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Sediment-Associated Elements in a Constructed Wetland Treatment System: Distribution, Characterization, and Toxicity

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ABSTRACT

In a pilot-scale constructed wetland treatment system, Hg, Se, and As were removed from flue gas desulfurization wastewater through a combination of physical, chemical, and biological processes that resulted in their accumulation in the sediment. The first objective of this research was to characterize sediment-associated Hg, Se, and As into operationally-defined fractions using sequential extraction procedures. All measurements were taken after 17 months of wastewater treatment. Mercury concentrations in the sediment varied from 0.025 to 0.035 mg kg^{-1} in the treatment system, while concentrations of Se and As in the sediment increased from 3.57 to 8.79 mg kg^{-1} and 11.91 to 14.08 mg kg^{-1}, respectively. Results suggest that Hg and As are stable, immobile, and non-bioavailable in the sediment, as long as current sediment conditions such as pH and redox potential remain stable. Approximately half of the total selenium in the sediment is mobile and bioavailable, while the other half is stable and not bioavailable to plants and organisms. Identification of the main phase associations of Hg, Se, and As in sediments helps to understand the biogeochemical processes involved and to evaluate the risk and remobilization potential of these elements in the constructed wetland. A toxicity assessment of this sediment was then performed to obtain the information needed to support environmental management decisions related to mitigating risks associated with FGD wastewater. The second objective of this research was to evaluate the toxicity of these sediments to *Hyalella azteca*. Despite the use of this constructed wetland treatment system to treat simulated FGD wastewater for over a year, the sediment was not toxic to *H. azteca*. It was apparent from this research that measuring sediment toxicity and total concentrations of Hg, Se, and As in sediment cannot provide the required information about mobility, bioavailability, and the potential impact on the aquatic system.

Keywords: arsenic, constructed wetlands, fractionation, *Hyalella azteca*, mercury, selenium

Abbreviations: As, arsenic; **CWTS**, constructed wetland treatment system; **Eq-P**, equilibrium partitioning; **FGD**, flue gas desulfurization; **Hg**, mercury; **PEL**, probably effect level; **Se**, selenium; **TEL**, threshold effect level; **TU**, toxic unit

INTRODUCTION

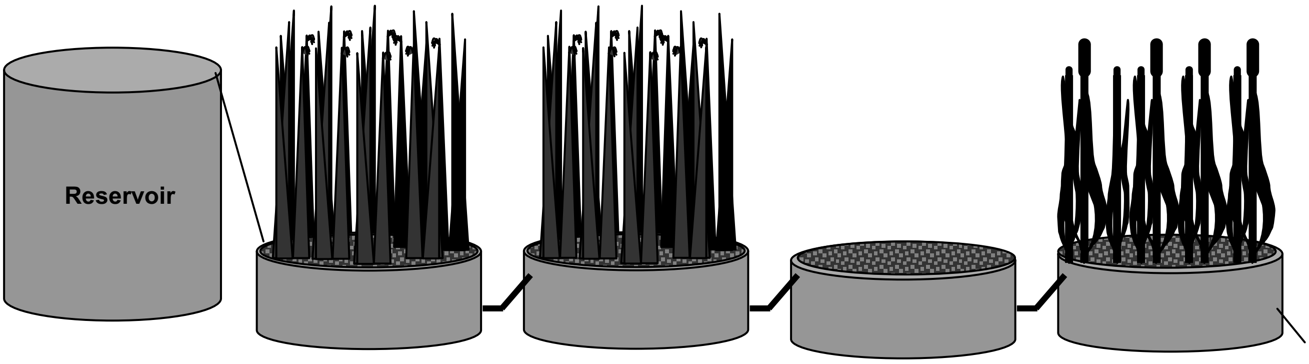
The Clean Air Act of 1963, followed by the Clean Air Act Amendments of 1990, addresses a number of air quality problems in the United States. These problems include that of acid rain resulting partially from fossil-fueled power plant emissions. The process of combusting coal to generate power transforms sulfur that naturally occurs in coal to gaseous sulfur dioxide that combines with water vapor to produce acid rain. In an effort to decrease the amount of sulfur dioxide released into the air, fossil-fueled power plants have installed flue gas desulfurization (FGD) systems, or scrubber systems. In this system, a mixture of water and pulverized limestone is sprayed on the flue gas discharged from the boiler. The limestone slurry absorbs and reacts with the sulfur dioxide to produce calcium sulfite, which then reacts with oxygen and is removed as gypsum (EPRI 1999). The FGD process results in wastewater containing high concentrations of mercury, selenium, and arsenic. This wastewater must then be treated to eliminate these contaminants in order to achieve discharge limitations established under the National Pollution Discharge Elimination System (NPDES) and Clean Water Act (EPRI 1999). Constructed wetland treatment systems (CWTSs) have considerable potential to treat targeted constituents of FGD wastewater.

Prior to field application, the efficiency of these systems must be evaluated in pilot-scale studies. A pilot CWTS was constructed at Clemson University consisting of an equalization basin followed by four wetland cells in series (**Fig. 1**). Wastewater was mixed in the basin and pumped into the first wetland cell at a constant flow rate. The hydraulic retention time (HRT), the time it takes wastewater to flow through the system, was 24 hours per wetland cell for a total HRT of 96 hours. This HRT time was chosen due to land constraints, wastewater flow rates, and costs at industrial sites where the wetland system will be constructed at full-size (Hawkins *et al*. 1997). After treatment, it was not necessary to pump the water back into the constructed wetlands. The first two wetland cells were planted with *Schoenoplectus californicus* (giant bulrush) and were designed to maintain a reducing sediment environment. The third wetland cell was filled with granite gravel and contained no sediment or vegetation. This cell was designed to aerate the water as it falls onto the gravel under the cell inflow. Finally, the fourth wetland cell was planted with *Typha angustifolia* (narrow leaf cattail) and was designed to maintain an oxidized sediment environment prior to the final outflow from the CWTS. Basic design parameters were also chosen based on a CWTS previously designed to remove copper from wastewater (Huddleston 2001; Murray-Gulde 2002).

In a CWTS used to treat constituents of FGD wastewater, sediment likely becomes a sink for contaminants such as Hg, Se, and As. Sediments commonly constitute reservoirs of contaminants that can pose potential hazards to sediment-dwelling organisms (such as epibenthic and infaunal invertebrate species), aquatic wildlife species (inc-

Received: 18 December, 2006. Accepted: 24 January, 2007.

Original Research Paper



	1st Wetland cell	2nd Wetland cell	3rd Wetland cell	4th Wetland cell
Contents	*Schoenoplectus californicus*	*Schoenoplectus californicus*	Granite gravel	*Typha angustifolia*
pH[a]	6.7	6.6	NA[d]	6.8
Redox[b]	- 410 ± 31 mV	- 337 ± 21 mV	NA	- 25.5 ± 37 mV
CEC[ac]	6.8	4.6	NA	5.1
% Organic Matter[a]	3%	2%	NA	2%
% Solids[a]	79%	79%	NA	75%
Particle Size Distribution[a]				
% Sand	91%	92%	NA	89%
% Clay	6%	6%	NA	6%
% Silt	3%	3%	NA	5%

[a] Measurements from composited sediment samples after collection (n=1).
[b] Redox potential (average ± standard deviation, n=2), measured in millivolts. Recorded before sediment collection.
[c] Cation exchange capacity (meq $100g^{-1}$)
[d] NA = not available since the wetland cell contained only granite gravel.

Fig. 1 Schematic of the pilot constructed wetland system for evaluating treatment of simulated flue gas desulfurization wastewater and characterristics of corresponding wetland cells after 17 months of wastewater treatment.

luding fish, amphibians, reptiles, birds, and mammals), and human health.

Element fractionation in sediment

Determining total concentrations of these constituents in sediment cannot provide the required information about mobility, bioavailability, and the potential impact on the aquatic system (Michalke 2003). The distribution of Hg, Se, and As in the CWTS depended on their speciation and propensity for particular biogeochemical reactions. Speciation defines a specific form of an element as to isotopic composition, electronic or oxidation state, or molecular structure (MITE 2003). Any change in environmental conditions or sediment characteristics, such as pH, redox potential, organic matter content, cation exchange capacity (CEC), and particle size distributions, can potentially cause changes in elemental speciation, mobility, and bioavailability (Lin and Chen 1998; Sanchiz *et al*. 2001).

Speciation can greatly affect the fate and behavior of elements in a constructed wetland treatment system, especially of those elements that exist in multiple oxidation states such as Hg, Se, and As. Identification of the main phase associations of these trace elements in sediments helps to understand the biogeochemical processes to evaluate the risk and remobilization potential of these elements in the constructed wetland treatment system.

Mercury has long been recognized as a constituent of concern due to its toxicity. In aqueous environments, Hg often exists as a cationic metal complexed by a variety of inorganic and/or organic ligands. Therefore, its distribution in soils is dependent not only on its speciation but also on soil pH, redox potential, and ligand availability. Mercurous (Hg(I)) and mercuric (Hg(II)) cations can be adsorbed by clay minerals, oxides, and organic matter, with adsorption increasing with increasing pH. These Hg species are also immobilized by forming various precipitates with chloride, phosphate, carbonate, hydroxide, and sulfide. Under reducing conditions, organic-bound Hg and inorganic Hg may be degraded to elemental Hg (Fitzgerald and Lamborg 2003; McLean and Bledsoe 1992). However, elemental Hg is volatile and may escape aquatic systems. In the CWTS designed to treat FGD wastewater, the primary targeted processes to decrease Hg concentrations in the water column were precipitation of insoluble Hg forms and sorption to organic material in wetland sediment. These processes increased residence times in the sediment, thereby hindering resuspension of the metals in the overlying water, and reduced the availability of Hg for methylation (Faust and Osman 1981; King *et al*. 2002). The potential formation of monomethyl-mercury (CH_3Hg^+) is a concern when using constructed wetlands to decrease Hg concentrations. Methylmercury is a lipophilic, organic form of mercury that is highly toxic and readily bioconcentrated by aquatic organisms (King *et al*. 2002). The aqueous production of elemental Hg (Hg^0) competes for reactants with the production of monomethyl-mercury (MMHg); thus, water systems with a large production of Hg^0 will have less Hg available for methylation and therefore smaller amounts of MMHg in biota and sediment (Fitzgerald and Lamborg 2003). However, a large production of Hg^0 may also create a source of atmospheric Hg due to its volatility. In reduced sediments (such as in the first two wetland cells; **Fig. 1**), the activity of Hg^{2+} and other dissolved forms of Hg(II) is also partially controlled by sulfide. It has been suggested that the presence of Fe(II) in reduced, sulfidic soils will complex with S^{2-} to form FeS(s). If mercuric ions are retained by FeS(s), which is known to retain trace elements by both adsorption and co-precipitation, the extent of mercury methylation will be decreased by decreasing Hg(II) solubility and bioavailability (Mehrotra *et al*. 2003). Mercury methylation will be avoided by providing sufficient amounts of iron to form strong complexes with sulfide, which retains mercuric ions thereby rendering them unavailable for methylation. Competition between Hg^{2+} and Fe^{2+} for sulfide is not likely to be an issue in this CWTS due to the abundant presence of S.

