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Removal of Arsenic from Simulated Groundwater Using a Pilot-Scale Constructed Wetland Treatment System

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REMOVAL OF ARSENIC FROM SIMULATED GROUNDWATER USING A PILOT-SCALE CONSTRUCTED WETLAND TREATMENT SYSTEM

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Hydrogeology

by
Jeffrey Paul Schwindaman
August 2013

Accepted by:
Dr. James W. Castle, Committee Chair
Dr. John H. Rodgers, Jr.
Dr. Eric H. Snider
ABSTRACT

A pilot-scale constructed wetland treatment system (CWTS) was designed and built to produce biogeochemical conditions that promoted targeted processes for removal of arsenic from simulated groundwater. Two series were designed to promote coprecipitation and sorption of arsenic with iron oxyhydroxides under oxidizing conditions, and two series were designed to promote precipitation of arsenic sulfide and coprecipitation of arsenic with iron sulfide under reducing conditions. The two major objectives were to (1) assess arsenic removal performance and (2) determine the fate and distribution of arsenic in each series. Results indicate that arsenic removal performance was greater in series designed to promote oxidizing conditions than in series designed to promote reducing conditions (mean removal extent of 64 and 108 µg L\(^{-1}\), respectively). Arsenic removal performance was significantly greater (α = 0.05) in the oxidizing series amended with zero-valent iron (ZVI) than in the other series, with removal extents, efficiencies, and rate coefficients ranging from 6-79 µg L\(^{-1}\), 40-95 %, and 0.13-0.77 d\(^{-1}\), respectively. The majority of inflow arsenic retained in the first reactor of each series partitioned to the sediment (88-99 %), while the remainder partitioned to *Typha latifolia*. A greater percentage of inflow arsenic was retained in the sediment of the first reactor of the two oxidizing series (20 and 13 %) than in the two reducing series (6 and 7 %). Addition of ZVI enhanced arsenic removal from the aqueous phase in both oxidizing series and reducing series and increased the percentage of inflow arsenic partitioned to sediment. A vertical concentration gradient developed over time in the sediment, with 74-85 % of sediment-bound arsenic accumulated in the upper 6 cm and the remaining
percentage below 6 cm. Results of this study demonstrate that a CWTS can be used successfully to decrease the concentration of arsenic in simulated groundwater to below the World Health Organization (WHO) drinking water quality guideline primarily by transferring arsenic from the aqueous phase to the sediment.
DEDICATION

I dedicate this thesis to my parents, Dale and Jill, my brothers, Darren and Greg, and my entire family for their endless love, encouragement, and support.
ACKNOWLEDGMENTS

I am grateful to my advisor Dr. James Castle for his guidance and motivation. I would also like to thank my committee members, Dr. John Rodgers Jr. and Dr. Eric Snider. Dr. Rodgers, thank you for teaching me “to say what I mean and to mean what I say”. I want to thank Dr. Brian Powell and his students for their help with equipment and for showing me how to multi-task. Thank you to Dr. Wayne Chao for help with methods and for always being a smiling face in the halls of Lehotsky. Lastly, I would like to thank my fellow EE&ES graduate students for their camaraderie, advice and friendship.
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CHAPTER ONE
INTRODUCTION

Arsenic contamination in groundwater occurs in several countries including Bangladesh, where approximately 1.5 million groundwater wells, used for drinking water and irrigation, contain arsenic concentrations in excess of 50 µg L$^{-1}$, affecting over 35 million people (Kinniburgh et al., 2002; Lièvremont et al., 2009; Rahman et al., 2006; Ryker, 2002). Chronic exposure to arsenic concentrations greater than 10 µg L$^{-1}$ can lead to negative health effects, including cardiovascular, pulmonary, nervous, and endocrine system disorders, skin lesions, and cancer (Hughes et al., 2009; WHO, 2011). A low-cost, low-maintenance approach is needed, especially in developing countries, to decrease the concentration of arsenic in drinking water. Several strategies exist for removal of arsenic from water, including precipitation, co-precipitation, sorption, ion exchange, and membrane filtration (Meng et al., 2001; USEPA, 2002), however, these strategies may be cost-prohibitive. Constructed wetland systems for treatment of arsenic-contaminated water may offer a low-cost, low maintenance alternative. Constructed wetland treatment systems (CWTSs) target specific biogeochemical processes (i.e. sorption, precipitation, co-precipitation, and volatilization) to remove constituents of concern (COCs) from the aqueous phase or transform COCs into less bioavailable forms (Dorman et al., 2009; Rodgers and Castle, 2008).

Arsenic is present naturally in a variety of geologic media, but is typically associated with sulfide mineral deposits or bound to iron oxyhydroxides (Henke, 2009;
Rahman et al., 2006). Arsenic can enter groundwater both by the oxidation of sulfide minerals and by the reductive dissolution of iron oxyhydroxides (Ford et al., 2006; Henke, 2009). Therefore, the hypothesis of this investigation is that arsenic can be removed from groundwater in a CWTS by precipitation of sulfide minerals and by sorption and co-precipitation of arsenic with iron oxyhydroxides. A CWTS can be designed to facilitate these processes by promoting the necessary biogeochemical conditions, including sediment oxidation-reduction (redox) potential, dissolved oxygen (DO) concentration, and pH (Dorman et al., 2009; Eggert et al., 2008; Spacil et al., 2011). Research presented in this thesis investigates arsenic removal performance and the fate and distribution of arsenic in a pilot-scale CWTS designed to target specific processes for arsenic removal. The two major objectives were to (1) assess the performance of a pilot-scale CWTS designed to decrease the concentration of arsenic in simulated Bangladesh groundwater, and (2) determine the fate and distribution of arsenic in a pilot-scale CWTS designed to target specific processes for arsenic removal. Two series were designed to promote co-precipitation and sorption of arsenic with iron oxyhydroxides under oxidizing conditions, and two series were designed to promote precipitation of arsenic with sulfide and co-precipitation of arsenic with iron sulfide under reducing conditions.

1. **Assess treatment performance of a pilot-scale CWTS designed to decrease the concentration of arsenic in simulated Bangladesh groundwater**

   The purpose of this study was to evaluate the performance of a CWTS for the removal of arsenic from simulated Bangladesh groundwater. The specific objectives of this study were to (1) design and construct a pilot-scale CWTS to compare the removal of
arsenic in oxidizing series with the removal of arsenic in reducing series, and (2) assess arsenic removal performance in each series by determining removal extents, efficiencies, and rates.

2. **Determine the fate and distribution of arsenic in a pilot-scale CWTS designed to target specific processes for arsenic removal**

The purpose of this study was to determine the fate and distribution of arsenic in a pilot-scale CWTS. The specific objectives of this study were to (1) measure biogeochemical conditions in a CWTS designed to target specific processes for the removal of arsenic from arsenic-contaminated water, (2) assess arsenic removal from the aqueous phase in each series by determining removal extents, efficiencies, and rates, and (3) determine the fate and distribution of arsenic in each treatment series.

3. **Thesis organization**

This thesis is organized into four chapters including an introduction (Chapter 1) and conclusions (Chapter 4). The two body chapters are written as independent manuscripts intended for submission to peer-reviewed scientific journals; therefore, some material is repeated in both chapters. The two body chapters are:

- Chapter 2: Design and Performance of a Pilot-Scale Constructed Wetland Treatment System for Arsenic Removal from Simulated Bangladesh Groundwater, prepared for submission to *Chemosphere*

- Chapter 3: Fate and Distribution of Arsenic in a Pilot-Scale Constructed Wetland Treatment System, prepared for submission to *Ecological Engineering*
Collectively, this research demonstrated the effective removal of arsenic from arsenic-contaminated water in a pilot-scale CWTS designed to target specific biogeochemical processes.
References


Design and Performance of a Pilot-Scale Constructed Wetland Treatment System for Arsenic Removal from Simulated Bangladesh Groundwater

