Elemental composition of native wetland plants in constructed mesocosm treatment wetlands

Beverly S. Collins *, Rebecca R. Sharitz, Daniel P. Coughlin

Savannah River Ecology Laboratory, PO Drawer E, Aiken, SC 29802, USA

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Abstract

Plants that accumulate a small percentage of metals in constructed treatment wetlands can contribute to remediation of acidic, metal contaminated runoff waters from coal mines or processing areas. We examined root and shoot concentrations of elements in four perennial wetland species over two seasons in mesocosm wetland systems designed to remediate water from a coal pile runoff basin. Deep wetlands in each system contained Myriophyllum aquaticum and Nymphaea odorata; shallow wetlands contained Juncus effusus and Pontederia cordata. Shoot elemental concentrations differed between plants of deep and shallow wetlands, with higher Zn, Al, and Fe concentrations in plants in shallow wetlands and higher Na, Mn, and P concentrations in plants in deep wetlands. Root and shoot concentrations of most elements differed between species in each wetland type. Over two seasons, these four common wetland plants did help remediate acidic, metal-contaminated runoff from a coal storage pile.

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1. Introduction

Runoff waters from coal stock-piled for coal-fired power plants are acidic and contain high concentrations of Fe, Mn, and Al, as well as trace metals such as As, Co, Cr, Cu, Ni, and Zn (Thomas et al., 2000). Regulatory compliance difficulties and public environmental concern about acidic, metal-contaminated drainage from mining, processing, and storing coal have contributed to research and development of remediation technologies (e.g., Guthrie and Cherry, 1979; Mudroch and Capobianco, 1979; Gavrilenko and Zolotukhina, 1990; Wieder et al., 1990; Hopkins et al., 1999). Constructed treatment wetlands (CTWs) are one attractive option. This passive technology uses natural chemical and biological processes to treat metal-contaminated, acidic drainage, and is a relatively low-cost, efficient choice (Ye et al., 2001).

Designs for CTWs differ, and can include anaerobic wetlands and vegetated or unvegetated aerobic wetlands (Gazea et al., 1996). Many include a successive alkalinity producing (SAP) system to remediate the low pH. Several chemical processes, including oxidation and hydrolysis in aerobic wetlands, and reduction in sediments and anaerobic wetlands, contribute to metal removal in CTWs (Gazea et al., 1996). In planted aerobic wetlands, decaying plant litter provides sites for metal adsorption and exchange, as well as nutrients for bacteria (Gazea et al., 1996; Jackson, 1998). Rooted macrophytes often diffuse oxygen into the surrounding substrate, which creates local oxidizing zones (Gazea et al., 1996). In addition, plants can accumulate metals in tissues, although accumulation often contributes only a minor fraction of metal removal (Gazea et al., 1996; Ye et al., 2001). Identifying and using plant species that can survive, produce biomass, and accumulate metals...
can facilitate remediation of coal runoff. The general objective of our research was to examine growth and accumulation of elements (phosphorus and metals) in four southeastern wetland plant species planted into mesocosm CTWs designed to remediate acidic metal contaminated water.

In general, elemental uptake by wetland plants varies among species, and is related to rooting depth and plant life form (Guilizzoni, 1991). Species that root in the upper layers of the substrate typically have higher root absorption due to greater exposure to concentrated elements at the sediment–water interface than do plants rooted more deeply (Mudroch and Capobianco, 1978). Submerged and floating-leaved species, which can absorb soluble elements directly from the water into shoots, often have greater accumulation of metals than emergent species (Mudroch and Capobianco, 1979; Sparling and Lowe, 1998; Mays and Edwards, 2001). Allocation of elements within plants can vary with the uptake pathway and the physiological function of the element in the plant tissues (Qian et al., 1999). Most trace elements show an order of magnitude greater concentration in roots than shoots (Qian et al., 1999). Exceptions are elements, such as boron, that move with the transpiration stream or are metabolized or stored in aboveground tissues (Qian et al., 1999).

