CONTRIBUTIONS OF *Schoenoplectus californicus* IN A CONSTRUCTED WETLAND SYSTEM RECEIVING COPPER CONTAMINATED WASTEWATER

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Abstract. Functional roles of *Schoenoplectus californicus*, giant bulrush, were evaluated in an 3.2 ha (8-acre) constructed wetland treatment system receiving copper-contaminated water. The constructed wetland used in this research was designed to decrease bioavailable copper concentrations in a wastestream and eliminate associated toxicity to downstream biota by exploiting the thermodynamic processes responsible for copper speciation. This was achieved by integrating carbon, sulfur and copper biogeochemical cycles. In this system, *S. californicus*, which represents an integral part of the carbon cycle, provides a physical, chemical and biological role in removing metals from the aqueous phase. The specific contributions of *S. californicus* in this system are to provide a sustainable carbon source for removal of copper by (1) provision of an organic ligand for sorption of copper entering the system, (2) production of organic ligands through growth of *S. californicus*, (3) accretion of organic ligands over time due to decomposition of *S. californicus* detritus, and (4) use of organic carbon as an energy source for dissimilatory sulfate production. Shoots and roots of viable *S. californicus* sorbed 0.88% and 5.88%, respectively, of copper entering the system. The half-life of *S. californicus* detritus in the constructed wetland system was approximately 184 d, indicating that sufficient detritus will accrete over time, providing binding sites for copper and an energy source for bacterial metabolic processes that contribute to copper immobilization in wetland systems.

Keywords: constructed wetland, copper, coupled biogeochemical cycles, giant bulrush, *Schoenoplectus californicus*,

1. Introduction

Roles of plants in constructed wetlands designed to remove metals (e.g. Cu, Pb, Zn) from wastewaters have not been studied extensively. In many cases, the roles of vegetation in removing metals from wastewater have been limited to or attributed to adsorption onto plant surfaces (Sinicrope *et al*., 1992; Gillespie *et al*., 1999), adsorption to detritus (Jenne, 1995), plant uptake by absorption (Gupta *et al*., 1994; Vymazal *et al*., 1998) and "hyperaccumulation" or phytoextraction (Rai *et al*., 1995; Salt *et al*., 1995; Sharma and Guar, 1995; Cheng *et al*., 2002). In addition to using vegetation as sources of organic ligands, constructed wetland treatment systems can

be specifically designed to further utilize other functional roles of macrophytes for promoting transfer and transformation processes that allow metals such as copper to be removed from the water column and sequestered in wetland sediments in non-bioavailable forms (Sinicrope *et al*., 1992; Hawkins *et al*., 1997; Gillespie *et al*., 1999, 2000).

A primary function of macrophytes in wetlands is to photosynthetically produce organic carbon. This carbon, when introduced through the degradation of plant tissue, provides a major carbon and energy source for sulfate reducing microbes that exist within the system (Moshiri, 1993). By using plants as an organic carbon source within a constructed wetland, metals, such as copper, can be precipitated from the water column through formation of stable metal sulfide minerals. The reduced sulfur for this reaction is provided by dissimilatory sulfate reduction (Vymazal *et al*., 1998). Therefore, a plant species with a biomass production rate exceeding the plant detritus decomposition rate, can be selected so that organic soil or "peat" will accumulate within the constructed wetland over time (Johnston, 1991; Vymazal *et al*., 1998). In aquatic systems such as wetlands, "peat" (i.e. partially decayed organic matter, mainly of plant origin) is formed through the decay of organic matter with emergent macrophytes supplying a significant source of organic material to the system (Wetzel, 1983). In effect, this produces an accreting sediment or hydrosoil in the constructed wetland system. In a constructed wetland designed to remove metals such as copper or zinc, an accreting system will provide an organic carbon source for sulfate reducing bacteria over time and allow the mass of sediments within the system to grow at a rate that exceeds the mass of metal entering and sequestered within the system. Since the metals are precipitating from the water column and are sequestered in the sediments, an accreting system will prevent the concentrations of copper or zinc in the sediments from increasing to concentrations that may be toxic to the vegetation or benthic organisms associated with the sediments. These novel functional roles provide the theory behind the use of *Schoenoplectus californicus* (C.A. Meyer) Palla in the constructed wetland designed for this research.

In 1999, a revised National Pollutant Discharge Elimination System (NPDES) permit established new outfall limits for a wastestream located on the Savannah River Site in Aiken, South Carolina. The wastestream formed an unnamed tributary into Tim's Branch, which discharges into the Savannah River. At that time the new limits were proposed (1998), samples collected from the wastestream failed to meet the proposed permit limits for copper and chronic toxicity (U.S. EPA, 1998). A toxicity identification evaluation (TIE) determined that copper was the causative agent of the observed toxicity. Therefore, a constructed wetland treatment system was designed to decrease total copper concentrations in the wastestream to $\leq$22 μg/L and eliminate the associated toxicity as required by the new NPDES permit. In aquatic systems, copper bioavailability and associated toxicity are largely dependent on the chemical forms or species of this metal (Deaver and Rodgers, 1996). Therefore, this constructed wetland treatment system was designed to mitigate effects of copper using characteristics that exploit the thermodynamic processes responsible for copper

speciation. Specifically, this constructed wetland was designed to couple carbon, sulfur and copper biogeochemical cycles so that inorganic copper complexes are formed in an environment poised for dissimilatory sulfate reduction (Leckie and Davis, 1979; Brookins, 1988; Morel and Hering, 1993; Morse, 1995). In this environment, copper precipitates from the water column and is sequestered in the wetland sediment in non-bioavailable forms (e.g. HS and H_2S) (Brookins, 1988; Ankley *et al*., 1996; Berry *et al*., 1996).

As described previously, the functional contributions of *S. californicus* in this constructed wetland include: (1) providing organic ligands for initial adsorption and absorption of copper entering the system, (2) continuous and sustainable production of organic ligands through the growth of *S. californicus*, (3) production of organic carbon as an energy source for dissimilatory sulfate reduction, and (4) accretion of organic matter in sediment over time due to decomposition and accumulation of detritus. To provide these functional contributions, *S. californicus* was selected for this constructed wetland treatment system based on published and experimental data that demonstrate (1) the plant's ability to maintain a reduced hydrosoil necessary for dissimilatory sulfate reduction (Josselyn *et al*., 1990; Sinicrope *et al*., 1992; Hawkins *et al*., 1997; Gillespie *et al*., 1999; 2000); (2) its ability to provide an organic carbon source for sulfate reducing bacteria (Sinicrope *et al*., 1992; Araujo de Oliveira *et al*., 1994; Richardson *et al*., 1995); and (3) a sufficiently slow detritus degradation rate for increasing organic binding sites over time (Smith, 1979) and production of peat sediments. In addition to *S. californicus*, other volunteer plants that colonized the constructed wetland cells will contribute organic carbon to the wetland providing additional binding sites and energy source for sulfate reducing bacteria.

The purpose of this research was to determine the ability *S. californicus* to accomplish the desired roles in a constructed wetland treatment system specifically designed to decrease copper bioavailability to downstream aquatic biota. Specific objectives were: (1) to measure the extent of adsorption and absorption of copper by *S. californicus* over time; (2) to contrast the concentrations and distributions of copper sorbed by *S. californicus* and other volunteer plant species found in the wetland; (3) to measure production of *S. californicus* within the constructed wetland over time; (4) to determine the rate of *S. californicus* detritus decomposition within the constructed wetland treatment system over time; and (5) to measure the oxidation-reduction potential over time in constructed wetland sediments planted with *S. californicus*.

