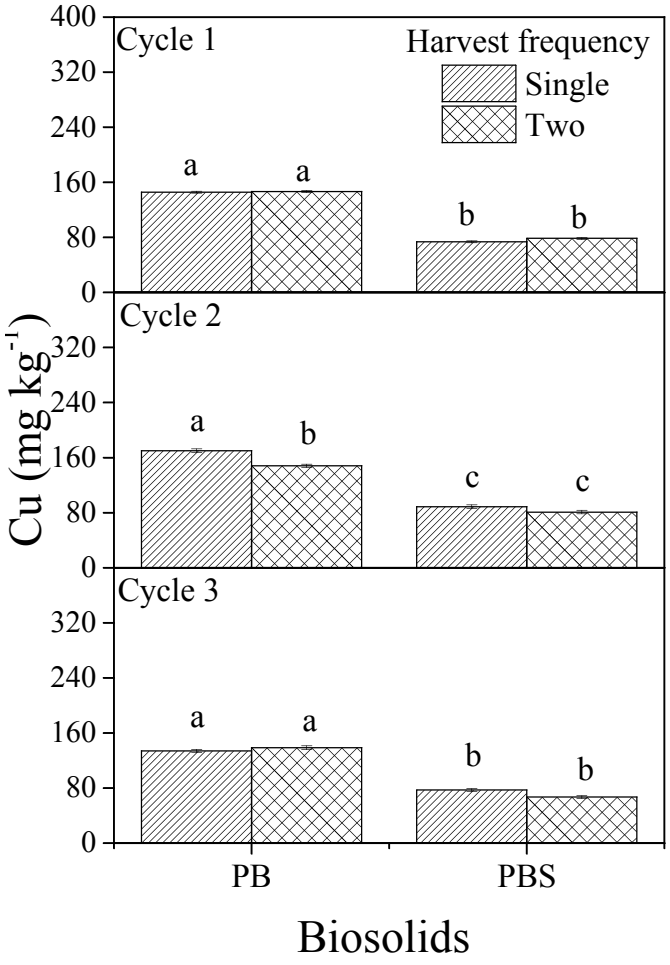

Figure 3.13 Biosolids Cu concentration as affected by growth cycle, harvest frequency, and biosolids. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

3.5.12.5 Chromium

The effect of harvest frequency on Cr concentration in biosolids varied with biosolids treatment and growth cycle, as indicated by the significant ($P = 0.003$) harvest frequency × cycle × biosolids interaction (Table 3.5). At the end of Cycle 1, there was no significant difference in Cr concentration between harvest frequencies, regardless of biosolids

treatment (Fig 3.15). At the end of Cycle 2, the single harvest resulted in a significantly greater Cr concentration in the biosolids compared with two harvests, regardless of biosolids treatment. At the end of Cycle 3, Cr concentration in PBS was ~150% greater with a single harvest than with two harvests, whereas harvest frequency had no significant effect on Cr concentration in PB.

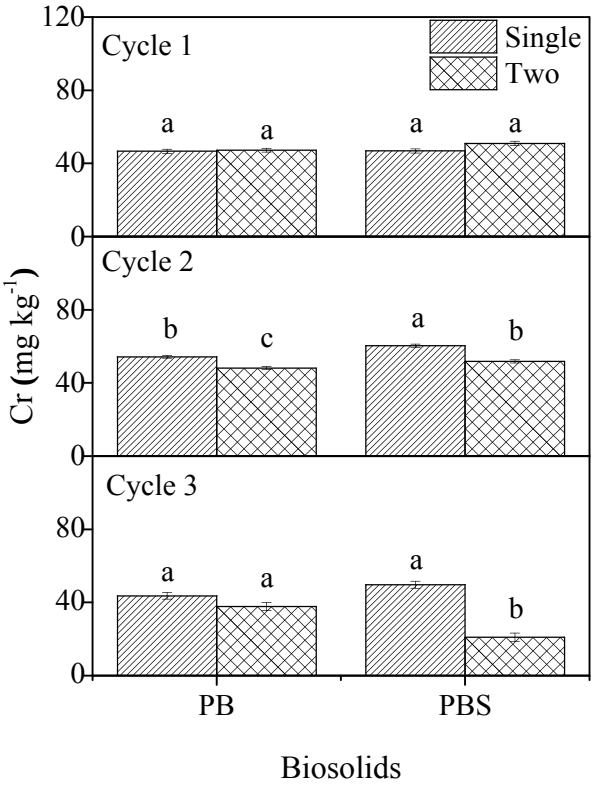

Figure 3.14 Biosolids Cr concentration as affected by growth cycle, harvest frequency, and biosolids treatment. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

3.5.12.6 Zinc concentration

Averaged over growth cycles, the effect of harvest frequency on Zn concentration differed with biosolids treatment, as shown by the significant ($P = 0.03$) biosolids ×

harvest frequency interaction (Table 3.5). Zinc concentration was significantly greater in PB than in PBS regardless of harvest frequency (Fig. 3.16). Zinc concentration was also greater under a single harvest compared with two harvests in the PB. In the PBS, there was no significant difference in Zn concentration between harvest frequencies.

