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BIOGEOCHEMICAL PROCESSES IN HYDROSOIL OF PILOT-SCALE CONSTRUCTED WETLAND TREATMENT SYSTEMS DESIGNED FOR TREATMENT OF SELENIUM

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BIOGEOCHEMICAL PROCESSES IN HYDROSOIL OF PILOT-SCALE CONSTRUCTED WETLAND TREATMENT SYSTEMS DESIGNED FOR TREATMENT OF SELENIUM

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ABSTRACT

Two pilot-scale wetland treatment system cells (nutrient amended and unamended) were designed and constructed to reduce aqueous Se concentrations in simulated energy-derived water. Specific objectives of this study were: (i) measure and correlate hydrosoil conditions with Se concentrations vertically through the hydrosoil; (ii) investigate Se-accumulating biogeochemical processes (dissimilatory Se reduction and sorption) operating in the hydrosoil; and (iii) evaluate the effect of a nutrient amendment on hydrosoil conditions, Se accumulation, and Se-sequestering biogeochemical processes in the hydrosoil. Se accumulation (i.e. total Se concentration) and hydrosoil conditions were measured with depth in the hydrosoil. Se-sequestering biogeochemical processes were investigated by counting Se-reducing microbial colony forming units (CFUs) and identifying Se geochemical fractions at various depths in the hydrosoil. The detritus (0-21 cm in nutrient amended cell and 0-14 cm in unamended cell) contained greater Se concentrations (308-830 μg/g and 138-569 μg/g) and greater CFUs (2,700-22,000 CFUs/mL pore water and 9,300-15,000 CFUs/mL pore water) than the underlying sandy sediment. Correlation of organic matter content with Se concentration ($r = 0.95$; $p<0.00001$ in nutrient amended and $r = 0.87$; $p<0.00002$ in unamended) suggests organic matter influences Se-accumulating biogeochemical processes. In detritus, mean hydrosoil conditions (redox: -2 to -173 mV, pH: 6.20-6.46, and organic matter: 52-86%) were more favorable for sorption than for dissimilatory Se reduction; however, the majority of Se measured in the detritus was elemental (52.1%-58.0% in the nutrient amended cell and 21.1%-62.6% in the unamended cell) suggesting that dissimilatory Se

reduction is the dominant biogeochemical process sequestering Se in the detritus. T-tests indicate significant difference in pH ($t = 2.87$, $p = 0.0132$) of the hydrosoil between the nutrient amended cell and unamended cell, but no significant differences ($p<0.005$) in redox potential, organic matter content, and Se concentration. Greater Se concentrations and percent of elemental Se in the nutrient amended cell than the unamended cell suggests that the nutrient amendment enhanced dissimilatory Se reduction and therefore Se accumulation in the hydrosoil.

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CHAPTER I

INTRODUCTION

1. Background

Selenium-contaminated waters (agricultural and energy-derived) are a growing environmental concern due to their deleterious effects on aquatic biota and waterfowl (Ohlendorf *et al*. 1990, Lemly 2004, Janz *et al*. 2010). Although selenium (Se) is an essential micronutrient for basic cellular function (Zayed *et al.* 1998, Carlson *et al*. 2004), the range in concentrations in which Se is essential or toxic is very narrow (e.g., bioconcentration, toxicity) (Oremland 1994, Lemly 2004, Selinus *et al*. 2005, Young *et al*. 2010). Treatment of Se-contaminated waters can be difficult; however, constructed wetland treatment systems (CWTSs) offer a treatment option for the aforementioned waters (Rodgers and Castle 2008; Spacil *et al*. 2011a, Spacil *et al.* 2011b). Over the past few decades, water contaminated with Se has been treated using CWTSs with varying degrees of performance (Gao *et al.* 2000, Gao *et al.* 2003, Sundberg-Jones and Hassan 2007, Spacil *et al.* 2011a, Spacil *et al.* 2011b). CWTSs remediate Se-contaminated waters by altering (mainly reducing) the oxidation state (VI, IV, 0, and -II) of Se via biogeochemical processes. The majority of biogeochemical processes that can remove selenate and selenite from contaminated waters occur within the hydrosoil (Trudinger and Swaine 1979, Kadlec and Wallace 2009). The hydrosoil in CWTSs contains two zones: detritus (partially decomposed and compacted plant matter) and sediment (added during CWTS construction) (Gao *et al.* 2003). Hydrosoil in a CWTS can be designed to produce conditions (e.g. pH, redox potential, and organic matter content) that promote

specific biogeochemical processes (Kanagy *et al.* 2008, Rodgers and Castle 2008, Dorman *et al*. 2009, Horner *et al*. 2011, Spacil *et al*. 2011a, Beebe *et al*. 2015).

Many CWTSs target dissimilatory Se reduction facilitated by anaerobic Se-reducing bacteria to sequester Se into the hydrosoil. Dissimilatory reduction of Se is a biogeochemical process that occurs in natural systems (Oremland *et al.* 1990). Anaerobic bacteria can transform selenate (SeO_4^{-2}) and selenite (SeO_3^{-2}) to elemental Se (Se^0) through dissimilatory Se reduction in a CWTS (Frankenberger and Arshad 2001). During dissimilatory Se reduction, Se-reducing bacteria utilize selenate and selenite as electron acceptors for microbial respiration resulting in insoluble elemental Se (Oremland *et al.* 2004). To promote and enhance dissimilatory Se reduction, organic carbon amendments have been added to CWTSs as an additional energy source and electron donor for Se reducing bacteria (Zhang and Frankenberger 2005). Although dissimilatory Se reduction is often the targeted pathway in CWTSs, recent studies have suggested that sorption, particularly with organic matter, also influences Se accumulation in the hydrosoil (Pezzarossa *et al.* 1999, Lin *et al.* 2010, Gonzalez-Acevedo *et al.* 2012). Sorption is another natural biogeochemical process that can reduce selenate (SeO_4^{-2}) and selenite (SeO_3^{-2}) to selenides (Se^{2-}), but does not naturally produce elemental Se (Se^0) (Stolz and Oremland 1999). Dissimilatory Se reduction is often preferred over sorption in CWTSs because elemental Se is insoluble, the most stable and least bioavailable form of Se (Sundberg-Jones and Hassan 2007).

2. Research Significance and Objectives

The effect of carbon amendments on Se-reducing bacteria and horizontal variation in treatment performance of Se in CWTSs has been studied previously (de Souza *et al.* 1999, Gao *et al.* 2000, Gao *et al.* 2003, Zhang and Frankenberger 2005, Zhang *et al.* 2008, Spacil *et al.* 2011a, Spacil *et al* 2011b, Van Heest 2012). However, few have investigated the effect of a carbon amendment on the vertical variation in hydrosoil conditions, biogeochemical processes, and Se accumulation. Because hydrosoil plays an essential role in Se treatment within CWTSs, studies of the vertical variation in hydrosoil conditions are needed. Therefore, objectives of this study were: (I) measure and correlate hydrosoil conditions (pH, organic matter content, and redox potential) and Se concentration vertically through hydrosoil; (II) investigate two major Se-sequestering biogeochemical processes (dissimilatory Se reduction and sorption); and, (III) evaluate the effect of a nutrient amendment on hydrosoil conditions, Se accumulation, and Se-accumulating biogeochemical processes vertically through the hydrosoil in a pilot-scale CWTS cell designed to treat Se in simulated energy-derived water (EDW).

3. Organization of Thesis

This thesis consists of four chapters including Introduction (Chapter I) and Conclusions (Chapter IV). The two body chapters of this thesis are written and formatted as independent manuscripts; consequently, some material is repeated in both chapters. The two body chapters include;

Chapter II: Nutrient Amendment Effect On Hydrosoil Conditions and Selenium-Accumulating Biogeochemical Processes in Pilot-Scale Constructed Wetland Treatment Cells

Chapter III: Evidence of Selenium-Accumulating Biogeochemical Processes In Pilot-Scale Constructed Wetland Treatment Cells

Chapter II investigates hydrosoil conditions (pH, redox potential, and organic matter content) and Se accumulation vertically through the hydrosoil as well as the potential for Se-accumulating biogeochemical processes to occur in pilot-scale CWTS cells designed to treat Se in simulated EDW. Chapter III examines Se-accumulating biogeochemical processes operating in the hydrosoil of a nutrient amended and unamended pilot-scale CWTS cell through the use of a modified sequential extraction procedure. Collectively, this research evaluated the effect of a nutrient amendment on the vertical variation in hydrosoil conditions and Se-sequestering biogeochemical processes.

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CHAPTER II

NUTRIENT AMENDMENT EFFECT ON HYDROSOIL CONDITIONS & SELENIUM-ACCUMULATING BIOGEOCHEMICAL PROCESSES IN PILOT-SCALE CONSTRUCTED WETLAND TREATMENT CELLS

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Abstract

Two pilot-scale wetland treatment system cells (nutrient amended and unamended) were designed and constructed to reduce aqueous Se concentrations in simulated energy-derived water. Specific objectives of this study were: (i) measure and correlate hydrosoil conditions with Se concentrations vertically through the hydrosoil; (ii) investigate potential for Se-accumulating biogeochemical processes operating in the hydrosoil; and (iii) evaluate effect of a nutrient amendment. Se concentration in the hydrosoil was 138-830 µg/g in organic detritus and 0.540-6.77 µg/g in sandy sediment. Correlation of organic matter content with Se concentration ($r = 0.95$; $p<0.00001$ in nutrient amended and $r = 0.87$; $p<0.00002$ in unamended) suggests organic matter influences Se-accumulating biogeochemical processes. In detritus, mean hydrosoil conditions (redox: -2 to -173 mV, pH: 6.20-6.46, and organic matter: 52-86%) were more favorable for sorption than for dissimilatory Se reduction, suggesting that sorption may be the dominant biogeochemical process resulting in accumulation of Se in hydrosoil of wetland cells studied. T-tests indicate significant difference in pH ($t = 2.87$, $p = 0.0132$) of the hydrosoil between the amended and unamended cells, but no significant differences ($p<0.005$) in redox potential, organic matter content, and Se concentrations.

1. Introduction

Selenium-contaminated waters (e.g. agricultural and energy-derived) are a growing environmental concern due to their deleterious effects on aquatic biota and waterfowl (Ohlendorf *et al*. 1990, Lemly 2004, Janz *et al*. 2010). Although selenium (Se) is an essential micronutrient for basic cellular function (Zayed *et al.* 1998, Carlson *et al*. 2004), the range in concentrations in which Se is essential or toxic is very narrow (e.g., bioconcentration, toxicity) (Oremland 1994, Lemly 2004, Selinus *et al*. 2005, Young *et al*. 2010). Treatment of Se-contaminated waters can be difficult; however, constructed wetland treatment systems (CWTSs) offer a treatment option for the aforementioned waters (Rodgers and Castle 2008; Spacil *et al*. 2011a, Spacil *et al.* 2011b). Over the past few decades, water contaminated with Se has been treated using CWTSs with varying degrees of performance (Gao *et al.* 2000, Gao *et al.* 2003, Sundberg-Jones and Hassan 2007, Spacil *et al.* 2011a, Spacil *et al.* 2011b). CWTSs remediate Se-contaminated waters by altering (mainly reducing) the oxidation state (VI, IV, 0, and -II) of Se via biogeochemical processes. The majority of biogeochemical processes that can remove selenate and selenite from contaminated waters occur within the hydrosoil (Trudinger and Swaine 1979, Kadlec and Wallace 2009). The hydrosoil in CWTSs contains two zones: detritus (partially decomposed and compacted plant matter) and sediment (added during CWTS construction) (Gao *et al.* 2003). Hydrosoil in a CWTS can be designed to produce conditions (e.g. pH, redox potential, and organic matter content) that promote specific biogeochemical processes (Kanagy *et al.* 2008,

Rodgers and Castle 2008, Dorman *et al*. 2009, Horner *et al*. 2011, Spacil *et al*. 2011a, Beebe *et al*. 2015).

Dissimilatory Se reduction facilitated by Se reducing bacteria can be targeted in CWTSs to accumulate Se in the hydrosoil (Zhang and Frankenberger 2005). Dissimilatory Se reduction is enhanced by addition of organic carbon to CWTSs as an energy source and electron donor for Se-reducing bacteria (Zhang and Frankenberger 2005, Spacil *et al.* 2011a). The effect of carbon amendments on Se-reducing bacteria and horizontal variation in treatment performance of Se in CWTSs has been studied previously (de Souza *et al.* 1999, Gao *et al.* 2000, Gao *et al.* 2003, Zhang and Frankenberger 2005, Zhang *et al.* 2008, Spacil *et al.* 2011a, Spacil *et al* 2011b, Van Heest 2012). However, few have investigated the effect of a carbon amendment on the vertical variation in hydrosoil conditions, biogeochemical processes, and Se accumulation. Because hydrosoil plays an essential role in Se treatment within CWTSs, studies of the vertical variation in hydrosoil conditions are needed. Therefore, objectives of this study were: (i) measure and correlate hydrosoil conditions (pH, organic matter content, and redox potential) and Se concentration vertically through hydrosoil; (ii) investigate potential for Se-accumulating biogeochemical processes (dissimilatory Se reduction and sorption) operating in hydrosoil at various depths; and (iii) evaluate the effect of a nutrient amendment on hydrosoil conditions, Se concentrations, and Se-accumulating biogeochemical processes.

2. Materials and Methods

2.1 Description of Pilot-Scale CWTSs

Two pilot-scale CWTSs were designed and constructed by Spacil *et al*. (2011a, 2011b) to investigate treatment of aqueous Se concentrations (50 μg/L) in simulated energy-derived water (EDW). EDWs are generated during fossil fuel extraction, fossil fuel energy production, and refining processes (Kanagy *et al.* 2008, Spacil *et al.* 2011a). Se can occur in petroleum effluents in several oxidation states (VI, IV, 0, and –II) (Zhang *et al.* 2004) and in a variety of compounds and ionic forms such as selenides (e.g., H_2Se^-), selenites (e.g., H_2SeO_3, $HSeO_3^-$, SeO_3^{-2}), and selenates (e.g., $HSeO_4^-$, SeO_4^{-2}) (Zhang and Moore 1996). Spacil *et al.* (2011a, 2011b) characterized various EDWs and used the results to develop a representative, simulated EDW for experimentation (Table 1). The predominant ions in EDWs are sodium, calcium, magnesium, chloride, and sulfate. For this investigation, the simulated EDW composition used by Spacil *et al.* (2011a, 2011b) was replicated, and the water prepared in a 5,678-L polypropylene carboy holding tank. The simulated EDW was mixed for a minimum of 24 hours with a 1-hp submersible pump.

The simulated EDW was treated in two pilot-scale CWTSs designed, constructed, and studied by Spacil *et al.* (2011a, 2011b). Each CWTS consisted of a 5678-L polypropylene carboy retention basin to hold simulated EDW and four 378-L Rubbermaid containers (124 cm long by 77 cm wide by 61 cm deep) arranged in series. In each CWTS, PVC pipe fittings connected the four cells approximately 6 cm below the top of each cell to allow gravity flow of water through the series. Each cell was filled to

a depth of approximately 30 cm with sand from 18-mile Creek in Clemson, SC. The cells were planted with *Typha latifolia* (broadleaf cattail) at a density of approximately 25-30 plants per cell. During construction of both CWTSs, 1,000 g of ground oyster shells were added to each treatment cell to maintain a circum-neutral pH (6.5-8) and increase alkalinity. Other additions to each cell included 100 g of zero-valent iron (Fe^0) to maintain reducing conditions and 12 g of 19-6-12 Osmocote® fertilizer to provide nitrogen, phosphorous, and potassium as nutrients for microbes and plants. To achieve a nominal 24-hr hydraulic retention time (HRT) per cell (96-hr per CWTS), a piston pump (FMI QG400) delivered 128 mL/min of simulated EDW to each system. One CWTS received a nutrient amendment (AquaSmart™, consisting of fermented yeast, organic carbon, and nutrients) to promote dissimilatory selenium reduction by microbial activity, while the other CWTS was used as a control and received no amendments (Spacil *et al.* 2011a, Spacil *et al.* 2011b). A 35 g AquaSmart/L solution was delivered at a rate of 1 mL/min by a FMI QG20 piston pump into the inflow of the nutrient amended CWTS. AquaSmart solution was pumped from a 19 L reservoir that was renewed weekly. The CWTSs were constructed and operated inside a greenhouse with natural (i.e. solar) photoperiod and temperature ranging from 20 to 30°C. Simulated EDW was first introduced into the CWTSs in April 2009 (Spacil *et al.* 2011a, Spacil *et al.* 2011b) and continuously flowed through the systems for approximately 2 years during the time when the systems were studied by Spacil *et al.* (2011a, 2011b) and throughout the current investigation.