The behavior and speciation of Se in wetlands have received considerable attention in recent years. The attention was most likely prompted by the high incidence of deformity and mortality of waterfowl at the Kesterson National Wildlife Refuge in California as a result of the input of selenium-rich agricultural drainage water (McLean and Bledsoe 1992). The nonmetal Se, which is somewhat chemically similar to sulfur, can exist in nature in four oxidation states. Changes in the oxidation state of Se can greatly affect solubility, mobility, fate, transport, and effects of selenium species in the wetland environment. Selenium may exist as selenate (Se(VI)), selenite (Se(IV)), elemental Se (Se^0), and selenide (Se(-II)) in soil environments. Reduction of Se(VI) to Se(IV), and then to insoluble elemental Se or selenide (Se(-II)) is expected to occur under the reducing conditions of the sediment in the first two wetland cells (**Fig. 1**). Selenate is the more mobile form of Se in soils, and is adsorbed by weak exchange mechanisms. Selenite tends to bind to iron oxides, which has been shown to increase with decreasing pH. In studies of competitive adsorption, selenite adsorption was not affected by the presence of sulfate or chloride (McLean and Bledsoe 1992). The primary targeted processes for decreasing Se concentrations in the water column in this CWTS are precipitation and adsorption of the insoluble forms (Masscheleyn and Patrick 1993; Masscheleyn *et al*. 1990).

The metalloid As, which is somewhat chemically similar to phosphate, can exist in nature in five oxidation states, and most commonly as either arsenate (As(V)), or as arsenite (As(III)), in sediment. In reduced sediment, arsenite, which is the more toxic and more soluble form of arsenic (McLean and Bledsoe 1992), will rapidly and strongly sorb to sulfide minerals, reacting most strongly with iron sulfides. Arsenic concentrations in the water column may also be decreased by oxidation, which occurs in the final wetland cell of the CWTS (**Fig. 1**). In oxidized sediment, arsenite can be oxidized to arsenate, which forms insoluble precipitates with Fe(II). Iron in soils is most effective in controlling arsenate's mobility. Adsorption of arsenate by iron oxides is maximized at a pH of 3-4, with decreasing adsorption with increasing pH. Precipitation and adsorption to organic matter are most effective in controlling arsenate's mobility in constructed wetland treatment systems (McLean and Bledsoe 1992). Therefore, the primary targeted processes for decreasing arsenic concentrations in the water column in the CWTS are precipitation and adsorption.

Sequential extraction procedures have been widely used to provide conceptual information on the fate of trace elements in aquatic systems (Tessier *et al*. 1979). These procedures extract trace elements from sediment with increaseing reagent strengths to break down sediment matrices and release bound metals into soluble forms with the extractant used in each step. Therefore, while this process cannot be used to identify the actual form of a given metal in sediment, sequential extractions are useful in categorizing elements into operational geochemical fractions (McLean and Bledsoe 1992). Operationally-defined species characterization is the characterization of molecule groups (not single species) according to their similar behavior during an analytical procedure, such as extraction (Michalke 2003). Sequential extraction procedures have been developed that can be applied to a number of elements, in particular to divalent cations. The five-step method of Tessier *et al*. (1979) and the three-step BCR (Community Bureau of Reference; Ko *et al*. 2005) method are among the most commonly used. However, the feasibility of applying these common schemes to the study of soil fractionation of some elements, such as Hg, Se, and As, has been questioned (Bloom *et al*. 2003; Gleyzes *et al*. 2002). These elements can exist under different oxidation states, each with a particular behavior. The use of reagents in the common extraction schemes can induce changes in oxidation state and therefore modify extraction results (Gleyzes *et al*. 2002). As a result, it was necessary for researchers to develop sequential extraction procedures specifically for Hg, Se, and As.

Sequential extraction procedures have been developed for Hg (Beldowski and Pempkowiak 2003; Bloom *et al*. 2003; Lechler *et al*. 1997; Wallschläger *et al*. 1998), Se (Chao and Sanzolone 1989; Wang and Chen 2003), and As (Bird *et al*. 2003; Miller *et al*. 1986; Schank 2003; Wenzel *et al*. 2001), utilizing operationally-defined geochemical fractions to define the distribution of these metals in sediment. After a review of the literature, sequential extraction procedures were chosen for use in this study based on the fractions identified by the method, the reagents used for each extraction step, the recovery of elements, and whether the fractions were comparable to those chosen for the other elements.

The Hg extraction method presented by Lechler *et al*. (1997) was developed for use with soils in western Nevada. Although the semi-arid soils to which the extraction procedure was applied were quite different from the sediments collected from the CWTS, the procedure identified fractions that were important to this system and were comparable to procedures used for Se and As. Lechler *et al*. (1997) utilized five fractions: elemental, exchangeable, strongly-bound, organic, and residual. Elemental Hg is volatile and can be expected to vary in space and time in response to changes in the forces that drive oxidation and reduction reactions in sediments (Fitzgerald and Lamborg 2003). Exchangeable Hg includes Hg sorbed to ion exchange complexes on soil surfaces and held there by electrostatic forces. Hg in this fraction is weakly bound and may be replaced by other ions by exchange processes and released to the water-soluble forms. Generally, Hg in the elemental and exchangeable fractions is considered to be most mobile and potentially bioavailable (Lechler *et al.* 1997; Filgueiras *et al.* 2002). Strongly-bound Hg reflects Hg from several soil/sediment components, such as Fe(III) and Mn(IV) oxyhydroxides and other mineral surface sites where Hg is strongly adsorbed (Lechler *et al.* 1997). Organic-bound Hg includes that which may be associated with various forms of organic matter through complexation. The degradation of organic matter, including detritus, using an oxidizing compound can lead to the release of organic-bound Hg. Finally, residual Hg accounts for Hg associated with structures of primary minerals and silicates. However, this residual fraction may also include Hg bound to sulfides (Letchler *et al.* 1997; Filgueiras *et al.* 2002).

The sequential extraction procedure for Se used in this study was modified from Chao and Sanzolone (1989). These authors used the procedure for Se fractionation in 22 different sediment samples, yielding selenium recoveries ranging from 81 to 109.7% while utilizing five fractions: soluble, exchangeable, oxide-bound, organic- and sulfide-bound, and residual. The soluble fraction includes nonspecifically adsorbed Se (McLean and Bledsoe 1992), species made up of free ions, soluble inorganic complexes, and soluble organic complexes. The exchangeable fraction includes specifically adsorbed Se (McLean and Bledsoe 1992) and other species sorbed to an ion exchange complex in the soil. Selenium in these two fractions is the most mobile and potentially the most bioavailable species (Chao and Sanzolone 1989; Filgueiras *et al.* 2002). The fraction bound to oxides is sensitive to pH changes, and release is achieved through dissolution (Gleyzes *et al.* 2002). Selenium recovered in this fraction may be present as co-precipiated with carbonate minerals, specifically sorbed to clay surfaces, associated with oxide minerals (Fe/Mn oxyhydroxides), and acid hydrolizable organic matter (Chao and Sanzolone 1989; Gleyzes *et al.* 2002). The organic- and sulfide-bound fraction can include Se complexed with humified organic matter and sulfide minerals, and the residual fraction is primarily composed of Se contained in siliceous materials (Chao and Sanzolone 1989).

Schank (2003) conducted studies comparing the efficiency and extractability of As using several previously-developed procedures, including Miller *et al.* (1986) and Wenzel *et al.* (2001). Based on this author's findings, a procedure modified from Schank (2003) and Wenzel *et al.* (2001) was chosen to extract As from FGD-contaminated sediment. The following fractions were extracted using this procedure: water soluble, exchangeable, amorphous and poorly-crystallized Fe/Mn hydrous oxides, well-crystallized Fe/Mn hydrous oxides, and residual. The water soluble, exchangeable, and residual fractions are similar to those described for Se. The other two fractions collectively include As associated with Fe(III) and Mn(IV) oxides; the reduction and dissolution of oxides will release adsorbed trace elements (Filgueiras *et al.* 2002; Gleyzes *et al.* 2002).

By categorizing Hg, Se, and As into these operational geochemical fractions, hypotheses of reactions occurring in sediment in the constructed wetland treatment system can be evaluated. Furthermore, extractions of these element species are crucial for understanding metal biogeochemical behavior in constructed wetlands for wastewater treatment. The first objective of this research was to characterize sediment-associated Hg, Se, and As into operationally-defined fractions using sequential extraction procedures.

Sediment toxicity

A toxicity assessment of sediment in this constructed wetland treatment system was necessary to obtain the information needed to support environmental management decisions related to mitigating risks associated with FGD wastewater. Aquatic organisms, such as *Hyalella azteca*, are able to take up trace elements from water, food, and sediment. One of the major pathways for uptake of elements by aquatic organisms is directly from the surrounding water through permeable surfaces, including gills. In addition, organisms that burrow in sediments may ingest that sediment as a food source. Since aquatic organisms are often a principle food source for predatory fish and birds, there is potential for elements accumulated by organisms to be transferred along the food chain (Rainbow 1997; Marsden and Rainbow 2004).

Threshold effect levels (TELs) and probable effect levels (PELs) are commonly used to predict the toxicity of contaminated sediment. TELs represent the sediment concentration below which adverse effects are unlikely to occur, and PELs represent the sediment concentration above which adverse biological impacts are likely to occur due to exposure to that contaminant alone (Smith *et al.* 1996; Ingersoll *et al.* 2000; MacDonald *et al.* 2000). However, important limitations of the use of TELs and PELs to predict sediment toxicity to aquatic organisms are that they may not be predictable for contaminants present in mixtures, and the issue of bioavailability is not considered (McCauley *et al.* 2000).

Swartz *et al.* (1995) developed a model to quantitatively predict the toxicity of PAH mixtures in field sediments based on the notion that effects of individual PAHs are additive in mixtures. This model was based on the 10-day LC_{50} values measured in spiked-sediment toxicity tests. Assuming that the interstitial water concentration reflected sediment exposure through the use of equilibrium partitioning (Eq-P), an LC50 for interstitial water ($LC50_{iw}$) was predicted based on a log $LC50_{iw}$ – log K_{ow} relationship. The predicted LC50s values for interstitial water were then used to calculate toxic units (TU) for individual PAHs, the sum of which was used to predict toxicity of field-collected sediments. Di Toro and McGrath (2000) and Di Toro *et al.* (2000) expanded this PAH model using species-specific, water-only LC50 values to develop sediment guidelines for PAH mixtures. This approach was based on the Eq-P theory that interstitial water LC50 values are the same as water-only LC50 values.