2. Materials and Methods

2.1. STUDY SITE

The constructed wetland used in this research is located at the U.S. Department of Energy's Savannah River Site, which occupies approximately 777 km^2 (300 $mile^2$)

in Aiken and Barnwell counties of South Carolina. The wastestream treated by this constructed wetland system was comprised of process wastewater from technical facilities (58% of total flow) and stormwater runoff (42% of total flow) from a 77-ha watershed. Land coverage of the drainage area included industrial development (47%), forest (42%), grass (9%), pavement (1%), and gravel roads (1%). Average daily process flow was approximately 0.04 m^3/s (0.97 million gpd), with low flow of 0.01 m^3/s (250,000 gpd) and peak flow of 0.11 m^3/s (2.6 million gpd). The constructed wetland treatment system was comprised of an upstream retention basin to control water flow to the constructed wetlands, and eight wetland cells, from which the outflow is coalesced and flows into the receiving stream (Figure 1). Capacity of the retention basin was based on one day's process flow and the runoff from a 25-year storm event (79540-m^3 or 21 million gallons). All flow from the

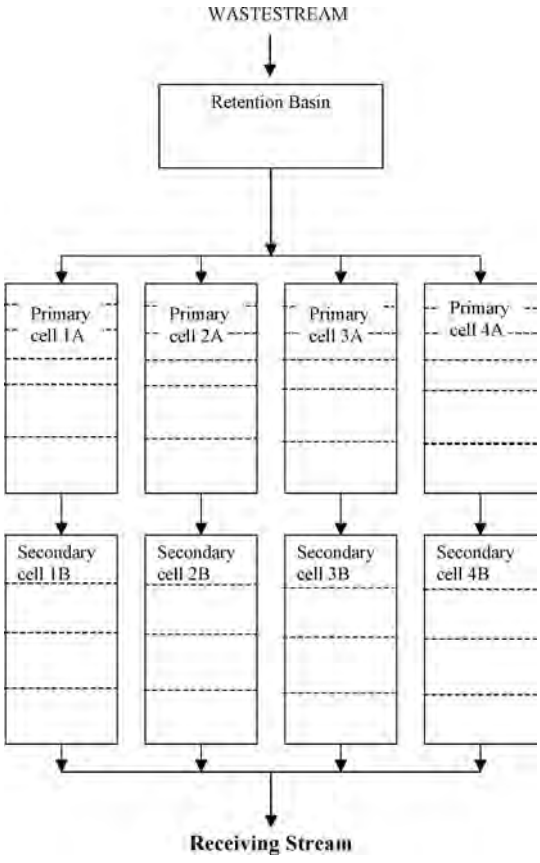

Figure 1. Flow diagram for constructed wetland treatment system designed to remove copper from a wastestream. The sampling locations are the same for each series. The dashed lines represent the assumed HRT (0, 4, 8, 12, 16, 20, 28, 36, 44, 52, and 60) where the sample was collected based on approximate linear flow.

wastestream to the retention basin and wetland cells was by gravity. Discharge from the retention basin was split into four streams, and each stream sequentially flowed into a series of two 0.4-ha wetland cells. Each series of wetland cells consists of a primary and a secondary cell for a total of eight cells (each cell 130 m $\times$ 31 m) forming the constructed wetland treatment system. The nominal HRT per series was 64 h at normal daily flow. The system was designed to provide a 48-h HRT at normal flow with one series of cells off line (in case of needed maintenance).

Hydrosoil for the full-scale system consisted of local soils ($\sim$85% sand, 15% silt and clay, 1.5% organic matter content) amended with pine mulch to achieve 3 to 5% organic matter by weight, agricultural lime to neutralize pH, gypsum as a source of sulfur, and Osmocote® time-release fertilizer. Wetland cells were planted from May to July 2000 with *S. californicus* on approximately 1-m centers. Plants were obtained from an aquatic plant nursery as root stock with shoots cut to approximately 30 to 45 cm in length. Water flow was established in wetland cells just prior to planting and water depth was maintained near the hydrosoil surface for two to four months to allow vegetation to become established. Following this time water depth was increased to approximately 30 cm. For purposes of this research, evaluation of this constructed wetland treatment system began on January 1, 2001.

2.2. SORBED COPPER ANALYSIS

S. californicus shoots and associated roots were harvested after 259, 294, 343, 416, and 569 d of copper loading to the system to determine the adsorbed and absorbed fractions of copper. Plants were collected from 7 locations from inflow to outflow of the primary wetland cell (Figure 1) using a shovel and stored in labeled plastic bags at 4 °C for transport to the laboratory at Clemson University. Vegetation was collected from the primary cell for these measurements because previous research indicated that the majority of copper in the wastestream was removed within the primary cell [average inflow concentrations and outflow concentration from the primary cells are 31 ($\pm$10) μg/L total copper and 6 ($\pm$4) μg/L total copper, respectively (Murray-Gulde *et al.*, 2005)]. For analysis, plants were rinsed with deionized water to remove sediment and were separated into aerial shoots, submerged shoots (from roots to water's surface) and roots using stainless steel scissors. Adsorbed copper was determined by rinsing submerged shoots and roots with 15% trace metal grade nitric acid. Rinsate was collected, brought to a known volume with Milli-Q water, filtered (0.45μm filter) and stored until analysis (Sinicrope *et al.*, 1992; Gillespie *et al.*, 2000). Absorbed copper was measured by drying the rinsed plant segments at 100 °C for 24–48 h, grinding the tissue into small pieces ($<$2 mm) and digesting 0.5–1.0 g in concentrated trace metal grade nitric acid using a Milestone ETHOS® 900 Microwave Labstation (U.S. EPA, 1996). Digested material was passed through a 0.45 μm filter, diluted to a known volume with Milli-Q water and stored for analysis. Copper concentrations were measured by flame atomic absorption (AA) spectroscopy using a Perkin-Elmer Aanalyst 800.

Background copper concentrations were measured using vegetation collected in November 2000, approximately 1 month prior to establishment of a 30 cm flow depth.

Following 518 d of copper loading to the constructed wetland cells, samples of plant species that voluntarily colonized the constructed wetland treatment system were collected from (1) within 25 m from the inflow structure of the primary cell, (2) the middle of the primary cell (approximately 48 to 66 m from the inflow) and (3) within 25 m of the outflow structure of the primary cell. Upon returning to Clemson University the plants were rinsed in deionized water and identified to species. Following identification, the plants were separated into seeds, flowers, aerial shoots, submerged shoots (from roots to water's surface) and roots as described above. Adsorbed copper was measured by rinsing submerged sections of the roots and shoots with 15% nitric acid. Each section of the plants was then digested using the absorbed copper procedure described previously. Copper concentrations were measured by flame AA spectroscopy using a Perkin-Elmer Aanalyst 800.