2.2 Measurement of Hydrosoil Conditions and Correlation with Selenium Concentration

The vertical variation in hydrosoil conditions (redox potential, pH, organic matter content) and Se concentration were investigated in the first cell of each of the two pilot-scale CWTSs. Hydrosoil conditions were measured and samples collected during one sampling event conducted from January to February 2011 approximately 2 years after the construction of the CWTSs. Two distinct zones (detritus and sediment) were present in the hydrosoil of both cells studied (Figure 1). The detritus consisted of *T. latifolia* plant debris including fallen leaves and stems that were compacted and partially decomposed and contained numerous, fibrous roots. Thickness of the detritus was 21 cm in the nutrient amended cell and 14 cm in the unamended cell. The underlying sediment consisted of sand added during the pilot-scale CWTS cell construction and few (approximately 10-15) *T. latifolia* roots.

At 3 locations in each of the two cells, redox potential in the hydrosoil was measured with a GDT-11 Multi-meter connected to in-situ platinum-tipped electrodes and an Accumet® calomel reference electrode (Faulkner *et al*. 1989) placed at 7-cm intervals in the detritus and 3-cm intervals in the sediment (Table 2 and Figure 2A, 2B, and 2C). After measuring redox potential and thickness of the detritus in each cell (Figure 1), 7-cm thick detritus grab samples (approximately 500 cm^3) were obtained from each sampling location and immediately placed into plastic freezer bags, from which air was quickly removed prior to sealing and freezing. For each cell, grab samples collected from the

same depth interval below the surface water-detritus interface (0 cm) were combined from the 3 sample locations to form a single composite sample for each depth interval.

After grab samples were obtained from the detritus, a chlorinated polyvinyl chloride (CPVC) pipe, 15 cm in length and 2.54 cm in diameter, was hammered into the sediment at each sampling location (Figure 2). The CPVC pipe, containing a sediment core, was then pulled up and capped with CPVC end caps. The caps and pipe were taped to preserve the core's conditions and minimize exposure to air. The sealed CPVC pipe and core were immediately frozen. After freezing, the pipe and core were removed from the freezer and placed in an anaerobic chamber (COY Laboratory Products, Inc.). After thawing for approximately ten minutes at room temperature (~25 ^{o}C), the sediment core was extruded (intact) from the CPVC onto a clean plastic tray and divided into five 3-cm long segments. Sediment segments from the 3 cores that were collected at the same depth interval below the surface water-detritus interface (0 cm) were composited into one representative sample for each depth interval. This process created a single vertical profile of the hydrosoil for each cell. The vertical hydrosoil profile for the nutrient amended cell consisted of three 7-cm thick detritus samples (0-21 cm) and five 3-cm thick sediment samples (21-36 cm). The profile for the unamended cell consisted of two 7-cm thick detritus samples (0-14 cm) and five 3-cm thick sediment samples (14-29 cm) (Figure 2). pH, organic matter content, and Se concentration were measured in each composited sample.

pH was measured by combining 2 g of sample (sediment or detritus) with 10 mL of deaerated distilled-deionized (DDI) water (1:5 dilution) in a 50-mL centrifuge tube. The sample slurry was agitated on a C10 Platform Shaker (New Brunswick Scientific Classic Series) for approximately 12 hours (Singh *et al.*, 1998), after which pH was measured with an Orion Model 420A pH meter (Table 2). Organic matter content was determined by loss-on-ignition (LOI) in which samples were dried at 105^{o}C to a constant weight and ignited in a muffle furnace (Type 6000 Furnace; Thermolyne Corporation) at 550^{o} C for four hours (Heiri *et al.* 2001).

In preparation for measuring Se concentrations, each sample was treated using a strong acid (Aquaregia) and microwave digestion (CEM 1997). In a microwave digestion vessel (Standard Advanced Composite Vessel; CEM Corporation), 0.5 grams of dry sample were combined with 1 mL trace metal grade (37%) HCl (Fisher Scientific), 4 mL trace metal grade (48%) HF (Fisher Scientific), 5 mL trace metal grade (70%) HNO_3 (Fisher Scientific), and 10 mL DDI water (Super-Q Plus, MilliPore). The vessel was then sealed and placed in a microwave digester at 170^{o}C (MDS-2000; CEM Corporation) for 30 minutes. After digestion, approximately 2g of H_3BO_3 crystals were added to neutralize the acid mixture. Digested samples were pipetted into separate 15-mL centrifuge tubes and diluted to 2% HNO_3 concentration by volumetric addition of deionized water. Total Se concentration in each solubilized sample was measured using an inductively coupled plasma-mass spectrometer (ICP-MS) (Thermo ICP-Mass Spectrometer X Series II) following standard method EPA 200.8 (USEPA 1994). The Se concentration measured for each sample represents the total amount of Se (all species and

forms) solubilized during this extraction procedure. Multi-elemental standards, ranging in concentration from 0.005 to 100 µg/L, were made by diluting stock solutions containing Ag, Au, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Th, Th, Tl, U, V, Zn, Cl, Ca, Fe, K, Mg, Na, P, S, and C in 2% Aristar Optima HNO_3. Volume additions were verified gravimetrically. Multi-element standards were used to calibrate the ICP-MS for analysis of Se. Rhenium and Scandium were selected as internal standards and used for sample recovery [internal standard recoveries were within the 80 to 120% standard Quality Assurance/Quality Control (QA/QC)]. Detection limit using this technique was approximately 0.1 µg/L in each solubilized sample. Additionally, QA/QC check samples made from stock solutions with known concentrations were analyzed in approximately 20-sample intervals.

For each cell, the vertical variation in hydrosoil conditions was compared to the vertical variation in measured Se concentrations using a Pearson correlation (measure of the strength of linear dependence between two variables). The significance of each correlation was determined by calculating a Pearson correlation coefficient (p-value).

2.3 Se-Sequestering Biogeochemical Processes in Hydrosoil

Biogeochemical processes by which Se may be removed from Se-contaminated waters in CWTSs and the ranges in hydrosoil conditions that favor these processes were identified from a literature review. Measured values of hydrosoil conditions were compared to the ranges of values at which two major Se-accumulating processes (dissimilatory Se reduction and sorption) occur (Table 3). These two processes can

transfer Se from impaired waters to less bioavailable and less toxic forms, sequestering them in the hydrosoil of a CWTS. Pearson correlation coefficients between the vertical variation of hydrosoil conditions and Se concentration at the same depth in each cell were calculated to determine which conditions were associated with Se accumulation in the hydrosoil and to provide insight into which Se-removal processes may be occurring.

2.4 Effect of Nutrient Amendment

Hydrosoil conditions (pH, redox potential, organic matter content) and Se concentration were compared between the nutrient amended cell and the unamended cell by: 1) observing numerical differences in hydrosoil conditions and Se concentration between the nutrient amended and unamended cell in both detritus and sediment; and 2) using a two-sample t-test assuming equal variance. The mean for each hydrosoil condition and the mean Se concentration were calculated for the entire sampling depth (0-36 cm in amended cell and 0-29 cm in unamended cell). Two-sample t-test assuming equal variance was then used to determine if the mean for each hydrosoil condition and the mean Se concentration were statistically different (alpha < 0.05) between the nutrient amended cell and unamended cell.

3. Results

3.1 Measurement of Hydrosoil Conditions and Correlation with Selenium Concentration

Redox potential in the detritus decreased with depth (-65 mV at 7 cm to -173 mV at 21 cm) in the nutrient amended cell and increased with depth in the unamended cell (-110 mV at 7 cm to -2 mV at 14 cm) (Figure 3; Table 4). In sediment, redox potential decreased in the nutrient amended cell below 24 cm (57 mV at 24 cm to -163 mV at 33 cm) and in the unamended cell below 20 cm (-48 mV at 20 cm to -145 mV at 29 cm). For both cells, organic matter content was greater in the detritus than in the sediment. In the nutrient amended cell, organic matter content ranged from 52% (14-21 cm) to 88% (0-7 cm) in detritus and 1% (33-36 cm) to 51% (21-24 cm) in sediment. In the unamended cell organic matter content ranged from 64% (7-14 cm) to 79% (0-7 cm) in detritus and 1% (26-29 cm) to 6% (14-17 cm) in sediment. pH was circum-neutral ranging from 6.29 to 6.76 in the nutrient amended cell and 6.20 to 6.47 in the unamended cell.

Selenium concentration decreased with depth in both the nutrient amended cell (830 μg/g at 0-7 cm to 0.97 μg/g at 33-36 cm) and the unamended cell (569 μg/g at 0-7 cm to 0.71 μg/g at 26-29 cm) (Figure 3; Table 4). In both cells, Se concentration was greater in detritus (308-830 μg/g in nutrient amended and 138-569 μg/g in unamended) than in sediment (0.89-212 μg/g in nutrient amended and 0.54-6.77 μg/g in unamended). The Pearson correlation performed on hydrosoil conditions and Se concentrations indicated that vertical variation of measured organic matter content significantly correlated to vertical variation of measured Se concentrations ($r = 0.95$; $p<0.00001$ in nutrient amended cell and $r = 0.87$; $p<0.00002$ in the unamended cell) (Table 5). Vertical variation in Se concentrations did not correlate to vertical variation in pH ($r = -$

0.47, $p<0.07$ in amended cell; $r = -0.47$, $p<0.04$ in unamended cell) or redox potential ($r = -0.02$ $p<0.94$ in amended cell; $r = 0.09$, $p<0.37$ in unamended cell).

3.2 Se-Sequestering Biogeochemical Processes in Hydrosoil

Based on results of the literature review, four biogeochemical processes that can remove selenate and selenite from contaminated waters were identified: dissimilatory Se reduction, sorption, bioconcentration, and volatilization (Trudinger and Swaine 1979, Shamberger 1983, Selinus *et al.* 2005, Torres *et al.* 2011). Under typical wetland soil conditions elemental Se and organic matter-bound Se are the forms of Se most likely found in hydrosoil (Nakamaru and Altansuvd 2014) indicating that dissimilatory Se reduction and sorption are the major biogeochemical processes occurring within the hydrosoil. In specifically designed CWTSs, selenate and selenite can be reduced to elemental Se through dissimilatory Se reduction (Stolz and Oremland 1999). Dissimilatory Se reduction is facilitated by anaerobic Se-reducing bacteria and hydrosoil conditions that are mildly reducing and circum-neutral pH (Brookins 1988, Siddique 2005). Although it was not a targeted process, sorption occurs over a broad range of hydrosoil conditions and has the ability to transfer and accumulate Se in the hydrosoil. Sorption is defined as adsorption or absorption of Se to abiotic or biotic sorption sites including organic matter (e.g. detritus), oxides, hydroxides and iron sulfides (Han *et al.* 2011, Gonzalez-Acevedo *et al.* 2012). Recent studies have suggested that sorption, particularly adsorption to organic matter in the hydrosoil, plays a major role in Se accumulation in hydrosoil (Pezzarossa *et al.* 1999, Lin *et al.* 2010, Gonzalez-Acevedo *et al.* 2012, Nakamaru and Altansuvd 2014). Selenium (mainly selenite) can also be

adsorbed onto particulate matter (both mineral and organic) suspended in inflow water to the CWTS (Christense, *et al.* 1989). Sorption can reduce selenate and selenite to selenides via complexation with humic acids, but does not readily produce elemental Se (Stolz and Oremland 1999). Previous studies (Hansen *et al.* 1998, Gao *et al.* 2003) have suggested that bioconcentration and volatilization can remove Se from contaminated waters. However, bioconcentration and volatilization were not targeted processes in the current study because they do not accumulate Se in the hydrosoil.

In detritus of the nutrient amended cell, one (0-7 cm) of three mean redox potential values was within the range favorable for dissimilatory Se reduction (Table 3 and 4). In the unamended cell, mean redox potential values at both depth intervals of the detritus were within the range favorable for dissimilatory Se reduction. Measured pH values were not within the range favorable for dissimilatory Se reduction in detritus of either the nutrient amended cell or the unamended cell. In sediment of the amended cell, 2 (24-27 and 27-30 cm) of 5 mean redox values and 3 (24-27, 27-30, and 30-33 cm) of 5 pH values were favorable for dissimilatory Se reduction. Four (all except 14-17 cm) of 5 mean redox values and no pH values measured in sediment of the unamended cell were favorable for dissimilatory Se reduction. All measured values of organic matter content were within the range favorable for dissimilatory Se reduction in both sediment and detritus of both cells at all depth intervals. In the nutrient amended cell and unamended cell, mean redox potential and pH values measured at all depth intervals (detritus and sediment) were favorable for sorption. All values of organic matter content measured in detritus of both cells were favorable for sorption; however, in the sediment, only 1 of 5

(21-24 cm) in the amended cell and 1 of 5 (23-26 cm) in the unamended cell were within the favorable range. Significant correlation of organic matter content with Se concentration ($r = 0.95$, $p<0.00001$ in amended cell and $r = 0.87$, $p<0.00002$ in unamended cell) suggests that Se-accumulating biogeochemical processes are influenced by the amount of organic matter present (Table 5).

3.3 Effect of Nutrient Amendment

Both organic matter content and Se concentration in the detritus were greater in the amended cell than in the unamended cell (e.g. 88% at 0-7 cm in amended cell vs. 79% at 0-7 cm in unamended cell; and 830 µg/g Se at 0-7 cm in amended cell vs. 569 µg/g Se at 0-7 cm in unamended cell). However, two-sample t-tests (equal variance) indicated that mean of redox potential values ($t = 0.214$, $p = 0.834$), mean of organic matter content values ($t = 0.690$, $p = 0.502$), and mean of Se concentration values ($t = 0.989$, $p = 0.341$) in the hydrosoil were not statistically different ($p < 0.05$) between the nutrient amended cell and the unamended cell, while mean of pH values ($t = 2.87$, $p = 0.0132$) was statistically different between the two cells (Table 6). The mean of pH values for hydrosoil was 6.54 in the nutrient amended cell and 6.30 in the unamended cell.

4. Discussion

Vertical variation of conditions that influence biogeochemical processes in the hydrosoil may affect the vertical distribution of Se, as suggested by the significant correlation between organic matter content and Se concentration in both cells studied. A correlation between the distribution of Se and total sediment carbon was identified by Lin

et al. (2010) in 4-year-old constructed wetlands treating agricultural drainage and by Tokunaga *et al.* (1991) in the Kesterson Reservoir. Lin *et al.* (2010) determined that 90% of the total Se retained in wetland hydrosoil was partitioned to the top 10 cm of the hydrosoil. The 15 samples analyzed in this investigation displayed an approximately bimodal distribution of organic matter: those with organic matter > 50% (6 samples) and those with organic matter < 6.1% (9 samples). Selenium concentration in the samples with organic matter greater than 50% ranged from 138 to 830 µg/g, while Se concentration in the samples with organic matter less than 6.1% ranged from 0.54 to 6.77 µg/g. Five of the six samples with > 50% organic matter and high Se concentration were from the detritus and one was from the uppermost sediment interval of the nutrient amended cell.

Values of mean redox potential, pH, and organic matter content for all 6 of the samples with high Se concentration ($\geq$ 138 µg/g Se) were within the ranges favorable for sorption. For hydrosoil samples with low Se concentration ($\leq$ 6.77 µg/g Se), organic matter content in 8 of 9 samples was outside the range favorable for sorption. Conditions were less favorable for dissimilatory Se reduction than for sorption for the 6 samples having high Se concentration. Of these 6 samples, both pH and mean redox potential were outside the range favorable for dissimilatory Se reduction for 2 samples; only pH was outside the range for 3 samples, and only redox for 1 sample. Measured values of both redox potential and pH were favorable for dissimilatory Se reduction in only 2 of the 15 samples analyzed, and both samples contained $\leq$ 3.28 µg/g Se. Hydrosoil pH and mean redox mean values for the other 13 samples were only slightly outside (redox: $\pm$ 50

mV and pH: ± 0.5 S.U.) the range favorable for dissimilatory Se reduction, and it is possible that hydrosoil conditions may have been within the range favorable for dissimilatory Se reduction prior to the time of this study. However, comparison of measured conditions with conditions favorable for dissimilatory Se reduction and sorption suggests that during this study the predominant process for moving Se from inflow water to the CWTS hydrosoil was more likely sorption to organic matter than dissimilatory Se reduction.

During the 4 month study by Spacil (2010), sediment redox potential in the nutrient amended cell was within the range favorable for dissimilatory Se reduction during January and February 2010 (-135.2 and -112.2 mV), but outside the favorable range in March and April 2010 (-154.8 and -157.5 mV). Sediment redox in the unamended cell from January to April 2010 (-208.6 to -170.3 mV) was outside the range favorable for dissimilatory Se reduction (Spacil 2010).

Mean organic matter content in the detritus and general thickness (cm) of detritus were greater in the nutrient amended cell than in the unamended cell (Table 5). Addition of the nutrient amendment may have promoted *T. latifolia* growth increasing the amount of plant litter falling to the hydrosoil. As suggested by the significant correlation of Se concentration with organic matter content, the greater Se concentration in detritus of the nutrient amended cell than in the unamended cell may be the result of greater organic matter content enhancing Se-accumulating biogeochemical processes, specifically sorption. Van Heest (2012) observed greater total Se concentrations in the top 2 cm of hydrosoil in a nutrient amended (AquaSmart^{TM}) CWTS cell than in the top 2 cm of an

unamended CWTS cell, but did not measure organic matter content. Organic matter in the hydrosoil can promote sorption by providing sorption sites for Se, as well as promoting dissimilatory Se reduction by adding carbon sources and electron donors for Se-reducing microbes (Zhang and Frankenberger 2005).