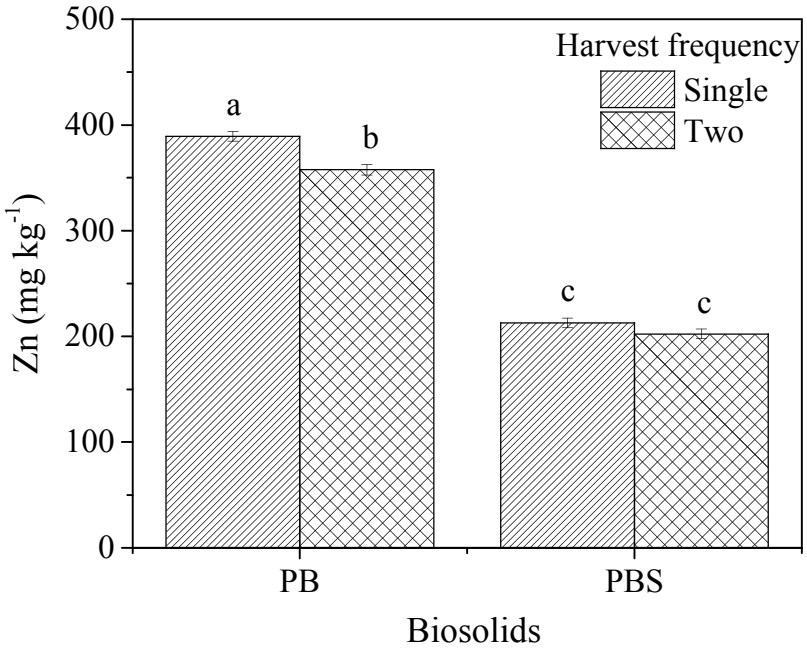

Figure 3.15 Biosolids Zn concentration, averaged across growth cycles, as affected by harvest frequency and biosolids treatment.. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

There was a significant biosolids × growth cycle interaction ($P < 0.001$) for Zn concentration in biosolids, averaged across harvest frequencies (Table 3.5). Zinc concentration in PB was significantly greater at the end of Cycle 2 than at the end of Cycle 3 but did not differ significantly between Cycle 1 and Cycle 2 and between Cycle 1 and Cycle 3 (Fig. 3.17). By comparison, there was no significant difference among cycles in PBS. Zinc concentration was significantly higher in PB than in PBS, regardless of growth cycle.

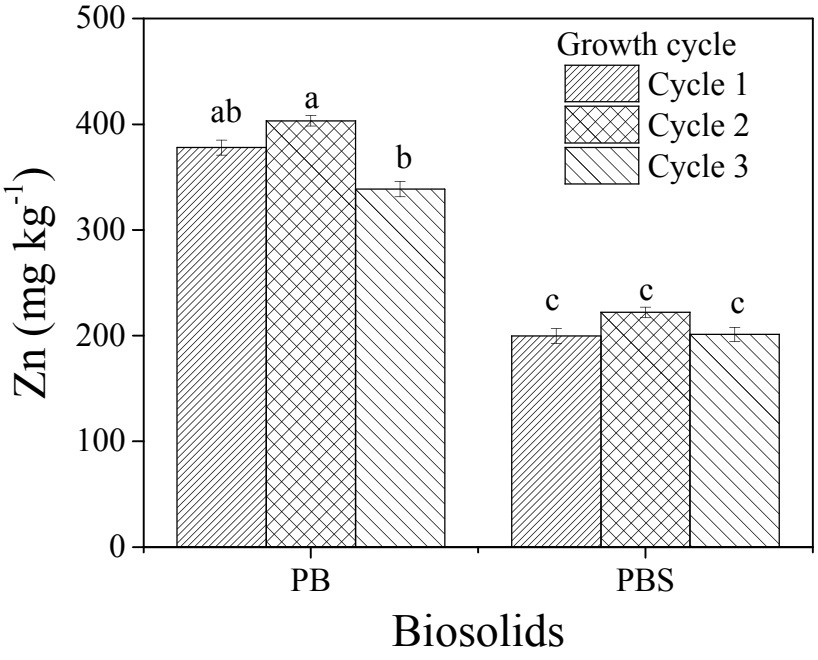

Figure 3.16 Biosolids Zn concentration as affected by growth cycle and biosolids treatment. Bars with the same letter are not significantly different ($P \geq 0.05$) according to the Tukey multiple comparison procedure.

3.5.13 Electrical conductivity, pH and Eh of water column

The effect of harvest frequency on wetland microcosm water EC varied with biosolids treatment and time, as indicated by the significant ($P < 0.001$) biosolids × harvest frequency × sampling time interaction (Table 3.7). Throughout the sampling period, higher EC values were measured in unvegetated than in vegetated PB and PBS regardless of harvest frequency (Fig. 3.17). Electrical conductivity under two harvests was significantly greater than that under a single harvest of cattail in PB and PBS. While the EC in the unvegetated biosolids tended to increase with sampling time, the EC in vegetated biosolids decreased during the first 5 wk of sampling in both biosolids. Water pH ranged from 7.8 to 8.5 (Fig 3.18). There was a spike in the sediment redox potential in the vegetated pots from Week 6 (-100 mV) to Week 7 (-40 mV), after which there was a gradual fluctuation in the Eh (Fig 3.19).

There was a significant harvest frequency × sampling time interaction ($P < 0.001$) for water redox potential (Eh) (Table 3.7). This was due to larger and significant differences in water Eh between harvest frequencies in weeks 17-19, whereas Eh differences at previous sampling times were not significant (Fig. 3.20). Averaged over harvest frequencies, the effect of biosolids treatment on redox potential in the water column varied with time, as indicated by the significant biosolids × time interaction ($P = 0.001$) (Table 3.7). Differences in Eh among biosolids treatments were significant only after week 16 (Fig 3.21).

Averaged over biosolids treatments, the effect of time on DO differed with harvest frequency, as indicated by the significant harvest × time interaction ($P = 0.01$) (Table

3.7). From Week 4 to Week 7, DO concentration in the control was significantly greater than in the vegetated pots (Fig 3.22). From Week 11 to Week 15, DO was significantly greater in the single harvest vegetated pots than in the control pots and the two harvest pots. After 20 wk, DO concentration in the control was significantly greater than that in the vegetated pots. Dissolved oxygen concentration was significantly greater under two harvests per cycle than DO under a single harvest.