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ABSTRACT

A pilot-scale constructed wetland treatment system (CWTS) was designed and built to produce biogeochemical conditions that promoted targeted processes for removal of arsenic from simulated Bangladesh groundwater. Two CWTS series were designed to promote co-precipitation and sorption of arsenic with iron oxyhydroxides under oxidizing conditions, and two series were designed to promote precipitation of arsenic sulfide and co-precipitation of arsenic with iron sulfide under reducing conditions. Results indicate that arsenic removal performance was greater in series designed to promote oxidizing conditions than in series designed to promote reducing conditions (mean removal extent of 64 and 108 µg L\(^{-1}\), respectively). The addition of zero-valent iron (ZVI) to oxidizing series and to reducing series enhanced arsenic removal (mean removal efficiency of 72 and 42 %, respectively) compared to unamended series (27 and 20 %, respectively). Arsenic removal performance was significantly greater (\(\alpha = 0.05\)) in the oxidizing series amended with ZVI than in the other series, with removal extents, efficiencies, and rate coefficients ranging from 6-79 µg L\(^{-1}\), 40-95 %, and 0.13-0.77 d\(^{-1}\), respectively. Results of this pilot-scale study demonstrated that a CWTS can decrease the concentration of arsenic in arsenic-contaminated water to below the World Health Organization (WHO) drinking water quality guideline of 10 µg L\(^{-1}\).
1. Introduction

The greatest extent of arsenic contamination in the world is in the Bengal Basin, where approximately 1.5 million groundwater wells, used for drinking water and irrigation, contain arsenic concentrations in excess of 50 µg L\(^{-1}\), affecting approximately 35 million people in Bangladesh (Kinniburgh et al., 2002; Smedley and Kinniburgh, 2002). Chronic exposure to arsenic concentrations greater than 10 µg L\(^{-1}\) can lead to negative health effects, including cardiovascular, pulmonary, nervous, and endocrine system disorders, skin lesions, and cancer (Hughes et al., 2009; WHO, 2011). A low-cost, low-maintenance approach is needed, especially in developing countries, to decrease the concentration of arsenic in drinking water. Several strategies exist for removal of arsenic from water, including precipitation, co-precipitation, sorption, ion exchange, and membrane filtration (Meng et al., 2001; USEPA, 2002), however, these strategies may be cost-prohibitive. Constructed wetland systems for treatment of arsenic-contaminated water may offer a low-cost, low maintenance alternative. Constructed wetland treatment systems (CWTSs) target specific biogeochemical processes (i.e. sorption, precipitation, co-precipitation, and volatilization) to remove constituents of concern (COCs) from the aqueous phase or transform COCs into less bioavailable forms (Dorman et al., 2009; Rodgers and Castle, 2008).

Arsenic is present naturally in a variety of geologic media, but is typically associated with sulfide mineral deposits or bound to iron oxyhydroxides (Henke, 2009; Rahman et al., 2006). Arsenic commonly exists in natural waters as arsenite, [As (III)], or as arsenate, [As (V)] (Francesconi and Kuehnert, 2002). Arsenite is the predominant
species of arsenic in groundwater and is more toxic to humans than arsenate (Sharma and Sohn, 2009). Under reducing conditions and near-neutral pH, arsenite most often occurs as H$_3$AsO$_3$. Arsenite can bind with sulfide under reducing conditions to form the insoluble minerals orpiment (As$_2$S$_3$), realgar (α-As$_4$S$_4$), and arsenopyrite (FeAsS) (Cheng et al., 2009; Lizama et al., 2011). Under oxidizing conditions and near-neutral pH, arsenate most often occurs as H$_2$AsO$_4^-$ and HAsO$_4^{2-}$ (Henke and Hutchison, 2009). The form of arsenate as an oxyanion allows for electrostatic interaction with constituents in soil and sediment, such as iron oxyhydroxides. Arsenic can enter groundwater both by the oxidation of sulfide minerals and by the reductive dissolution of iron oxyhydroxides (Ford et al., 2006; Henke, 2009). Therefore, the hypothesis of this investigation is that arsenic can be removed from groundwater in a CWTS by precipitation of sulfide minerals and by sorption and co-precipitation of arsenic with iron oxyhydroxides. A CWTS can be designed to facilitate these processes by promoting the necessary biogeochemical conditions, including sediment oxidation-reduction (redox) potential, dissolved oxygen (DO) concentration, and pH (Dorman et al., 2009; Eggert et al., 2008; Spacil et al., 2011). The objectives of this study were to (1) design and construct a pilot-scale CWTS to compare the removal of arsenic in oxidizing series with the removal of arsenic in reducing series, and (2) assess arsenic removal performance in each series by determining removal extents, efficiencies, and rates. Two series were designed to promote co-precipitation and sorption of arsenic with iron oxyhydroxides under oxidizing conditions, and two series were designed to promote precipitation of arsenic with sulfide and co-
precipitation of arsenic with iron sulfide under reducing conditions. Zero-valent iron (ZVI) was added to one oxidizing series and to one reducing series.

2. Materials and methods

2.1 Design and construction of pilot-scale CWTS

Targeted biogeochemical processes for arsenic removal and the conditions that promote these processes were determined from a review of published literature (Table 1). The pilot-scale CWTS was specifically designed and constructed to produce these ranges of conditions.

Water for the CWTS was simulated based on the composition of actual arsenic-contaminated groundwater from the Lakshmipur District in southern Bangladesh (Table 2) (BGS, 2001). Use of simulated water provides greater experimental control over water characteristics and eliminates the economic burden of acquiring, shipping, and storing large volumes of actual groundwater. The concentration of chemical constituents in the Lakshmipur District were within the range of other villages in central and southern Bangladesh and were therefore considered to be representative of arsenic-contaminated groundwater from the shallow Holocene aquifer, which is the source of most drinking water in Bangladesh (BGS, 2001).

Simulated Bangladesh groundwater was created by mixing chemical constituents with municipal water from Clemson, SC, in a 5678 L (1500 gal) detention basin. Arsenic was added as arsenic (III) oxide (As$_2$O$_3$ $\geq$ 99 \%) (Sigma-Aldrich) at 236 µg L$^{-1}$ and arsenic (V) oxide (As$_2$O$_5$ $\geq$ 99 \%) (Sigma-Aldrich) at 518 µg L$^{-1}$. Major cations and
anions (Ca$^{2+}$, Mg$^{2+}$, K$^+$, Na$^+$, Cl$^-$, HCO$_3^-$, PO$_4^{2-}$, SO$_4^{2-}$, SiO$_3^{2-}$) were added as calcium chloride (CaCl$_2$) (North American Salt Company) at 140 mg L$^{-1}$, magnesium chloride (MgCl$_2$) (North American Salt Company) at 170 mg L$^{-1}$, potassium chloride (KCl) (Diamond Crystal) at 24 mg L$^{-1}$, sodium bicarbonate (NaHCO$_3$) (Eastern Minerals, Inc.) at varying concentrations, magnesium sulfate (MgSO$_4$) (Aaron Industries) at 15 mg L$^{-1}$, Iron (II) sulfate (FeSO$_4$) (Sigma-Aldrich) at 15 mg L$^{-1}$, sodium metasilicate (Na$_2$SiO$_3$) (Fisher Scientific) at 60 mg L$^{-1}$, and potassium phosphate (K$_2$PO$_4$) (Fisher Scientific) at 1.4 mg L$^{-1}$. To investigate the effects of competitive anions on arsenic removal in a CWTS, the concentration of bicarbonate was adjusted from ~300 mg L$^{-1}$ to ~30 mg L$^{-1}$.

2.2 Measurement of CWTS conditions and performance

Dissolved oxygen (DO) concentration, pH, and sediment oxidation-reduction (redox) potential were measured bi-weekly in each reactor (Figure 1). DO concentration and pH were measured using YSI® (model 55) and Orion® (model A325) field instruments, respectively. Alkalinity was measured using standard methods (APHA, 2005) and converted to bicarbonate concentration using the carbonate-speciation method (Rounds, 2006). To measure sediment redox potential, one platinum-tipped electrode was installed approximately 2 cm into the sediment in the front (inflow area) and one in the back (outflow area) of each reactor. Electrodes remained in-situ for the duration of the experiment. Sediment redox potential was measured using a GDT-11 Multi-meter connected to in-situ electrodes and an Accumet® calomel reference electrode (Faulkner
et al., 1989). Statistical differences were determined by ANOVA and post-hoc t-tests with \( \alpha = 0.05 \).