5. **Conclusions**

Redox potential, pH and organic matter content varied with depth in hydrosoil of the two CWTS cells studied. Statistical analysis indicates that Se concentration in the hydrosoil correlates with organic matter content, but not with pH or redox potential. In detritus, hydrosoil conditions were within the range favorable for sorption, but not for dissimilatory Se reduction, suggesting that sorption was the dominant biogeochemical process accumulating Se during this investigation. Conditions in only 2 of the 15 samples analyzed were within the range favorable for dissimilatory Se reduction. However, it should be noted that hydrosoil condition values for the other 13 samples were only slightly outside (redox: $\pm$ 50 mV and pH: $\pm$ 0.5 S.U.) the range favorable for dissimilatory Se reduction. T-tests indicated no significant differences in mean redox potential, organic matter, and Se concentration in hydrosoil between the nutrient amended cell and unamended cell. However, greater mean organic matter content and mean Se concentration in detritus of the nutrient amended cell than in the unamended cell suggests that nutrient amendment (AquaSmart^{TM}) can be added to a CWTS to increase organic matter content and Se-accumulation in the hydrosoil.

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Figure 1. Schematic diagram of CWTS cell showing 2 zones of hydrosoil: detritus and sediment. The detritus consists of plant debris that has compacted and partially decomposed and contains numerous fibrous *Typha latifolia* roots. The sediment consists of medium-coarse (0.25 to 1.0 mm diameter) sand and fewer, but larger, *T. latifolia* roots compared to those in the detritus zone.

Figure 2. A.) Overhead schematic of sampling locations (X) in the nutrient amended and unamended CWTS cells. At each sampling location, 7-cm thick grab samples (7.6-cm diameter ring x 7-cm thick intervals) of the detritus and a sediment core (2.54-cm diameter and 15-cm long starting from the top of the sediment) were collected. Detritus could not be cored because of numerous roots. Each 15-cm long sediment core was sectioned in 3-cm intervals to obtain a vertical profile. B.) Vertical profile from the nutrient amended cell. C.) Vertical profile from the unamended cell.

Figure 3. Measured values of hydrosoil conditions (mean redox potential, pH, and percent organic matter) and Se concentration with depth through hydrosoil of the nutrient amended (A) and unamended (B) CWTS cells. Redox potential is a mean of measured redox for each depth interval measured at 3 locations within each cell. pH, organic matter content, and Se concentration were measured in each composited sample. Surface water/detritus interface is at 0 cm. The detritus/sediment interface is at 21-cm depth in the nutrient amended cell and at 15-cm depth in the unamended cell. Redox potential range of values and mean values from the same depth intervals at the 3 sampling

locations in each cell are plotted at the point of measurement in the hydrosoil. Organic matter, pH, and Se concentration values are plotted at the center of sample intervals.

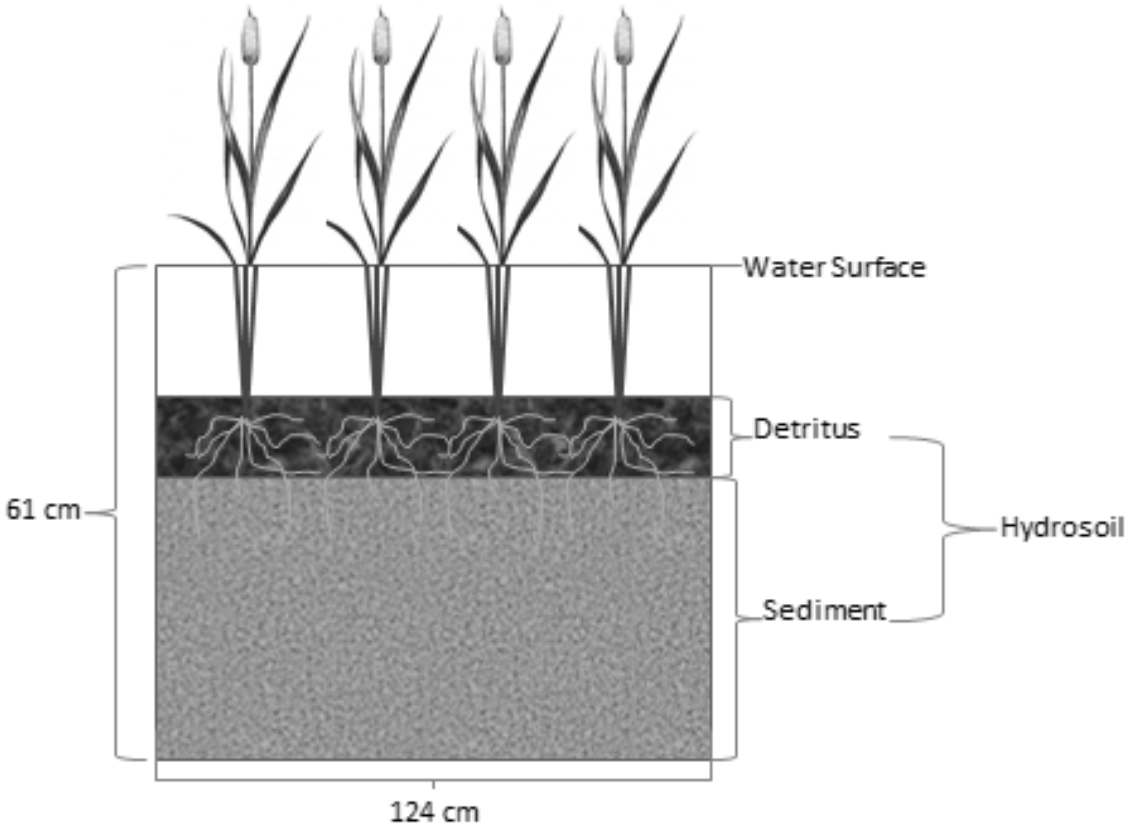

Figure 1.

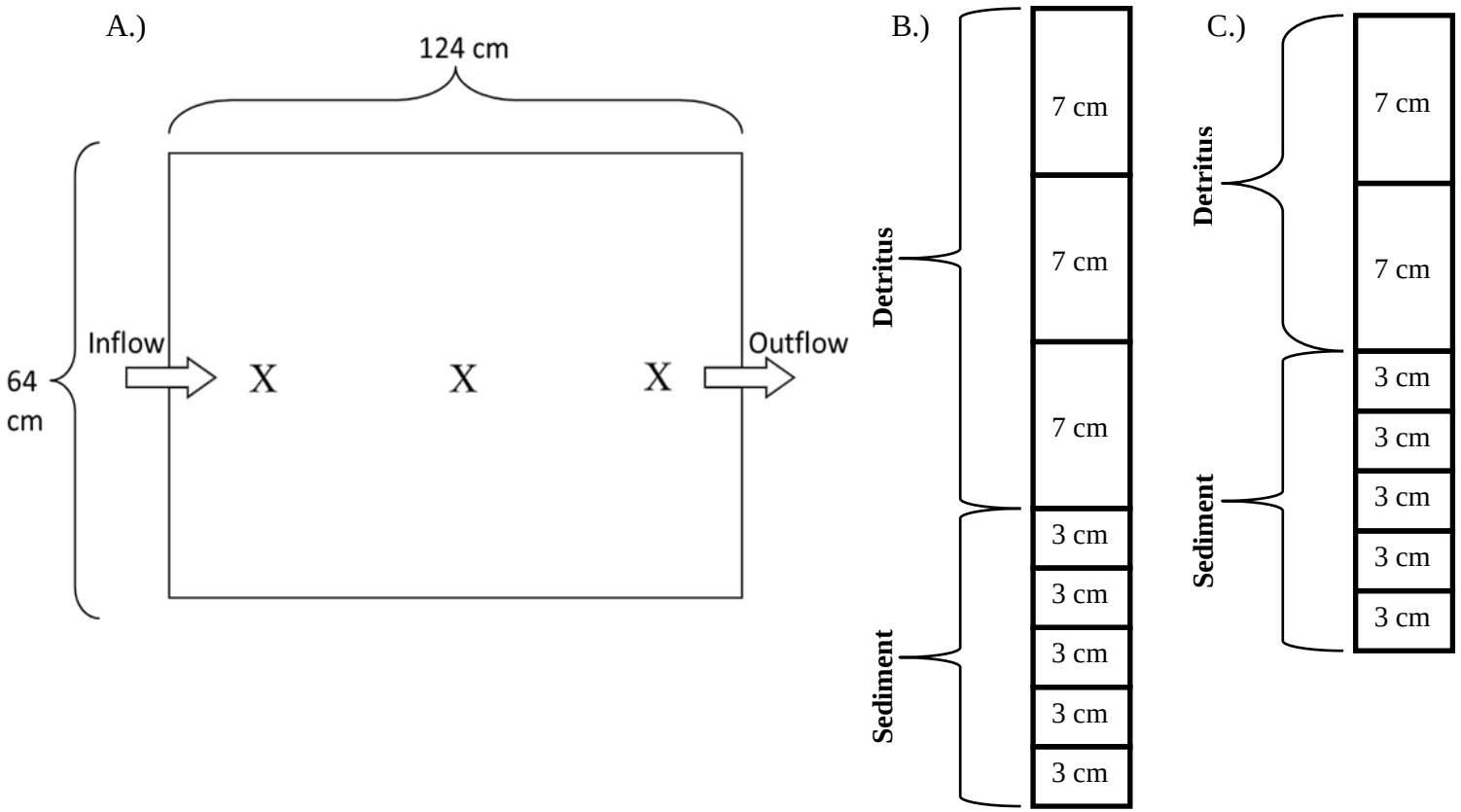
A.)
124 cm
Inflow
Outflow
64 cm
X
X
X
B.)
Detritus
Sediment
7 cm
7 cm
7 cm
3 cm
3 cm
3 cm
3 cm
3 cm
C.)
Detritus
Sediment
7 cm
7 cm
3 cm
3 cm
3 cm
3 cm
3 cm

Figure 2.

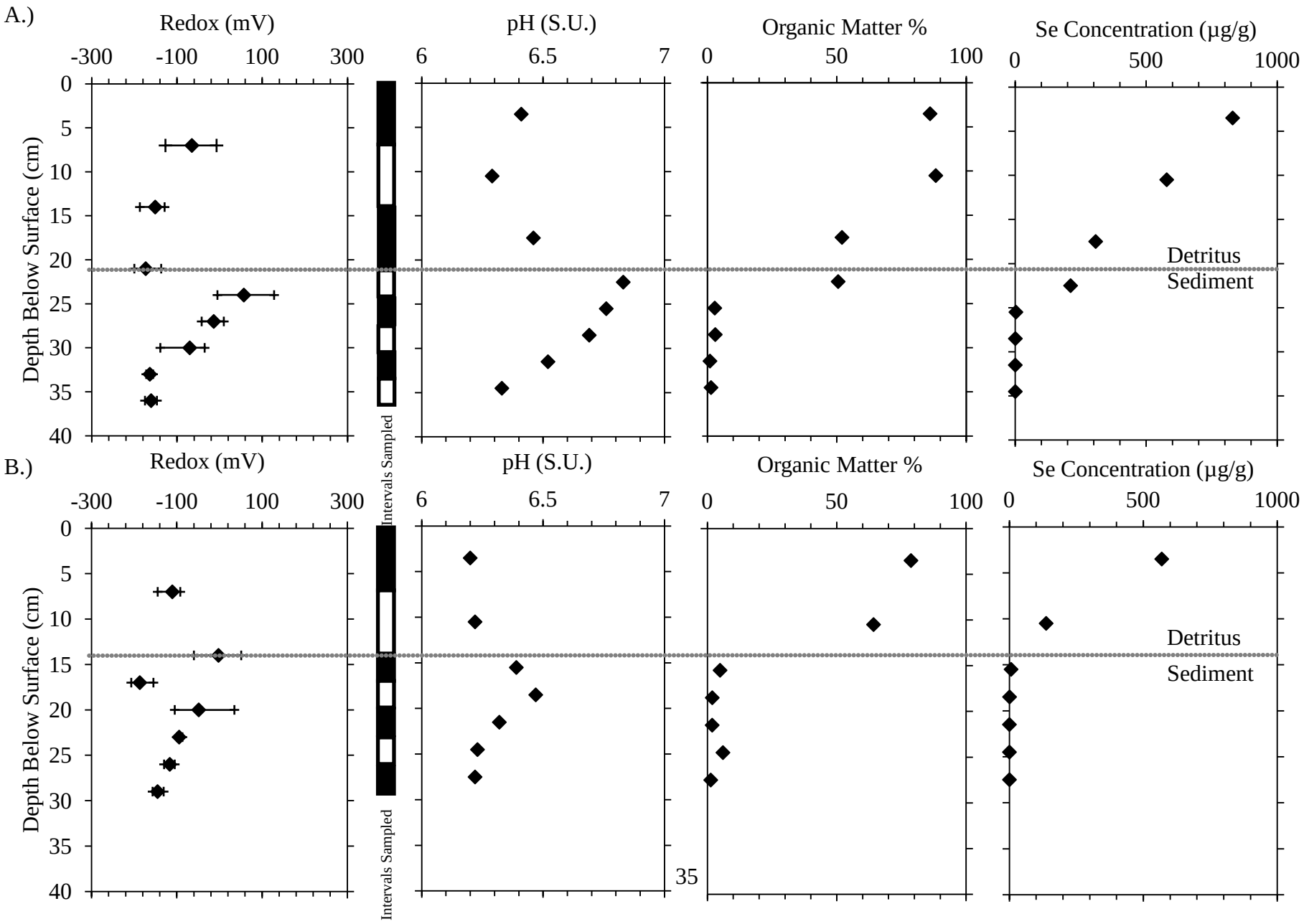
A.)
Redox (mV)
-300
-100
100
300
pH (S.U.)
6
6.5
7
Organic Matter %
0
50
100
Se Concentration (µg/g)
0
500
1000
Depth Below Surface (cm)
0
5
10
15
20
25
30
35
40
Detritus
Sediment
Intervals Sampled
B.)
Redox (mV)
-300
-100
100
300
pH (S.U.)
6
6.5
7
Organic Matter %
0
50
100
Se Concentration (µg/g)
0
500
1000
Depth Below Surface (cm)
0
5
10
15
20
25
30
35
40
Detritus
Sediment
Intervals Sampled
35

Table 1. Simulated energy-derived water formulation and characteristics.

	Constituent or Parameter	Concentration or Value
Formulation [a]		
	Calcium chloride dehydrate ($CaCl_2 \cdot H_2O$)	205 mg/L
	Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	355 mg/L
	Sodium chloride (NaCl)	1,230 mg/L
	Sodium selenite (Na_2SeO_3)	0.109 mg/L
Characteristics [a, b]		
	Selenium	~50 µg/L
	pH	6.5-8.0 S.U.
	Alkalinity	~42 mg/L as CaCO3
	Hardness	~140 mg/L as CaCO3
	Conductivity	~2,300 µS/cm
	Dissolved Oxygen	~8.4 mg/L

[a.] Spacil *et al.* (2011a, 2011b) characterized various EDWs and used the results to develop a representative, simulated EDW for experimentation.

[b.] Average values for simulated EDW characteristics (i.e. explanatory parameters) and Se concentration (Spacil 2010) Characteristics and Se concentration measured from pilot-scale CWTS inflow.

Table 2. Analytical methods for determining hydrosoil conditions.

Parameter	Method	Detection Limit
pH	Direct Instrumentation: Orion Model 420A (Singh *et al*., 1998)	0.01 S.U.
Redox Potential	Modified standard method 2580B: GDT-11 Multi-meter, in-situ platinum-tipped electrode (Faulkner *et al.* 1989)	10 mV
Organic Matter	Loss on ignition (Heiri et al. 2001)	0.1 mg

Table 3. Ranges of hydrosoil conditions favorable for biogeochemical processes that can result in Se accumulation in hydrosoil of CWTSs. Biogeochemical process operation is limited or nonexistent outside the ranges listed.

Process	Organic Matter	pH	Redox Potential
Dissimilatory Se Reduction [a, c, e, f, g, h, i]	> 0.1% [e, g, h]	Circum-Neutral (6.5-8) [b, i]	Reducing (-150mV to +50mV) [b, f, i]
Se Sorption (organic & inorganic) [a, d, e, h , j]	> 6% [d, e]	Acidic to Neutral (3-7) [b, e]	Reducing to Oxidizing (-400mV to +700mV) [b, e]

a. Trudinger and Swaine (1979)
b. Brookins (1988)
c. de Souza *et al.* (1999)
d. Pezzarossa *et al.* (1999)
e. Selinus *et al.* (2005)
f. Siddique *et al.* (2005)
g. Zhang *et al.* (2008)
h. Kadlec and Wallace (2009)
i. Spacil *et al.* (2011a, 2011b)
j. Nakamaru and Altansuvd (2014)

Table 4. Measured hydrosoil conditions and Se concentrations in the hydrosoil of nutrient amended and unamended CWTS cells.