Table 3.7 Biosolids treatment, harvest frequency, and sampling time effects on EC, pH, redox potential, and dissolved oxygen concentration in the wetland microcosms.

Effect	EC (dS m^{-1})	pH	Eh (mV)	Eh_s† (mV)	DO (mg L^{-1})
Biosolids treatment‡					
PB	1.66	8.08a¶	91.6	-130b	13.4a
PBS	1.03	8.17a	93.2	-99.5a	12.9a
Harvest					
Single	0.89	8.02a	88.0	-107a	11.0
Two harvest	1.03	8.02a	93.0	-98.7a	10.7
Control	2.84	8.34a	95.7	-139a	17.9
			P value		
Biosolids treatment (B)	0.02	0.16	0.36	0.04	0.95
Harvest (H)	0.06	0.99	0.35	0.49	0.72
Time(T)	<0.001	<0.001	<0.001	0.001	<0.001
B × H	0.72	0.81	0.95	0.55	0.81
B × T	0.04	0.11	<0.0001	0.53	0.08
H × T	<0.0001	0.08	0.0001	0.83	0.01
B × H × T	<0.0001	0.59	0.54	0.88	0.33

Eh_s† oxidation-reduction potential in biosolids treatment. Time (T) was excluded due to excess levels.

‡ PB = biosolids from the primary cell; PBS = 1:1 mixture of PB and soil.

¶ Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

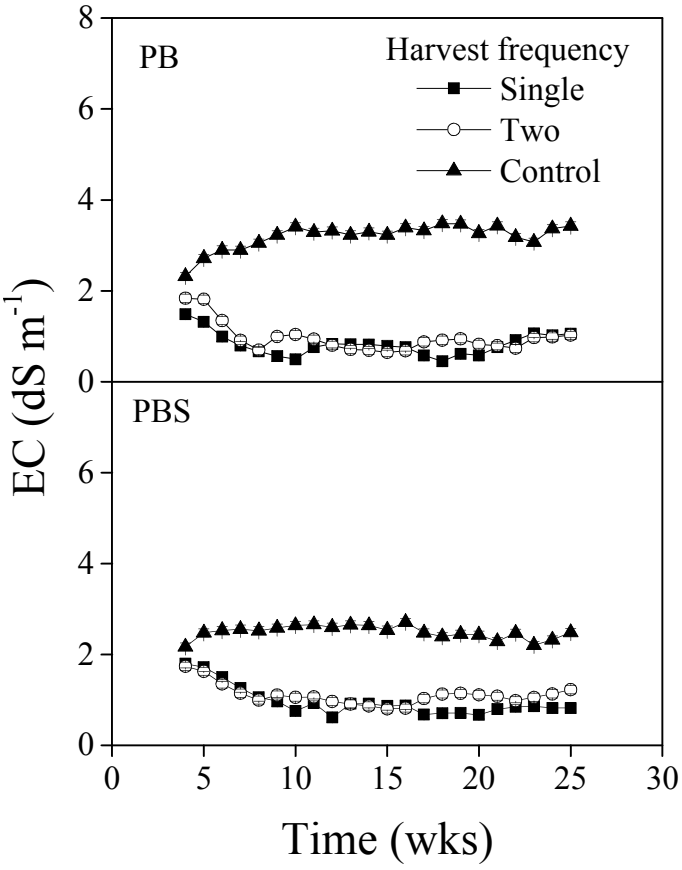

Figure 3.17 Microcosm water EC as affected by biosolids treatment, sampling time, and harvest frequency. Error bars represent standard errors of means.

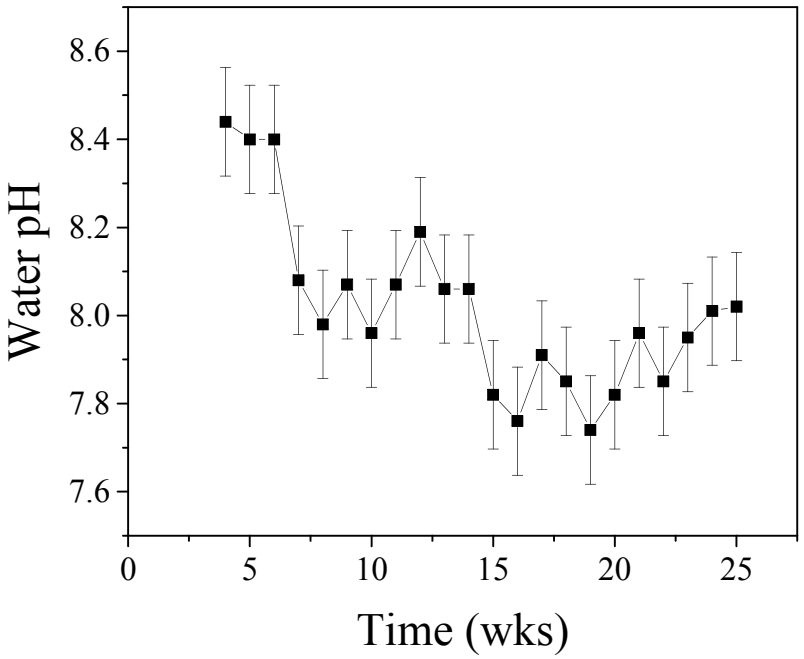

Figure 3.18 Effect of sampling time on water pH averaged over biosolids treatment and harvest frequency. Error bars represent standard errors of means.