2.3. PLANT PRODUCTION

Within each wetland cell, transect sampling and the harvest method (Rodgers *et al*., 1973; Smeins and Slack, 1978) were used to estimate primary production of *S. califonicus* in the wetland. On designated sampling dates, standing crop density was measured using a 125 ft line transect and counting all visible shoots within 10 cm of the transect (Smeins and Slack, 1978). Monthly sampling conducted during the initial growing season was decreased to bi-monthly sampling as plant growth and density began to stabilize. Shoot height was also measured on each sampling date at three locations within each cell ($n = 24$). Root-shoot samples for biomass analysis were collected on September 17, November 28, 2001, February 21, and July 24, 2002, by removing above and below-ground plant material from each wetland cell ($n = 8$). *S. californicus* roots and shoots were rinsed to remove sediment and algae, placed in a labeled plastic bag and stored on ice for transfer to the laboratory. In the laboratory, plants were sorted into roots (including rhizomes) and shoots where appropriate, and dried to a constant weight at 100 °C. Dry weight was determined using a Mettler Toledo PG503-S mass balance. Estimates of the change in biomass (g m^{-2}) over time were accomplished by determining above ground and below ground mass and multiplying by the mean standing crop density for each sampling date (Henson, 1987).

2.4. DECOMPOSITION

In situ decomposition of *S. californicus* was estimated by using nylon net litter bags and measuring mass loss over time of dried plant material (Rodgers *et al*., 1975; Hammerly *et al*., 1989). Decomposition was measured using two sizes of mesh, 1 mm and 3 mm. These mesh sizes were chosen because they were sufficiently

small to prevent large fragments of plants from washing away and large enough to allow at least a portion of the grazing invertebrates and benthic fauna access to the plant material (Rodgers *et al*., 1975). Two to five grams of oven-dried plant material were placed in each bag. The bags were placed in each cell on three separate dates, April 26, June 14 and November 28, 2001. The bags were placed on the hydrosoil surface and secured in place using plastic tie wraps. The bags were secured at three locations in each cell (from inflow to outflow of the cells). On each monthly sampling trip, one to two bags were removed from each location in each of the cells, placed in labeled plastic bags and transported, on ice, to the laboratory. Remaining plant material was dried at 103–105 °C for 24 h and weighed to determine the fraction lost over time. The detritus decay rate was determined by comparing initial and final dry weights, calculating percent biomass loss, and plotting over time for each mesh size. Decay rates were compared for summer months and winter months using litter bags placed in the wetland cells on June 14 and November 28, 2001, respectively.

2.5. Oxidation-reduction potential

Oxidation-reduction (redox) potential for wetland hydrosoil was measured monthly to indicate the status of the system for dissimilatory sulfate reduction. Redox was monitored monthly at four sampling sites within each constructed wetland cell, for a total of thirty-two redox measurements each sampling date. Platinum tipped redox probes were positioned along a diagonal in each wetland cell in November 2000 and remained *in situ* for the duration of the study with periodic maintenance as needed. Redox measurements were made against an Accumet® calomel reference electrode using a Fluke® 77 III voltage meter (Faulkner *et al*., 1989).

3. Results

3.1. Copper sorbed to submerged *Schoenoplectus californicus*

Background concentrations of copper adsorbed to submerged shoots and roots in November 2000 were 0.07 (±0.03) and 0.98 (±0.61) μg/g, respectively. Absorbed copper concentrations were 2.41 (±0.29) and 7.43 (±3.14) μg/g for shoots and roots, respectively. During the study, copper adsorbed to submerged *S. californicus* shoots ranged from 0.09 (±0.06) to 1.07 (±0.78) μg/g (Figure 2). Root adsorbed copper concentrations ranged from 0.98 (±1.25) to 5.64 (±2.73) μg/g (Figure 2A). Adsorbed copper concentrations were significantly greater on roots than shoots ($p = 0.04$). Absorbed copper concentrations ranged from 3.14 (±0.29) to 9.31 (±4.73) μg/g in shoots, compared to 9.34 (±5.14) to 51.32 (±32.13) μg/g absorbed

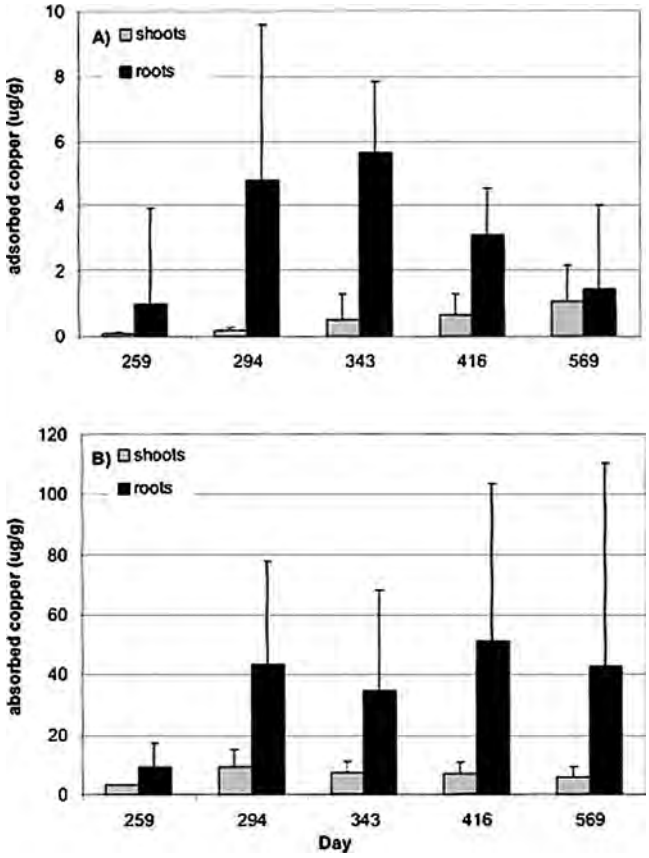

Figure 2. Average sorbed copper concentration measured in *Schoenoplectus californicus* shoots and roots collected from the first cell of the constructed wetland treatment system. Error bars represent upper range of concentrations.

by roots (Figure 2B). Similar to the adsorbed copper, copper absorbed by roots was significantly greater than copper absorbed by shoots ($p = 0.01$). Overall, adsorbed copper concentrations measured on roots and shoots throughout the study were significantly less than absorbed concentrations (7.07 ± 2.33 μg/g and 39.39 ± 17.09 μg/g, respectively; $p = 0.008$)

When comparing the concentration of copper sorbed by roots and shoots of plants located from inflow to outflow of the constructed wetland cell, a decreasing trend is observed in both the concentration of copper adsorbed and adsorbed by *S. californicus* (Figure 3) corresponding with a decrease in total copper concentrations observed in the surface water of the wetland cells from inflow to outflow (Figure 4). As stated previously, the absorbed copper concentration is significantly greater than the adsorbed concentrations and the concentrations of copper sorbed by the roots are statistically greater than the shoots.