	Depth (cm)	Redox (mV)[c]	pH (S.U.)	Organic Matter (%)	Se Concentration (µg/g)
Nutrient Amended Cell[a,b]					
	0-7	-65	6.41	86.1	830
	7-14	-151	6.29	88.3	579
Detritus	14-21	-173	6.46	52.0	308
Sediment	21-24	57	6.83	50.6	212
	24-27	-13	6.76	2.89	3.28
	27-30	-70	6.69	3.08	1.17
	30-33	-163	6.52	1.04	0.890
	33-36	-160	6.33	1.45	0.970
Unamended Cell[a,b]					
	0-7	-110	6.20	78.7	569
Detritus	7-14	-2	6.22	64.3	138
Sediment	14-17	-187	6.39	4.94	6.77
	17-20	-48	6.47	1.92	1.12
	20-23	-94	6.32	1.89	0.540
	23-26	-116	6.23	6.05	0.650
	26-29	-145	6.22	1.34	0.710

[a.] Conditions favorable for dissimilatory Se reduction are: redox potential= -150 to +50 mV, pH= 6.5-8 S.U., organic matter= >0.1%. (Trudinger and Swaine 1979, Brookins 1988, de Souza *et al.* 1999, Selinus *et al.* 2005, Siddique *et al.* 2005, Zhang *et al.* 2008, Kadlec and Wallace 2009, Spacil *et al.* 2011a)

[b.] Conditions favorable for Se sorption/complexation (organic & inorganic) are: redox potential= -400 to +700 mV, pH= 3-7 S.U., organic matter= >6%. (Trudinger and Swaine 1979, Brookins 1988, Pezzarossa *et al.* 1999, Kadlec and Wallace 2009, Selinus *et al.* 2005, Nakamaru and Altansuvd 2014)

[c.] Mean of three measurements.

Table 5. Pearson correlation coefficient and p-value of significance between hydrosoil conditions (mean redox potential, pH and organic matter content) and Se concentration.

	Redox Potential	pH	Organic Matter Content
Nutrient Amended Cell			
Pearson correlation coefficient "r"	-0.0214	-0.467	0.948
P-value of significance	0.938	0.0689	0.00001
Unamended Cell			
Pearson correlation coefficient "r"	0.0949	-0.470	0.875
P-value of significance	0.374	0.0450	0.0000210

Table 6. Results of a two-sample t-test assuming equal variance used to determine the statistical difference (alpha < 0.05) between the nutrient amended cell and unamended cell hydrosoil conditions (redox potential, pH and organic matter content) and Se concentration.

	Redox Potential	pH	Organic Matter Content	Se Concentration
t-statistic	0.214	2.87	0.690	0.989
p-value	0.834	0.0132	0.502	0.341
Degrees of freedom (df)	13	13	13	13

CHAPTER III

EVIDENCE OF SELENIUM ACCUMULATING BIOGEOCHEMICAL PROCESSES IN PILOT-SCALE CONSTRUCTED WETLAND TREATMENT CELLS

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Abstract

Two pilot-scale wetland treatment system cells (nutrient amended and unamended) were designed and constructed to reduce aqueous Se concentrations in simulated energy-derived water (EDW). Specific objectives of this study were: (i) measure vertical variation in Se concentration in the hydrosoil; (ii) investigate two major Se-sequestering biogeochemical processes (dissimilatory Se reduction and sorption); and (iii) evaluate the effect of a nutrient amendment on Se accumulation and Se-sequestering biogeochemical processes in the hydrosoil. Se-sequestering biogeochemical processes were investigated by counting Se-reducing microbial colony forming units (CFUs) and identifying Se geochemical fractions at various depths in the hydrosoil. The detritus (0-21 cm in nutrient amended cell and 0-14 cm in unamended cell) contained greater Se concentrations (308-830 μg/g and 138-569 μg/g) and greater CFUs (2,700-22,000 CFUs/mL pore water and 9,300-15,000 CFUs/mL pore water) than were present in the underlying sediment. The majority of Se measured in the detritus was elemental (52.1%-58.0% in the nutrient amended cell and 21.1%-62.6% in the unamended cell) suggesting that dissimilatory Se reduction is the dominant biogeochemical process sequestering Se in the detritus. Greater Se concentrations and percent of elemental Se in the nutrient amended cell than the unamended cell suggests that the nutrient amendment potentially enhanced dissimilatory Se reduction and therefore Se accumulation in the hydrosoil.

1. Introduction

Selenium-contaminated waters (e.g. agricultural and energy-derived) are a growing environmental concern due to their deleterious effects on aquatic biota and waterfowl (Lemly 2004). Although selenium (Se) is an essential micronutrient for basic cellular function (Zayed *et al.* 1998, Carlson *et al*. 2004), the range in concentrations in which Se is essential or toxic is very narrow (e.g., bioconcentration, toxicity) (Oremland 1994, Lemly 2004, Selinus *et al*. 2005). Treatment of Se-contaminated waters can be difficult; however, constructed wetland treatment systems (CWTSs) offer a treatment option for the aforementioned waters (Rodgers and Castle 2008; Spacil *et al*. 2011a, Spacil *et al.* 2011b). Over the past few decades, water contaminated with Se has been treated using CWTSs with varying degrees of performance (Gao *et al.* 2000, Gao *et al.* 2003, Sundberg-Jones and Hassan 2007, Spacil *et al.* 2011a, Spacil *et al.* 2011b). CWTSs remediate Se-contaminated waters by altering (mainly reducing) the oxidation state (VI, IV, 0, and -II) of Se via biogeochemical processes. The majority of biogeochemical processes that can remove selenate and selenite from contaminated waters occur within the hydrosoil (Trudinger and Swaine 1979, Kadlec and Wallace 2009). The hydrosoil in CWTSs contains two zones: detritus (partially decomposed and compacted plant matter) and sediment (added during CWTS construction) (Gao *et al.* 2003). Hydrosoil in a CWTS can be designed to produce conditions (e.g. pH, redox potential, and organic matter content) that promote specific biogeochemical processes (Kanagy *et al.* 2008, Rodgers and Castle 2008).

Many CWTSs target dissimilatory Se reduction facilitated by anaerobic Se-reducing bacteria to sequester Se into the hydrosoil. Dissimilatory reduction of Se is a biogeochemical process that occurs in many natural systems (Oremland *et al.* 1990). Anaerobic bacteria transform selenate (SeO_4^{-2}) and selenite (SeO_3^{-2}) to elemental Se (Se^0) through dissimilatory Se reduction in a CWTS (Frankenberger and Arshad 2001). During dissimilatory Se reduction, Se-reducing bacteria utilize selenate and selenite as electron acceptors for microbial respiration resulting in insoluble elemental Se (Oremland *et al.* 2004). To promote and enhance dissimilatory Se reduction, organic carbon amendments have been added to CWTSs as an additional energy source and electron donor for Se reducing bacteria (Zhang and Frankenberger 2005). Although dissimilatory Se reduction is often the targeted pathway in CWTSs, recent studies have suggested that sorption, particularly with organic matter, also influences Se accumulation in the hydrosoil (Pezzarossa *et al.* 1999, Lin *et al.* 2010, Gonzalez-Acevedo *et al.* 2012). Sorption is another natural biogeochemical process that can reduce selenate (SeO_4^{-2}) and selenite (SeO_3^{-2}) to selenides (Se^{2-}), but does not naturally produce elemental Se (Se^0) (Stolz and Oremland 1999). Dissimilatory Se reduction is often preferred over sorption in CWTSs because elemental Se is insoluble, the most stable and least bioavailable form of Se (Sundberg-Jones and Hassan 2007).

The effect of carbon amendments on Se-reducing bacteria and horizontal variation in treatment performance of Se in CWTSs has been studied previously (de Souza *et al.* 1999, Gao *et al.* 2000, Gao *et al.* 2003, Zhang and Frankenberger 2005, Zhang *et al.* 2008, Spacil *et al.* 2011a, Spacil *et al* 2011b, Van Heest 2012). However, few have

investigated the effect of a carbon amendment on the vertical variation in biogeochemical processes and Se accumulation in the hydrosoil. Therefore, specific objectives of this study were: (i) measure vertical variation in Se concentration in the hydrosoil of a pilot-scale CWTS cell designed to treat Se in simulated energy-derived water (EDW); (ii) investigate two major Se-sequestering biogeochemical processes (reduction and sorption); and (iii) evaluate the effect of a nutrient amendment on Se accumulation and Se-sequestering biogeochemical processes in the hydrosoil.

2. Materials and Methods

2.1 Description of Pilot-Scale CWTSs

Two pilot-scale CWTSs were designed and constructed by Spacil *et al*. (2011a, 2011b) to investigate the treatment of aqueous Se concentrations (50 μg/L) in simulated EDW. EDWs are generated during fossil fuel extraction, fossil fuel energy production, and refining processes (Kanagy *et al.* 2008, Spacil *et al.* 2011a). Se can occur in petroleum effluents in several oxidation states (VI, IV, 0, and –II) (Zhang *et al.* 2008) and in a variety of compounds and ionic forms such as selenides (e.g., H_2Se^-), selenites (e.g., H_2SeO_3, $HSeO_3^-$, SeO_3^{-2}), and selenates (e.g., $HSeO_4^-$, SeO_4^{-2}) (Zhang and Moore 1996). The characterization of various EDWs was conducted by Spacil *et al.* (2011a, 2011b) and utilized to develop a representative, simulated EDW for experimentation. The predominant ions in EDWs are sodium, calcium, magnesium, chloride, and sulfate. For this investigation, the simulated EDW created by Spacil *et al.* (2011a, 2011b) was replicated and prepared in a 5,678-L polypropylene carboy holding tank. The solutes

used to formulate the simulated EDW included calcium chloride dihydrate ($CaCl \cdot 2H_2O$) at 205 mg/L, magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) at 355 mg/L, sodium chloride (NaCl) at 1,230 mg/L, and sodium selenite (Na_2SeO_3) at 0.109 mg/L (Spacil *et al.* 2011a, Spacil *et al.* 2011b, Van Heest 2012). The simulated EDW was mixed for a minimum of 24 hours with a 1-hp submersible pump. The Se in this simulated EDW was treated in two pilot-scale CWTSs designed, constructed, and studied by Spacil *et al.* (2011a, 2011b).

Each CWTS consisted of a 5678-L polypropylene carboy retention basin to hold simulated EDW and four 378-L Rubbermaid containers (124 cm long by 77 cm wide by 61 cm deep) arranged in series. In each CWTS, PVC pipe fittings connected the four cells approximately 6 cm below the top of each cell to allow gravity flow of water through the series. Each cell was filled to a depth of approximately 30 cm with sand from 18-mile Creek in Clemson, SC. The cells were planted with *Typha latifolia* (broadleaf cattail) at a density of approximately 25-30 plants per cell. During construction of both CWTSs, 1,000 g of ground oyster shells were added to each treatment cell to maintain a circum-neutral pH (6.5-8) and increase alkalinity. Other additions to each cell included 100 g of zero-valent iron (Fe^0) to maintain reducing conditions and 12 g of 19-6-12 Osmocote® fertilizer to provide nitrogen, phosphorous, and potassium as nutrients for microbes and plants. To achieve a nominal 24-hr hydraulic retention time (HRT) per cell (96-hr per CWTS) a piston pump (FMI QG400) delivered 128 mL/min of simulated EDW to each system. One CWTS received a nutrient amendment (AquaSmart™, consisting of fermented yeast, organic carbon, and nutrients)

to promote dissimilatory selenium reduction by microbial activity, while the other CWTS was used as a control and received no amendments (Spacil *et al.* 2011a, Spacil *et al.* 2011b). A 35 g AquaSmart/L solution was delivered at a rate of 1 mL/min by a FMI QG20 piston pump into the inflow of the nutrient amended CWTS. AquaSmart solution was pumped from a 19 L reservoir that was renewed weekly. The CWTSs were constructed and operated inside a greenhouse with natural (i.e. solar) photoperiod and temperature ranging from 20 to 30°C. Simulated EDW was first introduced into the CWTSs in April 2009 (Spacil *et al.* 2011a, Spacil *et al.* 2011b) and continuously flowed through the systems for approximately 2 years during the time when the systems were studied by Spacil *et al.* (2011a, 2011b) and throughout the current investigation.

Two distinct zones (detritus and sediment) were present in the hydrosoil of both cells studied (Figure 1). The detritus consisted of *T. latifolia* plant debris including fallen leaves and stems that were compacted and partially decomposed and contained numerous, fibrous roots. Thickness of the detritus was 21 cm in the nutrient amended cell and 14 cm in the unamended cell. The underlying sediment consisted of sand added during the pilot-scale CWTS cell construction and few (approximately 10-15) *T. latifolia* roots.

2.2 Measurement of Selenium Concentrations in Hydrosoil

Selenium concentrations were measured in samples collected approximately 2 years after construction of the CWTS. The vertical variation in Se concentration was investigated at 3 locations in the first cell of each of the two pilot-scale CWTSs (Figure

2A, 2B, and 2C). After measuring thickness of the detritus in each cell (Figure 2), 7-cm thick detritus grab samples (approximately 500 cm^3) were obtained from each sampling location and placed immediately into plastic freezer bags, from which air was removed quickly prior to sealing and freezing. For each cell, grab samples collected from the same depth interval below the surface water-detritus interface (0 cm) were combined from the 3 sample locations to form a single composite sample for each depth interval.

After grab samples were obtained from the detritus, a chlorinated polyvinyl chloride (CPVC) pipe, 15 cm in length and 2.54 cm in diameter, was hammered into the sediment at each sampling location (Figure 2). The CPVC pipe, containing a sediment core, was then pulled up and capped with CPVC end caps. The caps and pipe were taped to preserve the core's conditions and minimize exposure to air. The sealed CPVC pipe and core were immediately frozen. After freezing, the pipe and core were removed from the freezer and placed in an anaerobic chamber (COY Laboratory Products, Inc.). After thawing for approximately ten minutes at room temperature (~25 ^oC), the sediment core was extruded (intact) from the CPVC onto a clean plastic tray and divided into five 3-cm long segments. Sediment segments from the 3 cores that were collected at the same depth interval below the surface water-detritus interface (0 cm) were composited into one representative sample for each depth interval. This process created a single vertical profile of the hydrosoil for each cell. The vertical hydrosoil profile for the nutrient amended cell consisted of three 7-cm thick detritus samples (0-21 cm) and five 3-cm thick sediment samples (21-36 cm). The profile for the unamended cell consisted of two 7-cm thick detritus samples (0-14 cm) and five 3-cm thick sediment samples (14-29 cm)

(Figure 2). The composited samples were used for measuring Se concentrations and analyzing the Se geochemical fraction.

In preparation for measuring Se concentrations, each sample was treated using a strong acid (Aquaregia) and microwave digestion (CEM 1997). In a microwave digestion vessel (Standard Advanced Composite Vessel; CEM Corporation), 0.5 grams of dry sample were combined with 1 mL trace metal grade (37%) HCl (Fisher Scientific), 4 mL trace metal grade (48%) HF (Fisher Scientific), 5 mL trace metal grade (70%) HNO_3 (Fisher Scientific), and 10 mL DDI water (Super-Q Plus, MilliPore). The vessel was then sealed and placed in a microwave digester at 170^{o}C (MDS-2000; CEM Corporation) for 30 minutes. After digestion, approximately 2g of H_3BO_3 crystals were added to neutralize the acid mixture. Digested samples were pipetted into separate 15-mL centrifuge tubes and diluted to 2% HNO_3 concentration by volumetric addition of deionized water. Total Se concentration in each solubilized sample was measured using an inductively coupled plasma-mass spectrometer (ICP-MS) (Thermo ICP-Mass Spectrometer X Series II) following standard method EPA 200.8 (USEPA 1994). The Se concentration measured for each sample represents the total amount of Se (all species and forms) solubilized during this extraction procedure. Multi-elemental standards, ranging in concentration from 0.005 to 100 µg/L, were made by diluting stock solutions containing Ag, Au, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Th, Th, Tl, U, V, Zn, Cl, Ca, Fe, K, Mg, Na, P, S, and C in 2% Aristar Optima HNO_3. Volume additions were verified gravimetrically. Multi-element standards were used to calibrate the ICP-MS for analysis of Se. Rhenium and Scandium were selected as internal standards and used for

sample recovery [internal standard recoveries were within the 80 to 120% standard Quality Assurance/Quality Control (QA/QC)]. Detection limit using this technique was approximately 0.1 µg/L in solubilized samples. Additionally, QA/QC check samples made from stock solutions with known concentrations were run in approximately 20-sample intervals.