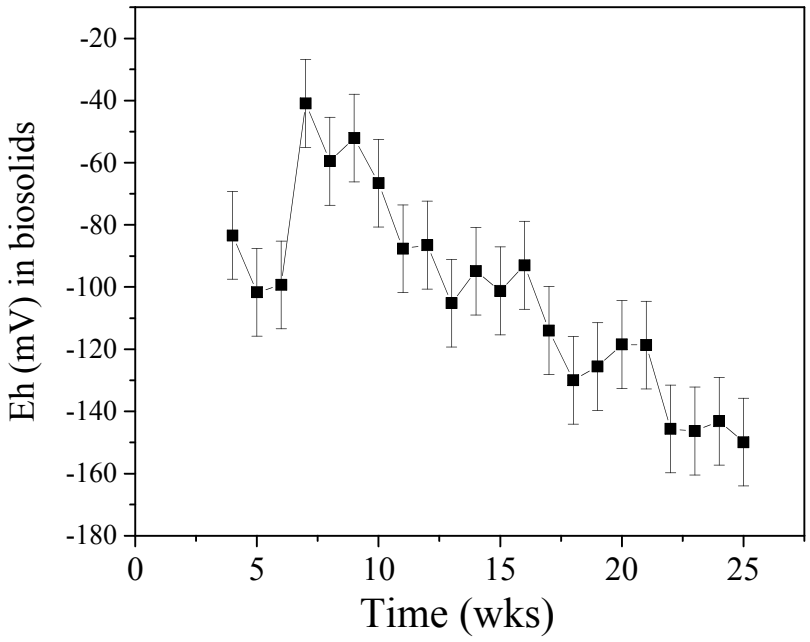

Figure 3.19 Effect of sampling time on water Eh averaged over biosolids treatment and harvest frequency. Error bars represent standard errors of means.

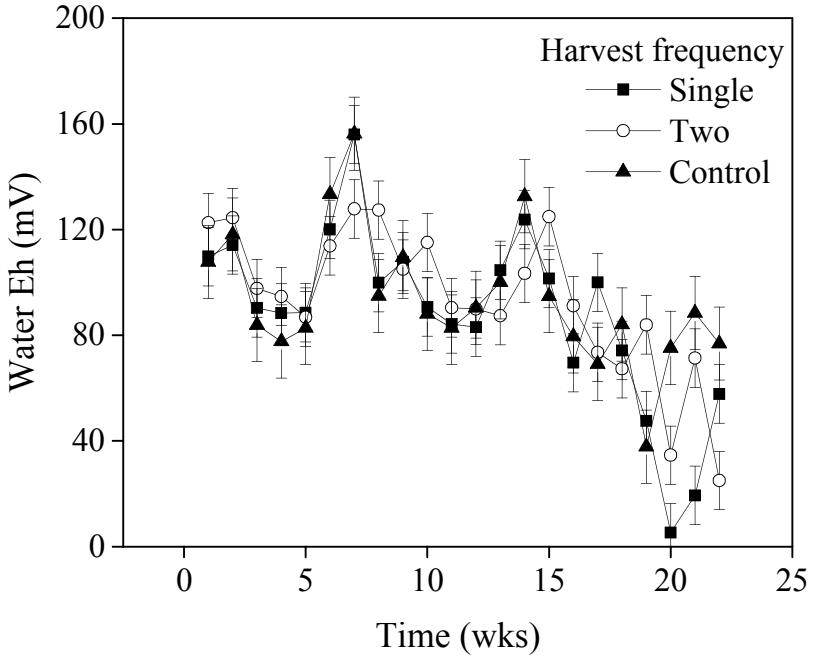

Figure 3.20 Microcosm water redox potential, averaged across biosolids treatments, as affected by sampling time and harvest frequency. Error bars represent standard errors of means.

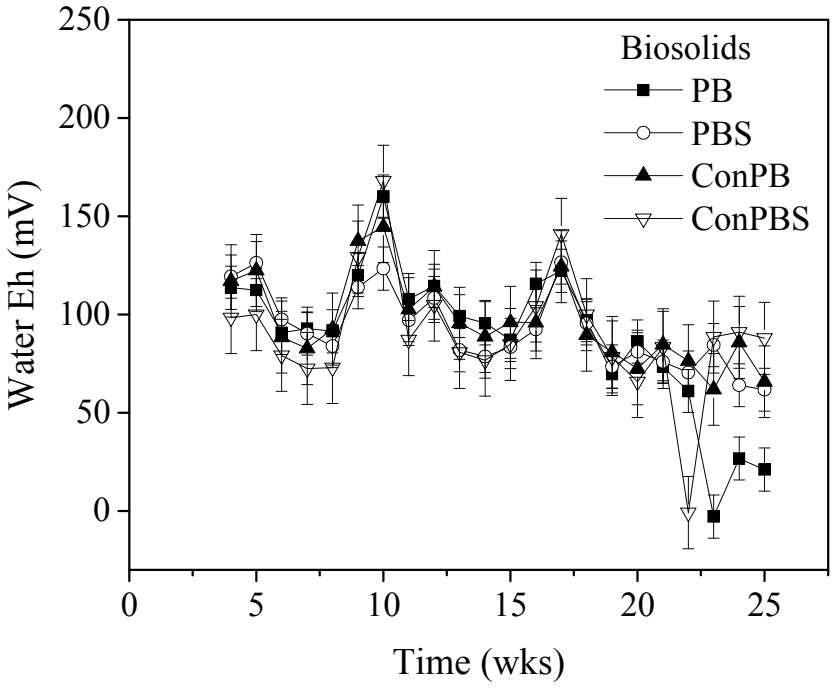

Figure 3.20 Microcosm water redox potential, averaged across harvest frequencies, as affected by sampling time and biosolids treatment. Error bars represent standard errors of means.