Assuming that the interstitial water concentrations in sediment reflects sediment exposure (Swartz *et al.* 1995), and that interstitial water 10-day LC50 values are the same as water-only 10-day LC50 values (Di Toro and McGrath 2000; Di Toro *et al.* 2000), toxic units (TU) can be calculated (Lee *et al.* 2001) to predict the toxicity of sediment containing a mixture of elements:

$$TU = \frac{\text{Conc. in sediment (mg/kg)}}{\text{Water only } LC_{50} \text{ (mg/L)}}$$

If the percents of total sediment concentrations that are potentially bioavailable to organisms are known, toxic units can calculated to represent only the potentially bioavailable portion of elements in the sediment:

$$TU = \frac{\text{Conc. in sediment (mg/kg)} \times \text{\% bioavail.}}{\text{Water only } LC_{50} \text{ (mg/L)}}$$

In this equation, the % bioavailable refers the fraction of the total quantity or concentration of a contaminant in sediment that is potentially available for biological action, such as uptake by an organism (Rand 1995).

In a CWTS that receives inflows such as FGD wastewater, sediments are expected to contain several contaminants, resulting in unique mixtures for which there are no accurate predictive tools.

The second set of objectives of this research were to: 1) characterize the partitioning of total Hg, Se, and As between contaminated sediment and water, 2) characterize the responses of *H. azteca* exposed to sediment collected from the pilot-scale constructed wetland, 3) determine the 10-day aqueous LC50 values for Hg, Se, and As for *H. azteca*, and 4) calculate toxic units to determine the predictability of sediment toxicity to *H. azteca*.

MATERIALS AND METHODS

Pilot constructed wetland treatment system

The pilot-scale CWTS was constructed at a Clemson University facility in Pendleton, South Carolina. Briefly, this system consists of a 6,800-L upstream retention basin followed by three parallel treatment systems (**Fig. 1**). Each wetland treatment system consisted of four wetland cells in series, including two cells planted with *S. californicus* (bulrush; 1st and 2nd wetland cells), a gravel cell (3rd wetland cell), and a final cell planted with *T. angustifolia* (cattails; 4th wetland cell).

Cattails and bulrushes are among the most researched plant species for wetlands treatment systems. Both are capable of growing in shallow and relatively deep waters, achieve similar heights, and form very dense stands and well developed litter zones.

Cattails respond favorably after a disturbance, such as construction activities and altered hydrology, and spread rapidly by vegetative growth. However, cattails do not compete well with established plant communities where conditions tend to inhibit cattail seed germination. Bulrushes have the ability to compete with cattails in that bulrush will grow and persist even when flooded in deep waters, and they are less of a threat to contiguous natural wetland communities than the more aggressive, invader cattail species. Bulrushes also tend to be more aesthetically pleasing than cattails (Kent 1994).

Vegetation (*S. californicus* and *T. angustifolia*) was specifically chosen for this constructed wetland treatment system. *S. californicus* was selected based on: 1) the plant's ability to maintain reduced hydrosoil; 2) providing organic ligands for sorption of contaminants entering the system; 3) continuous production of organic ligands through plant growth; 4) production of organic carbon as an energy source for dissimilatory sulfate reduction; and 5) accretion of organic ligands and sediments over time due to decomposition and accumulation of detritus (Murray-Gulde 2002). *S. californicus* was planted in the first two wetland cells.

T. angustifolia, on the other hand, was selected based on: 1) the plant's ability to transfer oxygen from the atmosphere into the root zone, termed radial oxygen loss, to maintain an oxidized hydrosoil; 2) providing organic ligands for sorption of contaminants entering the system; 3) continuous production of organic ligands through plant growth.; and 4) minimal accretion of organic ligands and sediments over time due to decomposition and accumulation of detritus. *T. angustifolia* was planted in the fourth, or last, wetland cell.

The gravel cell was used to aerate the water; water fell onto the gravel as it flowed into the cell from the single-point inflow. For the purpose of this research, FGD wastewater was simulated based on compositions of wastewaters from power plants in the U.S. currently operating FGD systems (Sundberg 2006). Therefore, the targeted inflow concentrations of these elements in the simulated FGD wastewater ranged from 0.002 to 0.16 mg Hg L^{-1}, 1.8 to 8.5 mg Se L^{-1}, and 0.002 to 0.34 mg As L^{-1}. These three constituents of FGD wastewater are recognized as toxicants of concern, and their discharge into the environment is monitored by the NPDES.

Water and sediment collection

Redox potential was measured using a digital millivolt meter and platinum-tipped electrodes (Zobell 1946; Faulkner *et al*. 1989) prior to removing sediment samples from the wetland cell to avoid false measurements due to sediment disturbance. Two electrodes were permanently placed in the front and back of each wetland cell containing sediment at a depth of three inches when the CWTS was first built. Each electrode was inspected and tested using a standard reference solution (Zobell 1946). Electrodes were considered acceptable if measurements were within 5% of the expected value. Prior to sediment collection, redox was measured using each electrode and recorded.

Water and sediment were collected from the planted wetland cells of the CWTS after 7 months (water) and 17 months (sediment) of treating simulated FGD wastewater. These samples were collected as representative samples, and characteristics may have slightly fluctuated throughout the year. All samples were collected and stored in high density polyethylene bottles that were presoaked in a 50% concentrated trace metal grade nitric acid for 24 hours and rinsed thoroughly with Milli-Q (18 MΩ cm) water. Water blanks were used to ensure the bottles were not contaminated. Wetland cells from which sediment and water were collected included the 1st, 2nd, and 4th wetland cells.

Sediment grab samples were taken from the front, middle, and back of each planted wetland cell of one treatment train in the upper 15 cm of sediment, which represents the most biologically active portion of the sediment (Burton 1991). The three grab samples were combined into one homogenized composite sample per planted wetland cell and stored wet in a plastic bag (double bagged) at 4°C in the dark (Burton 1991; USEPA 2001).

Aliquots of the composited samples were dried at 25°C until dry and sieved through a 2-mm sieve in preparation for total extractions. For the purpose of this research, "total" refers to the recoverable amount using an *aqua regia* (1:3 v/v concentrated HNO_3 and HCl) extraction. Three 1.5 ± 0.1 g of dried sediment (exact weight recorded) were transferred to 12-mL glass digestion vials. Three mL concentrated HNO_3 and 9 mL concentrated HCl (Gleyzes *et al*. 2002) were added to the vial and inverted to ensure all sediment was exposed to acid. Vials were placed in a dry incubator to digest for four hours. The digestates were diluted and brought to a known volume with Milli-Q water and filtered with separate Kimwipes® (Ellington and Evans 2000) to remove any undigested material. After each sample passed though its Kimwipe®, Milli-Q water acidified with concentrated trace metal grade nitric acid to a pH < 2 was used to rinse the Kimwipe® of any residual elements. The rinsate was then added to the corresponding sample and was brought to a known volume. The sample was further filtered through a 0.45 µm pore size Millipore 25 mm syringe filter. Samples were stored at 4°C in the dark until analysis. Sediments were previously characterized (Sundberg 2006).

Water samples were collected from the wetland cell outflows by gravity flow into the sample bottles and filled to the brim. The samples were acidified with concentrated trace metal grade nitric acid to a pH < 2 and stored at 4°C. Prior to analysis, water samples were filtered though a 0.45 µm filter.

Sediment characterization

Sediment samples were characterized within one week of collection. Particle size distribution, percent solids, cation exchange capacity (CEC), percent organic matter, and pH were determined for the composited sediment samples collected from each wetland cell. Sediment pore water pH, which is often one of the most important factors controlling speciation and equilibria for many contaminants, was measured using an YSI pH meter. Particle size distribution analyses yielded three fraction sizes: sand, silt, and clay. The hydrometer method was used, as described by Gee and Gauder (1986). Percent solids of the sediment were measured according to Black (1986) by drying a pre-weighed sediment sample in a drying oven and calculating the weight difference between the wet and dry sample. The percent of organic matter in the sediment was measured using the lost-on-ignition method, as described by Nelson and Sommers (1996). Sediment CEC is a measure of the number of cation binding sites in the sample. CEC was determined by the ammonium acetate method described by Plumb (1981) and Hendershot *et al*. (1993).

Sediment chemical extractions

Sediments collected from the constructed wetland treatment system were separated into operationally-defined geochemical fractions of Hg, Se, and As (refer to **Fig. 2** for procedure summaries). The extraction procedure for Hg was modified from Lechler *et al*. (1997). This procedure utilized five fractions: elemental, exchangeable, strongly-bound, organic, and residual Hg. The extraction procedure for Se was modified from Chao and Sanzolone (1989). This procedure utilized five fractions: soluble, exchangeable, oxide-bound, organic- and sulfide-bound, and residual Se. The extraction procedure for As was modified from Wenzel *et al*. (2001) and Schank (2003). This procedure utilized five fractions: soluble, exchangeable, amorphous and poorly-crytallized Fe/Mn hydrous oxides, well-crystallized Fe/Mn hydrous oxides, and

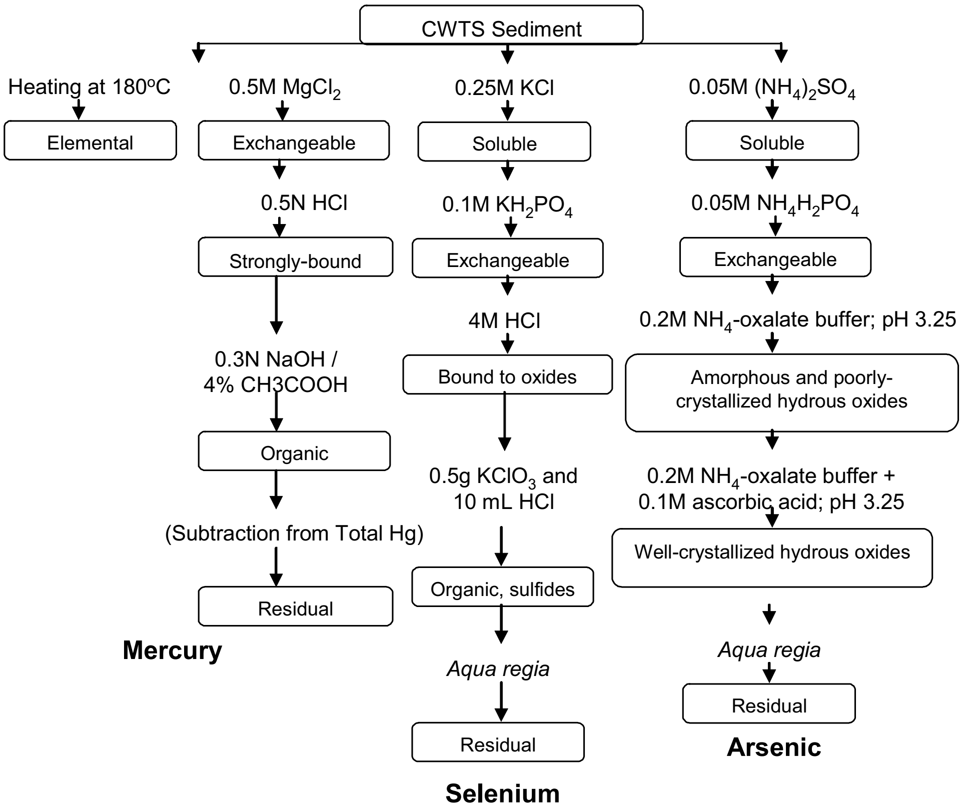


Fig. 2 Flow diagram of sequential extraction procedures used for Hg, Se, and As in sediment collected from the CWTS.

residual As.