2.3 Investigation of Major Se Biogeochemical Processes

Sequential extraction has been used successfully by many researchers to determine fractionation of Se in sediment (Zhang and Moore 1996, Gao *et al.* 2000). A modified sequential extraction procedure (SEP) was utilized in this study to identify and measure the following six Se associated geochemical fractions: soluble/exchangeable, adsorbed, organic, elemental, recalcitrant organic/selenides, and residual (Chao and Sanzolone 1989, Zhang and Moore 1996, Wright *et al*. 2003) (Table 1). This particular procedure was selected based on the fractions identified, reagents used for each extraction step, and recovery of Se (Wright *et al*. 2003) (Table 1). Reagents were chosen for their ability to solubilize specific Se-associated fractions and species and included the following: 0.25M KCl, 0.1M K_2HPO_4, 0.1M NaOH, 0.25M Na_2SO_3/0.25M sulfite solution, 5% NaOCl, and aquaregia. For each depth interval in the nutrient amended cell and unamended cell, 0.5 grams of dry composited hydrosoil sample was used for the SEP. The SEP included six extraction steps, which released Se into the six aforementioned, operationally defined, geochemical fractions. Extractions were carried out in one 50-mL Teflon centrifuge tubes to minimize hydrosoil loss. Each extraction

step comprised the addition of a reagent followed by shaking (Table 1). After each extraction: 1) the sample was centrifuged (10,000G for 15 min), 2) the supernatant was collected and pippetted into another centrifuge tube, 3) the sample was rinsed with 5mL of DDI water, 4) the sample was centrifuged a second time (10,000G for 15 min), and 5) the remaining supernatant was collected and pipetted into the supernatant centrifuge tube. During the residual step of the SEP, the remaining sample residue was digested using the microwave digestion procedure discussed previously for Se concentration analysis (Table 1). Supernatant of individual samples collected from each extraction step was filtered (0.45um). A subsample of each of these volumes was pipetted into individual 15-mL centrifuge tubes and acidified to 2% HNO_3. Total concentration of Se solubilized in the supernatant subsamples collected from each extraction step was measured using an ICP-MS with standard method EPA 200.8 (USEPA 1994). ICP-MS Se standards, samples of known Se concentration, and replicates were used for QA/QC as described previously. Se concentration measured in the supernatant from each extraction step (i.e. geochemical fraction of Se) was expressed as a percentage of the total Se extracted from each original composite sample by the SEP. Sequential extractions identify phase associations of Se which relate to biogeochemical processes occurring in the hydrosoil (Sundberg-Jones and Hassan 2007). The geochemical fractions of Se measured during this SEP provided insight into biogeochemical processes occurring at each depth interval in the CWTS cells studied.

Selenium reducing microbe abundance was quantified using a modified method utilizing a hard agar made from simulated EDW containing 100 ug/L selenate and

amended with AquaSmart™ (200 mg/L) as an energy source (Zhang *et al.* 2008, Spacil 2010). Sediment pore water samples were collected in 7-cm intervals from the detritus (0-21cm in nutrient amended and 0-14 cm in the unamended) and 3-cm intervals from the sediment (21-30 cm in the nutrient amended and 14-23 cm in the unamended) using a sterile needle and syringe. Using sterile technique, 0.00312 mL aliquots of each sample, with replication (n=3), were dispersed in 50 mL sterile (i.e. autoclaved at 121ºC for 15 minutes) water in a Nalgene® vacuum funnel and filtered through a 0.45 μm gridded membrane filter. These volumes were found by Spacil (2010) and Van Heest (2012) to yield countable numbers of microbial colonies. The filters were placed in individual 4-cm diameter petri plates filled with the modified Se agar and incubated in a GasPak® anaerobic vessel for two days (48 hours) at room temperature (approximately 22^{o}C). On the third day of incubation, the vessel lid was loosened to allow a small amount of air into the vessel simulating slightly reducing conditions. After seven days (168 hours) of incubation, the petri plates containing each filter were removed from the vessel and the filters were examined for Se-reducing colony forming units (CFUs). The presence of a Se-reducing CFU was indicated by a red, circular microbial colony on the filter paper. This hue is characteristic of precipitated elemental Se and CFUs (Oremland *et al.* 2004, Zhang *et al.* 2008). The CFUs on each filter for every sample were identified visually and counted utilizing the gridded pattern on the filters. The aforementioned procedure was conducted on two separate sampling events (May and June). During each sampling event, pore water samples were replicated (n=3) resulting in a total of 6 petri plates for each interval sampled. Se-reducing CFUs/mL of pore water from the 6 petri plates were

averaged to generate a mean Se-reducing CFUs value for each depth interval sampled (Table 2).

For each cell, the vertical variations between mean Se-reducing CFUs, Se geochemical fraction percentages, and total Se concentrations were compared using a Pearson correlation (measure of the strength of linear dependence between two variables). The significance of each correlation was determined by calculating a Pearson correlation coefficient (p-value). Pearson correlations were utilized to determine whether a potential relationship exists between Se-reducing bacteria, Se geochemical fractions, and Se concentrations vertically through the hydrosoil; thus, providing insight into biogeochemical processes.

2.4 Effect of Nutrient Amendment

Se concentrations, Se-reducing CFUs, and Se geochemical fractions were compared between the nutrient amended cell and the unamended cell by 1) observing differences in the Se concentrations, mean Se-reducing CFUs counted, and Se geochemical fraction percentages in detritus and sediment of each cell; and, 2) using a two-sample t-test (equal or unequal variance) to determine the significance of differences observed. Prior to the statistical comparison, the mean of Se concentrations and mean of each Se geochemical fraction were calculated for the entire sampling depth (0-36 cm in amended cell and 0-29 cm in unamended cell). The mean of Se-reducing CFUs was calculated for 0-30 cm in the nutrient amended cell and 0-23 cm in the unamended cell. Two-sample t-test assuming equal or unequal variance was then used to determine if the

mean Se concentration, mean of Se CFUs values, and mean of each Se geochemical fraction were statistically different (alpha < 0.05) between the nutrient amended cell and unamended cell in order to evaluate the effect of a nutrient amendment on Se accumulation and hydrosoil biogeochemical processes.

3. Results

3.1 Measurement of Selenium Concentrations in Hydrosoil

Selenium concentrations decreased with depth in both the nutrient amended cell (830 μg/g at 0-7 cm to 0.97 μg/g at 33-36 cm) and the unamended cell (569 μg/g at 0-7 cm to 0.71 μg/g at 26-29 cm) (Figure 3; Table 3). In both cells, mean Se concentrations were greater in detritus (308-830 μg/g in nutrient amended and 138-569 μg/g in unamended) than in sediment (0.89-212 μg/g in nutrient amended and 0.54-6.77 μg/g in unamended).

3.2 Investigation of Major Se Biogeochemical Processes

Mean Se-reducing CFUs in the nutrient amended cell increased with depth through the detritus (2,700 CFUs/mL pore water at 0-7 cm to 22,000 CFUs/mL pore water at 14-21 cm) and ranged from 2,800 to 4,800 CFUs/mL pore water in the sediment (Table 2; Figure 3). In the unamended cell, Se-reducing CFUs decreased with depth through the detritus (15,000 CFUs/mL pore water at 0-7 cm to 9,300 CFUs/mL pore water at 7-14 cm) and the sediment (6,300 CFUs/mL pore water at 14-17 cm to 1,300 CFUs/mL pore water at 20-23 cm). In both cells, the mean Se-reducing CFUs were

greater in the detritus than in the sediment, indicating greater potential for dissimilatory Se reduction in the detritus (Table 2). The greater number of Se-reducing CFUs may be attributed to the organic-richness of the detritus providing an energy source and electron donors for Se-reducing microbes.

The geochemical fractions of Se were measured in each composite sample from both the nutrient amended and unamended cells (i.e. soluble/exchangeable, adsorbed, elemental, recalcitrant organics/metal selenides, and residual) (Table 3; Figure 4). For all depth intervals in the nutrient amended cell, the largest fraction occurred as elemental Se (44.0% to 58.0%), with the percent as elemental Se decreasing with depth. In the unamended cell, the largest fraction occurred as soluble/ exchangeable Se (10.5% to 63.1%) except for the 0-7cm interval in which the largest fraction occurred as elemental Se (62.6%). In both the nutrient amended and unamended cells, residual Se was the smallest fraction ranging from 0.290% to 2.09% and 0.320% to 2.09%, respectively. Residual Se was below method detection limit (~0.1 ug/L in solubilized samples) in 1 unamended cell sediment sample (26-29 cm). Organic Se was below method detection limit (~0.1 ug/L in solubilized samples) in 2 nutrient amended sediment samples and 4 unamended sediment samples.

The abundance of elemental Se in the nutrient amended cell suggests that dissimilatory Se reduction was the dominant biogeochemical process operating throughout the hydrosoil. In the uppermost interval of the unamended cell, the largest geochemical fraction was elemental Se suggesting that dissimilatory Se reduction was the

dominant process from 0-7 cm. The presence of other geochemical fractions (soluble/exchangeable Se, adsorbed Se, organic associated Se, and recalcitrant organic/metal selenides) in samples collected from throughout the nutrient amended cell and 0-7 cm in the unamended cell suggest that Se sorption is also operating, but to a lesser degree than dissimilatory Se reduction. In samples deeper than 7 cm in the unamended cell, the high percentage of soluble/exchangeable Se suggests that Se sorption was the dominant biogeochemical process. The presence of organic-associated Se in the detritus (measured in 5 out of 5 samples) compared to organic-associated Se in the sediment (measured in 4 out of 10 samples) of both cells suggests that sorption associated with organic matter is more likely to occur in the detritus than in the sediment.

Pearson correlations performed on mean Se-reducing CFUs, Se concentrations, and Se geochemical fraction percentages indicate that vertical variation of mean CFUs correlates significantly ($p<0.05$) to vertical variation of Se concentrations and each of the geochemical fractions in the unamended cell (Table 4). Of the correlations between Se-reducing CFUs and geochemical fractions in the unamended cell; soluble/exchangeable Se, adsorbed Se, and residual Se were negative correlations, while organic Se, elemental Se, and recalcitrant Se were positive correlations. In the nutrient amended cell, vertical variation of mean CFUs did not significantly correlate to vertical variation of Se concentrations or to any of the geochemical fractions. All 6 geochemical fractions correlated significantly to vertical variation of Se concentrations in the nutrient amended cell (Table 5). Of the correlations between Se concentration and geochemical fractions in the nutrient amended cell; soluble/exchangeable Se, adsorbed Se, and residual Se were

negative correlations, while organic Se, elemental Se, and recalcitrant Se were positive correlations. Se concentrations in the unamended cell showed significant positive correlation to organic Se and elemental Se and significant negative correlation to soluble/exchangeable and adsorbed Se.

3.3 Effect of Nutrient Amendment

In the detritus, mean Se concentration was greater in the nutrient amended cell than in the unamended cell, while the mean value of Se-reducing CFUs was greater in the unamended cell than in the nutrient amended cell (Figure 3; Table 2). Although in both cells dissimilatory Se reduction occurs throughout the hydrosoil, the mean percentage of the elemental Se geochemical fraction for the nutrient amended cell (52 %) is greater than for the unamended cell (29 %) suggesting that dissimilatory Se reduction is more dominant in hydrosoil of the nutrient amended cell than in the unamended cell. Unlike the nutrient amended hydrosoil, soluble/exchangeable Se comprised the greatest percentage of total Se from 7 to 29 cm suggesting that sorption is the dominant process below 7 cm in the unamended hydrosoil.

For the entire hydrosoil depth interval analyzed, two-sample t-tests (equal or unequal variance) indicated soluble/exchangeable Se ($t = -3.36$, $p = 0.00514$), adsorbed Se ($t = -2.55$, $p = 0.0241$), and elemental Se ($t = 3.49$, $p = 0.010$) were statistically different ($p<0.05$) between the nutrient amended cell and unamended cell (Table 6). Organic Se ($t = 0.996$, $p = 0.338$), recalcitrant Se ($t= 2.06$, $p = 0.0600$), residual Se ($t =$

0.779, p = 0.450), Se-reducing CFUs (t = 0.0571, p = 0.956), and Se concentration (t = 0.989, p = 0.341) were not statistically different between the two cells.

4. Discussion

In hydrosoil of the nutrient amended cell, greater percentage of elemental Se than any other geochemical fraction measured suggests that dissimilatory Se reduction was more dominant than sorption throughout the hydrosoil. In the unamended cell, soluble/exchangeable Se was prevalent (below 7 cm) indicating sorption was the dominant process in the unamended hydrosoil. Greater Se concentrations in detritus compared to sediment suggest that more Se-accumulating biogeochemical processes were operating and/or at a greater rate in the detritus of both cells. Greater mean CFUs and percent of elemental Se in the detritus than in the sediment of both cells studied suggest that dissimilatory Se reduction was more likely to occur in the detritus than in the sediment. However, the presence of elemental Se and other geochemical fractions at every interval sampled suggests that sorption and dissimilatory Se reduction were occurring throughout the hydrosoil in both cells. Statistical differences in soluble/exchangeable Se, adsorbed Se, and elemental Se between the two cells provides evidence for differences in the major biogeochemical processes in the two cells. Furthermore, the observed differences between the nutrient amended cell and unamended cell suggest that addition of a nutrient amendment can enhance Se-accumulating biogeochemical processes operating in hydrosoil of CWTSs.

This study has found that the addition of nutrients to a pilot-scale CWTS designed to treat Se in EDW may enhance Se sequestration into the hydrosoil by promoting Se-sequestering biogeochemical processes operating in the hydrosoil, particularly dissimilatory Se reduction. In the detritus, Se accumulation was greater in the nutrient amended cell than the unamended cell. This may be attributed to greater number of Se-reducing CFUs in the detritus of the nutrient amended cell (22,000 CFUs/mL pore water) than in detritus of the unamended cell (15,000 CFUs/mL pore water). Although the top two depth intervals (0-7 cm and 7-14 cm) in the nutrient amended hydrosoil had higher Se concentrations and fewer CFUs than the same depths in the unamended hydrosoil, dissimilatory Se reduction through Se reducing microbes may be operating at a greater rate in the nutrient amended detritus than the unamended detritus due to the nutrient addition.

This study showed that a sequential extraction procedure can be utilized to investigate whether dissimilatory Se reduction and sorption are occurring at different depths in hydrosoil of a pilot-scale CWTS. In the areas of the nutrient amended hydrosoil where Se was accumulating, the percent of Se (>44.0%) that was elemental suggested that dissimilatory Se reduction was the dominant biogeochemical process. The information obtained from the SEP can also help evaluate the risk, potential remobilization, and bioavailability of Se (Sundberg-Jones and Hassan 2007). This study has shown that the addition of a nutrient amendment may have promoted dissimilatory Se reduction and therefore the sequestration of Se in less bioavailable forms throughout the hydrosoil.

5. Conclusions

This study found that Se concentration and Se-sequestering biogeochemical processes vary with depth in hydrosoil of a pilot-scale CWTS and that the detritus is the key interval of the hydrosoil for Se-sequestering biogeochemical processes and the resulting Se accumulation. Data collected suggests that dissimilatory Se reduction and sorption were operating at all sampled intervals in the hydrosoil of the nutrient amended and unamended cells. Differences in Se accumulation, CFUs, and Se geochemical fractions suggest that the addition of nutrients to a CWTS can affect Se-sequestering biogeochemical processes operating in the hydrosoil of CWTSs and potentially affect the rate at which those processes accumulate Se. Specifically, data obtained during this study suggest that nutrient amendments added to CWTSs designed to treat Se-contaminated waters can enhance Se sequestration into the hydrosoil by promoting dissimilatory Se reduction and increasing Se-reducing bacteria abundance in the detritus. Dissimilatory Se-reduction is often the targeted pathway in CWTSs because it produces elemental Se, which is the insoluble, stable, and least bioavailable form of Se. This study provides evidence suggesting that addition of a nutrient amendment (AquaSmart^{TM}) not only enhanced Se accumulation in the hydrosoil, particularly in the detritus, but also that the Se sequestered was elemental Se. In conclusion, the information gained in this study provides a better understanding of Se-sequestering biogeochemical processes in the hydrosoil and can be utilized by operational CWTSs and future CWTSs to improve performance and reduce hydrosoil toxicity.

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Figure 1. Schematic diagram of CWTS cell showing 2 zones of hydrosoil: detritus and sediment. The detritus consists of plant debris that has compacted and partially decomposed and contains numerous fibrous *Typha latifolia* roots. The sediment consists of medium-coarse (0.25 to 1.0 mm diameter) sand and fewer, but larger, *T. latifolia* roots compared to those in the detritus.

Figure 2. A.) Overhead schematic of sampling locations (X) in the nutrient amended and unamended CWTS cells. At each sampling location, 7-cm thick grab samples (7.6-cm diameter ring x 7-cm thick intervals) of the detritus and a sediment core (2.54-cm diameter and 15-cm long starting from the top of the sediment) were collected. Detritus could not be cored because of numerous roots. Each 15-cm long sediment core was sectioned in 3-cm intervals to obtain a vertical profile. B.) Vertical profile from the nutrient amended cell. C.) Vertical profile from the unamended cell. Thickness of detritus was 21 cm in the amended cell and 14 cm in the unamended cell.

Figure 3. Se concentrations and mean Se-reducing CFUs with depth through hydrosoil of the nutrient amended (A) and unamended (B) CWTS cells. Surface water/detritus interface is at 0 cm. The detritus/sediment interface is at 21-cm depth in the nutrient amended cell and at 14-cm depth in the unamended cell. Se concentration and CFU values are plotted at the center of sample intervals.

Figure 4. Geochemical fractions expressed as a percentage of total Se extracted from each sample using the sequential extraction procedure in the nutrient amended cell (A) and unamended cell (B).