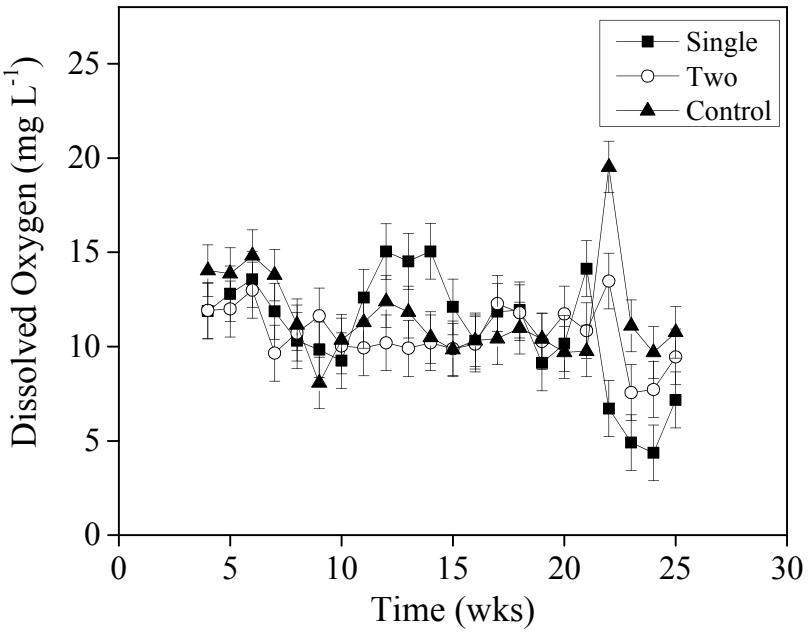

Figure 3.21 Effects of harvest frequency and sampling time on DO concentration, averaged over biosolids treatments, over a 22-week period. Error bars represent standard errors of means.

3.6 Discussion

3.6.1 Biosolids Characterization

The higher concentrations of trace elements and nutrients (N and P) measured in PB than in PBS was likely due to the dilution effect of the soil mixed with the PB. On average, nutrient and trace element concentrations measured in this study were ~100-300% lower than those measured in a decommissioned wastewater treatment lagoon in Steinbach, MB (Sahulka and Keam, 2013). This difference is likely due to the much larger service/retail industries, which include a large automobile retail industry, in the City of Steinbach.

More than 98% of available N in the biosolids was in the NO_3 form, implying that plant uptake of N was mostly in the NO_3 form. This result is consistent with Liphadzi et al. (2002), who observed that the concentration of NH_4-N in an animal lagoon soil decreased once lagoon operation ceased and the water dried out. The drying out improves aeration in the biosolids, resulting in NH_4-N being converted to NO_3-N by nitrification. Contrasting results have been reported for biosolids in an end-of-life municipal lagoon in Steinbach, MB, in which a greater percentage of available N was NH_4-N (Sahulka and Keam, 2013). Nitrogen is a big concern in decommissioned lagoons because it can contaminate ground water.

Chromium concentration in this study was ~23% lower than in the afore-mentioned biosolids from Steinbach. The disparities may be due in part to the larger population of Steinbach (13,500 vs. 3,500 for Niverville as of 2011) and its large automobile retail industry.

Total Kjedahl nitrogen and total P were above the lowest effect level (LEL) expected of sediments in aquatic life, which is 600 mg kg^{-1} for TKN and 500 mg kg^{-1} for total P (Persaud et al., 1993). Cadmium (1.16 mg kg^{-1}), Cu (119 mg kg^{-1}), Cr (45 mg kg^{-1}), and Zn (123 mg kg^{-1}) concentrations in PB exceeded the CCME sediment quality guidelines for aquatic life (0.6 mg kg^{-1} for Cd, 35.7 mg kg^{-1} for Cu 37.3 mg kg^{-1} for Cr, and 123 mg kg^{-1} for Zn) (CCME 1998).

3.6.2 Cattail Biomass

The higher cattail biomass measured in the PB than in the PBS reflected the higher nutrient concentrations in the PB. The fast growing cattail has high nutrient requirements

to sustain its vigorous growth (Grosshans et al., 2011; Miao, 2004). However, cattail also has a low nutrient efficiency (Lorenzen et al., 2001; Miao, 2004), which likely restricted biomass yields in the low-nutrient PBS.

In two (Cycles 1 and 3) of the three growth cycles examined in the present study, biomass yield was greater with two harvests than with a single harvest. This is consistent with a previous study that showed that cattail had the ability to regrow immediately after harvest when it was harvested two times in a growing season, with an interval of 4 weeks between harvests (USFWS, 2009).

The progressive decrease in biomass yield in successive growth cycles was likely due to the weakened state of the plants after repeated harvesting. By contrast, Grosshans et al., (2011) reported that harvesting of cattail stimulated further growth in the next season. This contradiction reflects the limited volume of the containers used in the present microcosm study, which meant that there was a limited amount of nutrients available to sustain continued growth after repeated harvesting. Additionally, unlike in the study by Grosshans et al. (2011) where the cattail plants had been established for years, our plants were established from seed and had a limited amount of time to build belowground biomass and store nutrient reserves necessary for sustained regrowth.

Harvesting cattail twice in a growth cycle produced significantly greater biomass yields than harvesting once, possibly because harvesting of cattail can stimulate further growth of plants since the new regrowth would emerge from an already established rhizome. Grosshan et al. (2011) reported cattail biomass yields of 1.5 kg DW m^{-2} in a coastal wetland compared with just 0.6 kg m^{-2} in PB and 0.4 kg m^{-2} in PBS in the present study,

which translates to ~2.5 times greater biomass yield in the field than in the growth room study. Similarly, Maddison et al. (2009) in Estonia reported 1.76 kg DW m^{-2} in autumn and 1.38 kg DW m^{-2} in winter, while Wild et al. (2002) reported 1.45 kg DW m^{-2} for cattail. The limited volume of biosolids (hence nutrients) in the present study and the lack of an off-season (winter) break may explain the lower biomass yields in the present study.