Toxicity evaluation

A series of 10-day toxicity experiments were conducted to measure the toxicity of contaminated sediment collected from each planted wetland cell as well as from an uncontaminated source. Prior to initiation of toxicity experiments, sediment was collected from the pilot CWTS and from an uncontaminated river bed in Pendleton, South Carolina where the original sediment for the pilot CWTS was collected. *Hyalella azteca* is an EPA test organism, and is used widely for sediment toxicity testing. This organism is an epibenthic detritivore that burrows in the upper 0.5 to 2 cm of the sediment surface. *Hyalella azteca* are commonly used as a toxicity test organism due to their sensitivity to aquatic contaminants and pollutants, close association with the sediment, ease of identification and handling, and extensive prior use in toxicity testing (USEPA 2000). This aquatic invertebrate is routinely cultured at a Clemson University aquatic research facility according to methods described by de March (1981). Organisms 2 to 3 weeks of age (those organisms that pass through a 1.0-mm mesh sieve but are retained by a 0.6-mm mesh sieve) were used in toxicity tests. For all tests, temperature, pH, dissolved oxygen, alkalinity, hardness, and conductivity were measured at test initiation and test termination (**Table 1**). The experimental design consisted of three replicate test chambers (250-mL beakers) for each sediment and a control. Test chambers contained a water to sediment ratio of 1:4 (v/v) (Deaver and Rodgers 1996; Riba *et al*. 2004). Following the addition of sediment and water, test chambers were allowed to settle for 24 hrs prior to test initiation. Each test chamber contained 10 juvenile (2-3 weeks in age) *H. azteca* at test initiation. Test chamber temperature was maintained at 25±1°C in a temperature-controlled testing room with a light:dark photoperiod of 16 hr:8 hr. Experiments containing control survival of ≥80% were considered valid. A reference toxicity experiment using copper sulfate was conducted to ensure the *H. azteca* were responding normally. The endpoints measured in all toxicity experiments were mortality and growth (dry weight) of test organisms.

Table 1 Characteristics of overlying water used for sediment toxicity tests measured at test conclusions (Day 10).

Sample	Temp (°C)	pH	DO[a]	Alkalinity[b]	Hardness[b]	Conductivity
Moderately hard laboratory water	24.10	7.83	7.28	30	60	355 mS
1st Wetland Cell	23.74	7.30	8.33	28	80	6.14 mS
2nd Wetland Cell	23.86	6.93	8.48	14	84	3.35 mS
4th Wetland Cell	23.96	7.56	8.48	46	95	2.73 mS

[a] Dissolved oxygen, mg L^{-1}
[b] mg L^{-1} as $CaCO_3$

In another series of 10-day toxicity experiments, *H. azteca* were exposed to known concentrations of Hg, Se, and As in aqueous exposures to measure the responses of the organisms to individual constituents. Reagent grade mercuric nitrate ($Hg(NO_3)_2•H_2O$), sodium selenate (Na_2SeO_4), and sodium arsenite ($NaAsO_3$) obtained from Sigma Chemical Company (St. Louis, Missouri) were used for this series of tests. All stock solutions were prepared with moderately hard laboratory water shortly before initiation of tests. Stock solutions were used to prepare test solutions ranging from 1 μg L^{-1} to 1000 μg L^{-1} for sodium selenate and sodium arsenite. A stock solution of mercuric nitrate was used to prepare test solutions ranging from 5 μg L^{-1} to 100 μg L^{-1}. Three controls were used in this series of tests. Testing conditions and parameters followed those used for sediment toxicity experiments.

Analytical and statistical procedures

Ten-mL water and digested sediment samples were analyzed for total Hg, Se, and As. Analyses were performed at the Laboratory for Environmental Analysis at the University of Georgia according to the standard method EPA 200.8 (USEPA 1994) using a Sciex Elan 9000 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Perkin-Elmer, Norwalk, CT). Three levels of standards for Hg (0.1, 0.5, and 1.0 μg L^{-1}) and three levels for Se and As (50, 100, 250 μg L^{-1}) were used for calibration. Quality control (QC) checks were run in 20-sample intervals, prepared from a different stock at a concentration level in the middle of the calibration standards. QC checks were considered acceptable if concentrations were within three standard deviations of the average recovery (USEPA 1994). Method blanks (Milli-Q water acidified with trace metal grade nitric acid to a pH < 2) were run after calibration and before QC checks. Fifty-μL aliquots of a 10 mg L^{-1} solution of Rh

Table 2 Summary of characteristics of water collected from the constructed wetland treatment system.

Water	pH	Alkalinity[a] (mg L^{-1})	Hardness[a] (mg L^{-1})	Cond[b] (mS)	TSS[c] (mg L^{-1})	TDS[d] (mg L^{-1})	Cl^{-} (mg L^{-1})	SO$_4^{-2}$ (mg L^{-1})
Retention basin	6.88	92.32	6737	11.12	246	11354	4051	801
1st Wetland Cell	6.97	83.09	5831	10.93	55	10957	4006	817
2nd Wetland Cell	7.20	91.53	5850	10.56	53	10301	3770	800
4th Wetland Cell	7.15	91.79	5958	10.78	61	10469	3847	787

[a] mg L^{-1} as CaCO$_3$
[b] Conductivity
[c] Total suspended solids
[d] Total dissolved solids

in a 5% nitric acid solution were added as an internal standard to each 10-ml sample prior to analysis. The isotopes quantified were ^{202}Hg, ^{82}Se, and ^{75}As, with method detection limits of 0.02, 0.107, and 0.05 µg L^{-1}, respectively.

Significant differences in average percent survivals and dry weights per organism were determined by Tukey's Multiple Comparison Test using the computer software program GraphPad Prism, Version 3.0 (GraphPad Software Inc., San Diego, CA).

RESULTS AND DISCUSSION

Sediment characteristics

Prior to sediment collection, oxidation-reduction potentials of sediment pore water in the first and second wetland cells were -410 ± 31 mV and -337 ± 21 mV (average ± standard deviation, n=2), respectively, indicating that the sediments were anoxic and probably undergoing sulfate reduction at the time of collection (**Fig. 1**). Sulfate is reduced to sulfide when sediment redox reaches approximately -75 to -150 mV (Cronk and Fennessy 2001). Sediment in the fourth wetland cell had a redox potential of -25.5 ± 37.5 mV. Measurements of sediment pore water pH ranged from 6.6 to 6.8, and the CEC of sediment ranged from 4.6 me 100g^{-1} to 6.8 me 100g^{-1}. Percent organic matter and percent solids in all sediments ranged from 2% to 3%, and 75% to 79%, respectively. Sediments were very sandy (>89%) with small fractions of clay and silt. With the exception of redox potential, sediment characteristics were measured using the composited samples (n=1) collected from the three wetland cells with sediment (the third wetland cell was granite gravel, and was therefore not included). It is likely that these characteristics varied spatially within each wetland cell; therefore, these measurements were considered estimates.

Partitioning of elements in water and sediment

Water collected from the CWTS had chloride and sulfate levels averaging 4,000 and 800 mg L^{-1}, respectively (**Table 2**). Water hardness reached about 6,000 mg L^{-1} as CaCO$_3$, and alkalinity averaged about 90 mg L^{-1} as CaCO$_3$. Total suspended and dissolved solids in the water were approximately 55 and 10,000 mg L^{-1}, respectively. Such a large concentration of suspended and dissolved solids may influence the bioavailability of Hg, Se, and As in the CWTS by providing additional sorption sites in the water column (Baker *et al.* 2003). Organisms that swim freely in the water column can take up these solids via ingestion. Conductivity ranged from 10 to 11 mS cm^{-1} in water collected from the CWTS.

Concentrations of Hg, Se, and As in water and sediment are shown in **Fig. 3** through **Fig. 5**. Water concentrations of Hg decreased from 0.028 ± 0.006 mg L^{-1} in the first wetland cell to 0.007 ± 0.003 mg L^{-1} in the second wetland cell and 0.001 ± <0.001 mg L^{-1} in the last wetland cell. Selenium and As concentrations in water also decreased from the first to last wetland cell. Selenium concentrations in water were 1.67 ± 0.58 mg L^{-1}, 0.47 ± 0.17 mg L^{-1}, and 0.045 ± 0.015 mg L^{-1} in the first, second, and fourth wetland cells, respectively. Arsenic concentrations in water were 0.118 ± 0.05 mg L^{-1}, 0.08 ± 0.04 mg L^{-1}, and 0.012 ± 0.004 mg L^{-1} in the first, second, and fourth wetland cells. It is apparent from this data that Hg, Se, and As were efficiently removed from flue gas desulfurization wastewater in the CWTS.

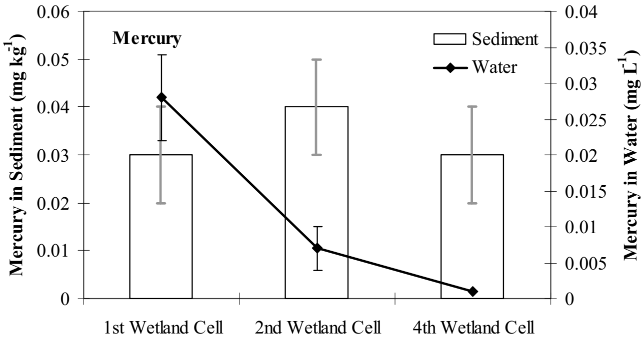


Fig. 3 Concentrations of Hg in water (lines) and sediment (bars) collected from the first, second and fourth wetland cells. Error bars represent standard deviations (n=3).