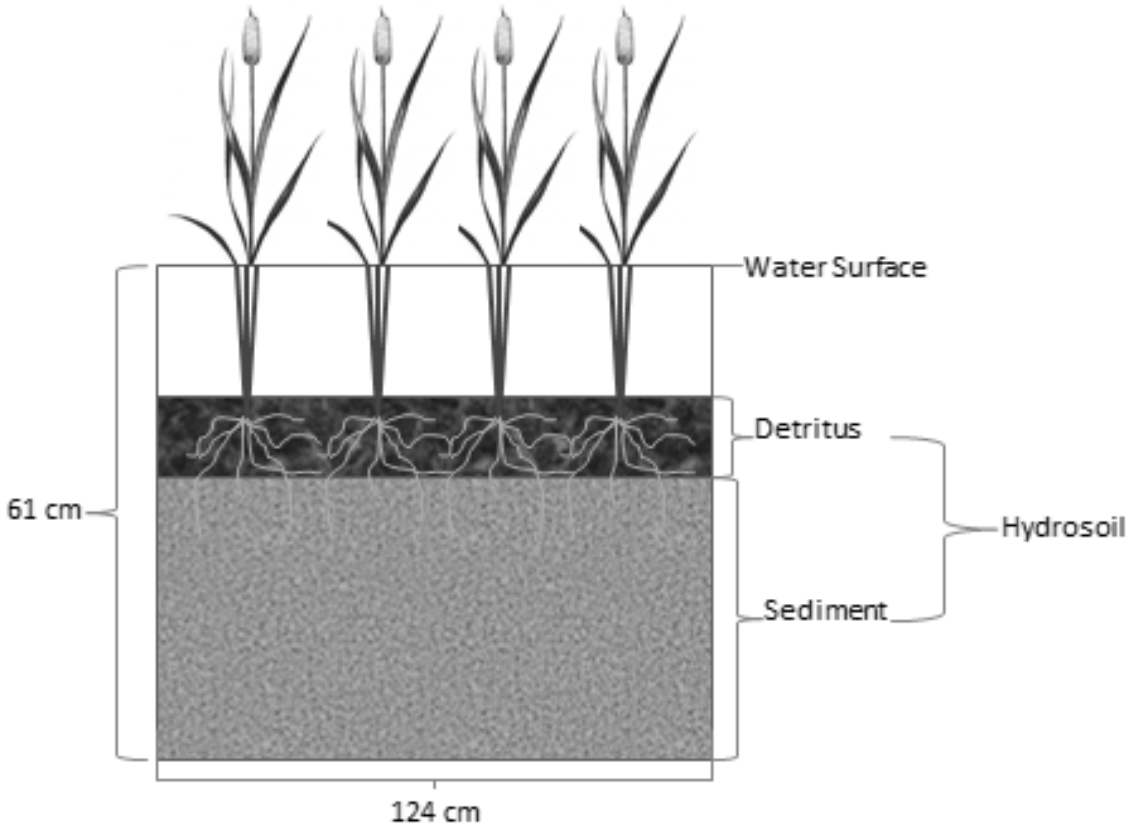


Figure 1.

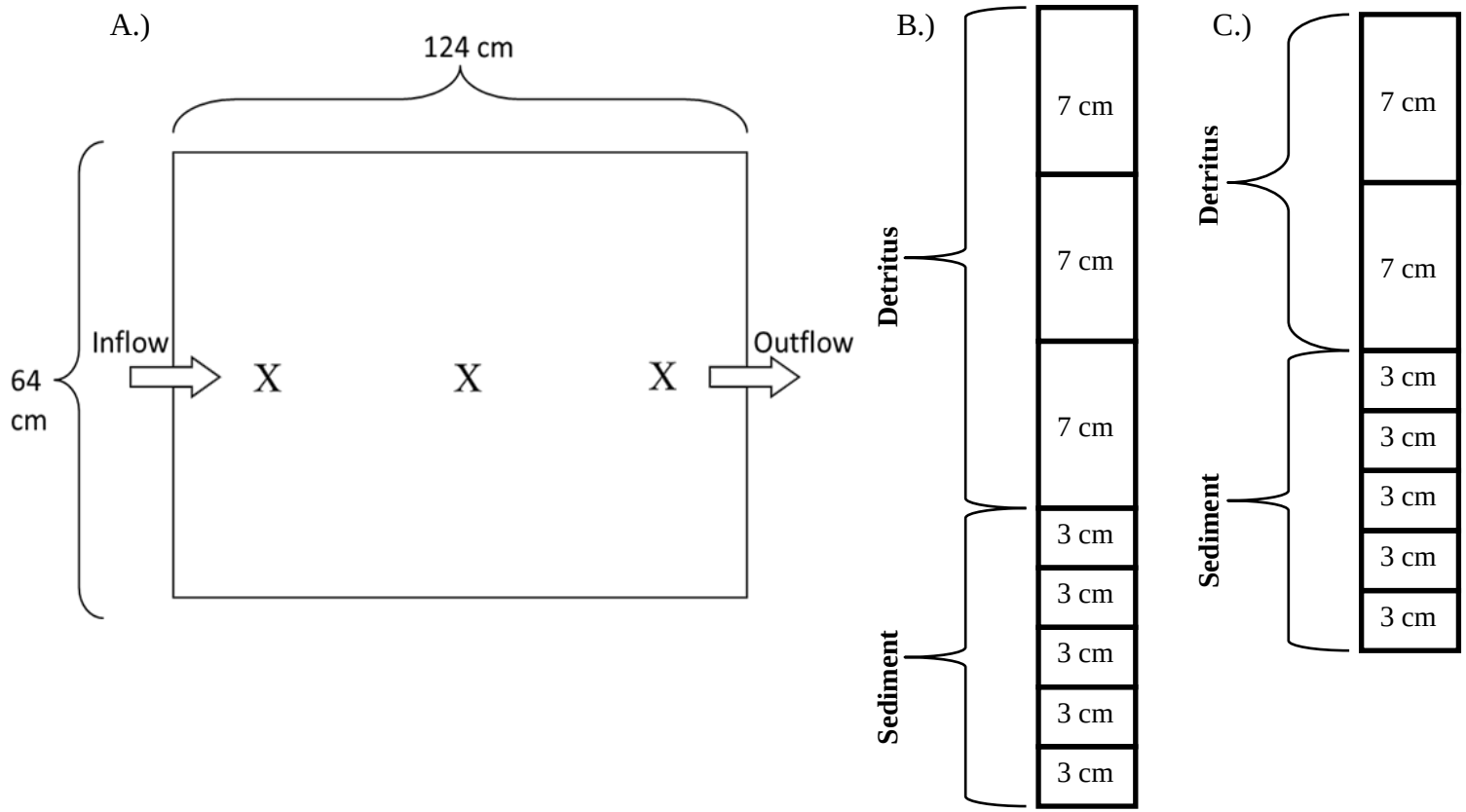

A.)
124 cm
64 cm
Inflow
Outflow
X
X
X
B.)
Detritus
Sediment
7 cm
7 cm
7 cm
3 cm
3 cm
3 cm
3 cm
3 cm
C.)
Detritus
Sediment
7 cm
7 cm
3 cm
3 cm
3 cm
3 cm
3 cm

Figure 2.

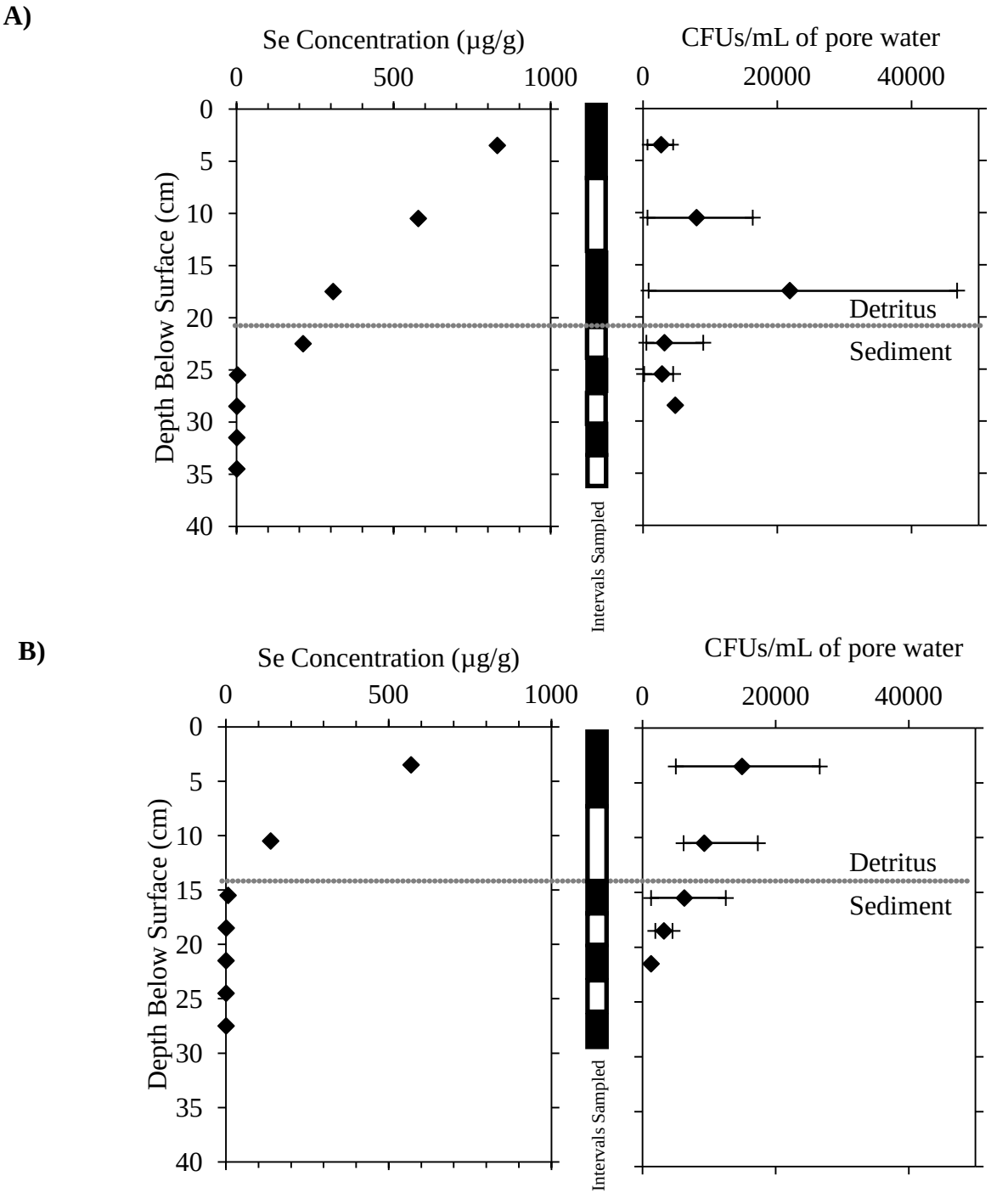
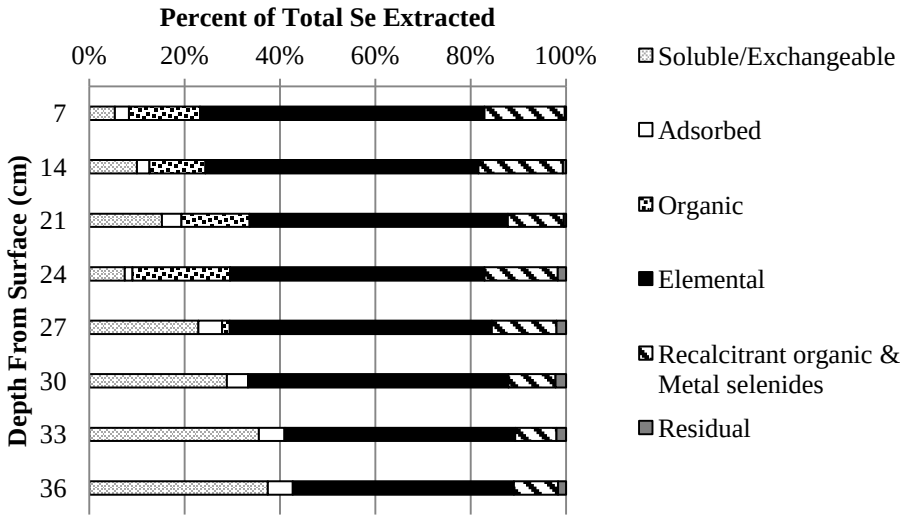

A)
Se Concentration (µg/g)
0
500
1000
CFUs/mL of pore water
0
20000
40000
Depth Below Surface (cm)
0
5
10
15
20
25
30
35
40
Detritus
Sediment
Intervals Sampled
B)
Se Concentration (µg/g)
0
500
1000
CFUs/mL of pore water
0
20000
40000
Depth Below Surface (cm)
0
5
10
15
20
25
30
35
40
Detritus
Sediment
Intervals Sampled

Figure 3.

A)


Percent of Total Se Extracted
0%
20%
40%
60%
80%
100%
Depth From Surface (cm)
7
14
21
24
27
30
33
36
Soluble/Exchangeable
Adsorbed
Organic
Elemental
Recalcitrant organic & Metal selenides
Residual

B)

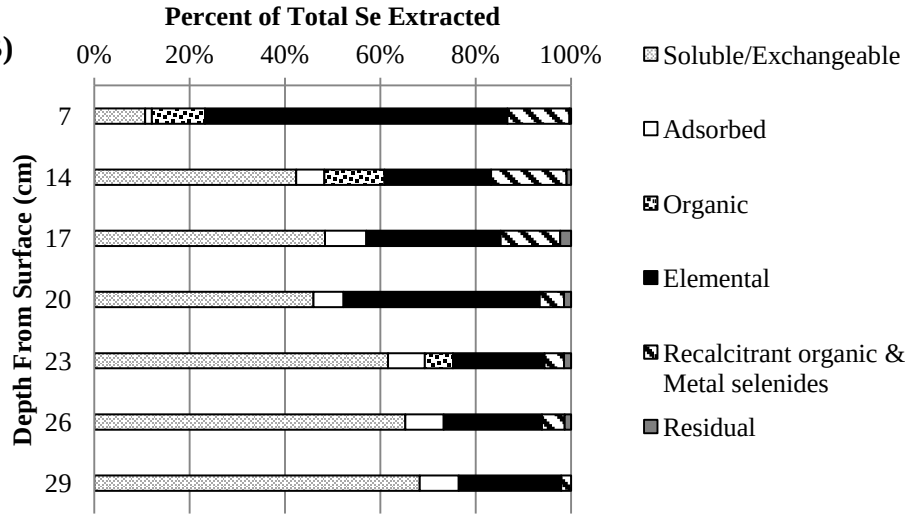

Percent of Total Se Extracted
0%
20%
40%
60%
80%
100%
Depth From Surface (cm)
7
14
17
20
23
26
29
Soluble/Exchangeable
Adsorbed
Organic
Elemental
Recalcitrant organic & Metal selenides
Residual

Figure 4.

Table 1. Summary of modified sequential extraction procedure for Se.

Step	Phase/Association of Se	Extract and Conditions
I	Soluble/Exchangeable	0.25M KCl,10:1 solution:solid, mixed 2h at 25^{o}C
II	Adsorbed	0.1M K_2HPO_4, pH 8.0, 10:1 solution:solid, mixed 2h at 25^{o}C, repeated once
III	Organic Associated	0.1M NaOH, 10:1 solution:solid, mixed 4h at 25^{o}C
IV	Elemental	0.25MNa_2SO_3, pH 7.0, 10:1 solution:solid, ultrasonic bath for 4h, rinsed twice with 0.25M sulfite solution (2:1 solution: solid)
V	Recalcitrant Organic/Selenides	5% NaOCl, pH 9.5, 4:1 solution solid, shaken 30 min at 90^{o}C, repeated once
VI	Residual	Microwave digestion using aquaregia (1mL HCl, 4mL HF, 5mL HNO_3, 10mL DDI water) on remaining sample residue

-Sequential extraction procedure modified from Chao and Sanzolone (1989), Zhang and Moore (1996), and Wright *et al*. (2003).

Table 2. Measured total Se concentration and mean Se-reducing microbial colony forming units (CFUs) with depth in the nutrient amended and unamended CWTS cells.

	Depth (cm)	Total Se (µg/g)	CFUs/mL pore water
Nutrient Amended Cell			
	0-7	830	2700
	7-14	579	8000
	14-21	308	22000
	21-24	212	3200
	24-27	3.28	2800
	27-30	1.17	4800
	30-33	0.890	NM
	33-36	0.970	NM
Unamended Cell			
	0-7	569	15000
	7-14	138	9300
	14-17	6.77	6300
	17-20	1.12	3200
	20-23	0.540	1300
	23-26	0.650	NM
	26-29	0.710	NM

-NM = Not measured. Pore water samples not collected due to lack of pore water and/or depth >30 cm.

Table 3. Geochemical fraction percentage of total Se extracted from each sample using the sequential extraction procedure in the nutrient amended cell and unamended cell.

