Biomass, Nutrient, and Trace Element Accumulation and Partitioning in Cattail (Typha latifolia L.) during Wetland Phytoremediation of Municipal Biosolids

Nicholson N. Jeke, Francis Zvomuya,* Nazim Cicek, Lisette Ross, and Pascal Badiou

Abstract

Biomass and contaminant accumulation and partitioning in plants determine the harvest stage for optimum contaminant uptake during phytoremediation of municipal biosolids. This wetland microcosm bioassay characterized accumulation and partitioning of biomass, nutrients (N and P), and trace elements (Zn, Cu, Cr, and Cd) in cattail (Typha latifolia L.) in a growth room. Four cattail seedlings were transplanted into each 20-L plastic pail containing 3.9 kg (dry wt.) biosolids from an end-of-life municipal lagoon. A 10-cm-deep water column was maintained above the 12-cm-thick biosolids layer. Plants were harvested every 14 d over a period of 126 d for determination of aboveground biomass (AGB) and belowground biomass (BGB) yields, along with contaminant concentrations in these plant tissues. Logistic model fits to biomass yield data indicated no significant difference in asymptotic yield between AGB and BGB. Aboveground biomass accumulated significantly greater amounts of N and P and lower amounts of trace elements than BGB. Maximum N accumulation in AGB occurred 83 d after transplanting (DAT), and peak P uptake occurred at 86 DAT. Harvesting at maximum aboveground accumulation removed (percent of the initial element concentration in the biosolids) 4% N, 3% P, 0.05% Zn, 0.6% Cu, 0.1% Cd, and 0.2% Cr. Therefore, under the conditions of this study, phytoremediation would be most effective if cattail is harvested at 86 DAT. These results contribute toward the identification of the harvest stage that will optimize contaminant uptake and enhance in situ phytoremediation of biosolids using cattail.

Core Ideas

• Wetland system using cattail can remove contaminants from biosolids.
• Wetland-based phytoremediation is more effective with two harvests than one harvest.
• Phytoextraction is optimized if plants are harvested at maximum aboveground contaminant accumulation.

Many small communities in Canada and the United States use lagoons or stabilization ponds for wastewater treatment. In the United States, over 7000 facultative lagoons are in use (USEPA, 2002). The disposal or management of biosolids in an economical and environmentally sustainable manner after operation of these lagoons cease is a challenge for many municipalities. In the United States and Canada, about 60% of biosolids produced are spread on agricultural lands (Cogger et al., 2006).

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

Using constructed wetlands for in situ remediation of end-of-life lagoons contributes to a wider effort to limit potential nutrient losses, especially P, which has caused serious eutrophication of freshwater bodies such as Lake Winnipeg in Manitoba, Canada. Erosion and surface runoff from nutrient-rich agricultural soils, including those receiving biosolids application, are important contributors to high N and P in river systems in western Canada (Manitoba Conservation, 2002). With in situ wetland phytoremediation, contaminants are sequestered within the wetland, reducing contamination of surrounding environments, whereas harvesting plants permanently removes contaminants from the biosolids. Harvesting high-biomass plants such as

N.N. Jeke and F. Zvomuya, Dep. of Soil Science, 362 Ellis Bldg., Univ. of Manitoba, Winnipeg, MB, Canada R3T 2N2; N. Cicek, Biosystems Engineering, Univ. of Manitoba, Winnipeg, MB, Canada R3T 2N2; L. Ross, Native Plant Solutions, Ducks Unlimited Canada, Unit A, 1238 Chevrier Blvd., Winnipeg, MB R3T 1Y3; P. Badiou, Ducks Unlimited Canada, Institute for Wetland and Waterfowl Research, Stonewall, MB, Canada R0C 2B0. Assigned to Associate Editor Karen Bradham.