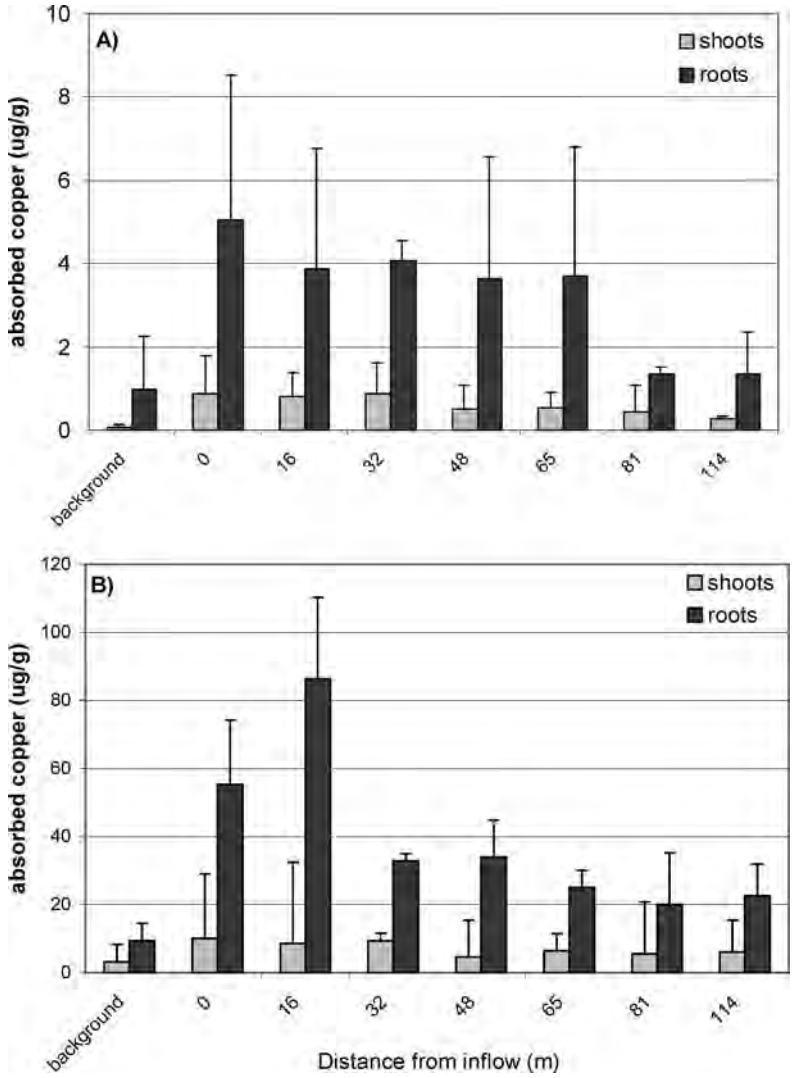

Figure 3. Sorbed copper measured in *Schoenoplectus californicus* shoots and roots collected October 2001, December 2001, February 2002 and July 2002 from inflow to outflow of the first cell of the constructed wetland treatment system. Error bars represent standard deviation.

3.2. COPPER SORPTION TO OPPORTUNISTIC SPECIES

During the period of the study, several species of vegetation other than *S. californicus* were observed in the wetland cells, however, *S. californicus* remains the clearly dominant species. Colonization of other species was likely the result of seeds or other propagules entering the wetland through sources such as wildlife, wind and

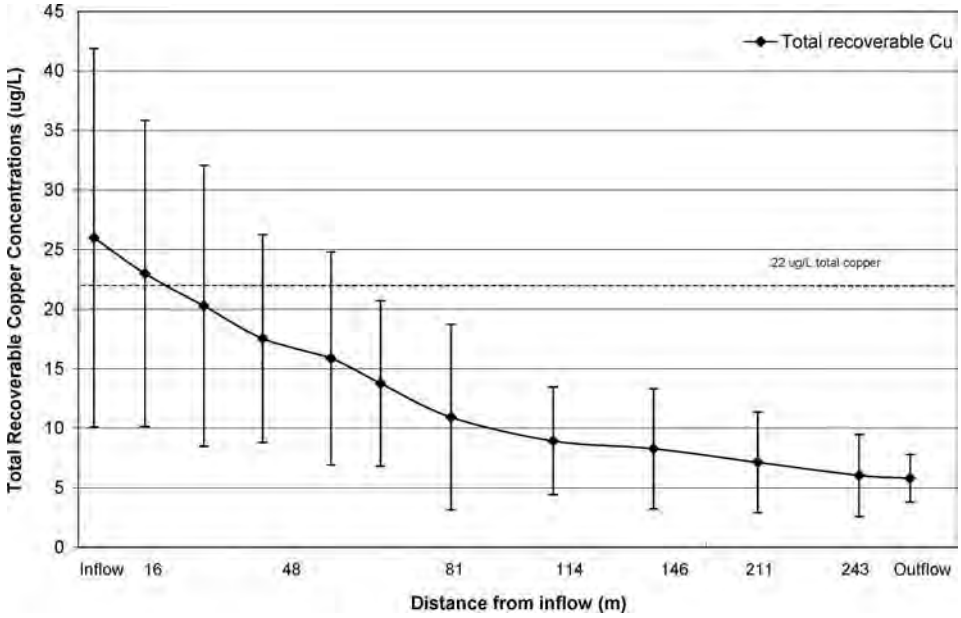

Figure 4. Mean total recoverable copper concentrations measured in the Constructed Wetland Treatment System (March 2001 to July 2002). Error bars represent standard deviation. Dashed line represents NPDES permit limit of 22 μg Cu/L.

transplanting. Table I lists the species identified in the wetland in June 2002. On June 29, 2002, (following 518 days of copper loading to the constructed wetlands) nine species were collected, identified and analyzed for copper. If possible, more than one sample of each species was collected from the wetland. The species collected and analyzed for copper analysis included *Schoenoplectus californicus* (C.A. Meyer) Palla, *Calamagrostis sp*. Adans., *Cyperus haspan* L., *Echinodorus cordifolius* L.C. Rich ex. Engelm., *Eleocharis obtusa* (Willd.) Schultes, *Hydrocotyle ranunculoides* L.f., *Juncus sp*. L., *Leersia hexandra* Sw., *Sagittaria subulata* (L.) Buchenau, and *Typha latifolia* L. The concentrations of total copper sorbed to roots, submerged shoots, aerial shoots and seeds, for each of the identified species are listed in Table II. Of the species analyzed, *Hydrocotyle ranunculoides* had the highest concentrations of copper sorbed to its roots, runner, leaves and seeds (Table II). Unlike other vegetation measured as part of this study, *Hydrocoty*le is a floating leaf macrophyte with long floating stems that form roots at the nodes. Often the roots float, which provides direct contact with the water. Therefore, both the structure and route of exposure (directly from the water column) of *Hydrocotyle* differs from the other species with roots immersed in the sediments. Due to these differences in structure or growth habit and exposure, *Hydrocotyle* was not included for comparisons of copper distribution with *S. californicus*. All the other species collected were emergent macrophytes and would have similar routes of exposure.

TABLE I

Aquatic macrophytes identified in constructed wetland treatment system designed to decrease copper concentrations in a wastestream

Scientific name	Common name
Azolla caroliniana	Carolina water fern
Alternantheria philoxeroides	Alligator weed
Calamagrostis sp.	Reed-bentgrass
Cyperus esculentus	Yellow nut-grass
Cyperus iria	Umbrella sedge
Cyperus haspan	Umbrella sedge
Echinodorus cordifolius	Creeping burhead
Eleocharis obtusa	Blunt spike rush
Hydrocotyle ranunculoides	Water pennywort
Juncus sp.	Rush
Leersia hexandra	Cutgrass
Lemna perpusilla	Duckweed
Ludwigia microcarpa	False Loosestrife
Pontederia cordata var. lancifolia	Pickerelweed
Sagittaria graminea	Grassy Arrowhead
Sagittaria subulata	Arrowhead
Salix sp.	Willow
Scirpus cyperinus	Woolgrass bulrush
Typha latifolia	Broadleaf cattail
Utricularia sp.	Bladderwort

A comparison of all emergent species collected approximately 45–60 m from the inflow to the primary wetland cell indicates the highest concentrations of copper are in the roots in all of the species analyzed except *E. cordifolius*, *Juncus sp.*, and *S. subulata* (Table II, Figure 5). These three species, were collected at a similar location within the wetland cell and had copper distributions in roots significantly less than *S. californicus* (approximately 16, 17, 35 and 64% total Cu, respectively). In addition, these three species also had the highest percent of sorbed copper measured in their aerial shoots and seeds (Figure 5). Of the nine emergent species analyzed, *Calamagrostis sp.*, *L. hexandra*, and *T. latifolia* sequestered the highest distribution of sorbed copper in their roots (approximately 87, 80 and 76 μg total Cu, respectively) and transported the least amount of copper to their shoots and seeds (Figure 5). However, the distributions of copper measured in the roots, shoots and seeds of *Calamagrostis sp.*, *L. hexandra*, and *T. latifolia* were not significantly different from the distribution measured in *S. californicus* ($P = 0.004$). The distribution of copper in *C. haspan* and *E. obtusa* most closely resembled the distribution in *S. californicus*.