	Depth (cm)	Soluble/ Exchangeable	Adsorbed	Organic	Elemental	Recalcitrant organic & metal selenides	Residual
Nutrient Amended							
	0-7	5.17	5.85	14.4	58.0	16.3	0.290
	7-14	9.72	5.09	11.5	55.9	17.2	0.660
	14-21	14.7	7.80	13.8	52.1	11.3	0.480
	21-24	7.30	3.26	20.1	52.5	15.1	1.70
	24-27	21.8	9.49	1.53	52.3	12.9	1.95
	27-30	27.6	8.55	NA	52.3	9.45	2.09
	30-33	33.7	10.3	0.200	45.7	8.23	1.88
	33-36	35.6	9.94	NA	44.0	8.80	1.63
Unamended							
	0-7	10.5	2.82	11.0	62.6	12.8	0.320
	7-14	40.0	11.1	12.0	21.1	15.0	0.880
	14-17	44.6	15.8	NA	26.0	11.5	2.09
	17-20	43.2	11.9	NA	38.7	4.83	1.37
	20-23	57.2	14.4	5.49	17.8	3.80	1.39
	23-26	60.4	14.9	NA	19.1	4.39	1.27
	26-29	63.1	15.1	NA	19.9	1.91	NA

-NA = Not applicable. Se concentration was below the method detection (~0.1 µg/L); therefore, a percentage could not be established.

Table 4. Pearson correlation coefficient and p-value of significance between Se concentrations and geochemical fraction percentages with mean Se-reducing colony forming units (CFUs).

	Total Se Concentration	Soluble/ Exchangeable	Adsorbed	Organic	Elemental	Recalcitrant organic & metal selenides	Residual
Nutrient Amended Cell							
Pearson correlation coefficient "r"	0.0258	0.0169	0.1810	0.1957	-0.3015	-0.3246	-0.4793
P-value of significance	0.9366	0.9584	0.5735	0.5422	0.3409	0.3033	0.1149
Unamended Cell							
Pearson correlation coefficient "r"	0.9140	-0.9361	-0.8355	0.6729	0.7089	0.8170	-0.7125
P-value of significance	0.0002	0.0001	0.0026	0.033	0.0217	0.0039	0.0208

Table 5. Pearson correlation coefficient and p-value of significance between mean CFUs and geochemical fraction percentages with Se concentrations.

	CFU's	Soluble/ Exchangeable	Adsorbed	Organic	Elemental	Recalcitrant organic & metal selenides	Residual
Nutrient Amended Cell							
Pearson correlation coefficient "r"	0.0258	-0.8121	-0.6443	0.6821	0.7829	0.8031	-0.8912
P-value of significance	0.9366	0.0001	0.0071	0.0036	0.0003	0.0002	0.0001
Unamended Cell							
Pearson correlation coefficient "r"	0.9140	-0.9080	-0.9603	0.7201	0.8563	0.5900	-0.4762
P-value of significance	0.0002	0.0001	0.0001	0.0037	0.0001	0.0264	0.0852

Table 6. Results of a two-sample t-test assuming equal or unequal variance used to determine the statistical difference (alpha < 0.05) between the nutrient amended cell and unamended cell for mean total Se concentrations, mean Se-reducing colony forming units (CFUs), and geochemical fraction percentages (soluble/exchangeable, adsorbed, organic, element, recalcitrant organic & metal selenides, and residual). Statistical differences used to evaluate the effect of a nutrient amendment on hydrosoil biogeochemical processes.

	Total Se Concentration	CFUs	Soluble/ Exchangeable	Adsorbed	Organic	Elemental	Recalcitrant organic & metal selenides	Residual
t-statistic	0.989	0.0571	-3.36	-2.55	0.996	3.49	2.06	0.779
p-value	0.341	0.956	0.00514	0.0241	0.338	0.0101	0.0600	0.450
Degrees of freedom (df)	13	9	13	13	13	7	13	13

CHAPTER IV

CONCLUSIONS

This study found that hydrosoil conditions (redox potential, pH and organic matter content), Se accumulation, and Se-sequestering biogeochemical processes vary with depth in the hydrosoil of pilot-scale constructed wetland treatments systems (CWTSs). The 15 samples analyzed in this investigation displayed an approximately bimodal distribution of organic matter: those with organic matter > 50% (6 samples) and those with organic matter < 6.1% (9 samples). Selenium concentration was greater in the samples with organic matter greater than 50% (138 to 830 µg/g) than in the samples with organic matter less than 6.1% (0.54 to 6.77 µg/g). In both the nutrient amended and unamended pilot-scale CWTS cells studied, hydrosoil conditions were within the range favorable for sorption and only slightly outside (redox: $\pm$ 50 mV and pH: $\pm$ 0.5 S.U.) the range favorable for dissimilatory Se reduction. A modified sequential extraction procedure (Chao and Sanzolone 1989, Zhang and Moore 1996, Wright *et al*. 2003) suggested that dissimilatory Se reduction and sorption were both operating at all sampled intervals based on the fact that elemental Se and other geochemical fractions were present at every interval sampled in the hydrosoil of the nutrient amended and unamended pilot-scale CWTS cells.

This research has also found that detritus is a key interval of the hydrosoil for Se accumulation and Se-sequestering biogeochemical processes. Greater Se concentrations were measured in detritus than in the sediment of both cells suggesting that more Se-accumulating biogeochemical processes were operating and/or at a greater rate in detritus

than in sediment. Statistical analysis indicated that Se concentration correlated with organic matter content, but not with pH or redox potential. Additionally, greater mean Se-reducing colony forming units (CFUs) and higher percentage of elemental Se in the detritus than in the sediment of both cells studied suggested that dissimilatory Se reduction is the dominant Se-accumulating biogeochemical process occurring in the detritus and is more likely to occur in detritus than in the sediment.

Differences in hydrosoil conditions, Se concentrations, and Se-accumulating biogeochemical processes were observed between the nutrient amended cell and unamended cell. Although, t-tests indicated no significant differences in mean redox potential, organic matter and Se concentration in hydrosoil, mean organic matter content and mean Se concentration were greater in detritus of the nutrient amended cell than in detritus of the unamended cell. In the nutrient amended cell, greater percentage of elemental Se than any other geochemical fraction measured suggested that dissimilatory Se reduction is more dominant than sorption throughout the nutrient amended cell hydrosoil. However in the unamended cell, soluble/exchangeable Se was most prevalent (below 7 cm) suggesting that sorption is more dominant in the unamended cell hydrosoil. Differences in hydrosoil conditions, Se accumulation, Se-reducing CFUs, and Se geochemical fractions suggest that the addition of nutrients to a CWTS can affect Se-sequestering biogeochemical processes operating in the hydrosoil of CWTSs and potentially affect the rate at which those processes accumulate Se. Specifically, data obtained during this study suggested that nutrient amendments added to a pilot-scale CWTSs designed to treat Se contaminated waters may enhance Se sequestration into the

hydrosoil by promoting dissimilatory Se reduction and increasing Se-reducing bacteria abundance.

Dissimilatory Se-reduction is often the targeted pathway in CWTSs because it produces elemental Se which is the insoluble, stable, and least bioavailable form of Se (Sundberg-Jones and Hassan 2007). This study provided evidence suggesting that addition of a nutrient amendment (AquaSmart^{TM}) not only enhanced Se accumulation in the hydrosoil, particularly in detritus, but also that the Se sequestered was elemental Se. In conclusion, the information gained in this study provides a better understanding of Se-sequestering biogeochemical processes in the hydrosoil and can be utilized by operational CWTSs and future CWTSs to improve performance and reduce hydrosoil toxicity.

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APPENDICES

Appendix A

Standard Operating Procedures for Hydrosoil Condition Analyses

The standard operating procedures used to measure hydrosoil conditions in detritus samples and sediment cores extracted from the pilot-scale constructed wetland treatment system designed to treat Se in impaired water are listed below and found on the pages indicated.

METHOD FOR EXTRACTING DETRITUS SAMPLES AND SEDIMENT CORES FROM A CONSTRUCTED WETLAND TREATMENT SYSTEM (CWTS) FOR MULTIPLE CHEMICAL ANALYSES

Kristen N. Jurinko, Christina Blaszkiewicz

1.0 OBJECTIVE

The objective of this standard operating procedure (SOP) is to clearly outline and define the requirements of sample collection and sectioning of the detritus and sediment.

2.0 HEALTH AND SAFETY

Proper personnel protective equipment will be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

One-liter plastic bags
1.91-cm chlorinated polyvinyl chloride (CPVC) pipe
2.54-cm CPVC pipe
50-mL polypropylene centrifuge tubes
15-mL polypropylene centrifuge tubes
Caps for 1.91-cm CPVC pipe

4.2 Equipment

Anaerobic chamber (98% N_2 (g)/2% H_2 (g) atmosphere)
Core sectioning tool

5.0 PROCEDURE

5.1Detritus Samples

Collect 7-cm thick samples of detritus from the surface water-detritus interface down to the detritus-sediment interface. Scoop each detritus sample (approximately 500cm3) into a one-liter plastic bag, seal underwater, double bag, and freeze immediately.

5.2 Sediment Core

5.2.1 Sampling

Sharpen one end of a 2.54-cm CPVC pipe. After the detritus samples were collected insert sharpened pipe with the aid of a mallet into the detritus-sediment surface to a depth of at least 15 cm. Insert 1.91-cm CPVC pipe into the 2.54-cm CPVC pipe while still in sediment. Create a vacuum and extract sediment core by pulling the 1.91-cm CPVC pipe upwards. Immediately cap, tape, and freeze the 1.91-cm CPVC pipe containing sediment core.

5.2.2 Sectioning

Construct a sediment core section tool by cutting a 2.54-cm x 30-cm CPVC pipe into 2 long halves. Screw each half to a 30-cm long wood piece and hinge the wood together. Sharpen and adhere at least 6, 2.5-cm washers in 3-cm increments onto one half of the CPVC pipe. Mark where the first washer meets the reciprocate half of CPVC pipe without washers. In an anaerobic chamber, let sediment core thaw in the 1.91-cm CPVC pipe until it can be pushed out of the pipe. Push sediment core onto the CPVC pipe with no washers, lining the top of the sediment with the mark. Section each frozen core into five 3-cm sediment intervals in an anaerobic chamber by closing the constructed sectioning tool. Collect surface water samples from each core pipe and place into 50-mL centrifuge tubes. Pipette pore water from between each set of washers into 15-mL centrifuge tubes, Subsample each detritus and sediment sample and test for hydrosoil conditions and selenium analyses.

Note: Homogenize samples from all three sampling locations within the CWTS cell to obtain a composite sample for later analyses of selenium and measurement of pH and organic matter content.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.

METHOD FOR MEASURING OXIDATION-REDUCTION POTENTIAL OF SURFACE WATER AND HYDROSOIL IN A CONSTRUCTED WETLAND TREATMENT SYSTEM

Sarah E. Sundberg, Derek Eggert, J. Chris Arrington, John H. Rodgers, Jr., Christina Blaszkiewicz

1.0 OBJECTIVE

Oxidation and reduction (redox) reactions mediate the behavior of many chemical constituents in wastewaters. The reactivities and mobilities of important elements in biological systems, as well as those of a number of other metallic elements, depend strongly on redox conditions. Like pH, Eh (redox) represents an intensity factor, it does not characterize the capacity of the system for oxidation or reduction. Measurements are made by potentiometric determination of electron activity (or intensity) with an inert indicator electrode and a suitable reference electrode. Electrodes made of platinum are most commonly used for Eh measurements. This protocol describes the method used to measure redox in the surface water and hydrosoil of a constructed wetland treatment system.

2.0 HEALTH AND SAFETY

Proper lab attire, including scrubs, lab coat, gloves and safety glasses must be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

Potassium ferrocyanide, $K_4Fe(CN)_6.3H_2O$
Potassium ferricyanide, $K_3Fe(CN)_6$
Potassium chloride, KCl

4.2 Equipment

pH or milivolt meter
Reference electrode
Oxidation-reduction indicator electrode
Beakers
Magnetic Stirrer

5.0 PROCEDURE

Prepare ZoBell's standard redox solution by adding 1.4080 g potassium ferrocyanide, 1.0975 g potassium ferricyanide, and 7.4555 g potassium chloride to 1000 mL of deionized water at 25^{o}C. These measurements must by as accurate as possible to result in a reliable solution. When stored in dark plastic bottles in a refrigerator, this solution is stable for several months.

Follow the manufacturer's instructions for using the pH/millivolt meter and in preparing electrodes for use. Immerse the reference electrode connected to the millivolt meter and the redox indicator electrode (platinum tip end) in the gently stirred, standard solution in a beaker. Connect the millivolt meter to the end of the indicator electrode opposite the platinum tip. Allow several minutes for electrode equilibration then record the reading to the nearest millivolt. If the reading is within $\pm$10mV from the theoretical redox standard value at 25^{o}C (+183 mV), record the reading. The indicator electrode is ready for placement in the hydrosoil. If the reading is not within $\pm$10mV, the indicator electrode must be re-made. Place the indicator electrode's platinum tip into the surface water or a specific hydrosoil depth making certain it is not near the plant roots. Allow the electrode to equilibrate for 24 hours prior to taking any readings. Connect the millivolt reader to the end of the indicator electrode opposite the platinum tip. Record the redox potential in mV. Repeat a second time by placing the reference electrode in another location in the hydrosoil. Successive readings that vary less than $\pm$10mV over 10 minutes are adequate for most purposes. Adjust the reading according to field corrections and electrode calibration corrections.

Example: The field redox measurement of a hydrosoil was -206mV. When the electrode was initially calibrated in the lab, the redox reading was +193mV, which is $\pm$10mV difference from the theoretical redox standard value of +183mV. The field redox measurement must be corrected for this difference by subtracting 10mV from -206mV. This gives a redox measurement of -216mV. The standard correction factor for field redox measurements for the millivolt reader is +240mV. Therefore, this correction factor is added to the redox measurement of -216mV to yield a final redox measurement of +24mV.

$$Eh_{system} = Eh_{observed} + Eh_{reference\ standard} - Eh_{reference\ observed} + Eh_{field\ correction}$$
$$Eh_{system} = -206\ mV + 183mV - 193mV + 240mV$$

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.

7.0 REFERENCES

Faulkner, S.P., Patrick, Jr., R.P., & Gambrell, W.H. (1989). Field techniques for meausuring wetland soil parameter. *Soil Science Society of America Journal, 53,* 883-890.

ZoBell, C.E. (1946). Studies on redox potential of marine sediments. *Bulletin of the American Association of Petroleum Geologists, 30,* 477-513.

METHOD FOR MEASURING ORGANIC MATTER CONTENT IN HYDOSOIL BY LOSS-ON-IGNITION METHOD

Kristen, N. Jurinko, Christina Blaszkiewicz

1.0 OBJECTIVE

Organic matter serves as sorption binding sites for selenium and an energy source for dissimilatory Se-reducing bacteria. Organic matter content can influence hydrosoil properties such as redox potential and pH of the hydrosoil, and contribute to selenium mobility. The Loss-On-Ignition method described is based on Heiri et al. (2001), which provides a reasonable estimate of the organic matter content in hydrosoil.

2.0 HEALTH AND SAFETY

Proper lab attire, including scrubs, lab coat, gloves and safety glasses must be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies
Porcelain crucibles (20 mL)

4.2 Equipment
Muffle furnace capable of $\pm 5^{o}$C temperature control
Analytical balance capable of weighing $\pm$0.1mg
Drying oven for sediment

5.0 PROCEDURE

1. Weigh empty crucible.
2. Add 1-3 g of wet hydrosoil to crucible. Dry hydrosoil at 105^{o}C in a drying oven for approximately 48 hours to a constant weight. Cool sample in crucible and weigh to 0.1mg.
3. Ignite samples in a muffle furnace at 550^{o}C for 4 hours. Cool crucibles and weigh with ignited sample to 0.1mg.

Calculations: The organic matter content is assumed to equal the LOI in most cases.

$LOI_{550} = ((DW_{105} - DW_{550})/DW_{105})*100$

where

LOI_{550} = the LOI at 550^oC (as a percentage)
DW_{105} = dry weight of the sample before organic matter combustion (g)
DW_{550} = dry weight of the sample after organic matter combustion at 550^oC (g)

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.

7.0 REFERENCES

Heiri, O., Lotter, A.F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonated content in sediments: Reproducibility and comparability of results. *Journal of Paleolimnology, 25*, 101-110.

METHOD FOR MEASURING pH IN HYDROSOIL

Kristen N. Jurinko, Christina Blaszkiewicz

1.0 OBJECTIVE

pH is an important controlling factor for transfer and transformation processes in CWTSs. For example, it affects speciation and mobility of selenium (Brookins, 1988). The hydrosoil pH method described below is based on Singh et al. (1998).

2.0 HEALTH AND SAFETY

Proper lab attire, including scrubs, lab coat, gloves and safety glasses must be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

De-aerated deionized water
50-mL polypropylene centrifuge tube
Pipettes
15-mL poly propylene centrifuge tubes

4.2 Equipment

Anaerobic chamber (98% N_2 (g)/2% H_2 (g) atmosphere)
Electronic pH meter
Accumet® liquid-filled pH/ATC epoxy body combination electrode (13-620-531: Fisher Scientific)
Orbit Shaker

4.3 Reagents

Standard buffer solutions of pH 7.0 and pH 4.0.