We selected four common southeastern wetland plant species that differ in growth form. *Juncus effusus* and *Pontederia cordata* are emergent perennials that root in the sediment and spread vegetatively. *Juncus* spp., including *J. effusus*, are used in wetlands designed to treat acid mine drainage (Mays and Edwards, 2001) or wastewater from animal production facilities (Hill et al., 1997; Clarke and Baldwin, 2002), although *J. effusus* may show poor growth in farm wastewaters (Tanner, 1996) and has not been shown to hyperaccumulate trace metals (Mays and Edwards, 2001). *Nymphaea odorata* is a floating-leaved perennial that roots in the sediment. *M. aquaticum* is a submerged species with little root development. Some *Myriophyllum* species, including *M. brasiliense*, *M. heterophyllum*, and *M. verticillatum*, have been shown to accumulate trace elements such as Cd, Cr, and Ni (Mudroch and Capobianco, 1978, 1979; Qian et al., 1999). *N. odorata* and *P. cordata* have been shown to accumulate trace metals to a lesser extent than *M. verticillatum* or *M. heterophyllum* (Mudroch and Capobianco, 1978, 1979). We compared P and metal concentrations in roots and shoots of the four wetland species over two seasons to determine if the plants differ in degree of accumulation or allocation of elements within the plant. We concentrated on P, Al, Fe, Mn, Na, and Zn because of their abundance in coal pile runoff and importance to plants.

2. Methods

2.1. Constructed treatment wetlands (CTWs) design

An array of mesocosm treatment wetlands was constructed to receive metal-contaminated effluent from a coal pile runoff basin (CPRB) on the Department of Energy’s Savannah River Site (SRS) near Aiken, South Carolina, USA. The CPRB functions as a sedimentation basin that receives 87.7 million L/yr of runoff water from a coal pile at a coal-fired power plant. The 5-ha CPRB is characterized by low pH (2.4) with high concentrations of sulfate and metals (Table 1) (WSRC, 2000).

The CTW array was a non-circulating, continuous flow system of 48 mesocosm wetland systems, with three wetlands (A, B, and C) per system (Fig. 1). Aerobic CPRB effluent flowed into anaerobic wetland A (0.73 m² surface area by 1.2 m deep), which was covered to prevent evaporation and contained (85 % by volume) a mixture (1:3) of limestone screenings and composted stable waste. Flow rates into wetland A ranged between 16 and 28 mL/min and averaged 20 mL/min (Thomas, 2003). The effluent from A flowed through a series of two aerobic wetlands (B and C), one deep (0.73 m² surface area by 1.2 m deep) and one shallow (0.73 m² surface area by 0.8 m deep), before returning to the CPRB (Fig. 1). Deep aerobic wetlands contained, by volume, 50% fine-grain sand and had 0.6 m water depth; shallow wetlands contained 75% fine-grain sand and had 0.2 m water depth. Water depths of the deep and shallow wetlands were based on rooting depths of the plant species and depths of natural wetlands where these species occur. To determine if the sequence of deep and shallow aerobic wetlands influenced effectiveness of metal removal, half the CTWs had a deep wetland B followed...
by a shallow wetland C; the other half had a shallow wetland B followed by a deep wetland C. Sixteen of the 48 wetlands in the array were used for this research.

Wetland plants were transplanted into the aerobic B and C wetlands in these 16 CTWs. Plants were collected during the dormant season, between January and February 1999, from ephemeral ponds and reservoirs on the SRS that did not receive coal runoff. The plants were stabilized in a greenhouse for 1–2 months before they were transplanted into the CTWs in March 1999. The surface area of each aerobic wetland to be planted was divided into 5 equal sections. An individual of each of the two emergent species, *J. effusus* and *P. cordata*, was planted into each section of the shallow aerobic wetlands, and an individual of the submergent species *M. aquaticum* and the floating-leaved species *N. odorata* was planted in each section of the deep aerobic wetlands. During March, the CTWs received water from a nearby stream that did not contain high concentrations of metals to allow the plants to become established.

### 2.2. Wetland functioning

The CTW array began receiving CPRB water in April 1999. From April 1999 until September 2000, water samples from the CPRB, within the A wetlands, and within the C wetlands were measured monthly for pH with a Model 250A Orion pH meter (Thermo Electron Cor., Waltham, MA), sulfate with a Dionex DX500 IC (Dionex Cor., Sunnyvale, CA), and metal concentrations with a Perkin Elmer Inc. (Wellesley, MA) ICP-MS. To determine if elements accumulated in the sediments, additional samples (sediment and accompanying pore water) were taken from 30 cm and 50 cm depths in both the shallow and deep aerobic wetlands in summer, 2000. pH and element composition of the sediment and pore water samples were determined as described above.

### 2.3. Plant analyses

Cuttings of roots (including rhizomes) and shoots (stems and/or leaves) of *Juncus, Pontederia, Myriophyllum* (shoots only) and *Nymphaea* individuals were analyzed for elemental composition in March 1999 before plants were transplanted to the CTWs. Shoots were additionally sampled and analyzed in July 1999. Roots were not sampled during the first growing season to allow the plants to stabilize and grow. Both roots and shoots were sampled and analyzed in December 1999 and July 2000. Shoots were collected for analyses by cutting 3–6 living shoots at the base of the stem (or leaf in *Nymphaea*) from each individual plant that contained leaves. Roots were collected for analyses by uncovering the buried root tissues and cutting a small representative portion of the root and rhizome.