3.6.3 Nutrients

3.6.3.1 Nitrogen

The lower NO_3-N concentrations measured in the wetland microcosm sediments at the end of the experiment compared with the initial concentration were likely partly due to plant uptake and denitrification. Bastviken et al. (2005) pointed to denitrification as the principal pathway of NO_3 removal in wetland sediments. Higher NH_4-N concentrations in the control microcosms compared with vegetated microcosms are an indication that nitrification was taking place in the planted pots. Similarly, higher NO_3-N concentrations in the control compared with the vegetated microcosms at the end of Cycle 1 indicates that the efficiency of NO_3-N removal was higher in wetlands with vegetation than in non-vegetated systems (Yang et al., 2001; Li et al., 2004) . At the end of the study, most of the N in the biosolids was in the NH_4 form, which was expected under the anaerobic conditions (e.g., Cameron et al., 2003).

The 1.27% N removal rate obtained per growth cycle in this study is within the range (1-5%) reported by Maddison et al. (2009) in a study examining the removal of nutrients by

aboveground cattail biomass from a wastewater treatment wetland. Higher percentages of 9% removal were reported by Birch et al., (2004).

Overall, N removal by cattail after three growth cycles (equivalent to three growing seasons) accounted for 3.8% of N initially present in PB and 3.3% of initial N content in PBS. Based on the initial total N concentration of 6000 mg kg^{-1} in PB, which was above the 4800 mg kg^{-1} severe effect level (SEL), it would take 71 years to achieve the Ontario aquatic sediment quality guideline of 550 mg N kg^{-1} for the lowest effect level (LEL, which defines sediments as clean to marginally polluted) (Persuad et al., 1993), assuming the phytoextraction rate remained relatively constant from one growing season to another. However, faster removal rates are expected under field conditions. As mentioned above (Section 3.5.2), biomass yields 2.5 times those from the present study have been measured under field conditions in Manitoba (Grosshans et al., 2011). Based on this, the number of years to achieve the LEL would be expected to be proportionately fewer and closer to the 30-year timeline often considered normal for 85% phytoremediation (USEPA, 2012).

3.5.3.2 Phosphorus

Harvesting cattail twice resulted in a higher P uptake than a single harvest. This is because periodic harvesting of the aboveground cattail biomass enhances plant growth, hence improves biomass yields (Grosshans et al., 2011), which in turn enhances the removal of nutrients and trace elements. Phosphorus in the media is likely to have undergone adsorption-precipitation reactions (Nichols, 1983).

After three growth cycles, the amount of P removed from biosolids was 2.1% of that initially present in the PB and 1.8% of initial PBS P content. This was quite low compared to Birch et al. (2004), who reported a 12% removal rate. As pointed out previously for N, the low removal rates reflect the low biomass under restrictive conditions in the pot experiment.According to Ontario guidelines for the protection and management of aquatic sediments, the LEL for P is 600 mg kg^{-1} while the SEL is 2000 mg kg^{-1} (Persuad et al., 1993). At 2730 mg kg^{-1}, the initial P concentration in PB was above the SEL. Based on the removal rates measured in this study, it would take 51 years to reduce P concentration in PB to the SEL. However, with as much as 2.5 times greater biomass expected under field conditions in Manitoba (Grosshan et al., 2011), the timeline would be proportionately shorter. Additionally, and consistent with results from previous studies (e.g., Grosshan et al., 2011), a large fraction of P absorbed by plant roots was sequestered in plant roots, adding to the total removed from biosolids and therefore unavailable to cause immediate harm to the environment.

3.6.4 Trace elements

Zinc was taken up in the largest amount of all trace elements, while Cd uptake was the lowest. The relative uptake rates of trace elements, with Zn taken up in the largest amount and Cd in the lowest amount, reflected their initial concentrations in the biosolids. The low concentrations (Table A.2), hence accumulation, of trace elements in the aboveground biomass was likely due to their preferential accumulation in plant roots. Using biosolids from the same lagoon cell as used in the present study, Jeke et al. (unpublished data, 2014) demonstrated that 59% of Zn, 64% of Cu, 97.7% of Cd, and 61% of Cr absorbed by cattail roots was sequestered in the belowground biomass.

Similarly, Deng et al. (2004) examined the accumulation of trace elements by 12 wetland plants and found that a greater percentage of trace element amounts absorbed by wetland plants such as cattail accumulated in the root system compared with the shoots. Although trace element concentrations in roots were not adequately examined in the present study, results from roots samples collected in the two-harvest treatment in Cycle 3 indicate significantly higher concentrations and accumulation of trace elements in the roots than in the shoots. The high pH (7.36 – 8.2) of biosolids in the present study also likely contributed to the low bioavailability, hence uptake, of the trace elements (Racz, 2006).

Initial Cu, Cr, Cd, and Zn concentrations in PB exceeded the CCME sediment quality guidelines for aquatic life, which are 35.7 mg kg^{-1} for Cu, 37.3 mg kg^{-1} for Cr, 0.6 mg kg^{-1} for Cd, and 123 mg kg^{-1} for Zn (CCME, 1998). The rate of trace element removal in this study was very low, ranging from 0.04-0.51% in PB and 0.01-0.08% in PBS. These results corroborate those from a study by Maddison et al. (2009), who reported that harvesting of aboveground cattail biomass had no significant effect on the removal of Cd, Zn, Cu, and Pb in a constructed wetland treating wastewater.

3.6.5 Wetland Chemistry

3.6.5.1 Electrical Conductivity

Electrical conductivity of the water column was higher in the control pots than in the vegetated microcosms regardless of biosolids treatment. This was due to the higher salt content in the vegetated pots compared to the control (Bulc and Ojstršek, 2008). The EC values of 1.66 dS m^{-1} in PB and 1.03 dS m^{-1} in PBS are within the range (1.9-3.0 dS m^{-1}) reported by Ye et al. (2001b) in a constructed wetland treating coal combustion by-product leachate.