5.0 PROCEDURE

Note: All steps were performed in an anaerobic chamber to maintain chemical conditions of the hydrosoil.

5.1 Calibration

Prior to taking hydrosoil pH reading, calibrate the pH meter per manufacturer's instructions. Insert the glass electrode into a buffer solution of pH 7.0. Adjust the pH meter to read pH 7.0. Rinse the electrode with distilled water and then place it into a buffer solution of pH 4.0. The meter should read pH 4.0. Rinse the electrode with deionized water.

5.2 Sample preparation and pH measurement

Weigh 2 g of detritus and sediments in separate clean 50-mL centrifuge tubes. Add 10 mL de-aerated deionized water and mix with an Orbit Shaker for at least 12 hours (Singh et al., 1998). Once the pH meter has been calibrated, place the glass electrode into the soil suspension. Read the pH measurement. Remove the electrode from the soil suspension, rinse with deionized water, and place it in the buffer solution of pH 7.0.

Note: The glass electrode requires a hydrated layer on the outer glass wall to accurately measure the hydrogen ion activity. To prevent the impairment of the electrode, it is important not to allow the electrode to dry out. The glass electrode should be stored in a buffer solution of pH 7.0.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.

7.0 REFERENCES

Brookins, D.G. (1988). Eh-pH Diagrams for Geochemistry. Springer, Berlin., 176.

Singh, S.P., Tack, F.M., & Verloo, M.G. (1998). Heavy metal fractionation and extractability in dredged sediment derived surface soils. *Water, Air, and Soil Pollution, 102,* 313-328.

Appendix B

Standard Operating Procedures for Hydrosoil Selenium Analyses

The standard operating procedures used to measure selenium concentrations and geochemical fractions in detritus samples and sediment cores extracted from the pilot-scale constructed wetland treatment system designed to treat Se in impaired water are listed below and found on the pages indicated.

METHOD FOR MEASURING SELENIUM CONCENTRATION USING INDUCTIVELY COUPLED PLASMA MASS-SPECTROMETER (ICP-MS)

Peter Van Heest, Dr. Brian Powell, Christina Blaszkiewicz

1.0 OBJECTIVE

The purpose of this protocol is to measure total selenium concentration in hydrosoil samples.

2.0 HEALTH AND SAFETY

Proper lab attire, including scrubs, lab coat, gloves and safety glasses must be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

50-mL polypropylene centrifuge tubes
15-mL polypropylene centrifuge tubes
Pipettes
1-L plastic Nalgene bottle
Multi-element standards containing Ag, Au, Al, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Th, Tl, U, V, Zn, Cl, Ca, Fe, K, Mg, Na, P, S, and C
HNO_3, trace metal grade concentrated (67%) nitric acid
HF, trace metal grade concentrated (48%) hydrofluoric acid
HCl, trace metal grade concentrated (37%) hydrochloric acid
H_3BO_3, ACS grade boric acid crystals
Deionized (DI) water
Tuning Solution containing 10 ppb Li, In, U, Ce, and Be
Internal standard solution containing Re and Sc

4.2 Equipment

CEM Microwave Sample Preparation System (includes turntable, pressure sensing line)
Advanced Composite Vessel Accessory Set (1 control vessel, 11 sample vessels, 1 collection vessel)
Thermo Scientific X Series ICP-MS

5.0 PROCEDURE

5.1 Sample Preparation

Remove organic matter from sample using the Loss-On-Ignition method described in the SOP for the measurement of hydrosoil organic matter content.

5.2 Microwave Digestion

Weigh 0.5 g of sample from 12 samples and place into a control vessel and 11 sample vessels. The control vessel should contain the sample that had the greatest organic matter content. Add 10 mL of deionized water, 5 mL HNO_3, 4 mL of HF, and 1 mL of HCl to each vessel. Seal all vessels except the one to be used for pressure control. Seal the control vessel with a modified cap assembly. Place all vessels into the turntable. Connect the vent tubes from all vessels to the collection vessel (collects sample if it overflows during digestion). Place the turntable into the system. Connect the pressure sensing line attached to the microwave system to the control vessel. Digest samples for 20 minutes at 170^{o}C. Cool samples for a minimum of 5 minutes. Remove all vessels from system and add approximately 2 g of H_3BO_3 crystals. Mix samples well to dissolve the boric acid crystals. Transfer the solution to a 50-mL centrifuge tube.

5.3 ICP-MS Analysis

Sample was pipetted into a 15-mL centrifuge tube and diluted to 2% HNO_3 concentration by volumetric addition of deionized water. Se concentration was measured in accordance with EPA Method 200.8 (USEPA 1994). The Se concentration measured for each sample represents the total amount of Se (all species and forms) solubilized during this extraction procedure.

1. Add 30 mL trace-metal grade HNO_3 to 1 L DI water contained in I L plastic Nalgene bottle
2. Create standards ranging from 0.0005 to 100µg/L by dilution of Multi-element standard in 20% HNO_3
3. Calculate concentrations of each element in each standard
4. Verify that there is sufficient Argon Supply for ICP-MS
5. Turn on chiller
6. Open Plasma Lab program on desktop computer
7. Select “Create new experiment”
8. Select Rhenium and Scandium as reference elements
9. In internal standard tab enter the calculated concentrations for each element ion each internal standard
10. In sample list, enter the 9 standards followed by the samples
11. Enter a standard between every 5-10 samples
12. Enter last 4 standards after the last sample on list
13. Put both intakes into the Nalgene bottle containing 20% HNO_3
14. On menu select instrument then connect to auto-sampler

15. Wait two minutes then place both intakes into the 10 ppb tuning solution containing Li, In, U, Ce and Be
16. Set argon gas to level 5
17. Adjust major setting to obtain Se counts of about 10 cps. Setting will vary for each analysis.
18. Adjust nebulizer to obtain Ce/O ratio of 0.02 or less
19. Place intake into internal standard solution contain Re and Sc
20. Place sample intake into auto-sampler arm
21. Go to menu and select experiment. Press Queue then select Vacuum from pull-down menu and select Append.
22. The auto-sampler will run the program. When sampling is complete check that internal standard recoveries are within the 80% to 120% standard Quality Assurance/Quality Control (QA/QC) protocol for the nutrient

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

Internal standard recoveries must be within the 80% to 120% standard Quality Assurance/Quality Control (QA/QC) protocol for the instrument. All procedures are subject to review by the Quality Assurance Unit.

7.0 REFERENCES

CEM Corporation (1991). Microwave Sample Preparation Note: OS-14, Applications and Manual. CEM Corporation, Matthews, NC.

United States Environmental Protection Agency (USEPA) (1994). EPA Method 200.8. Determination of trace elements in water and wastes by inductively coupled plasma-mass spectrometry, Revision 5.4, Methods for the Determination of Metals in Environemental Samples-Supplement 1, EPA/600/R-94-111.

SEQUENTIAL EXTRACTION PROCEDURE FOR THE IDENTIFICATION OF SELENIUM GEOCHEMICAL FRACTIONS

Christina Blaszkiewicz, Dr. Brian Powell

1.0 OBJECTIVE

Sequential extraction procedures (SEPs) are chemical analyses that access metal fractionation and potential mobility. SEPs have been used to interpret geochemical forms of selenium in wetland hydrosoil including soluble, adsorbed, associated with organic matter, elemental, selenides, and residual Se. SEPs provide insight into potential Se mobility and Se accumulation biogeochemical processes in hydrosoil. This SEP was based on the procedures of Chao and Sanzolone (1989), Zhang and Moore (1996), and Wright *et al*. (2003) with some modifications, and the procedures of CEM (1991; Microwave sample preparation note: OS-14).

2.0 HEALTH AND SAFETY

Proper lab attire, including scrubs, lab coat, gloves and safety glasses must be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

50-mL plastic centrifuge tubes
15-mL plastic centrifuge tubes
Pipettes
Deionized water

4.2 Equipment

Drying oven (105^{o}C) with $\pm$5^{o}C temperature control
Thermo Scientific X Series ICP-MS
Centrifuge
Ultrasonic bath
Temperature control Orbit Shaker
Analytical balance capable of weighing $\pm$ 0.1 mg
Anaerobic chamber (98% N_2(g)/2% H_2(g) atmosphere)

4.3 Reagents

0.25M KCl, ACS grade potassium chloride

0.1M K_2HPO_4, ACS grade potassium hydrogen phosphate
0.1M NaOH, ACS grade sodium hydroxide
0.25M Na_2SO_3, ACS grade sodium sulfate
0.25M sulfite solution
5% NaOCl, sodium hyperchlorite
HNO3, trace metal grade concentrated (67%) nitric acid
HF, trace metal grade concentrated (48%) hydrofluoric acid
HCl, trace metal grade concentrated (37%) hydrochloric acid
H_3BO_3, ACS grade boric acid crystals

5.0 PROCEDURE

5.1 Sample Preparation

Store samples in an anaerobic chamber until needed to maintain conditions similar to those from which the sediment were taken.

Prepare subsamples by weighing approximately 0.5 g of wet hydrosoil sample and placing in a 50-mL polypropylene centrifuge tube.

*Note: All glassware should be soaked in 10% HNO_3 for 24 hours and rinsed with deionized water prior to use for sequential extraction.

5.2 Extractions

Successive extractions are to be carried out in the same centrifuge tube in order to minimize the risk of contamination and losses through handing. After each extraction: 1) the sample was centrifuged (10,000G for 15 min), 2) the supernatant was collected and pippetted into another centrifuge tube, 3) the sample was rinsed with 5mL of DDI water, 4) the sample was centrifuged a second time (10,000G for 15 min), and 5) the remaining supernatant was collected and pipetted into the supernatant centrifuge tube. Weigh tubes prior to adding sediment, after adding sediment sample, following the addition of each extractant, and after the removal of each supernatant to determine true extraction volumes.

Fraction 1: Soluble/Exchangeable

Add 5 mL of 0.25 M KCl to 0.5 g of hydrosoil sample in an anaerobic chamber. Agitate continuously using Orbit Shaker for 2 hours at room temperature (~25^{o}C).

Fraction 2: Adsorbed

Add 5 mL of 0.1 M K_2HPO_4 (pH 8.0) to the residue from Fraction 1 (portion of sample remaining in the centrifuge tube after the extraction of Fraction 1) and agitate on Orbit Shaker for 2 hours at room temperature (~25^{o}C).

Fraction 3: Organic Associated
Add 5 mL of 0.1 M NaOH to the residue from Fraction 2 and agitate on Orbit Shaker for 4 hours at room temperature (~25^{o}C).

Fraction 4: Elemental
Add 5 mL of 0.25 M Na_2SO_3 (pH 7.0) to the residue from Fraction 3 and agitate in ultrasonic bath for 4 hours. Rinse twice with 1 mL 0.25 M sulfite solution.

Fraction 5: Recalcitrant Organic/Selenides
Add 2 mL of 5% NaOCl (pH 9.5) to the residue from Fraction 4 and agitate continuously for 30 minutes at 90^{o}C. Repeat once.

Fraction 6: Residual
Label and weigh a drying boat for each sample taken through the sequential extraction process. Spray 5-8 mL of deionized water into each tube to remove the pellet at the bottom of the tube (residue from Fraction 5) and place in separate drying boats. Dry the residue at 105^{o}C until a constant weight is maintained. Place dry residues (samples) into separate acid digestion vessels. Add 10 mL deionized water, 5 mL HNO_3, 4 mL HF, and 1 mL HCl to the acid digestion vessels containing the residue from Fraction 5. Seal vessels and place into the turntable. Heat vessels to 170^{o}C for 20 minutes using microwave heating with an appropriate laboratory microwave.

Allow vessels to cool at least 5 minutes before removing from the microwave. Once the vessels have cooled, manually vent the open vessel and add 2 g H_3BO_3 crystals to the acid mixture. Mix gently to dissolve the boric acid crystals. Transfer solution into centrifuge tubes using a pipette.

5.3 ICP-MS Se Concentration Analysis
Acidify each sample to 2% HNO_3, concentration by volumetric addition of trace metal grade concentrated (67%) nitric acid or deionized water. Measure Se concentration associated with each fraction using and ICP-MS according to EPA 200.8 (USEPA, 1994) and detailed in the SOP for measuring selenium concentration using inductively coupled plasma mass-spectrometer (ICP-MS).

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.

7.0 REFERENCES

Chao, T.T. & Sanzolone, R.F. (1989). Fractionation of soil selenium by sequential partial dissolution. *Soil Science Society of America Journal,* 53, 385-392.

Wright, M.T., Parker, D.R., & Amrhein, C. (2003). Critical evaluation of the ability of sequential extraction procedures to quantify discrete forms of selenium in sediments and soil. *Environmental Science Technology*, 37, 4709-4716.

United States Environmental Protection Agency (USEPA) (1994). EPA Method 200.8. Determination of trace elements in water and wastes by inductively coupled plasma-mass spectrometry, Revision 5.4, Methods for the Determination of Metals in Environemental Samples-Supplement 1, EPA/600/R-94-111.

Zhang, Y. & Moore, J.N. (1996). Selenium fractionation and speciation in a wetland system. *Environmental Science & Technology, 30*, 2613-2619.

Appendix C

Standard Operating Procedures for Quantifying Selenium Reducing Microbe Colony Abundance

The standard operating procedure used to quantify Se-reducing microbes in the hydrosoil of pilot-scale constructed wetland treatment system cells designed to treat Se in impaired water are listed below and found on the pages indicated.

METHOD FOR QUANTIFYING SELENIUM REDUCING MICROBE COLONY ABUNDANCE

Peter Van Heest, Mike Spacil, Christina Blaszkiewicz

1.0 OBJECTIVE

The objective of this procedure is to determine the numbers of culturable selenium reducing microbe colony forming units (CFUs) present in 1 mL of water gathered from hydrosoil

2.0 HEALTH AND SAFETY

Proper lab attire, including scrubs, lab coat, gloves and safety glasses must be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies

15-mL sterile plastic centrifuge tubes
AquaSmart™
Simulated Water (DI water $MgSO_4$, $CaCl_2$, NaCl, Na_2SeO_3)
Beakers
Stir Bar
Erlenmeyer Flask
0.45-um gridded membrane filter
GasPak® packets
2-L plastic bottles
5-cm plastic sterile petri dish
Tweezers
Agar
DI Water

4.2 Equipment

Autoclave
Stir-plate/heater
Sterile vacuum filter assembly
Bunsen burner
Anaerobic Chamber

Balance

5.0 PROCEDURE

5.1 Formulation of Simulated Water

For 500 mL simulated water

1. Weigh 177.5 mg $MgSO_4$, 102.5 mg $CaCl_2$, 615 mg NaCl, and 0.11 mg Na_2SeO_3
2. Mix salts into 500 mL DI water in 500 mL Erlenmeyer flask with stir bar
3. Stir for 15 minutes until salts dissolve

5.2 Formulation of Agar

1. Add 7 g Agar to flask containing simulated water
2. Add 100 mg AquaSmart to simulated water
3. Cover flask with aluminum foil
4. Heat on stir plate at heat setting 5 for 15 minutes and stir
5. Fill two 2-L plastic bottles with DI water
6. Transfer flask and plastic bottles to autoclave and set autoclave for 120^{o}C for 15 minutes
7. Remove flask from autoclave and put on stir plate for 20 minutes
8. Remove bottles from autoclave and set aside covered
9. Pour agar solution from flask (approximately 8 mL) into 5-cm plastic sterile petri dishes and cover dishes

5.3 Sampling

1. Using sterile syringes and 15-mL plastic centrifuge tubes collect water from the hydrosoil interval

5.4 Culturing CFUs

1. Set up sterile vacuum filter assembly with 0.45-um gridded membrane filter under air filter hood
2. Add 50 mL of sterile DI water from plastic bottle to funnel
3. Pipette 0.003 mL of sample into the 50 mL of sterile water. Swirl water in funnel gently (this volume yields a countable number of microbe colonies (Spacil, 2010)
4. Filter water through sterile assembly
5. Using tweezers sterilized in flame of Bunsen burner transfer filter membrane from filter assembly to agar containing petri dishes
6. Place membrane face up on agar and cover
7. Repeat process with other samples using sterilized tweezers and vacuum assembly
8. Create 3 replicates of each sample

5.5 Incubation

1. Transfer petri dishes to sterile anaerobic chamber
2. Place 3 GasPak packets into chamber and seal chamber

3. Leave chamber at room temperature for 48 hours
4. After 48 hours loosen top of chamber to allow air to enter chamber while still covered
5. Let petri dishes remain in chamber for another 120 hours

5.6 Quantification

1. Remove petri dishes from chamber
2. Visually identify CFU based upon red to reddish-brown color
3. Count number of CFUs per petri dish
4. Divide number of CFUs in each dish by 0.003 to extrapolate the number of CFUs per mL of pore water from the hydrosoil

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.

7.0 REFERENCES

Spacil, M.M. (August 2010). Constructed wetland treatment systems for risk mitigation of energy derived waters. *Thesis* . Clemson, South Carolina: Graduate School of Clemson University.