One sample from the upper 2 cm of sediment was collected from the front, one from the middle, and one from the back of the first reactor of each series on day 1 of the experiment (8/31/12), day 80 (11/19/12), day 126 (1/4/13), and day 174 (2/21/13). Samples were scooped with an acid-washed metal spatula into a 50 mL centrifuge tube and sealed underwater. The three samples from each reactor were composited and analyzed for acid volatile sulfide (AVS) using a modified diffusion method (Leonard et al., 1996). AVS is defined as the sulfide extracted from sediment by 1-N HCL (Di Toro et al., 1992; Leonard et al., 1996) and interpreted as the reactive fraction of sulfide available to bind metals (Di Toro et al., 1992; Keon et al., 2001; Wilkin and Ford, 2002). Sulfide was measured using an ion-selective electrode to determine the AVS concentration.

Aqueous samples were collected bi-weekly over 14 sampling periods (8/31/12 to 3/15/13) from each treatment reactor sequentially according to the hydraulic retention time (HRT; 24 h per reactor). For example, samples of inflow water entering the first reactor were collected at \( t = 0 \) h, samples of outflow water from the first reactor were collected at \( t = 24 \) h, from the second reactor at \( t = 48 \) h, from the third reactor at \( t = 72 \) h, and from the fourth reactor at \( t = 96 \) h. In this way, a single volume of water was theoretically sampled as it passed through a series. Samples were collected in acid-washed 50 mL polypropylene centrifuge tubes from sampling ports between each reactor. 25 mL of sample was centrifuged for 20 minutes at 8000 rpm. 1 mL of supernatant was
transferred gravimetrically to an acid-washed 15 mL polypropylene centrifuge tube and brought to a volume of 10 mL with 2% trace metal grade nitric acid solution. Samples were analyzed for arsenic using inductively-coupled plasma mass-spectrometry (ICP-MS) (Thermo Scientific X Series) according to EPA Method 200.8 (USEPA, 1994). Gallium and Yttrium internal standard recoveries were within 80 to 120% according to instrument quality assurance/quality control protocol.

Treatment performance of each series was assessed in terms of removal extent, removal efficiency, and removal rate coefficient. Removal extent is defined as the concentration of arsenic in the outflow (µg L⁻¹). Removal efficiencies were calculated using Eq. (1):

\[
\text{removal efficiency (\%)} = \frac{[C]_0 - [C]}{[C]_0} \times 100
\]

(1)

where \([C]_0\) is inflow concentration (µg L⁻¹) and \([C]\) is outflow concentration (µg L⁻¹). Removal rate coefficient (k, day⁻¹) was calculated using Eq. (2):

\[
k = \frac{-\ln([C]/[C]_0)}{HRT}
\]

(2)

where \(HRT\) is the nominal hydraulic retention time (i.e. the time between sampling inflow and outflow). First-order rate kinetics, assumed in Eq. (2), are often used to model removal of COCs in a CWTS (Eggert et al., 2008; Horner et al., 2012; Lizama et al., 2011; Wong et al., 2006). By rearranging Eq. (2), the theoretical HRT necessary to decrease an initial concentration of arsenic to a specific final concentration can be estimated based on calculated removal rate coefficients using Eq. (3):

\[
HRT = \frac{-\ln([C]/[C]_0)}{k}
\]

(3)
A conservative estimate of the aerial extent needed to treat water at a specific rate can be made by using the largest HRT from Eq. (3) in Eq. (4):

\[ A = \frac{Q \cdot HRT}{d} \tag{4} \]

where \( A \) is the aerial extent (m\(^2\)), \( Q \) is the flow rate of water through the CWTS (L day\(^{-1}\)), and \( d \) is water depth (m).

3. Results

3.1 Design and construction of pilot-scale CWTS

Processes identified for removal of arsenic are sorption and co-precipitation of arsenic with iron oxyhydroxides, precipitation of arsenic sulfide, and co-precipitation of arsenic with iron sulfide. Sorption and co-precipitation of arsenic with iron oxyhydroxides occurs under oxidizing conditions (sediment redox potential > -50 mV), high DO concentration (≥ 2 mg L\(^{-1}\)), and near-neutral pH (4-9), whereas precipitation of arsenic sulfide and co-precipitation of arsenic with iron sulfide occur under reducing conditions (sediment redox potential from -50 to -250 mV), low DO concentration (≤ 2 mg L\(^{-1}\)), and near-neutral pH (5-8) (Table 1; Buddhawong et al., 2005; Cheng et al., 2009; Lizama et al., 2011; Rahman et al., 2011).

Four pilot-scale CWTS series, specifically designed to promote identified processes for arsenic removal, were built and operated in a climate-controlled greenhouse in Clemson, SC (Figure 1). Two series were designed to promote oxidizing conditions, and two series were designed to promote reducing conditions. Each series consisted of four treatment reactors, with each reactor contained in a 265 L (70 gal) Rubbermaid®
utility tank (92 cm long by 74 cm wide by 61 cm deep). Each reactor, with the exception of the third reactor in each of the two oxidizing series, contained (1) a 30-cm thickness of river sand from 18-Mile Creek in Clemson, SC, (2) water at a depth of 25 cm, and (3) approximately 20 Typha latifolia plants harvested from an aquaculture pond in Clemson, SC. The third reactor in each of the two oxidizing series contained a 50-cm thickness of approximately 3-cm diameter granitic gravel, and water at a depth of 5 cm. In the two reducing series, the upper 5 cm of sediment in each reactor was amended with pelletized gypsum (1 % v/v) as a source of sulfate for dissimilatory sulfate reduction and with hay and shredded hardwood mulch (5 % v/v) to provide a nutrient source for sulfate-reducing bacteria (SRB). Water was transferred from the detention basin into the first reactor of each series by a piston pump (FMI® QG400) at a flow rate of 128 mL min⁻¹, resulting in a nominal hydraulic retention time (HRT) of 24 h per reactor or 96 h per series. Reactors were connected by PVC pipe fittings located at 4 cm below the top of each reactor. Reactors were arranged with decreasing elevation from the first to fourth reactor in each series to induce gravity flow.

Addition of ZVI to oxidizing environments has been shown in bench-scale batch equilibrium experiments to remove > 90% of total arsenic from water due to rapid coprecipitation with iron oxyhydroxides (Farrell et al., 2001; Kanel et al., 2005; Li et al., 2011; Manning et al., 2002; Su, 2007), while addition of ZVI to reducing environments has been shown to promote dissimilatory sulfate reduction (Karri et al., 2005). Therefore, to investigate the effects of ZVI on the performance of a CWTS, one oxidizing and one reducing series were amended with ZVI (Peerless Metal Powders & Abrasive, Detroit,
MI) by distributing 20 g per reactor by hand into the water column approximately once every 14 days.

3.2 CWTS conditions and performance

Sediment redox potential was within the targeted range (> -50 mV for oxidizing series; -50 to -250 mV for reducing series) in 63, 80, 55, and 64 % of measurements for series 1, 2, 3, and 4, respectively (n = 56) (Table 3). Mean sediment redox potential was lower in reducing series 3 and 4 (-77 and -79 mV, respectively) compared to oxidizing series 1 and 2 (45 and 51 mV, respectively). In a previous bench-scale batch reactor study of arsenic and ZVI (Su and Puls, 2001a), the addition of Peerless ZVI resulted in an initial decrease in redox potential followed by steady-state positive values. In the current study, the addition of ZVI had no significant effect (α = 0.05) on the sediment redox potential of two of the four reactors in each series to which ZVI was added. For both oxidizing and reducing series, the redox potential in reactor 1 and reactor 2 amended with ZVI was significantly lower than the unamended reactor 1 and reactor 2 (p = 1.7 x 10^{-3} and 9.0 x 10^{-4}, respectively for oxidizing series, and 3.7 x 10^{-7} and 5.1 x 10^{-5}, respectively for reducing series). Dissolved oxygen concentration was within the targeted range (≥ 2 mg L^{-1} for oxidizing series; ≤ 2 mg L^{-1} for reducing series) in 100, 98, 70, and 66 % of measurements for series 1, 2, 3, and 4, respectively (n = 56) (Table 4). Mean DO concentration was less in reducing series 3 and 4 (2.0 and 1.8 mg L^{-1}, respectively), compared to oxidizing series 1 and 2 (6.6 and 7.4 mg L^{-1}, respectively). Lower mean DO concentration and sediment redox potential in series 3 and 4 compared to series 1 and 2 are attributed to the consumption of oxygen by aerobic microorganisms during the
biodegradation of organic matter in the reducing series. Measured pH was within the targeted range (4-9 for oxidizing series; 5-8 for reducing series) in 93, 82, 89, and 93% of samples for series 1, 2, 3, and 4, respectively (Table 5).