The root and shoot tissues were washed by agitation in tap water, rinsed with distilled water to remove metal precipitates or epiphytic microorganisms that might have bound to the surfaces, dried, and ground in a Thomas Scientific Wiley mill (Philadelphia, PA). The tissues of plants from each of the five sections of each wetland were pooled by species into one root and shoot sample for analyses by species and wetland. Elemental composition was determined using a Model 965 Plasma Atomcomp Jarrell-Ash ICP (Thermo Electron Cor., Waltham, MA).

### 2.4. Data analyses

Analysis of variance (ANOVA, Proc GLM, SAS v. 8.1) was used to compare P and metal concentrations in plant roots and shoots before they were transplanted into the CTWs. Mixed-model ANOVA (Proc Mixed, SAS v. 8.1) was used to compare elemental compositions of plants between deep and shallow wetlands, between rows (positions B and C) of deep and shallow wetlands, and between species in deep and shallow wetlands. Wetland type (deep, shallow), row, and species were considered fixed; replicate wetland systems were considered random. Wetlands were the unit of observation, and elemental composition of species in wetlands was compared by planned contrasts. Separate analyses were conducted for each sampling date. Root and shoot samples were analyzed separately.
3. Results and discussion

3.1. Plant elemental composition in donor wetlands

Baseline (pre-CTW) elemental composition differed among species in plants taken from donor wetlands that do not receive coal runoff (Fig. 2). The two emergents, *Pontederia* (P) and *Juncus* (J), tended to concentrate elements more than the submerged and floating-leaved species, *Myriophyllum* (M) and *Nymphaea* (N). Compared among species, Al and Fe were highest in *Juncus* roots; Mn and Zn were highest in *Juncus* shoots. *Pontederia* had highest P concentrations in shoots, and highest Na concentrations in roots. *Nymphaea* and *Myriophyllum* had highest shoot Na concentrations. Within plants, Al and Fe tended to be more concentrated in roots than in shoots (Fig. 2), and Mn tended to be more concentrated in shoots (Fig. 2).

3.2. CTW function and plant elemental composition

In the CTWs, anaerobic A wetlands increased pH of the water to 6.4, and water exiting the aerobic wetlands was pH 6.9 (Table 1). The A wetlands reduced Al, Fe, and Zn by 90–99%. The aerobic wetlands further reduced Al and Fe by 40–88% (Table 1), but increased...
Zn (from 0.05 to 0.12 mg/L) and pH (from 6.4 to 6.9) (Table 1). Manganese concentrations averaged 3.9 mg/L entering the CTWs and 2.1 mg/L exiting the systems; sulfate showed little change (1.5–1.3 mg/L) (Table 1).

Consistent with the circumneutral pH, elemental concentrations in the water column and pore water of the aerobic wetlands remained low and concentrations increased in the sediments. In the deep wetlands, pore water at 30 cm substrate depth averaged 0.47 ± 0.41 μg/L Fe, 64 ± 16 μg/L Mg, 1.8 ± 0.59 μg/L Mn, and 0.11 ± 0.03 μg/L Zn; Fe and Mg pore water concentrations (0.73 ± 0.92 μg/L Fe, 67 ± 12 μg/L Mg) were significantly greater at 50 cm depth, and Mn and Zn concentrations were unchanged. In the shallow wetlands, pore water at 30 cm substrate depth averaged 6 ± 6 μg/L Fe, 58 ± 24 μg/L Mg, 1.9 ± 1.1 μg/L Mn, and 0.14 ± 0.05 μg/L Zn; metal concentrations did not differ.

Fig. 3. Elemental concentrations (mean ± 1 std. dev.) in shoots of all plants growing in deep (D) and shallow (S) wetlands in row B (closed circles, closed triangles) and row C (open circles, open triangles) of each wetland system. Asterisks indicate significant differences (P ≤ 0.05) between deep and shallow wetland species at each sampling date; “r” indicates significant differences between rows (wetlands B and C).
significantly between 30 and 50 cm depths. Iron and Mn in wetland B and C sediments increased from below detection limits to 3.17 and 15 μg/g dry soil, respectively; Al increased almost threefold, to 233 μg/g dry soil; and Zn increased from 0.01 to 0.46 μg/g dry soil. These values are comparable to concentrations of the elements in the CPRB effluent and indicate that the CTWs function like other treatment wetlands. Metals, including Fe, Mn, Co, and Ni also accumulated in sediments in a flow-through wetland system designed to treat coal combustion by-product leachate from a power station in Pennsylvania (Ye et al., 2001).