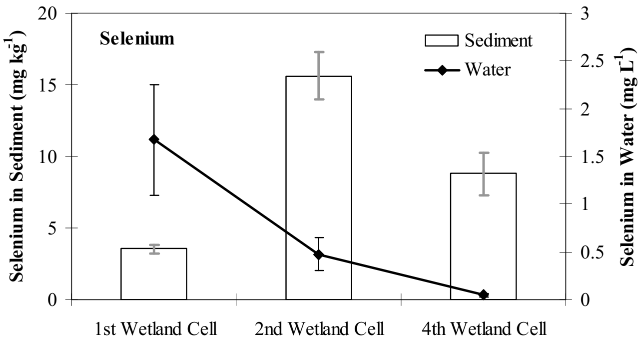


Fig. 4 Concentrations of Se in water (lines) and sediment (bars) collected from the first, second, and fourth wetland cells. Error bars represent standard deviations (n=3).

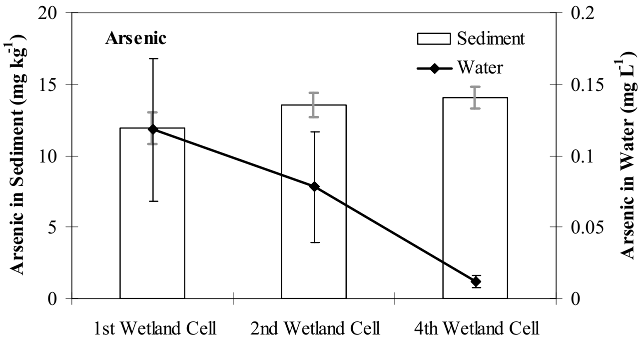


Fig. 5 Concentrations of As in water (lines) and sediment (bars) collected from the first, second, and fourth wetland cells. Error bars represent standard deviations (n=3).

Concentrations of Hg in sediments from the first, second, and fourth wetland cell digested for total Hg were 0.026 ± 0.001 mg kg^{-1}, 0.035 ± 0.006 mg kg^{-1}, and 0.025 ± 0.008 mg kg^{-1}, respectively (**Table 3**). Concentrations of Se in sediments from the first, second, and fourth wetland cell digested for total Se were 3.572 ± 0.271 mg kg^{-1}, 15.627 ± 1.617 mg kg^{-1}, and 8.790 ± 1.501 mg kg^{-1}. In sediments digested for total As, concentrations were 11.914 ± 1.081 mg

Table 3 Concentrations (dry weight basis) of Hg, Se, and As in the total digestions and the fractions of the samples extracted by sequential extraction procedures.

Sediment	Total Hg (mg kg^{-1})	Hg in extracted fractions (mg kg^{-1})						Total/∑ (%)
		F1: Elemental	F2: Exchangeable	F3: Strongly-bound	F4: Organic	F5: Residual	∑Hg in fractions	
1st Wetland Cell	0.0260 ± 0.001	0.0177	0.0000	0.0002	0.0023	0.0058	0.0260	100.0
2nd Wetland Cell	0.0350 ± 0.006	0.0295	0.0000	0.0002	0.0012	0.0041	0.0350	100.0
4th Wetland Cell	0.0250 ± 0.008	0.0221	0.0000	0.0006	0.0000	0.0023	0.0250	100.0
Sediment	**Total Se (mg kg^{-1})**	**Se in extracted fractions (mg kg^{-1})**						**Total/∑ (%)**
		F1: Soluble	**F2: Exchangeable**	**F3: Bound to oxides**	**F4: Organic and sulfides**	**F5: Residual**	**∑Se in fractions**	
1st Wetland Cell	3.5720 ± 0.271	3.3243	2.0810	1.4049	2.1235	0.4195	9.3533	38.2
2nd Wetland Cell	15.6270 ± 1.617	3.7608	3.4916	2.9580	8.4856	1.1952	19.8912	78.6
4th Wetland Cell	8.7900 ± 1.501	6.4808	3.5861	2.1056	3.8014	0.6352	16.6092	52.9
Sediment	**Total As (mg kg^{-1})**	**As in extracted fractions (mg kg^{-1})**						**Total/∑ (%)**
		F1: Soluble	**F2: Exchangeable**	**F3: PC^a Oxides**	**F4: WC^b oxides**	**F5: Residual**	**∑As in fractions**	
1st Wetland Cell	11.9140 ± 1.081	0.4594	0.4343	0.7731	0.3471	23.8554	25.8693	46.1
2nd Wetland Cell	13.5320 ± 0.834	0.1799	0.6041	2.5685	0.3867	21.1824	24.9215	54.3
4th Wetland Cell	14.0750 ± 0.767	0.2546	0.4350	0.8552	0.3183	23.9746	25.8378	54.5

[a] Amorphous and poorly-crystallized Fe and Al hydrous oxides
[b] Well-crystallized Fe and Al hydrous oxides

Table 4 Percents of Hg, Se, and As associated with each sediment fraction as determined by sequential extractions, expressed as percent of total sum of elements in the fractions. All data are reported on a dry weight basis.

Sediment	$[Hg]_{SUM}$ (mg kg^{-1})	Percent of $[Hg]_{SUM}$ in each fraction				
		F1: Elemental	F2: Exchangeable	F3: Strongly-bound	F4: Organic	F5: Residual
1st Wetland Cell	0.026	68.02	0.00	0.89	8.88	22.21
2nd Wetland Cell	0.035	84.26	0.00	0.53	3.48	11.73
4th Wetland Cell	0.025	88.20	0.00	2.50	0.00	9.30
Sediment	**$[Se]_{SUM}$ (mg kg^{-1})**	**Percent of $[Se]_{SUM}$ in each fraction**				
		F1: Soluble	**F2: Exchangeable**	**F3: Bound to oxides**	**F4: Organic and sulfides**	**F5: Residual**
1st Wetland Cell	9.353	35.54	22.25	15.02	22.71	4.49
2nd Wetland Cell	19.891	18.91	17.56	14.87	42.66	6.01
4th Wetland Cell	16.609	39.02	21.69	12.68	22.89	3.82
Sediment	**$[As]_{SUM}$ (mg kg^{-1})**	**Percent of $[As]_{SUM}$ in each fraction**				
		F1: Soluble	**F2: Exchangeable**	**F3: PC^a Fe/Al oxides**	**F4: WC^b Fe/Al oxides**	**F5: Residual**
1st Wetland Cell	25.869	1.77	1.68	2.99	1.34	92.21
2nd Wetland Cell	24.922	0.72	2.42	10.31	1.55	84.99
4th Wetland Cell	25.838	0.99	1.68	3.31	1.23	92.79

[a] Amorphous and poorly-crystallized Fe and Al hydrous oxides
[b] Well-crystallized Fe and Al hydrous oxides

kg^{-1} in sediment from the first wetland cell, 13.532 ± 0.834 mg kg^{-1} in sediment from the second wetland cell, and 14.075 ± 0.767 mg kg^{-1} in sediment from the fourth wetland cell.

Element distribution in sediment

Elemental Hg was defined as that which is volatilized upon heating the sample at >150°C (Bloom *et al.* 2003). Elemental Hg was therefore fractionated from sediment using pyrolytic extraction, and was quantified as the difference between total Hg and what was left after the pyrolytic extraction; the amount of Hg volatilized during extraction was not measured. A magnesium chloride solution was used to extract exchangeable Hg from sediment samples in the second fractionation step of this procedure (Lechler *et al.* 1997). Use of magnesium chloride combines the rather strong Mg^{2+} ion-exchange capacity with the weak complexing ability of Cl^-. Although this reagent does not attack organic matter, silicates, or metal sulfides, some slight dissolution of carbonates may occur (Gleyzes *et al.* 2002). Other neutral salts such as ammonium acetate and sodium- or calcium nitrate have been previously applied to displace elements sorbed onto soil surfaces (Schank 2003). Since only weak associations are formed in the exchangeable fraction, Hg can be replaced and released into solution by nitrate ions or chloride salts through ion exchange. Miller *et al.* (1986), Keon *et al.* (2001), and Tessier *et al.* (1979) also used magnesium chloride for this fraction. For extracting the strongly-bound (or reducible) fraction of Hg, a hydrochloric acid solution was used (Hirner *et al.* 1990) since it is capable of dissolving a variety of sediment constituents, including Fe, Mn, and Al oxides, amorphous materials, carbonates, and weaker sulfide minerals as well as portions of soil organic matter (Chao and Sanzolone 1989). A mixture of sodium hydroxide and acetic acid was used for the organic, or oxidizable, fraction of sediment. Some oxidizing agents tend to oxidize sulfides in addition to organic matter. For example, hydrogen peroxide was occasionally used for the oxidizable fraction; however, it was not very efficient in destroying organic matter and tends to partially dissolve sulfides (Gleyzes *et al.* 2002). Sodium hydroxide was previously used to extract humic-bound elements, and worked best for sediment with a higher organic matter content, such as sludge (Gleyzes *et al.* 2002). It was also a common extractant for Fe and Al oxides when a previous step targeting these constituents was not used (Gleyzes *et al.* 2002). Sediment-associated Hg left in the sample after the first four fractionation steps was considered to be residual Hg and included Hg encapsulated in silica or Hg sulfide (Lechler *et al.* 1997).

Based on results from the sequential extraction study, the fraction of elemental Hg increased from the first through fourth wetland cell, accounting for up to 88% of total Hg (**Tables 3, 4; Fig. 6**). However, pyrolytic extractions for sediment-associated Hg may be problematic by overestimating the amount of elemental Hg present in the sample (Sladek and Gustin 2003). Bloom *et al.* (2003) found that virtually all elemental Hg in sediment samples was volatilized. Although $HgCl_2$ and Hg bound to humic

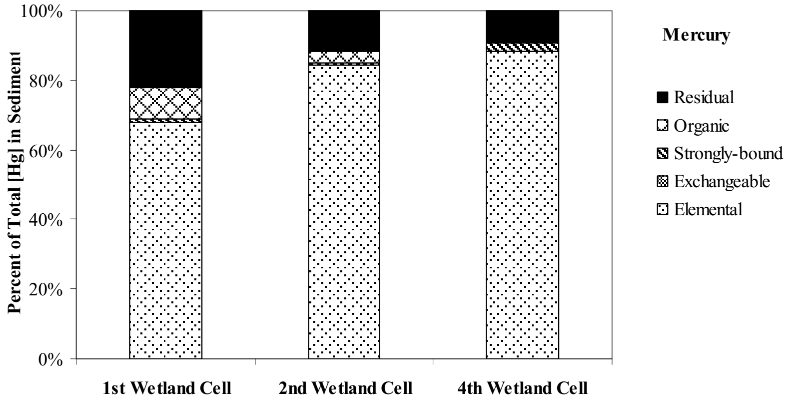


Fig. 6 Distribution of Hg in the fractions of the samples extracted by sequential extraction procedures. All data are reported on a dry weight basis.