In oxidizing series 1 and 2, mean sediment redox potential (344 and 227 mV, respectively) and mean DO concentration (9.3 and 11.5 mg L⁻¹, respectively) were significantly greater (α = 0.05) in the third oxidizing reactor than in any other reactor in the series (range from -78 to 38 mV and 5.6 to 6.3 mg L⁻¹). This is attributed to lower organic matter content in the substrate of the third reactor of each oxidizing series (unplanted granitic gravel) compared to other reactors (river sand planted with *Typha latifolia*) and to greater atmospheric oxygen diffusion as the result of shallower water depth in the third reactor of each oxidizing series (5 cm) than in the other reactors (25 cm). pH values in the planted reactors of series 1 and 2 (range of 6.9-8.4 and 6.9-8.7, respectively) were less than in the unplanted third reactor of these series (7.1-9.6 and 7.7-9.9, respectively). This is attributed to organic acids in planted reactors lowering the pH compared to unplanted reactors.

Throughout the experiment, AVS concentrations were greater by approximately two orders of magnitude in reducing series 3 and 4 (100-643 mg L⁻¹) than in oxidizing series 1 and 2 (0-8 mg L⁻¹) (Figure 2). Greater AVS concentrations in reducing series compared to oxidizing series are attributed to dissimilatory sulfate reduction in the reducing series. AVS concentrations in the reducing series amended with ZVI (range from 101-643 mg L⁻¹) were greater than in the unamended reducing series (100-234 mg
L\(^{-1}\)), which is attributed to the ZVI acting as an electron donor for dissimilatory sulfate reduction (Karri et al., 2005).

Concentrations of arsenic decreased between the inflow and outflow in all pilot-scale CWTS series with the exception of series 4 during the 8/31/12 sampling period, series 2 and 3 during the 2/1/13 sampling period, series 2 during the 2/15/13 sampling period, and series 3 during the 3/15/13 sampling period (Figure 3). Arsenic removal performance was significantly greater (α = 0.05) in the oxidizing series amended with ZVI than in any other series, with removal extents and efficiencies ranging from 6-79 µg L\(^{-1}\) and 40-95 %, respectively, compared to 49-162 µg L\(^{-1}\) and 0-79 %, respectively, in the oxidizing series not amended with ZVI (Table 6). Addition of ZVI significantly improved the removal extent and efficiency of oxidizing series (p = 1.7 x 10\(^{-6}\) and 1.2 x 10\(^{-7}\), respectively) and reducing series (p = 3.9 x 10\(^{-3}\) and 1.7 x 10\(^{-2}\), respectively).

Outflow concentrations of arsenic were less than the WHO drinking water quality guideline of 10 µg L\(^{-1}\) in the oxidizing series amended with ZVI during the 10/28/12, 11/13/12, and 11/29/12 sampling periods. Mean removal extent and removal efficiency were 89 µg L\(^{-1}\) and 42 %, respectively, in the reducing series amended with ZVI compared to 128 µg L\(^{-1}\) and 20 %, respectively, in the reducing series not amended with ZVI. The removal efficiency of series 1, 2, 3, and 4 ranged from 70-95 , 20-49 , 3-36 , and 31-69 % , respectively, during the period of low sodium bicarbonate loading (mean bicarbonate concentration ranged from 39-44 mg/L), compared to removal efficiencies of 44-89 , 0-79, 0-76 and 0-54 %, respectively, during periods of high sodium bicarbonate loading (mean bicarbonate concentration ranged from 278-294 mg/L).
Removal rate coefficients in oxidizing series 1 and 2, ranging from 0.13-0.77 d\(^{-1}\) and 0.00-0.39 d\(^{-1}\), respectively, were greater than removal rate coefficients in reducing series 3 and 4, ranging from 0.00-0.36 d\(^{-1}\) and 0.00-0.29 d\(^{-1}\), respectively. In the current pilot-scale study, the removal rate coefficient of the oxidizing series amended with ZVI (0.13-0.77 d\(^{-1}\)) was within the range of removal rate coefficients from previous bench-scale batch reactor studies with arsenic and ZVI under oxidizing conditions (Bang et al., 2005; Su and Puls, 2001b), in which pseudo-first-order rate coefficients (\(k = -d[As]/dt\)) ranged from 0.09-0.84 d\(^{-1}\) depending on pH, type of ZVI, and speciation of arsenic. Using the first-order rate coefficients calculated in this experiment, the calculated HRT necessary to decrease the concentration of arsenic from 200 to 10 µg L\(^{-1}\) ranged from 4-23, 8-150, 8-300, and 10-300 d for series 1, 2, 3, and 4, respectively.

4. Discussion

Results of this pilot-scale study demonstrate that a CWTS can be designed and built to produce conditions that promote targeted processes for removal of arsenic from water. In both oxidizing series, sediment redox potential and DO concentration were favorable for sorption and co-precipitation of arsenic with iron oxyhydroxides, but not for dissimilatory sulfate reduction. In both reducing series, sediment redox potential and DO concentration were favorable for dissimilatory sulfate reduction, but not for co-precipitation of arsenic with iron oxyhydroxides.

In this study, arsenic removal under oxidizing conditions was more effective than arsenic removal under reducing conditions (mean removal extent of 64 and 108 µg L\(^{-1}\), respectively.
respectively). First-order arsenic removal rate coefficients in the oxidizing series amended with ZVI (ranging from 0.13-0.77 d\(^{-1}\)) were consistent with the rapid removal associated with sorption and co-precipitation of arsenic to iron oxyhydroxides observed in previous bench-scale batch reactor studies (Bang et al., 2005; Lackovic et al., 2000; Su and Puls, 2001a). In reducing series, the targeted arsenic removal processes were precipitation of arsenic sulfide and co-precipitation of arsenic with iron sulfide. Previous research suggested that reducing wetland environments are most often undersaturated with respect to the arsenic sulfide minerals, orpiment and realgar, and that co-precipitation of arsenic with iron sulfide is more likely than direct precipitation of arsenic sulfide (Gammons and Frandsen, 2001; Kirk et al., 2010; Wilkin et al., 2003). In addition, arsenic removal during iron sulfide formation is associated primarily with pyrite formation rather than the formation of the initial iron sulfide phases mackinawite and greigite (Kirk et al., 2010; Wilkin and Ford, 2006). The rate of pyrite formation in reducing sediment of natural wetlands varies but generally occurs on a scale of years to tens of years (Wilkin and Ford, 2006), much longer than the HRT of a CWTS. Biogeochemical removal processes require sufficient contact time between arsenic in water and components of a CWTS such as the sediment and plants; therefore, HRT is an important factor affecting removal performance (Kadlec and Wallace, 2009). While all series were theoretically capable of achieving the performance goal of 10 µg L\(^{-1}\) based on calculated first-order rate coefficients, series 1 required the smallest HRT to achieve that goal (4-23 d).
Promotion of AVS production in reducing series 3 and series 4 is attributed to biogeochemical conditions favorable for dissimilatory sulfate reduction and to gypsum and organic matter amendments to the sediment. Pelletized gypsum provided a source of sulfate for dissimilatory sulfate reduction, while shredded hardwood mulch and hay increased the organic matter content of the upper portion of the sediment. Organic matter in sediment can (1) increase the sediment oxygen demand, which lowers the DO concentration and sediment redox potential, (2) serve as an electron-donor for sulfate reducing bacteria, and (3) provide a substrate to which microbes can attach.

Addition of ZVI significantly improved the performance of both oxidizing and reducing series. In oxidizing series, ZVI provided a source of iron for the formation of iron oxyhydroxides, while in reducing series, ZVI provided not only a source of iron for sorption and co-precipitation, but also served as an electron-donor for dissimilatory sulfate reduction. Results of this research indicate that ZVI is an important addition to a CWTS designed to treat arsenic-contaminated groundwater. ZVI in the form of cast-iron filings is a waste product of many industrial processes, and therefore can typically be obtained at a low cost.