Accumulation of elements in plant biomass is related to both pH (Jackson et al., 1993) and concentrations of the elements in the sediments or water column (Mudroch and Capobianco, 1979; Jackson et al., 1993; Spar-

Fig. 4. Shoot concentrations (mean ± 1 std. dev.) of elements in species in deep (M, N) and shallow (J, P) wetlands in rows B (closed circles, closed triangles) and C (open circles, open triangle) of the three-wetland systems. Asterisks indicate significant differences (P ≤ 0.05) between the species within deep or shallow wetlands at each sampling date; "r" indicates significant differences between rows (wetlands B and C).
ling and Lowe, 1998; Mays and Edwards, 2001). In general, elemental concentrations in biomass increase as concentrations in sediment (rooted plant species) or water column (non-rooted submerged and floating leaved species) increase. Raising the pH to circumneutral can promote precipitation and decrease bioavailability, or, as seen in the CTWs, shift concentrations from the water column to sediments (Jackson et al., 1993). By the middle of the first growing season (summer, 1999) in the CTWs, shoots of the submerged and floating leaved plants (*Myriophyllum*, *Nymphaea*) in deep wetlands had greater concentrations of Fe, Na, Mn, Al, and P, while emergent species (*Juncus*, *Potamogeton*) in shallow wetlands had greater concentrations of Zn (Fig. 3). Order of the wetland within the wetland system affected Zn concentrations in plant shoots in shallow and deep wetlands in the first season, with greater concentrations in row C (Fig. 3). Sodium, Mn, and P concentrations continued to be greater in shoots of plants in deep wetlands into the second growing season.
(summer, 2000; Fig. 3). The greater surface area and foliar uptake by the submerged and floating-leaved species could have facilitated accumulation of these more soluble elements (Mudroch and Capobianco, 1978; Guilizzoni, 1991). Zinc concentrations in plant shoots continued to be higher in shallow wetlands. Fe and Al concentrations in plant shoots also were greater in the shallow wetlands over winter \((P = 0.003, 0.0001 \text{ for Fe and Al, respectively})\) (Fig. 3). The shallow roots and exposed leaves of the emergent species may have facilitated uptake of these less soluble metals, which became concentrated in the aerobic wetland sediments and tend to be absorbed primarily from upper sediment layers via roots (Spence, 1964; De Marte, 1969). Root oxidation also could have contributed to formation of Fe plaques, which can absorb metals and are associated with divalent cations that can

Fig. 5. Root concentrations (mean ± 1 std. dev.) of elements in plant species (N, J, P) sampled in spring, 1999, from wetlands that have not received coal runoff (NCR), and in winter (Wn99) and summer (Su00) from deep (N) and shallow (J, P) wetlands of the CTWs. For each element, different letters indicate significant differences among sampling dates for each species. Asterisks indicate significant differences between species in shallow wetlands at each sampling date.
interfere with uptake of elements such as Na (Mitsch and Gosselink, 2000; Serrano and Rodriguez-Navarro, 2001).

Differences in uptake of elements among species and allocation of elements among plant tissues depend partly on plant life form, absorption site, and physiological function of the element in the plant tissues (Sculthorpe, 1967; Welsh, 1977; Bosserman, 1981; Guilizzoni, 1991; Jackson, 1998; Serrano and Rodriguez-Navarro, 2001). For most elements, concentrations in plant shoots and roots differed between species within both deep and shallow wetlands (Figs. 4 and 5). Alone among the elements examined, Zn concentrations were lower in roots of all species in the CTWs than in samples from uncontaminated wetlands (comparison among samples of each species from uncontaminated wetlands (NCR) and wetlands; Fig. 5).

In the deep wetlands, Myriophyllum shoots tended to be higher in Mn than shoots of Nymphaea in all sample periods, higher in P through the first winter, and higher in Zn after the first summer (Fig. 4). Sodium, however, became concentrated in Nymphaea shoots (Figs. 4 and 6); concentrations were more than twofold those in Myriophyllum shoots in the wetlands or in Nymphaea shoots from the donor wetlands (Fig. 2). Sodium has also been shown to be high in shoots of Nymphaea from natural, uncontaminated wetland (Bosserman, 1981). Nymphaea roots had greater concentrations of P and Na, and lower concentrations of Al and Mn compared to roots from reference wetland plants.
Table 2
Mean and range (in brackets) of concentrations (ppm) of metals in plants in the CTW’s and donor SRS wetlands compared to concentrations in these and other plant species in natural wetlands and wetlands receiving acidic, metal contaminated drainage