TABLE II

Distribution and concentration of copper (μg/g) sorbed to ten species of vegetation found in a constructed wetland designed to decrease bioavailable copper from a wastestream. Concentrations measured following 518 days of copper loading to the wetlands

	Concentration of copper (μg/g)									
Species	*S. californicus*	*Calamagrostis sp.*	*C. haspan*	*E. cordifolius*	*E. obtusa*	*H. ranunculoides*	*Juncus sp.*	*L. hexandra*	*S. subulata*	*T. latifolia*
Seed/flower	5.1–7.8 (±2.0)	5.5–8.9	14.7–16.7 (±1.4)	24.4	12.9	18.8–35.3 (±11.6)	9.5	NA	32.2	2.6–4.2 (±1.1)
Aerial shoot	1.8	NA	NA	78.9[a]	NA	23–114.4 (±50.8)[a]	4.4	2.1	NA	2.9–4.1 (+0.9)
Submerged shoot (adsorbed)	9.1–23.1 (±9.9)	14.3	18.6–75.4 (±40.1)	12.2[b]	1.3	14.4–34.4 (±14.2)[b]	3.8	4.3	15.5	4.6–4.9 (±0.2)
Submerged shoot (absorbed)	1.9–6.6 (±3.4)	16.9	19.5–20.1 (±0.4)	14.6[b]	11.3	33.5–38.8 (±3.8)[b]	5.2	1.8	35.6	2.2–2.5 (+0.2)
Root (adsorbed)	1.6–4.2 (±1.9)	17.4	2.1–7.4 (±3.8)	7.9	4.8	73.8–76.9 (±2.2)	0.7	1.0	14.0	1.1–1.5 (±0.2)
Root (absorbed)	28.4–79.8 (±36.3)	141.7	68.4–78.4	117.7	48.7	206–217 (±7.9)	3.6	57.1	32.8	23.8–118.3 (±66.8)

NA – not collected and measured.
[a]leaf; [b]runner.

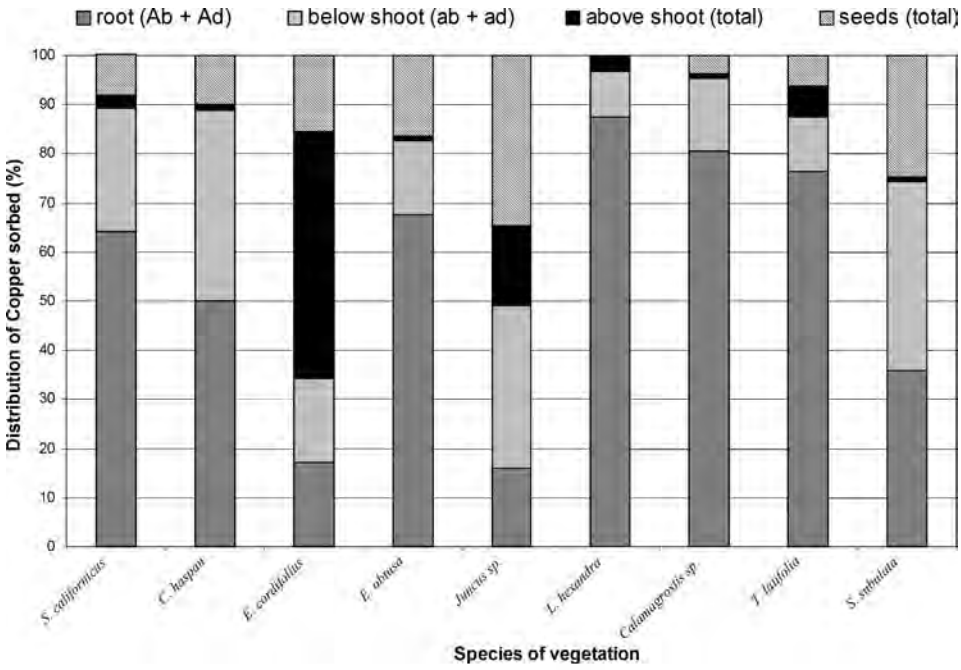

Figure 5. Distribution of copper measured in nine species of vegetation collected from 45–60 m from the inflow of the primary cell of the Constructed Wetland Treatment System (June 2002).

3.3. *SCHOENOPLECTUS CALIFORNICUS* PRODUCTION

The mean density of *S. californicus* increased from 100 shoots/m^2 to 500 shoots/m^2 in the first growing season, decreasing to 20 shoots/m^2 at the end of winter. During the second growing season, an increase to 770 shoots/m^2 was observed by July 2002 (Figure 6). Average plant height increased from 150 cm (range 105–214 cm) in March 2001 to 267 cm (228–330 cm) in July 2002. Analysis of the above ground and below ground biomass, indicates that the mean ratio of roots to shoots is approximately one (Figure 7). Based upon measured biomass and adsorbed and absorbed copper concentrations, *S. californicus* shoots accounted for $0.88 \pm 0.66\%$ of the copper loaded to the wetland system, while $5.88 \pm 3.61\%$ was associated with the roots (Table III). Based on the increase in above and below ground biomass observed throughout the study, no adverse effects due to copper exposure have been observed.

3.4. DECOMPOSITION OF *SCHOENOPLECTUS CALIFORNICUS*

As expected, decomposition rates of senesced *S. californicus* differed between the summer and winter months (Figure 8). The calculated decomposition rate coefficients (assuming first order decay) for litter bags *in situ* from April 2001

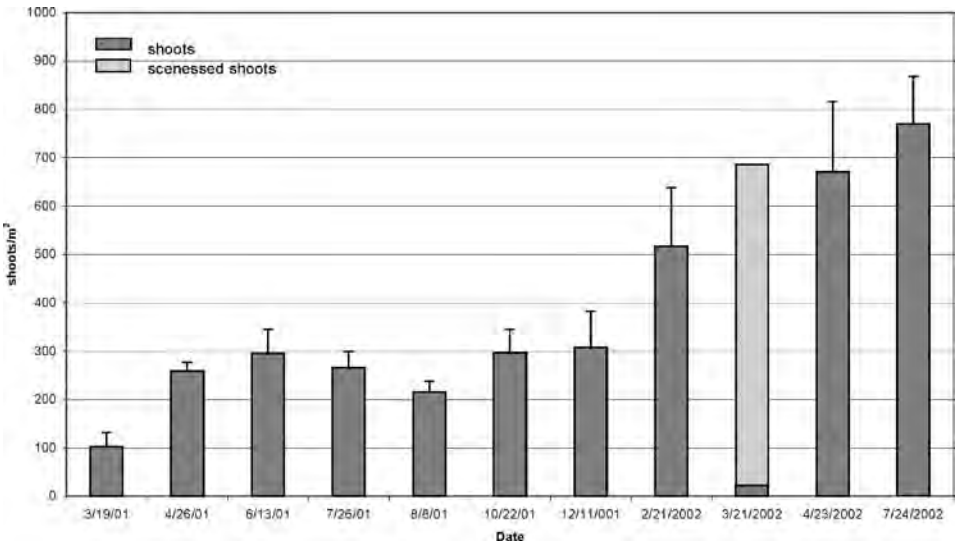

Figure 6. Mean stem density of *Schoenoplectus californicus* in a constructed wetland designed to decrease the concentration of bioavailable copper in a wastestream. Initial stem density at planting 1 plant per m^2 (May–July 2000).