Dissolved anions such as bicarbonate, phosphate, sulfate, and silicate have been shown to negatively affect sorption and ion exchange processes by competing with arsenic for binding sites (Henke, 2009; Leupin et al., 2005; Stollenwerk, 2002; USEPA, 2002). Therefore, it was hypothesized that high bicarbonate loading would result in competition of anions with soluble arsenic, primarily arsenate (H₂AsO₄⁻), for iron oxyhydroxide sorption sites. No negative effect of bicarbonate loading on arsenic
removal performance was observed during the experiment, however. Results of this study indicate that a CWTS can effectively treat arsenic in the presence of competitive anions.

Results demonstrate that a CWTS can decrease the concentration of arsenic in simulated Bangladesh groundwater to below the WHO drinking water quality guideline of 10 µg L$^{-1}$. Therefore, CWTSs could be an appropriate option for the effective treatment of arsenic-contaminated water in rural villages in Bangladesh. Components of the pilot-scale system used in this study could be adapted for implementation in a full-scale CWTS. For example, existing hand-pump tube wells in Bangladesh could be used to deliver arsenic-contaminated groundwater directly to an in-ground detention basin, while the natural topography could be utilized to induce gravity flow of water through a CWTS. Based on calculated arsenic removal rate coefficients from this pilot-scale study, it is estimated that daily water requirements (7.5 L per person; WHO, 2011) can be met for 1000 people using a CWTS with an aerial extent of 700 m$^2$ (0.17 acres).

5. Conclusions

This study represents a step in the process of developing a low-cost, passive, long-term solution to arsenic contamination of groundwater in Bangladesh. Results demonstrate that a pilot-scale CWTS can be designed and built to produce biogeochemical conditions that promote processes for the removal of arsenic from simulated Bangladesh groundwater to below the WHO drinking water quality guideline of 10 µg L$^{-1}$. In this study, arsenic removal in the CWTSs was more effective under oxidizing conditions than under reducing conditions (mean removal extent of 64 and 108
µg L\(^{-1}\), respectively). The addition of ZVI enhanced arsenic removal performance in oxidizing series and in reducing series (mean removal efficiency of 72 and 42 %, respectively) compared to unamended series (27 and 20 %, respectively). The design of a full-scale CWTS for the treatment of arsenic-contaminated water would benefit from the incorporation of features from this pilot-scale study including oxidizing conditions and amendment with ZVI. Results indicate that a CWTS is suitable for the treatment of arsenic-contaminated water containing competitive anions.
References


List of Figure Captions

Figure 1. Schematic diagram of pilot-scale constructed wetlands for treatment of arsenic-contaminated water. Series 1 and 2 were designed to promote oxidizing conditions, and series 3 and 4 were designed to promote reducing conditions. Series 1 and 4 were amended biweekly with 20 g ZVI per reactor. To promote a sediment redox potential > -50 mV, the third reactor in each of the two oxidizing series was unplanted and contained a 50-cm thickness of granitic gravel.

Figure 2. AVS concentration in the first reactor of each series. AVS concentrations in reducing series 3 and 4 are approximately two orders of magnitude greater than in oxidizing series 1 and 2. AVS concentration in the reducing series amended with ZVI (series 4) was greater than in the unamended series (series 3) during the 11/19/12, 1/4/13, and 2/21/13 sampling periods. Error bars represent standard deviation of three replicates.

Figure 3. Inflow concentration (white bars) and outflow concentration (black bars) of arsenic in (A) series 1, (B) series 2, (C) series 3, and (D) series 4. The inflow concentrations are similar in all series, whereas the outflow concentration is lower in series 1 than all other series.
Figure 1.
Figure 2.
Figure 3.
Table 1. Targeted biogeochemical conditions to promote processes for arsenic removal from simulated Bangladesh groundwater in pilot-scale constructed wetland treatment system.

<table>
<thead>
<tr>
<th>Removal process</th>
<th>Sediment Redox potential (mV)</th>
<th>Dissolved Oxygen (mg L(^{-1}))</th>
<th>pH (S.U.)</th>
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</thead>
<tbody>
<tr>
<td>Co-precipitation and sorption with Fe-oxyhydroxides</td>
<td>Oxidizing (^{a})</td>
<td>≥ 2</td>
<td>4.9 (^{b})</td>
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<tr>
<td>Precipitation of As-S and co-precipitation of As with Fe-S</td>
<td>Eh &gt;-50</td>
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<td></td>
<td>Reducing (^{d})</td>
<td>≤ 2</td>
<td>5.8 (^{c})</td>
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<td></td>
<td>Eh -50 to -250</td>
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\(^{a}\) Buddhawong et al. (2005)  
\(^{b}\) Cheng et al. (2009)  
\(^{c}\) Lizama et al. (2011)  
\(^{d}\) Rahman et al. (2011)
Table 2. Chemical characteristics of actual Bangladesh groundwater and simulated Bangladesh groundwater (All units in mg L\(^{-1}\) unless noted).

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Bangladesh groundwater(^a)</th>
<th>Simulated Bangladesh groundwater(^b)</th>
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<tbody>
<tr>
<td>As (_\text{Total})</td>
<td>0.16</td>
<td>0.30</td>
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<tr>
<td>As (_{\text{III}})</td>
<td>0.09</td>
<td>0.18</td>
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<tr>
<td>As (_{\text{V}})</td>
<td>0.07</td>
<td>0.12</td>
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<tr>
<td>Ca</td>
<td>55</td>
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<td>Mg</td>
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<td>Na</td>
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<tr>
<td>K</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>HCO(_3)</td>
<td>220</td>
<td>30-300</td>
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<tr>
<td>Cl</td>
<td>220</td>
<td>220</td>
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<tr>
<td>SO(_4)</td>
<td>18</td>
<td>18</td>
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<tr>
<td>Si</td>
<td>15</td>
<td>15</td>
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<tr>
<td>P</td>
<td>1.2</td>
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<tr>
<td>Fe</td>
<td>3.0</td>
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<tr>
<td>pH (S.U.)</td>
<td>7.1</td>
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<tr>
<td>Conductivity (µS/cm)</td>
<td>890</td>
<td>890</td>
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\(^a\) Values from shallow depth (<150 m) wells in the Lakshmipur District (n=59) (BGS, 2001)

\(^b\) Targeted values
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<tr>
<th>6.9</th>
<th>3.2</th>
<th>1.1</th>
<th>0.6</th>
<th>0.3</th>
<th>0.1</th>
<th>2.1</th>
<th>4.3</th>
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<td>0.1</td>
<td>0.3</td>
<td>0.5</td>
<td>0.7</td>
<td>0.9</td>
<td>1.3</td>
<td>1.7</td>
<td>2.2</td>
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<tr>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
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Table 3: Measured sediment redox potential (mv) for each sampling date. Values outside expected range are indicated by (†).
Table 4. Measured dissolved oxygen concentration (mg L⁻¹) for each sampling date. Values outside range are indicated by (•).
| 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 |
|-----|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 |

Table 5: Measured pH (S.U.) for each sampling date. Values outside targeted range are indicated by (*).
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<th></th>
<th>Series 1</th>
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<th>Series 3</th>
<th>Series 4</th>
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Fate and Distribution of Arsenic in a Pilot-Scale Constructed Wetland Treatment System

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ABSTRACT

The fate and distribution of arsenic was determined in a pilot-scale constructed wetland treatment system (CWTS) designed to promote specific processes for arsenic removal. Two CWTS series were designed to promote co-precipitation and sorption of arsenic with iron oxyhydroxides under oxidizing conditions, and two series were designed to promote precipitation of arsenic with sulfide and co-precipitation of arsenic with iron sulfide under reducing conditions. Conditions in the CWTS were within the range of targeted processes for arsenic removal. Arsenic removal performance was significantly greater ($\alpha = 0.05$) in the oxidizing series amended with zero-valent iron (ZVI) than in the other series, with removal extents, efficiencies, and rate coefficients ranging from 6-79 µg L$^{-1}$, 40-95 %, and 0.13-0.77 d$^{-1}$, respectively. The majority of inflow arsenic retained in the first reactor of each series partitioned to the sediment (88-99 %), while the remainder partitioned to Typha latifolia. A greater percentage of inflow arsenic was retained in the sediment of the first reactor of the two oxidizing series (20 and 13 %) than in the first reactor of the two reducing series (6 and 7 %). The addition of ZVI enhanced arsenic removal from the aqueous phase in both oxidizing series and reducing series and increased the percentage of inflow arsenic partitioned to sediment. A vertical concentration gradient developed over time in the sediment, with 74-85 % of sediment-bound arsenic accumulated in the upper 6 cm and the remaining percentage below 6 cm. Results of this study demonstrate that a CWTS can decrease the concentration of arsenic in simulated groundwater to below the World Health
Organization (WHO) drinking water quality guideline of 10 µg L$^{-1}$ primarily by transferring arsenic from the aqueous phase to the sediment.
1. Introduction

Constructed wetland treatment systems (CWTSs) may offer a low-cost, low-maintenance approach for treating arsenic-contaminated water. CWTSs target specific biogeochemical processes (i.e. sorption, precipitation, co-precipitation, and volatilization) to remove constituents of concern (COCs) from the aqueous phase or transform COCs into less bioavailable forms (Dorman et al., 2009; Rodgers and Castle, 2008).