3.6.5.2 pH

The pH was higher in the microcosm water column than in the biosolids sediment. This was probably due to the presence of CO_2 from the respiration of algae and the presence of atmospheric CO_2 in the water column (Reddy and DeLaune, 2008). At the end of the experiment, water pH in both biosolids was 8.1, which is consistent with the CCME water quality guideline (pH 6.5-9.0) for the protection of aquatic life (CCME, 1987). Ye et al. (2001b) also reported a mean pH of 7.2 in a constructed wetland treating coal combustion by-product leachate. Hansen et al. (1998) reported pH values in the range of 7.2-7.5 over a 16 week period in a constructed wetland treating selenium.

Biosolids pH changed little during the experiment, decreasing only slightly albeit remaining in the alkaline range. After the experiment, biosolids pH ranged from 7.2-7.5. Within this pH range, trace elements in sediments are expected to be less toxic and less available for plant uptake (Reddy and DeLaune, 2008). Within the pH and Eh (-63 to -154 mV) ranges of the present study, Cr speciation was likely dominated by the less soluble and less toxic Cr(III) form (Reddy and DeLaune, 2008).

3.5.5.3 Redox Potential

The higher sediment Eh in the vegetated than in the control microcosms was likely due to better aeration facilitated by the root system in the vegetated microcosms (Reddy and DeLaune, 2008). On average, the redox potential during the 22-week experiment was greater in the water column (+50 to 147mV) than in the biosolids (-63 to -154 mV). This was due to the higher DO concentration measured in the water column. The higher organic C content of the biosolids enhances microbial activities that deplete oxygen in the biosolids (Ponnamperuma, 1972). The redox potential ranges of the sediment and water

column are within the range (< 200 mV) at which denitrification is known to occur (Kashem and Singh, 2001). In the fifth week following flooding, Eh values in the biosolids had decreased to less than 100 mV. This is in line with Reddy and DeLaune (2008), who reported that for sediments that are organic in nature, Eh in the biosolids can get as low as -100mV within a very short period of time after flooding compared to mineral soils (Reddy and DeLaune, 2008). The biosolids Eh range observed in this study was narrower than the range of +700 to -300 mV expected in wetland soils) (Reddy and DeLaune, 2008). The low Eh values measured in the present study were conducive to Fe, Mn, and sulphate reduction. This would in turn limit the bioavailability of trace elements, since Fe and Mn oxides can immobilize trace elements and provide sorption sites, while sulphide can form complexes with the trace elements to form insoluble sulphide salts (Van den Berg et al., 1998).

3.7 Conclusion

Results from this study indicate that the biosolids tested can support cattail growth in a constructed wetland system without the addition of soil. This obviates the costly trucking of soil into the lagoon for biosolids dilution purposes. Nutrient and trace element removal rates were greater when cattail was harvested twice compared with a single harvest per growth cycle. Based on expected biomass yields in the field of ~2.5 times those obtained in this microcosm study, we estimate that satisfactory phytoextraction of N and P from the biosolids would take between 20 and 30 years, assuming little change in biomass yields and nutrient uptake. Wetland-based in situ treatment of biosolids is a promising approach for small municipalities where stabilization ponds are the only means of

domestic waste treatment and for communities with limited or no access to suitable agricultural land to absorb the biosolids.

3.8 References

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4. GENERAL SYNTHESIS

4.1 Summary of Research

Environmentally friendly, safer, and less expensive alternatives to the widespread disposal of biosolids on agricultural lands during municipal lagoon decommissioning are a growing area of interest to municipalities and environmentalists. This is partly due to the high cost of trucking and land-spreading the biosolids, the risk of spreading pathogens in the environment while trucking, and increasing pressure on suitable agricultural land for spreading the biosolids. Terrestrial phytoremediation and wetland-based phytoremediation approaches were considered in this research, and tested on biosolids from an end-of-life municipal lagoon system. Terrestrial phytoremediation is examined in Chapter 2, while Chapter 3 addresses wetland-based phytoremediation as a strategy for removing nutrients and trace elements from the biosolids. Despite their demonstrated effectiveness in cleaning up contaminated soil, water, wastewater, and sediments (e.g., Ciria et al., 2005; Karathanasis et al., 2003; Poe et al., 2003), these approaches have not been studied for their potential to remove contaminants from end-of-life municipal lagoons.

Results from both the terrestrial phytoremediation and the wetland-based phytoremediation experiments indicate that primary biosolids from a typical municipal lagoon does not need amendment with soil to enhance plant growth. Predictably, cattail was able to thrive better in the wetland system than in the terrestrial phytoremediation experiment, consistent with its high moisture requirement. In the terrestrial phytoremediation experiment, switchgrass had higher biomass yield, averaged over the two growth cycles studied, and took up more nutrients and trace elements (Zn, Cu, Cd

and Cr) than cattail. For both the terrestrial and the wetland-based approaches, contaminant removal was greater with two harvests than with a single harvest.

4.2 Limitations of Study

Since this study was carried out in a growth chamber, there were a number of limitations: (1) there was no seasonal breaks between growth cycles, which adversely affected biomass yields, hence phytoextraction; (2) in the wetland system, a 10–cm deep fixed water column was considered compared to the fluctuating water systems expected in real life wetlands; and (3) the small size of pots used meant that a small amount of biosolids could be accommodated, which limited the amount of nutrients available to support plant growth, thus restricting plant growth.

4.3 Practical Implications

Assuming biomass yields remain virtually unchanged during treatment, results from the terrestrial phytoremediation experiment reported in Chapter two suggest field-equivalent timelines of 13-20 years if N and P concentrations were to be reduced to LELs of these nutrients, based on Ontario guidelines. These results suggest that switchgrass would be a better candidate for terrestrial phytoremediation. Another critical consideration in favor of switchgrass is its adaptability to dry conditions that would adversely affect cattail growth and biomass yields. While wet conditions are expected within the first year or two of lagoon use cessation, biosolids are expected to be drier in subsequent years, depending on precipitation. Under the drier conditions, switchgrass would be a more effective phytoremediant than cattail, unless supplemental irrigation is applied to enhance cattail growth.