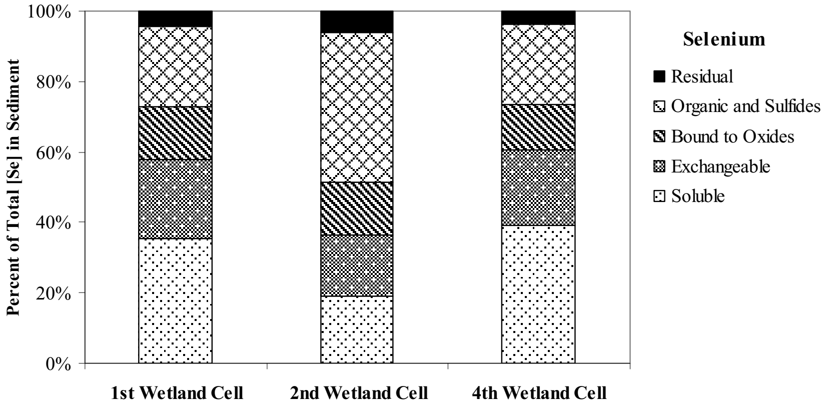


Fig. 7 Distribution of Se in the fractions of the samples extracted by sequential extraction procedures. All data are reported on a dry weight basis.

matter were also volatilized at high rates, none of the HgS (amorphous analog of cinnabar) was volatilized from the sample. Measured elemental Hg in sediment from the pilot-scale constructed wetland treatment system therefore may have included elemental, chloride-complexed, and humic-bound Hg.

Less than 2.5% of the total Hg was associated with the strongly-bound fraction, which reflected Hg from several sediment components such as Fe and Mn oxyhydroxides and mineral surface sites where Hg is strongly adsorbed (Lechler *et al.* 1997). Sediment from the first wetland cell contained 8.88% of the total Hg in the organic fraction, compared to 3.49% in the second wetland cell and no detectable amounts in the fourth wetland cell. Sediment in the first wetland cell did however contain the highest organic matter content (3%) of the three wetland cells. Over half of the total sediment Hg was found in the residual fraction which is known to include species associated with the structures of soils and minerals. There has been controversy about the stability of sulfide complexes and in which step the sulfides are targeted (Schank 2003). In some procedures sulfides are considered to be associated with the organic fraction, while other procedures target sulfides in the residual fraction due to their high stability. In the pilot CWTS, mercury sulfide complexes are expected to be abundant. Since mercury sulfides are unlikely to volatilize during pyrolytic extraction (Bloom *et al.* 2003), it may be extracted in the residual fraction which would explain the larger percentage of total Hg associated with this fraction. The fraction of residual Hg decreased from 22.21% to 9.3% of the total Hg from the first through fourth wetland cell. There was a clear inverse relationship between Hg fractions in the CWTS sediment; in the CWTS sediment, elemental and strongly-bound Hg increased though the system while organic and residual Hg decreased through the system. The increase in elemental Hg corresponded with an increase in sediment redox potential in the CWTS, suggesting that Hg became increasingly mobile and potentially bioavailable with more oxidizing conditions.

In the first Se fractionation step, a potassium chloride solution was used to bring water-soluble or nonspecifically adsorbed Se into solution. The sediment-associated Se can be replaced by the chloride ion through anion exchange. Potassium chloride was also used by Zhang and Moore (1996) for extracting soluble Se. Potassium dihydrogen phosphate was then used to extract exchangeable Se from soil. This extractant is effective in replacing selenite that was specifically adsorbed on oxide minerals. As described previously, hydrochloric acid was effective in dissolving an array of sediment constituents, thereby releasing strongly bound elements, and was therefore utilized to extract reducible sediment-associated Se. Hirner *et al.* (1990) also used hydrochloric acid for extracting this fraction. For the oxidizable or organic fraction, a combination of potassium chlorate and concentrated hydrochloric acid was used. This strong oxidizing agent was very effective in dissolving organic matter and sulfide minerals (Chao and Sanzolone 1989). Finally, for the residual Se fraction, concentrated nitric acid was used to destruct the silicate structure (Chao and Sanzolone 1989).

Selenium was more evenly distributed among the extracted fractions compared to Hg (**Fig. 7**). According to sequential extraction results, approximately 35-60% of the total Se concentrations in the constructed wetland sediment were in the most mobile and bioavailable fractions (soluble and exchangeable). These fractions included nonspecifically and specifically adsorbed Se. The remaining Se was stable in the sediment under the current redox potential conditions, residing in the oxide-bound, the organic- and sulfide-bound, and to a lesser degree, the residual fractions. Selenium residing in the oxide-bound fraction, accounting for 12 to 15% of total Se, likely included precipitates of iron minerals which was thought to be an important component in the CWTS for Se removal from wastewater. Selenium was also expected to bind to organic matter, which accounted for 22 to 43% of total Se. However, this fraction also included Se bound to sulfide minerals, which may also be important in Se removal. There was no clear spatial relationship between fractions, with the exception that the second wetland cell contained the highest amount of orga-

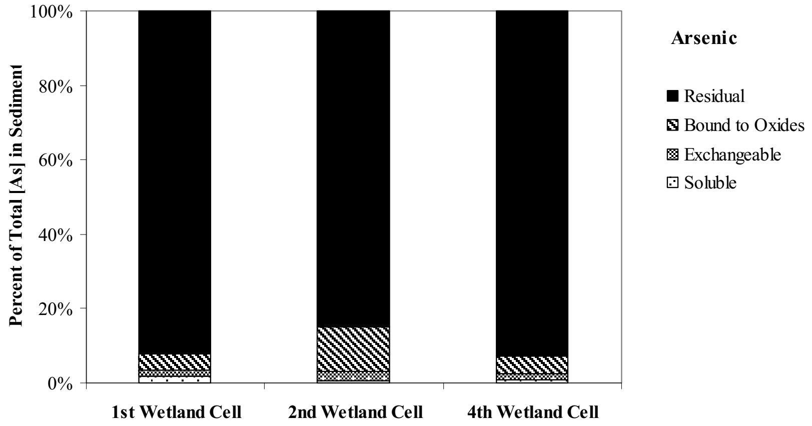


Fig. 8 Distribution of As in the fractions of the samples extracted by sequential extraction procedures (fractions for amorphous, well-crystallized, and poorly-crystallized Fe and Al oxides were combined in this graph). All data are reported on a dry weight basis.

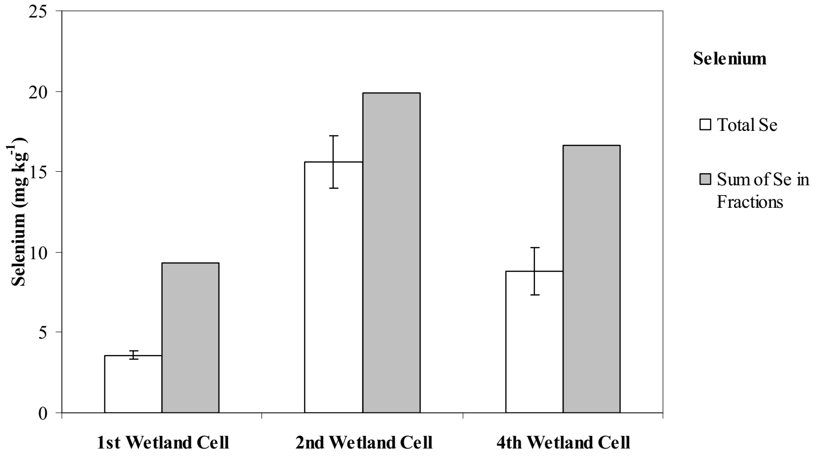


Fig. 9 Concentrations of total Se extracted by *aqua regia* versus the sum of Se concentrations sequentially extracted in the five fractions. Error bars represent standard deviations (n=3).

nic- and sulfide-bound Se while the first wetland cell contained the greatest amounts of soluble Se. Therefore, results suggested that Se in the second wetland cell was the most stable compared to the rest of the CWTS.

In the sequential fractionation procedure for As, soluble and nonspecifically sorbed As was extracted with ammonium sulfate. This extractant has been shown to extract As slightly more effectively than ammonium nitrate and ammonium acetate solutions, both of which have been used in other methods (Wenzel *et al.* 2001). In the next step of this procedure, ammonium phosphate was used to extract exchangeable and specifically-sorbed As from mineral surfaces. Phosphate outcompetes As for adsorption sites in sediment because of the smaller size and higher charge density of phosphates (Wenzel *et al.* 2001), making phosphate solutions effective extractants of As. Also, ammonium phosphate solutions extract relatively small amounts of Al and Fe as compared to other extractants such as ammonium hydroxide, indicating its selectivity for surface-bound As fractions. The amorphous and poorly-crystallized hydrous oxides of Fe and Al fraction of sediment-associated As was extracted with an ammonium oxalate buffer. Ammonium oxalate was efficient in destructing Fe and Al oxides to release bound As (Gleyzes *et al.* 2002). When mixed with ascorbic acid, which was used in the next step, ammonium oxalate more strongly targeted the hydrous oxides, thereby releasing the As associated with the well-crystallized hydrous oxides of Fe and Al. Finally, the residual fraction of sediment-associated As was extracted with concentrated nitric acid (Wenzel *et al.* 2001).

Results indicate that As was primarily in the residual fraction of the sediment in the CWTS, ranging from 85% to 93% of total As sediment concentrations (**Fig. 8**). Since organic-bound As was not targeted in previous extraction steps, it may have been extracted in the residual fraction. Nitric acid is a common reagent used for the destruction of organic matter (Gleyzes *et al.* 2002). Therefore, the residual fraction may have included As bound to the mineral and silicate matrices as well as organic-bound As, suggesting that As in the system is non-mobile and potentially non-bioavailable. Smaller amounts of As were found in the oxide-bound fractions (<10%), and negligible amounts were found in the remaining three fractions. Like Se, no clear spatial relationships were found between sediment fractions in the CWTS. However, if sediment extractions had been done on multiple samples collected from each wetland cell as opposed to one composited sample from each cell, spatial information regarding the fractions in which Hg, Se, and As were associated with may have been obtained.