Arsenic commonly exists in natural waters as arsenite, [As (III)], or as arsenate, [As (V)] (Francesconi and Kuehneit, 2002). Arsenite is the predominant species of arsenic in groundwater and is more toxic to humans than arsenate (Sharma and Sohn, 2009). Under reducing conditions and near-neutral pH, arsenite most often occurs as \( \text{H}_3\text{AsO}_3 \). Under oxidizing conditions and near-neutral pH, arsenate most often occurs as \( \text{H}_2\text{AsO}_4^- \) and \( \text{HAsO}_4^{2-} \) (Henke and Hutchison, 2009). The form of arsenate as an oxyanion allows for electrostatic interaction with constituents in soil and sediment, such as iron oxyhydroxides.

Arsenic can be retained in the sediment of a CWTS via precipitation, co-precipitation, and sorption (Lizama et al., 2011). Precipitation and co-precipitation can remove arsenic from the aqueous phase by direct formation of insoluble arsenic complexes or by incorporation of trace amounts of arsenic into newly formed insoluble compounds (Henke and Hutchison, 2009). Under reducing conditions, dissimilatory sulfate reduction results in production of hydrogen sulfide, which can react with dissolved arsenic to precipitate insoluble As-S complexes (Cohen, 2006; Kirk et al., 2010; Lizama et al., 2011), or co-precipitate arsenic with insoluble iron sulfide minerals.
(Ford et al., 2006; Henke, 2009; Paul et al., 2009). Oxidation of reduced iron can result in the formation of insoluble iron oxyhydroxides (e.g. ferrihydrite, goethite, etc.) into which trace amounts of arsenic can be incorporated (Henke and Hutchison, 2009). Arsenic also can be removed from the aqueous phase in a CWTS by sorption to iron oxyhydroxides or to organic detritus (Lizama et al., 2011; Sundberg et al., 2006).

Plants can retain arsenic in a CWTS via sorption to roots and submerged shoots, as well as translocation to emergent shoots and tips (An et al., 2011; Blute et al., 2004; Sundberg-Jones and Hassan, 2007). Many wetland plants, including *Typha latifolia* (broadleaf cattail), are known to translocate oxygen from the atmosphere to the rhizosphere via radial oxygen loss from roots (Doyle and Otte, 1997; Li et al., 2011). This process modifies the redox chemistry around the roots and promotes precipitation of iron oxyhydroxides. Ferric iron plaque, enriched in arsenic, has been observed on the roots of *Typha latifolia* in wetland sediments and is possibly the primary mechanism by which arsenic is sequestered by wetland plants (Blute et al., 2004). This hypothesis is supported by studies that found approximately an order of magnitude greater mass of arsenic associated with wetland plant roots compared with shoots (Buddhawong et al., 2005; Dushenko et al., 1995).

The hypothesis of this investigation is that arsenic can be removed from water by precipitation of sulfide minerals and by sorption and co-precipitation of arsenic with iron oxyhydroxides. A CWTS can be designed to facilitate these processes by promoting the necessary biogeochemical conditions, including sediment oxidation-reduction (redox) potential, dissolved oxygen (DO) concentration, and pH (Dorman et al., 2009; Eggert et
Arsenic removed from the aqueous phase in a CWTS will be retained most often in sediment, plants, or lost due to volatilization (Rahman et al., 2011). Previous studies have evaluated the performance of CWTSs designed to remove arsenic in terms of removal extents, removal efficiencies, and first-order removal rate coefficients (Dorman et al., 2009; Eggert et al., 2008; Spacil et al., 2011), but did not consider the fate and partitioning of arsenic in the CWTS. Therefore, the objectives of this study were to (1) measure biogeochemical conditions in a pilot-scale CWTS designed to target specific processes for the removal of arsenic from arsenic-contaminated water, (2) assess arsenic removal from the aqueous phase in the CWTS by determining removal extents, efficiencies, and rates, and (3) determine the fate and distribution of arsenic in the CWTS. Two series were designed to promote coprecipitation and sorption of arsenic with iron oxyhydroxides under oxidizing conditions, and two series were designed to promote precipitation of arsenic with sulfide and coprecipitation of arsenic with iron sulfide under reducing conditions.

2. Materials and methods

2.1 Pilot-scale CWTS and Measurement of Conditions

Targeted biogeochemical processes for arsenic removal and the conditions that promote these processes were determined from a review of published literature (Table 1; Buddhawong et al., 2005; Cheng et al., 2009; Lizama et al., 2011; Rahman et al., 2011). The pilot-scale CWTS was specifically designed and constructed to produce these ranges of conditions. Four CWTS series were built and operated in a climate-controlled...
greenhouse in Clemson, SC. Two series were designed to promote oxidizing conditions, and two series were designed to promote reducing conditions (Figure 1). Each series consisted of four treatment reactors, with each reactor contained in a 265 L (70 gal) Rubbermaid® utility tank (92 cm long by 74 cm wide by 61 cm deep). Each reactor, with the exception of the third reactor in each of the two oxidizing series, contained: (1) a 30-cm thickness of river sand from 18-Mile Creek in Clemson, SC, (2) water to a depth of 25 cm, and (3) approximately 20 Typha latifolia plants harvested from an aquaculture pond in Clemson, SC. The third reactor in each of the two oxidizing series contained a 50-cm thickness of approximately 3-cm diameter granitic gravel, and water to a depth of 5 cm. In the two reducing series, the upper 5 cm of sediment in each reactor was amended with pelletized gypsum (1 % v/v) as a source of sulfate for dissimilatory sulfate reduction and with hay and shredded hardwood mulch (5 % v/v) to provide a nutrient source for sulfate-reducing bacteria (SRB). To provide a source of iron for coprecipitation and sorption, one oxidizing series and one reducing series were amended with zero-valent iron (ZVI; Peerless Metal Powders & Abrasive, Detroit, MI) by distributing 20 g per reactor by hand into the water column approximately once every 14 days.

Simulated arsenic-contaminated water was created by mixing chemical constituents with municipal water in a 5678 L (1500 gal) detention basin (Table 2). Water was transferred from the detention basin into the first reactor of each series by a piston pump (FMI® QG400) at a flow rate of 128 mL min⁻¹, resulting in a nominal hydraulic retention time (HRT) of 24 h per reactor or 96 h per series. Reactors were connected by
PVC pipe fittings located at 4 cm below the top of each reactor. Reactors were arranged with decreasing elevation from the first to fourth reactor in each series to induce gravity flow.

Dissolved oxygen (DO) concentration, pH, and sediment oxidation-reduction (redox) potential were measured bi-weekly in each reactor. DO concentration and pH were measured using YSI® (model 55) and Orion® (model A325) field instruments, respectively. To measure sediment redox potential, one platinum-tipped electrode was installed approximately 2 cm into the sediment in the front and one in the back of each reactor. Electrodes remained in-situ for the duration of the experiment. Sediment redox potential was measured using a GDT-11 multi-meter connected to in-situ electrodes and an Accumet® calomel reference electrode (Faulkner et al., 1989). Statistical differences were determined by ANOVA and post-hoc t-tests with α = 0.05.