<table>
<thead>
<tr>
<th>Element/plant</th>
<th>Shoots</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTW</td>
<td>Other AMD</td>
</tr>
<tr>
<td>Al</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>271 [78–613]</td>
<td>74</td>
</tr>
<tr>
<td>Fe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>942 [222–2500]</td>
<td>164</td>
</tr>
<tr>
<td>Mn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. heterophyllum</em></td>
<td>18.6 [12–32]</td>
<td>64 [20–92]</td>
</tr>
</tbody>
</table>

In the shallow CTW wetlands, *Juncus* shoots had greater concentrations of Zn. *Pontederia* shoots were richer in P, Na, Mn (summer, 1999 and 2000) and Fe (by winter, 1999; Fig. 4). Higher concentrations of P and Na in *Pontederia* compared to *Juncus* shoots follows the trend in shoots of plants from the donor wetlands that do not receive coal runoff (Fig. 2). Iron and Al, which had greater concentrations in *Juncus* roots than in *Pontederia* roots of plants from wetlands that have not received coal runoff (Fig. 5), were also more concentrated in *Juncus* root samples from the shallow wetlands of the CTWs throughout the treatment period (significant difference between species in the shallow wetlands; Fig. 5).

Elemental partitioning within CTW plants (compared as the shoot:root (SR) ratio, Fig. 6) followed similar trends, but was less pronounced than that in samples from the donor wetlands. By the second season, Mn concentrations were higher in shoots than roots (SR > 1), and Fe and Al concentrations were higher in roots than shoots (SR < 1), in the emergent species (Fig. 6). Ye et al. (2001) also report higher concentrations of Fe in roots compared to shoots of cattail (*Typha latifolia*) in wetland cells used to treat coal combustion by-product leachate.

### 3.3. Comparisons with other wetlands

With the exception of *Juncus* spp., which has been used in wetlands designed to treat wastewater from animal production facilities (Hill et al., 1997; Clarke and Baldwin, 2002), the four wetland species planted in the CTWs are not commonly used in treatment wetlands, and have been sampled in contaminated wetlands infrequently. We surveyed the literature to compare elemental composition of plant species in the CTWs with related species in natural wetlands and wetlands contaminated with acid mine drainage (AMD). In general, Al and Fe concentrations in CTW plants were within the range for plants in SRS and other natural wetlands, and below the average for plants in contaminated wetlands. Shoot Mn concentrations ranged widely in plants from the CTWs and natural SRS wetlands, and averaged above those of plants from other natural or AMD-contaminated wetlands. Zinc concentrations in CTW plants were comparable to concentrations in the same or related species (*Myriophyllum heterophyllum*, *M. verticillatum*) growing in streams and lakes of a river system draining a mining/smelting region (Mudroch and Capobianco, 1979). Although Zn concentrations in surface sediments varied between 202 and 1270 µg/g dry sediment (mean = 402 calculated from Table 2, Mudroch and Capobianco, 1979) in these streams and lakes, concentrations of elements in the water were low (≤5 µg/L) and similar to those in the deep and shallow CTW wetlands (Table 1).

### 4. Conclusions

Wetland plants in deep and shallow aerobic wetlands of the mesocosm CTWs accumulated P and metals in biomass, which could contribute to removal of these elements from coal runoff waters. Concentrations of P and all metals except Zn increased in plant tissues over time in the CTWs, or were greater than background levels in plants before they were removed from uncontaminated donor wetlands.

As has been shown in other wetland plants, patterns of elemental uptake and concentration in the four planted species reflected plant life form, element concentrations and interactions in the system, and physiological roles of elements. In the shallow wetlands, emergent species *J. effusus* and *P. cordata* tended to have highest tissue concentrations of the less soluble Fe and Al, with greater concentrations in roots than in shoots by the second growing season. In the deep wetlands, the submerged plant *M. aquaticum* and floating-leaved *N. odorata* had highest tissue concentrations of the more soluble elements Na, Mn, and P. Sodium became concentrated in *Nymphaea* shoots over time in the CTWs; the large surface area of floating leaves may have contributed to uptake of this element.

In general, metal concentrations within the four plant species were more similar to those in plants from natural wetlands than in plants subjected to high concentrations of AMD (Table 2), which suggests the primary role of the plants in the CTW may be to help polish the discharge by accumulating a small percentage of metals. We conclude that the four common species of southeastern wetlands can function in this capacity for at least two growing seasons in CTWs designed to remediate acidic, metal-contaminated coal runoff.

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