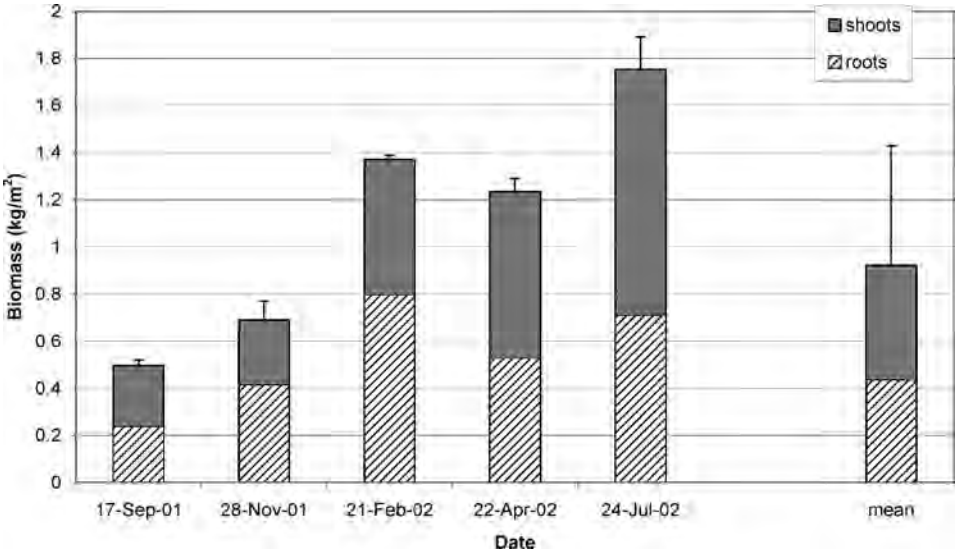

Figure 7. Shoot:root distribution of *Schoenoplectus californicus* biomass (kg/m^2) measured in a full-scale constructed wetland designed to remove copper from the water column. Error bars represent standard deviation.

TABLE III

Distribution of copper sorbed by viable *S. californicus* shoots and roots after 259, 294, 343, 416, and 569 days of copper loading to the constructed wetland treatment system

Day		Sorbed copper (μg/g)	Biomass (g/m^2)	Mass of copper sorbed (μg/m^2)	Mass of copper added to wetland system (g)	% copper added to wetland system sorbed by vegetation
259	Shoots	3.21	258	829.39		0.36
	Roots	10.32	237	2446.97	1850	1.07
294	Shoots	9.47	275	2604.70		1.00
	Roots	48.04	414	19886.7	2100	7.67
343	Shoots	8.04	574	4615.08		1.52
	Roots	40.16	795	31929.20	2450	10.55
416	Shoots	7.57	25	189.36		0.05
	Roots	54.43	262	14260.49	2971	3.88
569	Shoots	7.06	1045	7377.70		1.47
	Roots	44.03	708	31173.24	4064	6.21
Mean	Shoots				0.88 (s.d. 0.66)	
	Roots				5.88 (s.d. 3.61)	

until April 2002 were $k = 0.005/d$ and $k = 0.003/d$ for the 1 mm and 3 mm mesh size litter bags, respectively. The estimated average half-life of *S. californicus* detritus was 184 d, with approximately 23% of the organic matter remaining after one year. No significant difference was observed between the rates of decay measured using 1 mm and 3 mm mesh bags. However, a significant difference in decomposition rate coefficients was observed between litter bags placed in the system on June 2001 (monitored for 166 days) and litter bags placed in the system in November 2001 (monitored for 144 days) ($k = 0.01/d$ and $0.004/d$ for summer and winter, respectively).

3.5. Oxidation-reduction potential

Redox potential measured in the wetland hydrosoil provided evidence that translocation of oxygen to the rootzone by *S. californicus* was not sufficient to increase the average redox potential to levels greater than -75 mV over time. The overall average redox of the constructed wetlands was -133 (± 27) mV (range $= -17$ to -261 mV), which is within the desired range of -75 to –250 mV (Figure 9).

4. Discussion

The availability of trace metals to aquatic macrophytes is a complex process that is dependent on both specific chemical characteristics of the metal and the substrate in

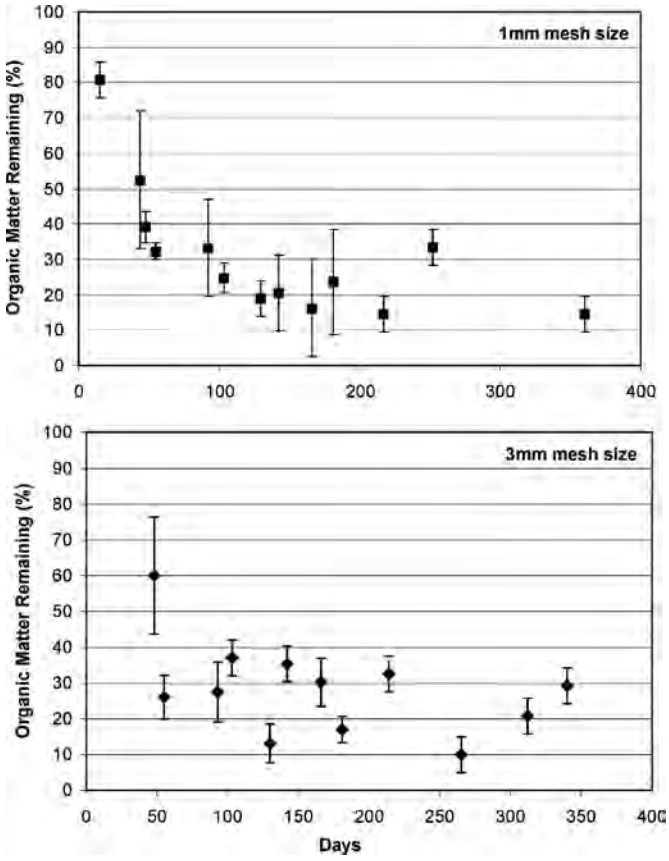

Figure 8. Decomposition of *Schoenoplectus californicus* detritus measured using 1 mm and 3 mm nylon mesh bags in a full-scale constructed wetland receiving copper-contaminated water, April 2001 through April 2002.