Abbreviations: AGB, aboveground biomass; BGB, belowground biomass; CCU, cumulative copper uptake; CCZU, cumulative chromium uptake; CNU, cumulative nitrogen uptake; CPU, cumulative phosphorus uptake; CZZU, cumulative zinc uptake; DAT, days after transplanting; DW, dry weight; LEL, lowest effect level; RO, reverse osmosis; TEL, threshold effect level.

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cattail removes contaminants from the wetland system and can provide feedstock for bioenergy production (Cieck et al., 2006; Grosshans, 2014), thereby addressing public concerns with human and environmental health issues arising from contaminants when biosolids are applied to agricultural land.

Harvesting of wetland plants is an important step in the phytoremediation of contaminated sediments. Harvesting removes contaminants taken up by the plants and prevents contaminant recycling in the wetland ecosystem from decaying plant tissues (Martin and Fernandez, 1992; Vymazal, 2007). It is therefore important to harvest plants when contaminant accumulation is optimal to effectively remove contaminants in the harvested aboveground plant tissues. The uptake of contaminants, and hence removal by harvesting, depends on biomass yield and contaminant concentration in the harvested biomass (Vymazal, 2007; Kadlec and Bevis, 2009). As plants mature, biomass and nutrient accumulation diminish, and nutrients and photoassimilates are retranslocated from aboveground to belowground tissues where they are stored for use during spring regrowth (Mitsch and Gosselink, 2007). Martin and Fernandez (1992) reported that 40 to 45% of N and P initially present in secondary effluent was removed when cattail growing in the effluent was harvested in November; by comparison, a late September to early October harvest (i.e., before nutrient retranslocation to the root biomass) could have potentially removed 70% of these nutrients. Retranslocation can reduce the concentration of nutrients accumulated in the aboveground biomass by more than 50% (Vymazal, 1995; Reddy and DeLaune, 2008; Grosshans, 2014). Therefore, harvesting before the onset of retranslocation is important to ensure optimum contaminant removal through harvesting aboveground biomass (AGB). Harvesting plants at maximum nutrient accumulation also increases rhizome uptake of nutrients such as P so that they can be stored for use the following season (Kim and Geary, 2001; Headley et al., 2003).

Removal of nutrients through harvesting macrophytes in wetlands has been reported to be efficient in wetlands with low inflow contaminant concentrations (Cieck et al., 2006). Vymazal (2007) reported <10% N removal through harvesting plants from various constructed wetlands for secondary wastewater treatment systems, whereas harvesting plants was reported to be more important in N removal when inflow N concentrations are low, such as in constructed wetlands for tertiary wastewater treatment. Other researchers have reported higher removal of N and P through harvesting of plants. For example, Martin and Fernandez reported 70% of N and P removal from secondary effluent that were filled with RO water. Seeds were sowed in plastic trays placed in seed trays washed with tap water and then rinsed with reverse osmosis (RO) water. Seeds that settled at the bottom of the blender were repeatedly extracted by blending cattail fruits in a Contrad deep. The municipality used a wastewater stabilization treatment system that included a primary cell (4.6 ha) as the treatment cell, which received raw effluent, and a secondary cell (8.8 ha) into which effluent was transferred for further treatment and storage. The volumes of biosolids at the time of lagoon closure were approximately 20,000 m³ for the primary cell and 28,000 m³ for the secondary cell. The lagoon operated for 37 yr (1971–2008). The lagoon treated predominantly domestic effluent and served a population of approximately 2500 in 2006 (Statistics Canada, 2006). Biosolids for this study were collected in the summer of 2011 from random sampling points in the primary cell. The biosolids had an average depth of 20 cm at the time of sampling.

**Materials and Methods**

**Biosolids**

Biosolids samples were collected from a primary cell of an end-of-life municipal lagoon in Niverville, Manitoba, Canada (49°35′42.7″ N, 97°02′50.3″ W). The municipality used a wastewater stabilization treatment system that included a primary cell into which effluent was introduced and a secondary cell into which effluent was transferred for further treatment and storage. The volumes of biosolids at the time of lagoon closure were approximately 20,000 m³ for the primary cell and 28,000 m³ for the secondary cell. The lagoon operated for 37 yr (1971–2008). The lagoon treated predominantly domestic effluent and served a population of approximately 2500 in 2006 (Statistics Canada, 2006). Biosolids for this study were collected in the summer of 2011 from random sampling points in the primary cell. The biosolids had an average depth of 20 cm at the time of sampling.