Using the wetland-based approach (Chapter 3), phytoremediating to the LEL endpoints for N and P would take 20-30 years, which is considered acceptable for phytoremediation. It is noteworthy, though, that these numbers are based on aboveground accumulation of these nutrients and likely represent half the total amount of each nutrient absorbed from biosolids by plant roots. Studies indicate that about 50% of nutrients and trace elements absorbed by roots accumulate in the belowground biomass (Jeke et al., unpublished data, 2014; Shahandeh and Hossner, 2000), which is another mechanism of phytoremediation.

Overall, nutrient and trace element removal rates from the biosolids were higher with switchgrass in the terrestrial phytoremediation experiment than with cattail. This reflects the higher biomass yields of switchgrass. The implication of this is that switchgrass is a better option for terrestrial phytoremediation. Additionally, in remediating biosolids, the use of switchgrass for terrestrial phytoremediation would be a cheaper approach compared to the use of a constructed wetland, considering the cost of wetland construction.

4.4 Recommendations for further study

In Chapter 2 of this thesis, concentrations of nutrients and trace elements in the plant roots were not considered. Further studies should be carried out on the below-ground portions of plants as this will give an insight into the total amount of trace elements and nutrients removed from biosolids.

In the wetland experiment, the cattail plants emerging from the control pots were uprooted. Similar studies in the future should consider allowing any plants emerging in

the control to develop since, as we later observed, a control wetland in a real lagoon would behave similarly.

While controlled environment experiments offer the advantage of low complexity and provide important insights into expected behavior of treatments in the field, results do not directly translate to field expectations. For example, lower yields were realized in these experiments compared with those reported for field situations. It is therefore advisable to conduct field experiments to ground-truth results from the growth room studies.

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5 APPENDICES

Appendix A: Supplementary Tables

Table A.1 Concentrations of nutrients and trace elements in the aboveground biomass as affected by harvest frequency, plant species, biosolids treatment and growth cycle.

Effect	N	P	Cd	Cu	Cr	Zn
	mg g^{-1}		mg kg^{-1}			
Cycle						
1	1.47	0.32	0.18	6.99	2.83	67.4a†
2	1.22	0.21	1.15	3.62	2.39	32.7b
‡Biosolids treatment						
PB	1.74	0.24	0.58	5.54	2.57b	79.7a
SB	1.06	0.34	1.15	4.58	3.15a	29.9c
PBS	1.23	0.21	0.27	5.80	2.12b	40.7b
Harvests						
Single	1.26	0.20	0.01	6.00	1.83	46.4
Two	1.44	0.33	1.32	4.62	3.39	53.8
Plant species						
Cattail	1.52	0.25	0.69	4.97	2.38	37.6b
Switchgrass	1.18	0.28	0.64	5.64	2.84	63.2a
	P value					
Cycle (C)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Biosolids (B)	<0.001	<0.001	<0.001	0.01	0.01	<0.001
Harvest (H)	0.02	<0.001	<0.001	0.003	<0.001	0.4
Plant species (P)	<0.001	0.06	0.65	0.79	0.27	0.01
C × B	0.17	<0.001	<0.001	0.03	0.72	0.8
C × P	0.04	0.48	0.01	0.64	0.61	0.73
C × H	<0.001	<0.001	<.001	0.10	<0.001	0.51
B × P	0.05	0.11	0.51	0.13	0.19	0.05
B × H	0.31	<0.001	0.02	0.12	0.50	0.48
P × H	0.36	0.37	0.64	0.02	0.11	0.69
C × B × P	0.24	0.35	0.71	0.15	0.92	0.72
C × B × H	0.46	<0.001	<0.001	0.02	0.99	0.83
C × P × H	0.01	0.30	0.01	0.0003	0.40	0.89
B × P × H	0.01	0.86	0.52	0.19	0.91	0.82
C × B × P × H	0.72	0.21	0.69	0.04	0.95	0.73

†Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.

‡PB = biosolids from the primary cell; SB = biosolids from the secondary cell; PBS = 1:1 mixture of PB and soil

Table A.2 Aboveground nutrient and trace element concentrations as affected by growth cycle, biosolids treatment and harvest frequency in the wetland microcosms.

Effect	N	P	Cd	Cu	Cr	Zn
	mg g^{-1}		mg kg^{-1}			
Cycle						
1	0.73	0.22b†	0.01	5.99	1.04	26.5a
2	1.00	0.17b	0.01	5.74	2.93	17.1b
3	1.18	0.24a	0.01	5.59	2.59	16.3b
‡Biosolids treatment						
PB	0.99	0.22ab	0.01	6.48a	2.23	21.7a
PBS	0.95	0.20	0.01	5.06b	2.15	18.2b
Harvest						
Single	0.87	0.19b	0.01	5.62	2.47	19.8
Two-harvest	1.07	0.23a	0.01	5.92	1.90	20.2
			P value			
Cycle(C)	<0.001	<0.001	0.01	0.55	<0.001	<0.001
Biosolids (B)	0.39	0.04	0.65	<0.001	0.58	0.004
Harvest(H)	0.001	0.002	0.27	0.19	0.002	0.99
C × B	0.47	0.36	0.02	0.58	0.35	0.63
C ×H	0.44	0.55	0.07	0.14	0.04	0.22
B × H	0.60	0.46	0.46	0.14	0.88	0.46
C × B × H	0.04	0.48	0.10	0.31	0.52	0.46

†Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.05$). Mean separation letters are applied to the main effects only in the absence of a significant interaction.
‡ PB = biosolids from the primary cell; PBS = 1:1 mixture of PB and soil.