which the metal is found (Taylor and Crowder, 1983; Jackson *et al.*, 1991; Sparling and Lowe, 1997; Zayed *et al.*, 1998). In most aquatic systems, the concentration of metal (such as copper) measured in rooted macrophytes is generally proportional to the concentration of metals in the surrounding sediment (Jackson *et al.*, 1991; Jackson, 1998). However, due to the complexation of metals to sulfides, organic matter, carbonate compounds and iron or manganese oxyhydroxides that exist in sediments, only a fraction of the total concentration of metal measured in a sediment is in the ionic form and available for uptake (Jenne, 1968; Anderson, 1975; Fitter and Hay, 1983; Jackson, 1998). Therefore, deviations from a 1:1 relationship of total metal concentration in the sediment and metal concentration in the rooted macrophytes are expected (Jackson, 1998). In this constructed wetland, the average mass of copper measured in the sediments decreased from 57 (+40) μg/g at the inflow of the primary cell to 6 (+2) μg/g at the outflow of the primary cell

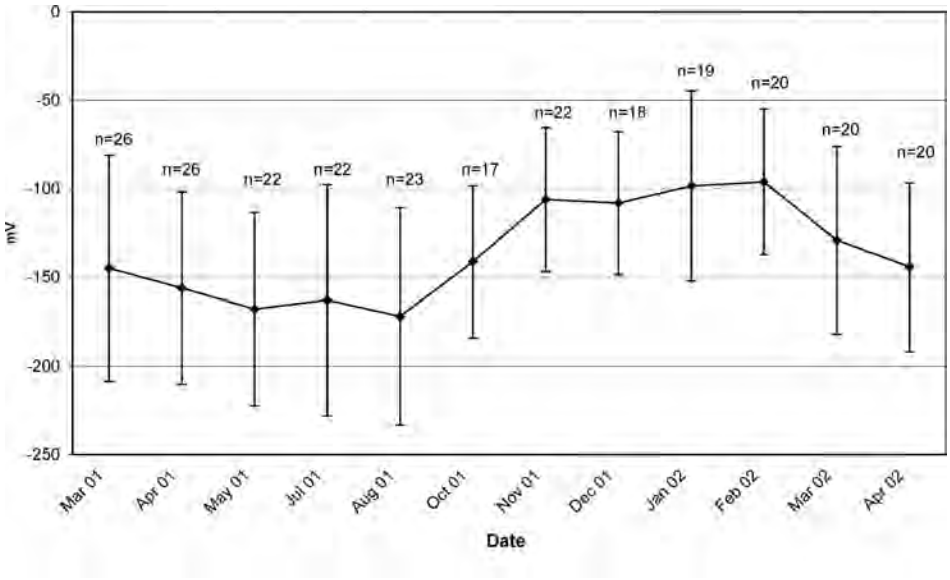

Figure 9. Average hydrosoil oxidation-reduction potential in a full-scale constructed wetland. Error bars represent the standard deviation.

corresponding to the decline in metal concentration in the overlying water (average aqueous total copper concentrations at the inflow and outflow of the primary cell are 31 μg/L and 9 μg/L, respectively). Similarly, the concentrations of copper measured in *S. californicus* root tissue ranges from 56 (+18) μg/g in samples collected from the inflow to 22 (+9) μg/g at the outflow of the primary cell. Therefore, on average for this constructed wetland, a 1:1 relationship was observed. These results differ from studies of constructed wetlands used to remove copper from acid mine drainage (Mays and Edwards, 2001), where plant metal concentrations were not significantly correlated with sediment or water concentrations of metals (i.e. the distance from the wetland inlet had no significant effect on the metal concentrations measured in plants). In the present constructed wetland system, a clear relationship was observed between the decrease in concentrations of copper sorbed by plant tissue from inflow to outflow and decreases in concentrations of copper measured in the sediments and overlying water.

In comparing the distribution of copper observed in *S. californicus* with other emergent aquatic species found in this constructed wetland, the highest copper concentrations were detected in the roots, with decreasing copper concentrations measured in the submerged shoots, aerial shoots and seeds for the species *Calamagrostis sp.*, *C. haspan, E. obtusa, L. hexandra*, and *T. latifolia*. These results are similar to other studies of the distribution of metals in macrophytes which have demonstrated a general decreasing trend of roots > rhizomes and immersed parts of leaves > aerial leaves (Taylor and Crowder, 1983; Vymazal, 1995). Of the species

analyzed in this study, *Calamagrostis sp*., *L. hexandra* and *T. latifolia* translocated the lowest concentrations of copper to above ground tissue. This is consistent with a study by Taylor and Crowder (1991), which demonstrated the ability of *T. latifolia* to exclude most copper from above ground tissue. In contrast, the concentrations of copper measured in the roots of *E. cordifolius*, *Juncus sp*., and *S. subulata* were not different from the concentrations of copper measured in their aerial shoots and seeds. Although the results from this study agree with those from studies that have concluded that the characteristics of the metal influence the distribution between roots, shoots and leaves (Cheng *et al*., 2002) it appears that metal characteristics alone do not regulate transport to above ground vegetation. Rather, the differences in the distribution of copper between roots, shoots and seeds may be more closely associated with growth form (emergent vs. submerged, floating species) and species of vegetation (Rai *et al*., 1995).

Studies have shown that emergent species have lower total metal concentrations than submerged or non-rooted, floating species (Sparling and Lowe, 1997) and that metal uptake by plants varies among species (Rai *et al*., 1995). Submerged plants generally have the ability to uptake higher concentration of metals from the water column compared to emergent macrophytes due to higher surface/biomass ratios observed with submerged vegetation (Rai *et al*., 1995). Macrophytes with floating roots generally absorb higher concentrations of metals compared to emergent vegetation due to the direct contact of roots with metals in water (Jackson, 1998). The *Hydrocotyle ranunculoides* analyzed as part of this study is a floating leaved aquatic macrophyte with long floating stems that form roots at the nodes allowing the leaves to emerge slightly above the surface of the water (Fernald, 1950). A comparison of *H. ranunculoides* to the other emergent macrophytes observed in this study (i.e. macrophytes having roots and rhizomes that exist predominately below the surface of the hydrosoil), demonstrates the difference in copper concentrations observed in emergent and floating-leaved vegetation. Of the species analyzed in this study, *H. ranunculoides* had the highest concentration of copper sorbed to its roots, runner, leaves and seeds of any of the species analyzed. As stated previously, this difference in observed concentrations is related to the notion that the water is the principle source of uptake (for elements such as copper) by floating aquatic macrophytes that possess roots but do not have close contact with sediments (Jackson, 1998).