**Wetland Microcosm Setup**

Seeds were extracted by blending cattail fruits in a Contrad toluene for 20 s in a blender (Model 54227C, Hamilton Beach) according to the method described by McNaughton (1968). Seeds that settled at the bottom of the blender were repeatedly washed with tap water and then rinsed with reverse osmosis (RO) water. Seeds were sowed in plastic trays placed in seed trays that were filled with RO water to supply water from the bottom. After 28 d, four uniform cattail seedlings were transplanted into each plastic pail (27.5 cm diameter × 32 cm height) to give a plant density of ~70 plants m⁻² (Kadlec and Bevis, 2009). Each pail contained 3.94 kg (dry weight [DW]) of biosolids ~12 cm deep. The experiment was arranged in a completely randomized design consisting of 24 planted pails (three replicates × eight sampling times) and three pails containing no plants (controls).

The pails were placed in a controlled-environment growth room under a 16-h photoperiod and day/night temperatures of 22/15°C. Relative humidity during the experiment was set at 65%. Daytime light intensity was 270 µmol photons m⁻² s⁻¹. Microcosms were weighed every other day and watered to replace water lost during evapotranspiration. Moisture content
was initially maintained at ~60% water-filled pore space and then increased by 20% 7 d after transplanting (DAT). The microcosms were flooded to a depth of 10 cm above the biosolids surface 14 DAT to provide wetland conditions.

### Sampling and Laboratory Analysis

Plants (AGB and BGB) were harvested from three pails 28 DAT and every 14 d thereafter for the rest of the 126-d experiment. Aboveground biomass was harvested by clipping plants at the surface of the biosolids after decanting the "wetland" water from each pail. Belowground (roots and rhizomes) biomass was recovered by washing the roots with tap water to remove biosolids and then rinsing with RO water. Harvested biomass samples were placed in paper bags and dried for 72 h in an oven at 60°C. The dry biomass samples were weighed for biomass yield determination and then ground (<0.2 mm) with a mixer/mill grinder (Model 8000D, Spex Sample Prep). Ground plant tissue samples from all sampling dates were analyzed for nutrient and trace element concentrations. Total Kjeldahl N concentrations were determined by an autoanalyzer (FIAlab 2500, FIAlab Instruments) after digestion with sulfuric acid in a block digester. For determination of P and trace element concentrations, a 0.5-g sample was digested in aqua regia for 2 h at 90°C using a microprocessor-controlled digestion block. Phosphorous concentration was then measured using a Varian 735 ES inductively coupled plasma mass spectrometer (Varian Inc.), and trace element concentrations were measured with a PerkinElmer SCIEX ELAN 6000 ICP-MS (Perkin-Elmer SCIEX Instruments).

### Statistical Analysis

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

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

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

Nutrient and trace element uptake (i.e., transport of elements through both the xylem and phloem) values were calculated by multiplying element concentrations by the corresponding biomass yields for every sampling date. In this paper, we define uptake as the amount of each element that was extracted from the biosolids in each pail. Similarly, cumulative uptake is the net cumulative uptake of the element (i.e., total uptake less any retranslocation to the BGB). Segmented regression models were fitted using PROC NLIN in SAS to predict the time at which peak nutrient and trace element accumulation occurred.

### Results and Discussion

#### Biosolids Properties

Selected chemical properties of the biosolids are presented in Table 1 along with applicable sediment quality guidelines. High N and P concentrations were the major concern in the biosolids. Although a wide range of trace elements were measured in the biosolids, only Zn, Cu, Cr, and Cd are reported because they were above the threshold effect level (TEL) set by the Canadian Sediment Quality Guidelines (CCME, 2001). The low concentrations of the rest of the trace elements, including nickel, mercury, lead, selenium, and arsenic, were expected because the biosolids were produced from domestic wastewater.