2.2 Determination of arsenic concentration and CWTS performance

Piston pumps were calibrated before collecting aqueous samples by measuring the flow rate in and out of the first reactor of each series with a graduated cylinder and stopwatch. Aqueous samples were collected bi-weekly during 14 sampling periods from each treatment reactor sequentially according to the HRT (24 h per reactor). For example, samples of inflow water entering the first reactor were collected at t = 0 h, samples of outflow water from the first reactor were collected at t = 24 h, from the second reactor at t = 48 h, from the third reactor at t = 72 h, and from the fourth reactor at t = 96 h. In this way, a single volume of water was theoretically sampled as it passed through a series.
Samples were collected in acid-washed 50 mL polypropylene centrifuge tubes from sampling ports between each reactor. 25 mL of sample was centrifuged for 20 minutes at 8000 rpm. 1 mL of supernatant was transferred gravimetrically to an acid-washed 15 mL polypropylene centrifuge tube and brought to a volume of 10 mL with 2% trace metal grade nitric acid solution. Samples were analyzed for arsenic using inductively-coupled plasma mass-spectrometry (ICP-MS) (Thermo Scientific X Series) according to EPA Method 200.8 (USEPA, 1994a).

Treatment performance of each series was assessed in terms of removal extent, removal efficiency, and removal rate coefficient. Removal extent is defined as the concentration of arsenic in the outflow (µg L\(^{-1}\)). Removal efficiency was calculated using Eq. (1):

\[
\text{removal efficiency (\%)} = \left( \frac{[C]_0 - [C]}{[C]_0} \right) \times 100
\]

where \([C]_0\) is inflow concentration (µg L\(^{-1}\)) and \([C]\) is outflow concentration (µg L\(^{-1}\)).

First-order rate kinetics are often used to model removal of COCs in a CWTS (Eggert et al., 2008; Horner et al., 2012; Lizama et al., 2011; Wong et al., 2006). Removal rate coefficient (\(k, \text{day}^{-1}\)) was calculated using Eq. (2):

\[
k = \frac{-\ln([C]/[C]_0)}{HRT}
\]

where \(HRT\) (day) is the nominal hydraulic retention time (i.e. the time between sampling series inflow and outflow).

2.3 Fate and distribution of arsenic

Sediment cores and plant samples were collected on day 1 of the experiment (8/17/12, prior to introduction of arsenic on the same day), day 81 (11/6/12), day 141
(1/5/13), and day 188 (2/21/13). One 30-cm long sediment core representing the entire sediment thickness was collected from the front (inflow area), one from the middle, and one from the back (outflow area) of the first reactor in each series with a 1.91-cm diameter coring device. Each core was sectioned into 6 cm intervals (Figure 2), and the three samples from each interval were combined and homogenized. This procedure resulted in 5 sediment samples from each series, for a total of 20 samples. One *Typha latifolia* plant was collected from the front and one from the back of the first reactor in each series. The number of *Typha latifolia* plants in the first reactor of each series was recorded at the time of sampling. Plants were rinsed with deionized water and sectioned with stainless steel shears into roots, submerged shoots, emergent shoots, and tips (Figure 2). Approximately 10 g of each plant sample and 30 g of each sediment sample (wet weight) were dried for 8 h at 100°C. The dry bulk density of each sediment sample was determined gravimetrically. Sediment and plant samples were stored at 4°C prior to chemical analysis. 0.5 g each of dried sediment and plant samples were placed in Teflon® MARS microwave digestion tubes (CEM Corporation) with 10 mL of trace metal grade nitric acid (67 %) (Fisher Scientific) and digested using EPA method 3051 (USEPA, 1994b). The digestate was transferred into a 50 mL centrifuge tube, and the volume brought to 25 mL with deionized water. Samples were centrifuged, diluted, and analyzed for arsenic using the same procedure that was used for aqueous samples.

Translocation factor (TF), which is the ratio of the concentration of arsenic in above-ground plant tissues (i.e. shoots and tips) to the concentration of arsenic in plant roots, was calculated using Eq. (3) (Li et al., 2011; Sundberg-Jones and Hassan, 2007):
where [\text{As}]_{\text{above ground}} is arsenic concentration in above-ground plant tissues (sum of concentrations in shoots and tips; mg kg\(^{-1}\) plant dry weight) and [\text{As}]_{\text{roots}} is arsenic concentration in the roots (mg kg\(^{-1}\) plant dry weight).

The aqueous bioconcentration factor (BCF), which is the ratio of the concentration of arsenic in plant tissue (i.e. roots, shoots, and tips) to the concentration of arsenic in the aqueous phase, was calculated using Eq. (4) (Sundberg-Jones and Hassan, 2007):

\[
\text{BCF} = \frac{[\text{As}]_{\text{plant}}}{[\text{As}]_{\text{water}}}
\]

where [\text{As}]_{\text{plant}} is arsenic concentration in plant tissue (sum of arsenic concentrations in roots, shoots, and tips; mg kg\(^{-1}\) plant dry weight) and [\text{As}]_{\text{water}} is arsenic concentration in the aqueous phase (mg L\(^{-1}\)).

One grab sample from the upper 2 cm of sediment was collected from the front, one from the middle, and one from the back of the first reactor of each series on day 14 of the experiment (8/31/12), day 94 (11/19/12), day 140 (1/4/13), and day 188 (2/21/13). Samples were scooped with an acid-washed metal spatula into a 50 mL centrifuge tube and sealed underwater. The three samples from each reactor were composited and analyzed for acid volatile sulfide (AVS) using a modified diffusion method (Leonard et al., 1996). AVS is defined as the sulfide extracted from sediment by 1-N HCL (Di Toro et al., 1992; Leonard et al., 1996) and interpreted as the reactive fraction of sulfide available to bind metals (Di Toro et al., 1992; Keon et al., 2001; Wilkin and Ford, 2002).
Sulfide was measured using an ion-selective electrode to determine the AVS concentration.

Previous studies (Dorman et al., 2009; Horner et al., 2012; Rahman et al., 2011; Rousseau et al., 2004; Spacil et al. 2011, Wong et al., 2006) have shown that the greatest percentage removal of inflow mass of a COC most often occurs in the first reactor of a CWTS series; therefore, the first reactor of each series was chosen as the system for a chemical mass balance (Eq. 5).

\[ M_{in} = M_{out} + M_{sed} + M_{plant} + M_{unacc} \]  \hspace{1cm} (5)

where \( M_{in} \) is mass of arsenic that entered the system as inflow (mg) during time \( t \) (the duration of the experiment over which the mass balance is applicable), \( M_{out} \) is mass of arsenic that exited the system as outflow (mg) during time \( t \), \( M_{sed} \) is mass of arsenic retained in sediment (mg) after time \( t \), \( M_{plant} \) is mass of arsenic retained in \textit{Typha latifolia} (mg) after time \( t \), and \( M_{unacc} \) is mass of arsenic unaccountable (i.e. a loss or gain from mass balance calculation) (mg). The \( M_{sed} \) term was calculated from arsenic concentrations from sediment samples according to Eq. (6):

\[ M_{sed} = \sum_{i=1}^{n} (C_{sed,i} \rho_{sed,i} V_{sed,i}) \]  \hspace{1cm} (6)

where \( C_{sed,i} \) is the concentration of arsenic in a sediment sample from depth interval \( i \), (mg arsenic g\(^{-1}\) sediment dry weight) after time \( t \), \( \rho_{sed,i} \) is the dry bulk density of a sediment sample from interval \( i \), (g cm\(^{-3}\)), and \( V_{sed,i} \) is the volume of sediment within interval \( i \), based on the dimensions of the reactor and thickness of the interval \( i \) (cm\(^3\)).