When assessing the influence of vegetation on performance of a constructed wetland, the roles of vegetation within the constructed wetland must be considered. Studies using *Lemna polyrrhiza* (duckweed) (Sharma and Gaur, 1995) and *Ceratophyllum demersum* (horntail) (Rai *et al*., 1995) have demonstrated that some plants species can be successfully used to remove metals from wastewater by hyperaccumulation. Other studies of constructed wetland systems have reported that uptake of metals by vegetation does not contribute significantly to the retention of metals in constructed wetlands treating acid mine drainage (Sencindiver and Bhumbia, 1988; Fennessy and Mitsch, 1989; Mitsch and Wise, 1998). The differences in metal accumulation by plants in these studies demonstrate the need to define the

contributions expected of vegetation when designing constructed wetland systems. In the constructed wetland used for this study, the primary roles of *S. californicus* are to provide an organic carbon source to the wetland over time to promote dissimilatory sulfate reduction and produce an accreting system. Therefore, as recognized in other constructed wetland systems designed in a similar manner, the total metal content in the plant component of this system accounts for only a small percentage of the annual metal load supplied to the wetland. In this study, only seven percent of the copper loaded into the wetland was removed by vegetation. A study by Sinicrope *et al*. (1992) reported that *S. californicus* shoots accounted for 0.4% of copper added and roots accounted for 7%. Gillespie *et al*. (1999) investigated the removal of zinc in similarly designed constructed wetland microcosms containing *S. californicus*, and determined that the plants accounted for approximately 2 % of the added zinc after 62 d. Similar findings were reported for copper removal from a mine drainage that was treated by a natural wetland (Eger and Lapakko, 1988) and for copper removal from municipal sewage treated by a constructed wetland (Gersberg *et al*., 1984). These studies, including the current investigation, demonstrate that in wetlands designed using vegetation that does not hyperconcentrate metals, the fraction of metal associated with living biomass is low compared to other partitioning phases such as detritus or mineral particles. Overall, total metal removal from the water by the constructed wetlands in these studies was greater than 70% with approximately 7% of the removed copper associated with the vegetation.

A primary function of the *S. californicus* in this constructed wetland is to produce organic carbon to provide a carbon and energy source for sulfate reducing bacteria in the system through the degradation of the *S. californicus*. Therefore, the productivity and decomposition of *S. californicus* are important in this system to promote dissimilatory sulfate reduction which enhances precipitation of copper as stable sulfide minerals. Productivity of emergent macrophytes is usually estimated by measuring changes in standing crop biomass over time. For many species, the production of underground root and rhizome tissue may equal or exceed that of the above ground standing crop. In this study, the average ratio of shoot biomass to root biomass was 1.1:1.0. However, over the eleven months monitored, shoot biomass to root biomass ranged from 1.0:1.6 to 1.4:0.7. These results are consistent with microcosm investigations conducted by this laboratory where the shoot to root biomass ratios were 1:1.02 and a study by Andrews and Pratt (1978) where the ratio of underground (root + rhizome) tissue to shoot tissues of cattail varied from 0.2 to 1.0.

Decomposition of *S. californicus* was measured for this system by determining the percent decrease in weight of senesced tissue. Using this method, detritus decomposition in this constructed wetland system was sufficiently slow that residual biomass from previous growth seasons (approximately 23%) will remain at the time of senescence of the present standing crop. Therefore, organic matter will accrete over time within the constructed wetland system (Johnston, 1991; Vymazal, 1998). Since this constructed wetland is designed to remove copper by precipitation from

the water column and sequestration in the sediment, an accreting system will insure the production of peat over time (Johnston, 1991; Moshiri, 1993; Mitsch and Wise, 1998) and allow for the mass of sediments within the system to grow at a rate that exceeds the mass of metal entering the system. If the detritus decay rate is too rapid [as with less lignin containing macrophytes such as *Typha spp*., which can completely decay in 180 d or less (Rodgers *et al*., 1975; Mitsch and Gosselink, 1993; Gambrell, 1994)] a sufficient reservoir of organic carbon may not be available to sustain the desired metabolic processes (e.g. dissimilatory sulfate reduction). While it is possible for macrophytes such *as S. californicus* to release sorbed metals during senescence, studies have shown that only 25–30% of the metal within macrophyte tissue is lost to surrounding water in dissolved forms (Blake *et al*., 1987; Jackson, 1994). Therefore, for the *S. californicus* in this system, the estimated amount of copper lost during decomposition of the above ground biomass is less than 1% of the copper loaded to the system.

Aquatic plants with roots in anaerobic sediments survive by transporting oxygen to their root zone. For this constructed wetland system, it was important to ensure that the sediment maintained a sufficiently negative redox to support the production of HS^- through dissimilatory sulfate reduction. Redox status of sediments is generally controlled by microbial oxidation of organic carbon (Grundl, 1995). As diatomic oxygen present in the hydrosoil is depleted, alternative electron acceptors are utilized in a well-defined sequence that results in decreasing redox potential (Pardue and Patrick, 1995). Sulfate is reduced to sulfides in this sequence between -75 and -250 mV (Mitsch and Gosselink, 1993; Morel and Hering, 1993). By maintaining a continuous organic energy source in the form of detritus, a reservoir of reduced sulfur, and hydrosoil redox conditions between -75 to -250 mV, this system will be poised to precipitate copper as a stable, non-bioavailable sulfide mineral (Morel and Hering, 1993; Morse, 1995; Deaver and Rodgers, 1996). Therefore, it is important to insure that the sediments in this system maintain reduced conditions in the presence of *S. californicus*. Because plants translocate oxygen from the shoots to the roots, the rhizosphere or root zone will offer an oxidized microenvironment in an otherwise anaerobic substrate (Gersberg *et al*., 1986).

Oxygen release rates from roots depend on the internal oxygen concentrations, the oxygen demand of the surrounding medium and the permeability of the root walls (Armstrong and Armstrong, 1988). The redox potential of hydrosoil measured within this system averaged -144 mV (range $= -17$ to -261 mV), which is within the desired range for dissimilatory sulfate reduction (i.e. utilization of sulfate as the terminal electron acceptor) (Brock and Madigan, 1988; Mitsch and Gosselink, 1993). Together, the redox and increasing biomass measured within this constructed wetland indicate that oxygen leakage of *S. californicus* occurs primarily in a microzone around the roots and serves only to oxidize and detoxify potentially harmful reducing substances within that microzone allowing the *S. californicus* to grow in a reduced environment (Brix, 1994). The multiple roles of *S. californicus* (i.e. sorption, production and decomposition) coupled with the biogeochemistry of

sulfur and copper in this constructed wetland allows for a sustainable system for decreasing the concentration and bioavailability of copper in wastewater.

5. Conclusions

Schoenoplectus californicus was utilized in this constructed wetland to promote processes that would allow copper to be removed from the water column and sequestered in wetland sediments in non-bioavailable forms. Unlike other studies that focus on removal of copper from the water column through sorption processes, a primary function of *S. californicus* in this wetland is to produce organic carbon that serves through degradation as a major carbon and energy source for microbes in the system. Copper concentrations associated with viable shoots and roots were similar to other studies involving *S. californicus* in constructed wetlands, accounting for approximately 7% of the mass of copper added to the system. As was observed in other studies, the highest concentration of copper sorbed to *S. californicus* was found in the roots. However, the distribution most commonly reported for metals in emergent macrophytes (root > rhizome > leaf tissue) was not observed in all of the species analyzed in this study. The production of *S. californicus* increased from 0.1 kg/m^2 in March 2001 to 0.77 kg/m^2 in July 2002. The half-life of *S. californicus* detritus in this constructed wetland was approximately 184 d, with approximately 23% of the biomass remaining after one year indicating that detritus will provide a continuous energy source for bacterial metabolic processes that contribute to copper transfers and transformations to less bioavailable forms. Thus, organic matter will accrete over time, also providing additional binding sites for copper. Overall, *S. californicus* met the functional roles required for removal of copper in this constructed wetland by: 1) providing organic ligands for sorption of copper entering the system, 2) continuously producing organic ligands through the growth of *S. californicus*, 3) producing organic carbon as an energy source for dissimilatory sulfate reduction, and 4) accretion of organic ligands over time due to decomposition of detritus.

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