### Table 1. Selected initial chemical properties of biosolids used in the microcosm study, along with applicable sediment quality guidelines.

<table>
<thead>
<tr>
<th>Property</th>
<th>Biosolids</th>
<th>Ontario SQG†</th>
<th>CCME SQG‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN,(\ S\ g \ kg^{-1})</td>
<td>7.8</td>
<td>0.55</td>
<td>4.8</td>
</tr>
<tr>
<td>Total P, g kg(^{-1})</td>
<td>3.4</td>
<td>0.6</td>
<td>2</td>
</tr>
<tr>
<td>(\text{NO}_3-N, \ mg \ kg^{-1})</td>
<td>119</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(\text{NH}_4-N, \ mg \ kg^{-1})</td>
<td>63</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Olsen P, mg kg(^{-1})</td>
<td>155</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cu, mg kg(^{-1})</td>
<td>143</td>
<td>–</td>
<td>35.7</td>
</tr>
<tr>
<td>Zn, mg kg(^{-1})</td>
<td>396</td>
<td>–</td>
<td>123</td>
</tr>
<tr>
<td>Cd, mg kg(^{-1})</td>
<td>1.4</td>
<td>–</td>
<td>0.6</td>
</tr>
<tr>
<td>Cr, mg kg(^{-1})</td>
<td>42.4</td>
<td>–</td>
<td>37.3</td>
</tr>
<tr>
<td>EC,(\ \Omega \ \text{cm}^{-1})</td>
<td>4.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

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

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

§ Total Kjeldahl nitrogen.

¶ Electrical conductivity.
Biomass Yield

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

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

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

The $k$ parameters from the three-parameter logistic model did not differ significantly between AGB and BGB, indicating similar growth rates for the two yield components. Aboveground biomass yield was greater than BGB yield until the end of the study period when a more even biomass partitioning occurred. If the experiment had continued for a longer period, it appears that belowground biomass (Fig. 1b) would have continued increasing, albeit at a slower rate.

### N Uptake

A linear–linear segmented model provided the best description for temporal changes in cumulative net N uptake by AGB (Fig. 2a). Cumulative N uptake (CNU) increased linearly (CNU = −386 + 17.4 DAT) with time for 28 ≤ DAT ≤ 86, peaking at 1114 mg pail$^{-1}$ (18.8 g N m$^{-2}$) on Day 86, after which it decreased linearly (CNU = 1644 - 6.2 DAT for DAT > 86) until the end of the experiment. The decrease in N uptake was likely due to retranslocation of the nutrient to BGB. The peak cumulative N uptake by AGB accounted for 72% of the total N uptake by the plant, indicating that phytoextraction, in conjunction with aboveground biomass harvesting at peak

### Table 2. Segmented and linear model parameters for nitrogen, phosphorus, and trace element uptake in aboveground and belowground biomass of cattail grown in biosolids for 126 d in a wetland microcosm.

<table>
<thead>
<tr>
<th>Element</th>
<th>Aboveground†</th>
<th>Belowground</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a$</td>
<td>$b_1$</td>
</tr>
<tr>
<td>N</td>
<td>−386</td>
<td>17.4</td>
</tr>
<tr>
<td>P</td>
<td>−115</td>
<td>6.1</td>
</tr>
<tr>
<td>Cu</td>
<td>0.034</td>
<td>0.04</td>
</tr>
<tr>
<td>Zn</td>
<td>−2</td>
<td>0.104</td>
</tr>
<tr>
<td>Cd</td>
<td>−0.002</td>
<td>9 × 10$^{-5}$</td>
</tr>
<tr>
<td>Cr</td>
<td>0.277</td>
<td>0.049</td>
</tr>
</tbody>
</table>

† $a$, intercept (mg kg$^{-1}$ pail$^{-1}$); $b_1$, rate of change of response variable when $x < x_1$ (mg kg$^{-1}$ pail$^{-1}$ d$^{-1}$); $b_2$, rate of change of response variable when $x > x_1$ (mg kg$^{-1}$ pail$^{-1}$ d$^{-1}$); $X_1$, number of days after transplanting; $x_1$, critical point at maximum nutrient and trace element uptake (d).