The sums of Se and As in the five fractions of each of the samples were compared to the total concentrations measured in separate samples by acid digestion (**Figs. 9, 10**). Since the concentrations of Hg in elemental and residual fractions were derived from total Hg concentrations, no comparison between total Hg and the sum of Hg in fractions could be made. The concentrations of Se from the total digestions were 38 to 78% lower than sum of Se in the five fractions. Similarly, As from the total digestions reflected only about 50% of the As from the five fractions. Since sediment for total concentrations was digested in *aqua regia* (5 mL concentrated HNO_3 and 15 mL concentrated HCl), as opposed to the recommended HF, the extractant may not have been strong enough to completely break down the silicate structure of the sediment (Chao and Sanzolone 1989). The use of several solvents increasing in acid strength during the sequential extraction procedures is likely more efficient in breaking down matrices and releasing bound elements, thereby providing more reliable concentrations

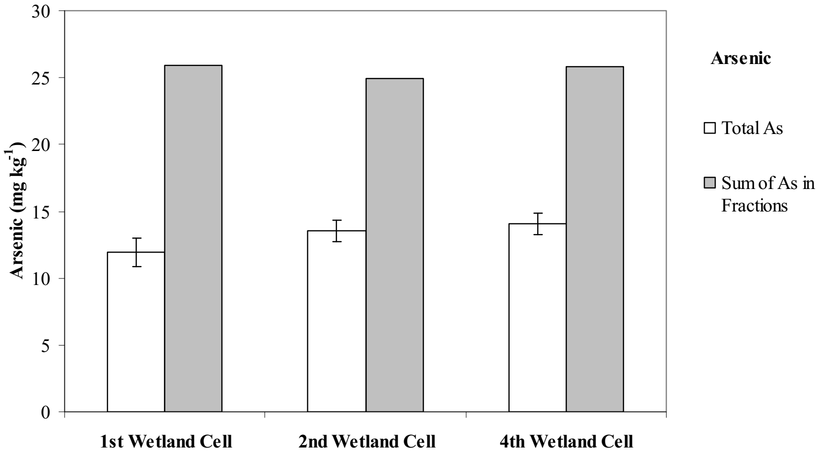


Fig. 10 Concentrations of total As extracted by *aqua regia* versus the sum of As concentrations sequentially extracted in the five fractions. Error bars represent standard deviations (n=3).

of sediment-associated Se and As.

There were several limitations associated with using sequential extraction procedures or techniques to define element distribution in sediment. It is well known that sediment collection, handling, and storage procedures may alter contaminant bioavailability and concentration by changing the physical, chemical, or biological characteristics. Manipulations such as mixing, homogenizing, and sieving may disrupt the equilibrium of contaminants in sediments. Excess exposure to air increases oxygen penetration, alters bacterial activity, and causes oxidation of porewater species (Simpson *et al.* 2004). Therefore, it was imperative that sediment preparation for sequential extraction procedures be as consistent as possible to minimize alteration of sediment characteristics. Researchers have concluded that extraction procedures should be applied to wet sediment immediately after sampling if environmentally-relevant information was to be obtained (USEPA 2001).

Ideally, reagents utilized in sequential extraction procedures are chosen to selectively dissolve a geological phase of sediment, inducing the solubilization of associated elements. However, it has been repeatedly demonstrated that reagents used in sequential extraction procedures often lack specificity, making it difficult to interpret results properly. Also, the lack of sensitivity of reagents can be responsible for element redistribution during the extraction procedures. For example, researchers have noticed that FeS dissolution during an acid-soluble step in some procedures led to the precipitation of dissolved metals with sulfide ions (Gleyzes *et al.* 2002).

While these procedures are useful in categorizing elements into operationally-defined geochemical fractions, sequential extraction procedures cannot identify competition between ions or species. Due to the constituents of FGD wastewater, sediment in this CWTS can be considered a complex mixture. Competition between Hg, Se, and As for binding sites and/or complexing compounds may occur in natural sediment systems. However, due to an abundance of iron and sulfur in this CWTS, interactions between Hg, Se, and As were unlikely to be significant.

Another limitation to using the selected sequential extraction procedure for mercury was that the first fraction, elemental Hg, was assumed to be the difference between total Hg and what was left over after the pyrolytic extraction in the first step of the procedure. If the recovery of total Hg in this procedure was inadequate, elemental Hg concentrations could be over or under estimated.

Predicting sediment toxicity

Typical freshwater sediment background levels of Hg, Se, and As according to Buchman (1999) are 0.004-0.051 mg Hg kg^{-1}, 0.29 mg Se kg^{-1}, and 1.1 mg As kg^{-1}. Concentrations measured in the control sediment were 0.007 mg Hg kg^{-1} and 0.004 mg As kg^{-1}. Selenium concentrations were below the detection limit (0.107 μg L^{-1}). Values of TELs and PELs have been estimated for Hg and As in freshwater sediment. The TEL and PEL concentrations (dry weight basis) for Hg are 0.174 mg kg^{-1} and 0.486 mg kg^{-1}, respectively, and the reported TEL and PEL concentrations for As are 5.9 mg kg^{-1} and 17.0 mg kg^{-1}, respectively (Buchman 1999; Ingersoll *et al.* 2000). TELs and PELs have not yet been established for Se in freshwater sediment.

Results indicate that after 17 months of treating simulated FGD wastewater containing Hg concentrations ranging from 0.001 to 0.16 mg L^{-1}, sediment Hg concentrations did not significantly exceed typical background levels in freshwater sediments. However, after additional years of wastewater treatment, it is likely that sediment concentrations of Hg will increase. Sediment concentrations of Se and As in the CWTS were well above typical background levels. Sediment-associated As, ranging from approximately 12 to 14 mg kg^{-1}, are within the range between threshold and probable effect levels reported by Buchman (1999) and Ingersoll *et al.* (2000). The gap between the As TEL and PEL represents the range of concentrations at which adverse biological effects may or may not occur in organisms exposed to sediment contaminated only with As. Based on individual TELs and PELs for Hg and As, sediment collected from the CWTS would not likely be toxic to *H. azteca*. However, these guidelines can not predict toxicity of sediment-associated contaminants in complex mixtures, such as the case with this CWTS. If toxic effects of sediment-associated Hg, Se, and As were assumed to be additive, then toxicity would have been expected to occur in organisms exposed to this sediment.

Using methods described by Swartz *et al.* (1995), Di Toro and McGrath (2000) and Di Toro *et al.* (2001), toxic units were calculated for Hg, Se, and As based on the assumptions that the interstitial water concentrations in sediment reflected sediment exposure and that interstitial water 10-day LC50 values were the same as water-only 10-day LC50 values. For this method, 10-day aqueous toxicity tests were conducted in which *H. azteca* were exposed to Hg (as mercuric nitrate), Se (as sodium selenate), and As (as sodium arsenite). Aqueous LC50 values for Hg, Se, and As were 41.49 μg L^{-1}, 176.4 μg L^{-1}, and 642.2 μg L^{-1}, respectively. These values, in addition to sediment concentrations (wet weight) adjusted for expected bioavailability, were used to calculate toxic units (TUs; **Table 5**). The percent bioavailability of Hg, Se, and As in sediment included those portions in elemental, soluble, and exchangeable fractions (**Table 4**), since elements associated with these particular fractions are considered to be potentially bioavailable to organisms. The sum of toxic units for sediment in the first, second, and fourth wetland cells are 10.5, 26.39, and 31.14, respectively. Individual toxic units for Hg and As were all less than 1 (a TU of 1 indicates 50% mortality ex-pected in sediment due

Table 5 Data used for toxic unit calculations for sediment toxicity prediction.

	Element	WW[a] concentration (mg/kg)	Bioavailable (%)	Adjusted WW concentration (mg/kg)	Aqueous LC50 (mg/L)	Toxic Unit (TU)	$\sum$ TU[b]
1st Wetland Cell	Mercury	0.020	68.02	0.014	0.042	0.32	10.50
	Selenium	2.944	57.79	1.701	0.176	9.64	
	Arsenic	9.902	3.45	0.342	0.644	0.53	
	Element	**WW concentration (mg/kg)**	**Bioavailable (%)**	**Adjusted WW concentration (mg/kg)**	**Aqueous LC50 (mg/L)**	**Toxic Unit (TU)**	**$\sum$ TU**
2nd Wetland Cell	Mercury	0.027	84.26	0.023	0.042	0.54	26.39
	Selenium	12.346	36.47	4.503	0.176	25.52	
	Arsenic	6.593	3.14	0.207	0.644	0.32	
	Element	**WW concentration (mg/kg)**	**Bioavailable (%)**	**Adjusted WW concentration (mg/kg)**	**Aqueous LC50 (mg/L)**	**Toxic Unit (TU)**	**$\sum$ TU**
4th Wetland Cell	Mercury	0.019	88.20	0.017	0.042	0.40	31.14
	Selenium	8.790	60.71	5.336	0.176	30.25	
	Arsenic	11.778	2.67	0.314	0.644	0.49	

[a] WW, Wet weight

[b] TU, Toxic unit

Table 6 Data used for expected mortality calculations for predicting sediment toxicity to *H. azteca*.

	Element	WW[a] concentration (mg/kg)	Bioavailable (%)	Adjusted WW concentration (mg/kg)	Expected mortality (%)	Total expected mortality (%)	Observed mortality (%)
1st Wetland cell	Hg	0.020	68.02	0.014	8.30	108.30	10
	Se	2.944	57.79	1.701	100		
	As	9.902	3.45	0.342	0		
	Element	**WW concentration (mg/kg)**	**Bioavailable (%)**	**Adjusted WW concentration (mg/kg)**	**Expected mortality (%)**	**Total expected mortality (%)**	**Observed mortality (%)**
2nd Wetland cell	Hg	0.027	84.26	0.023	17.70	117.70	3.33
	Se	12.346	36.47	4.503	100		
	As	6.593	3.14	0.207	0		
	Element	**WW concentration (mg/kg)**	**Bioavailable (%)**	**Adjusted WW concentration (mg/kg)**	**Expected mortality (%)**	**Total expected mortality (%)**	**Observed mortality (%)**
4th Wetland cell	Hg	0.019	88.20	0.017	11.23	111.23	3.33
	Se	8.790	60.71	5.336	100		
	As	11.778	2.67	0.314	0		

[a] WW, wet weight

to that contaminant), whereas Se toxic units were much greater than 1. According to these results, sediment from the three wetland cells should have been toxic, with Se contributing to organism toxicity the most.