‡ Parameters did not apply to the model, and simple linear regression analysis was conducted.
N accumulation, can effectively remove a large fraction of N absorbed by the plant. Unlike AGB, cumulative N uptake by BGB increased linearly with time (CNU = -178 + 7.1 DA T) over the entire study period (Fig. 2b). This was likely due in part to retranslocation of N from AGB to BGB and to absorption of N by plant roots, although BGB was senescing. However, a plateau in N uptake is expected at times beyond the sampling time used in this study. However, CNU was generally greater in AGB than in BGB throughout the study period. The rate of increase in CNU in AGB (17.4 mg pail\(^{-1}\) d\(^{-1}\)) was 2.5 times the rate of CNU increase in BGB (7.1 mg pail\(^{-1}\) d\(^{-1}\)) (Table 2), indicating that cattail readily translocated N to aerial parts to support shoot growth. Photosynthetic tissues require more N and other macronutrients than nonphotosynthetic roots and rhizome tissues (Baldantoni et al., 2004; Sharma et al., 2006).

Harvesting AGB at peak N accumulation on Day 86 resulted in the removal of 3.7% of TN initially present in the biosolids. Based on the Ontario sediment quality guidelines for the protection and management of aquatic sediment (Persaud et al., 1993), 25 harvest cycles, corresponding to 25 growing seasons, would be required to remediate the sediment to the lowest effect level (LEL) (550 mg kg\(^{-1}\)), which defines clean to marginally polluted sediment. Eleven harvest cycles would be required to remediate the sediment to the severe effect level (4800 mg kg\(^{-1}\)), which defines marginally to significantly polluted sediments. The estimates disregarded other N removal pathways, such as denitrification, and are based on the assumption that biomass production and uptake of N and other contaminants are relatively constant in each harvest cycle. However, contaminant uptake may differ with time as a result of repeated harvesting or changes in plant growth and development. Moreover, available nutrient concentrations in the biosolids decrease with time as they are taken up by plants, resulting in a decrease in uptake with time.

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

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

The percentage of initial biosolids N content removed through harvesting at peak N accumulation in our study is lower than the 20% (Kadlec and Bevis, 2009), 20 to 26% (Tanner, 1996), and 40 to 45% (Martin and Fernandez, 1992) reported in other studies for wastewater treatment wetlands. Higher plant N uptake in wastewater studies may be attributed to the readily bioavailable ammonia and ammonium forms of N, which are the predominant inorganic N forms in wastewater (Cronk and Fennessy, 2001). On the contrary, biosolids contain predominantly organic N, which must be mineralized before plant uptake. Moreover, wastewater treatment wetlands receive fresh supplies of nutrients, whereas, in our closed wetland microcosms, plants obtained a continuous supply of nutrients from the biosolids.

**P Uptake**

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

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

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

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

Maximum aboveground accumulation of N and P occurred 86 and 83 DAT, respectively (i.e., just before AGB accumulation reached the stationary phase). These results suggest that optimum harvesting of AGB should occur, at the latest, when the AGB accumulation curve reaches the stationary phase to prevent retranslocation of nutrients to roots and rhizomes. Although harvesting AGB at optimum nutrient uptake maximizes nutrient removal, Vymazal (2004) and Asaeda et al. (2006) suggested that harvesting plants before retranslocation of nutrients and assimilates to belowground tissues may cause serious damage to plant productivity. On the contrary, Kim and Geary (2001) and Headley et al. (2003) reported that harvesting plants at the time of peak nutrient accumulation increases nutrient uptake and storage for rapid spring growth. In a field study examining cattail biomass production, Pratt et al. (1988) showed that annual harvesting of cattail over three seasons did not have an adverse effect on plant productivity, whereas harvesting during peak nutrient uptake resulted in significant nutrient removal from rhizomes, thus necessitating fertilizer application to maintain biomass yield. Grosshans (2014) reported no significant change in belowground P reserves during a 4-yr field study, ostensibly due to readily available P from the sediment and litter.

**Trace Element Uptake**

Cumulative Zn uptake (CZU) by the aboveground biomass increased as a linear function of time (CZU = -2 + 0.104 DAT) for 28 ≤ DAT ≤ 99 and decreased linearly with time (CZU = 21 – 0.124 DAT) for DAT > 99 (Fig. 4a). Peak cumulative Zn uptake on Day 99 was 8.3 mg pail⁻¹ (0.14 g Zn m⁻²), accounting for 47% of total Zn uptake by the plant. Although Zn uptake was low, it was readily translocated to aboveground tissues, with about 50% of total plant Zn uptake partitioned to the AGB. Throughout the experiment, Zn accumulation in the BGB increased linearly (CZU = -1.32 + 0.1 DAT) with time (Fig. 4b). The rate of Zn accumulation was similar for AGB (0.10 mg pail⁻¹ d⁻¹) and BGB (0.11 mg pail⁻¹ d⁻¹) until 99 DAT, after which Zn accumulation in the AGB decreased by 3.3 mg pail⁻¹ d⁻¹ during the rest of the experiment, whereas that in the BGB continued to increase linearly. When harvesting coincided with peak cumulative Zn uptake, 0.05% of the Zn initially present in the biosolids was removed in the harvested biomass. This translates to 127 harvest cycles required to remediate to the TEL (123 mg kg⁻¹) of the CCME sediment quality guidelines for protection of aquatic life (CCME, 2001) under conditions of the present experiment, assuming that the phytoextraction rate remains constant season to season. Although this estimate cannot be directly translated...
to field conditions, it suggests that Zn phytoextraction may not be efficient enough to remove the contaminant from biosolids.

Small amounts of Cu were taken up by cattail plants, most of which were sequestered in the belowground plant tissues, with very little translocation to AGB. Temporal changes in cumulative Cu uptake (CCU) in the aboveground biomass were best described by an exponential–linear function (Fig. 5a). For 28 ≤ DAT ≤ 82, Cu accumulation in the aboveground tissues increased as an exponential function of time (CCU = 0.034e^{0.04DAT}), after which it decreased linearly (CCU = 1.4 - 0.006 DAT) for DAT > 82. The peak Cu accumulation of 0.87 mg pail\(^{-1}\) (0.01 g Cu m\(^{-2}\)) on Day 82 represented 34% of total plant Cu uptake at that stage. Retranslocation of Cu to BGB after peak uptake was not evident given the low concentrations translocated to aboveground plant tissues in the first place. Total Cu accumulation in the BGB was two to three times greater than that in the AGB before peak aboveground Cu accumulation and was five times greater by the end of the study period, indicating that roots continued to absorb Cu with no significant translocation to the shoots. Low Cu uptake by the plant was likely due to strong adsorption of the metal by organic matter (Laidlaw et al., 2012). When plants were harvested at the time of peak Cu accumulation in the AGB, 0.6% of Cu initially present in the biosolids was removed in the harvested biomass.

Cumulative Cu uptake (CCU) by the aboveground biomass increased as an exponential function (CCU = 0.277e^{0.04DAT}) of time for 28 ≤ DAT ≤ 80, after which it increased by 0.06 mg pot\(^{-1}\) (CCU = 0.2 + 0.001 DAT) for DAT > 80 (Fig. 6a). Peak Cr accumulation on Day 80 was 0.28 mg pail\(^{-1}\) (0.005 g Cr m\(^{-2}\)), which represented 44% of total plant accumulation by that day. Greater Cr accumulation in the AGB than in the BGB was likely due to strong adsorption of the metal by organic matter. When plants were harvested at the time of peak Cu accumulation in the AGB, 0.2% of the Cr initially present in the biosolids was removed in the harvested biomass.