Another method to predict sediment toxicity was the use of nonlinear regression models to predict toxicity attributed to each individual contaminant. This method was also based on the assumptions that the interstitial water concentrations in sediment reflect sediment exposure (Swartz *et al.* 1995), and that interstitial water LC50 values are the same as water-only LC50 values (Di Toro and McGrath 2000; Di Toro *et al.* 2000). Using data obtained from water-only exposures, concentration-response curves were generated in GraphPad Prism 3.0. Nonlinear regression (curve-fit) was then used to formulate polynomial equations (where $r^2 > 0.98$) for the concentration-response curves of each element (**Fig. 11**). In Microsoft Excel, the polynomial equations were used to calculate the mortality expected following exposure to element concentrations measured in sediment (wet weight; adjusted for potential bioavailability). Expected mortalities for individual elements were added, according to the assumption that the effects of elements in mixtures are additive (**Table 6**). According to these results, the total expected mortalities of organisms exposed to sediment from the first, second, and fourth wetland cells were greater than 100%.

Sediment toxicity to *Hyalella azteca*

Results of sediment toxicity tests using *H. azteca* as the test organism indicated that sediments collected from the CWTS were not toxic. Average percent survivals of test organisms exposed to contaminated sediment from this CWTS ranged from 90-96.67%, which were not significantly different ($p > 0.05$) from each other or the average survival of controls (**Fig. 12**). These percent survivals are within the range of test acceptability. Average dry weights per test organism ranged from 250 µg to 340 µg, which were not significantly different ($p > 0.05$) from each other or the dry weights for controls, which averaged 260 µg. Although toxicity was not observed in *H. azteca* in these short-term experiments, chronic toxicity tests (i.e. 28-day test) in which mortality, dry weight, and reproduction are the measured endpoints may provide different results.

The observed lack of toxicity in test organisms exposed to contaminated sediment from the CWTS could also be explained by the bioavailability of Hg, Se, and As in the sediment. The bioavailable fraction is the amount of a contaminant that is potentially available for biological action, such as uptake by an aquatic organism (Rand 1995). Benthic invertebrates can take up elements from sediment by the gut through ingestion or through the gills. For those organisms in direct contact with sediment, contaminants may also accumulate by direct adsorption to the body wall or by absorption through the integument (Rand 1995). If Hg, Se, and As were bioavailable in the CWTS, the concentrations may not have been high enough to elicit an effect in the organisms. On the other hand, if the constituents were not bioavailable, the lack of observed toxicity would not be correlated with the concentrations of Hg, Se, and As in sediment. It is widely accepted that determining total concentrations of these constituents in sediment may not

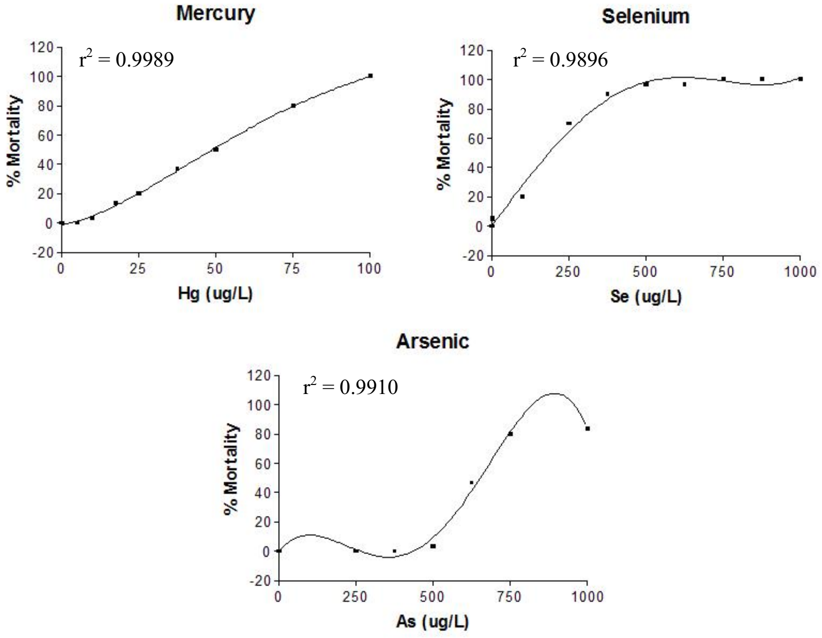


Fig. 11 Concentration-response curves generated for Hg, Se, and As following aqueous-only exposures to *H. azteca*. Equations and r^2 values were determined using nonlinear regression (curve-fit).

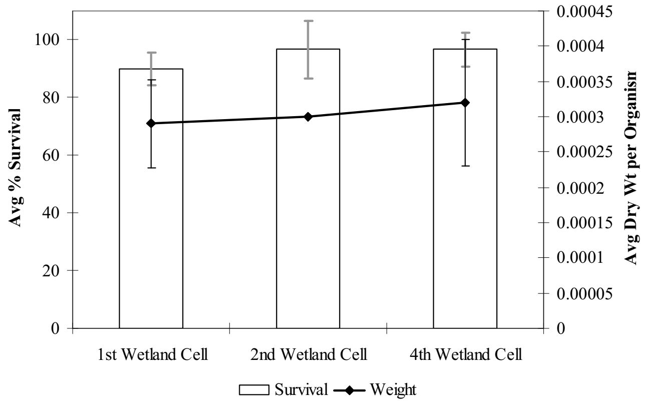


Fig. 12 Average percent survivals and dry weights (per organism) of test organisms exposed to contaminated sediment. Error bars represent standard deviation (n=3).

provide the required information about mobility, bioavailability, and the potential impact on the aquatic system (Michalke 2003). Speciation can greatly affect the fate and behavior of elements in a constructed wetland treatment system, especially of those elements that exist in multiple oxidation states. For example, Hg can exist in inorganic or organic forms. While Hg species are generally toxic, inorganic Hg salts are less toxic than organic, methylated forms. Organic Hg are generally more mobile, more toxic, and can be bioconcentrated up to 10,000-fold in fish (Michalke 2003).

The distribution of Hg, Se, and As in the constructed wetland treatment system depends on its speciation, which defines a specific form of an element as to isotopic composition, electronic or oxidation state, or molecular structure (MITE 2003). Mercury, Se, and As can exist in multiple oxidation states and tend to form species differing from those of other metals. Identification of the main phase associations of these trace elements in sediments would help in understanding the geochemical processes in order to evaluate the risk and remobilization potential of Hg, Se, and As in the constructed wetland treatment system.

In addition to speciation, the physico-chemical properties of the contaminated sediment (**Fig. 1**) may influence bioavailability or the behavior of Hg, Se, and As in the CWTS. For example, sediment in this CWTS is primarily composed of sand (89-92%) and to a much lesser extent, clay and silt (< 11%). According to Rand (1995), the coarser fragments of sediment, including sand, are not generally associated with chemical contaminants. Contaminants are more often associated with fine sediment particles with relatively large surface areas, which can increase the sorptive capacity of contaminants. Sediment pH can also affect sorption of elements; a lower pH lowers sorption, thus increasing pore water concentrations and subsequent water-borne exposure to aquatic organisms. The pH of sediment in the CWTS ranges from 6.6 to 6.8, which should not greatly affect sorption (Baker *et al.* 2003). Redox potential can drastically affect the chemical nature of Hg, Se, and As present in sediment. For example, a reduced redox condition (as present in the 1st and 2nd wetland cells with redox values of -410 and -337 mV) will reduce the external dose and uptake via the pore water by increasing the formation of stable precipitates (i.e. sulfides). However, for sediment-dwelling organisms, such as *H. azteca*, that are capable of digesting reduced materials, element bioavailability might increase with reduced redox conditions (Baker *et al.* 2003). The sample principle applies to the organic matter content of sediment; a great amount of organic matter will reduce the aqueous phase of many elements, but will increase the risk to sediment-dwelling organisms.

One limitation associated with the use of one test organism in sediment toxicity experiments is the relative sensitivity of that organism to elements. Due to the varying sensitivity of organisms to sediment-associated contaminants, a number of organisms would ideally be used in assessing sediment toxicity. Reish (1988) reported the relative toxicity of several elements to crustaceans, polychaetes, pelecypods, and fishes, and concluded that no one species or group of animals was the most sensitive to all the elements. Therefore, *H. azteca* was chosen as the representative organism since it is the most widely used test organism and allows for potential comparisons to similar future studies.

SUMMARY AND CONCLUSIONS

Mercury, Se, and As are three constituents of FGD wastewater that require treatment prior to discharge. Constructed wetland treatment systems have great potential to treat these constituents in wastewater. However, sediment in the constructed wetland can act as a sink for Hg, Se, and As during treatment processes. Since determining total concentrations of these constituents in sediment cannot provide the required information about mobility, bioavailability, and the potential impact on the aquatic system, sequential extraction procedures were employed to operationally-define the species of Hg, Se, and As in sediment. Results suggest that Hg and As are stable, immobile, and non-bioavailable in the sediment, as long as current sediment conditions such as pH and redox potential remain stable. Mercury was primarily in its elemental fraction in the sediment, which may also include chloride-complexed and humic-bound Hg. Mercury sulfide complexes were likely extracted in the residual fraction, which accounted for less than 22% of total sediment-associated Hg. Approximately half of the total Se in the sediment was found to be mobile and potentially bioavailable, while the other half was stable and not likely bioavailable to plants and organisms.

A toxicity assessment of sediment in a pilot-scale constructed wetland treatment system was performed to obtain the information needed to support environmental management decisions related to mitigating risks associated with flue gas desulfurization wastewater. Sediment in this treatment system contained Hg, Se, and As concentrations below reported probable effect levels, which identify the concentrations above which adverse biological impacts are likely to occur due to that contaminant alone. Sediments were predicted to be toxic to *H. azteca* based on calculated toxic units and nonlinear regression methods. However, sediment was not toxic to *H. azteca* despite the use of this constructed wetland treatment system to treat FGD wastewater for over a year.

Results obtained from measuring the distribution and characterization of Hg, Se, and As in this sediment support the observed lack of toxicity observed in *Hyalella azteca*. If sediment concentrations of Hg, Se, and As were high enough to elicit adverse effects, the lack of toxicity may be explained by the fractionation of these elements in non-bioavailable forms in the sediment. Identification of the main phase associations of Hg, Se, and As in sediments also helps to understand the biogeochemical processes involved and to evaluate the risk and remobilization potential of these elements in the constructed wetland treatment system. To completely assess ecological risk associated with the use of a CWTS, contaminant enrichment, bioavailability, and toxicity in sediment must also be monitored.

ACKNOWLEDGEMENTS

The authors thank Dr. Stephen Klaine and Dr. Mark Schlautman from Clemson University for their contributions to this research.

DISCLAIMER

The research described in this original research paper has been funded in part by the United States Environmental Protection Agency (EPA) under the Greater Research Opportunities (GRO) Graduate Program. EPA has not officially endorsed this publication and the views expressed herein may not reflect the views of the EPA.

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