Cadmium accumulation in the aboveground biomass was low, peaking at just 0.0075 mg pail\(^{-1}\) (0.0001 g Cd m\(^{-2}\)), or 7% of total plant uptake on Day 107 (Fig. 7a). When plants were harvested at the time of peak Cd accumulation in the AGB, 0.1% of the Cd initially present in the biosolids was removed in the harvested biomass. Greater accumulation of Cd in the BGB could be a result of Cd immobilization in the cell wall of roots as an exclusion mechanism to protect aerial plant tissues from Cd toxicity (Stoltz and Greger, 2002; Bah et al., 2011; Salem et al., 2014). Trace elements such as Cd and Cr generate free radicals and toxic reactive oxygen species in the cytoplasm, inducing oxidative stress to plants (Cho and Seo, 2005) that can
damage proteins, lipids, and DNA in plant cells (Fu and Huang, 2001). Bah et al. (2011) demonstrated the activation of the enzymatic antioxidants superoxide dismutase and peroxidase in *Typha angustifolia* to detoxify reactive oxygen species as a defense mechanism to protect the plant from oxidative stress induced by Cd and Cr.

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

The low trace element uptake in the present study may be due to low trace element concentrations or bioavailability. Bioavailability of trace elements in wetlands is affected by environmental conditions such as redox potential, pH, and salinity of the sediment and overlying water column (Reddy and DeLaune, 2008). Transport of trace elements from roots to shoots occurs in the xylem (Prasad, 2006); therefore, immobile nonessential elements such as Cd may have a greater dependence on the transpiration flux to reach aboveground biomass than mobile essential trace elements that can be assimilated into the plant at the root level. If vapor pressure demand and transpiration rates were lower in our growth room relative to summer field conditions, then transport of immobile nonessential elements to aboveground biomass could be greater in field conditions than reported in this study.

As a result of low trace element uptake in the present study, the time frame required to achieve trace element remediation goals by phytoextraction would be undesirably long. Phytoextraction is the dominant mechanism of phytoremediation for trace elements. The trace elements are immobilized in the roots and are therefore not available to affect sediment-dwelling organisms. Although the trace element concentrations in the biosolids used in this study were above TEL values of the CCME sediment quality guidelines for protection of aquatic life, the concentrations were below threshold values of all the four land uses in the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (CCME, 1999) and would therefore not pose a threat to humans and the environment under any of the land uses after remediating for N and P.

### Conclusion

Maximum biomass yields were similar between AGB and BGB. At peak N and P accumulation in AGB, greater proportions of N and P were partitioned to AGB than to BGB. We suggest that optimum removal of these nutrients from biosolids would occur if harvesting of cattail plants coincides with peak AGB nutrient accumulation. Harvesting at peak N and P accumulation removed 191 and 65 kg ha$^{-1}$ yr$^{-1}$, respectively, under the conditions of the experiment. Although the estimates from this study cannot be directly translated to field conditions, they demonstrate how harvesting cattail in an in situ wetland-based phytoremediation system reduces the potential of nutrient loading to surface water bodies that would occur if biosolids were spread on agricultural land. Harvested biomass can be used as a feedstock for bioenergy production. Phytoextraction was not effective in removing trace elements from biosolids because small amounts of trace elements were absorbed, most of which were partitioned to BGB. Findings from this study will inform policymakers and landowners on the timing of harvesting for maximum phytoremediation and removal of nutrients from biosolids. However, long-term studies are needed to determine the nutrient and biomass dynamics, and hence the timing, of harvesting under field conditions.

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### References