\( M_{plant} \) was calculated from the concentration of arsenic in \textit{Typha latifolia} according to Eq. (7):
\[ M_{\text{plant}} = \sum_{i=1}^{n} (m_{\text{plant},i} C_{\text{plant},i} n_{\text{plant}}) \]  \hspace{1cm} (7)

where \( m_{\text{plant},i} \) is dry mass of plant tissue (g), \( C_{\text{plant},i} \) is concentration of arsenic in plant tissue (mg arsenic g\(^{-1}\) plant dry weight) after time \( t \), and \( n_{\text{plant}} \) is number of plants per reactor. Substituting Eq. (6) and Eq. (7) into Eq. (5) and expressing the mass in and mass out of the system as a mass loading rate (\( Q_{\text{in}} C_{\text{in}} \) and \( Q_{\text{out}} C_{\text{out}} \), respectively) results in Eq. (8):

\[ Q_{\text{in}} C_{\text{in}} t = (Q_{\text{out}} C_{\text{out}} t) + \left( \sum_{i=1}^{n} (m_{\text{plant},i} C_{\text{plant},i} n_{\text{plant}}) \right) + \left( \sum_{i=1}^{n} (C_{\text{sed},i} \rho_{\text{sed},i} V_{\text{sed},i}) \right) + M_{\text{unacc}} \]  \hspace{1cm} (8)

where \( Q_{\text{in}} \) is volumetric flow rate of water entering the system as inflow (L d\(^{-1}\)), \( Q_{\text{out}} \) is volumetric flow rate of water leaving the system as outflow (L d\(^{-1}\)), \( C_{\text{in}} \) is inflow arsenic concentration (mg L\(^{-1}\)), \( C_{\text{out}} \) is outflow arsenic concentration (mg L\(^{-1}\)), and \( t \) is time (days) over which the mass balance is applicable.

3. Results and discussion

3.1 CWTS Conditions

Sediment redox potential was within the targeted range (\( \geq -50 \text{ mV} \) for oxidizing series; -50 to -250 mV for reducing series) in 63, 80, 55, and 64 % of measurements for series 1, 2, 3, and 4, respectively (\( n = 56 \)). Mean sediment redox potential was lower in reducing series 3 and 4 (-77 and -79 mV, respectively) compared to oxidizing series 1 and 2 (45 and 51 mV, respectively). In a previous bench-scale batch reactor study of arsenic and ZVI (Su and Puls, 2001a), addition of Peerless ZVI resulted in an initial decrease in redox potential followed by steady-state positive values. In the current study, addition of ZVI had a significant effect (\( \alpha = 0.05 \)) on sediment redox potential in two of
the four reactors in each series to which ZVI was added. For both oxidizing and reducing series, the redox potential in reactor 1 and reactor 2 amended with ZVI was significantly lower than the unamended reactor 1 and reactor 2 (p = 1.7 x 10⁻³ and 9.0 x 10⁻⁴, respectively for oxidizing series, and 3.7 x 10⁻⁷ and 5.1 x 10⁻⁵, respectively for reducing series). DO concentration was within the targeted range (≥ 2 mg L⁻¹ for oxidizing series and ≤ 2 mg L⁻¹ for reducing series) in 100, 98, 70, and 66 % of measurements for series 1, 2, 3, and 4, respectively (n = 56). Mean DO concentration was less in reducing series 3 and 4 (2.0 and 1.8 mg L⁻¹, respectively), compared to oxidizing series 1 and 2 (6.6 and 7.4 mg L⁻¹, respectively). Lower mean DO concentration and sediment redox potential in series 3 and 4 compared to series 1 and 2 are attributed to the consumption of oxygen by aerobic microorganisms during biodegradation of organic matter in the reducing series. Measured pH was within the targeted range (4-9 for oxidizing series and 5-8 for reducing series) in 93, 82, 89, and 93 % of samples for series 1, 2, 3, and 4, respectively.

In oxidizing series 1 and 2, mean sediment redox potential (298 and 201 mV, respectively) and mean DO concentration (9.0 and 11.6 mg L⁻¹, respectively) were significantly greater (α = 0.05) in the third oxidizing reactor than in any other reactor in the series (range from -53 to 39 mV and 5.5 to 6.1 mg L⁻¹). This is attributed to lower organic matter content in the substrate of the third reactor of each oxidizing series (unplanted granitic gravel) compared to other reactors (river sand planted with *Typha latifolia*) and to greater atmospheric oxygen diffusion as the result of shallower water depth in the third reactor of each oxidizing series (5 cm) than in the other reactors (25 cm). pH values in the planted reactors of series 1 and 2 (range of 6.9-8.4 and 6.9-8.7, respectively).
respectively) were less than in the unplanted third reactor of these series (7.1-9.6 and 7.7-9.9, respectively). This is attributed to organic acids in planted reactors lowering the pH compared to unplanted reactors.

3.2 Arsenic concentration and removal performance

Concentration of arsenic decreased from the inflow to the outflow in all pilot-scale CWTS series with the exception of series 4 during the 8/31/12 sampling period, series 2 and 3 during the 2/1/13 sampling period, series 2 during the 2/15/13 sampling period, and series 3 during the 3/15/13 sampling period. Arsenic removal performance was significantly greater (α = 0.05) in the oxidizing series amended with ZVI than in any other series, with removal extent, efficiency and rate coefficient ranging from 6-79 µg L⁻¹, 40-95 %, and 0.13-0.77 d⁻¹ respectively, compared to 49-162 µg L⁻¹, 0-79 %, and 0.00-0.39 d⁻¹, respectively, in the oxidizing series not amended with ZVI (Table 3).

Outflow concentration of arsenic was less than the WHO drinking water quality guideline of 10 µg L⁻¹ in the oxidizing series amended with ZVI during the 10/28/12, 11/13/12, and 11/29/12 sampling periods. Mean removal extent and removal efficiency were 89 µg L⁻¹ and 42 %, respectively, in the reducing series amended with ZVI compared to 128 µg L⁻¹ and 20 %, respectively, in the reducing series not amended with ZVI. The addition of ZVI significantly improved the removal extent and efficiency of oxidizing series (p = 1.7 x 10⁻⁶ and 1.2 x 10⁻⁷, respectively) and reducing series (p = 3.9 x 10⁻³ and 1.7 x 10⁻², respectively). Enhanced removal performance of the series amended with ZVI is attributed to a source of iron for co-precipitation and sorption of arsenic with iron.
oxyhydroxides in the oxidizing series, and a source of iron for co-precipitation of arsenic with iron sulfide in the reducing series.

### 3.3 Fate and distribution of arsenic

Over the duration of the experiment (188 days), the total inflow mass of arsenic ($M_{in}$, Eq.5) ranged from 4128-4949 mg among the four series, with the percentage of total inflow mass of arsenic removed ranging from 16-76 %. In the first reactor of each series, 2-30% of inflow arsenic was retained in sediment ($M_{sed}$, Eq. 5), retained in plants ($M_{plant}$, Eq. 5), or was unaccountable ($M_{unacc}$, Eq. 5), and the remainder was passed into the second reactor in the outflow ($M_{out}$, Eq. 5) (Table 4).

The majority of inflow arsenic retained in the first reactor of each series ($M_{sed} + M_{plant}$, Eq. 5) partitioned to the sediment (88-99 %), with the remainder partitioned to *Typha latifolia*. Mass of arsenic in sediment increased over the duration of the experiment from a range of 52-89 mg among the four series at the beginning of the experiment to a range of 270-809 mg among the four series at the end of the experiment.

Initial concentrations of arsenic in sediment, determined prior to introduction of arsenic into the system, were within a narrow range (0.2-0.7 mg arsenic kg$^{-1}$ sediment dry weight) (Figure 3). Over time, however, a concentration depth profile developed with a significant decrease ($\alpha = 0.05$) in arsenic concentration with depth (Figure 3). At the conclusion of the experiment, the upper 6 cm of sediment (upper sediment core interval) accounted for 74-85 % of total sediment-bound arsenic, with the remainder at 6 to 30 cm depth. The vertical arsenic concentration gradient is attributed to the majority of arsenic
removal having occurred upon initial contact with sediments at the sediment-water interface, with decreasing removal as water moved downward from the sediment-water interface. Conditions in the sediment may also play a role in the vertical distribution of arsenic. For example, a previous pilot-scale wetland study by Buddhawong et al. (2005) found the sediment redox potential at the top of the reactor bed to be approximately 250 mV greater than at the bottom. A decrease with depth in sediment redox potential of this magnitude in an oxidizing reactor could mobilize arsenic through the reductive dissolution of iron oxyhydroxides, and in a reducing reactor could result in redox conditions unfavorable for dissimilatory sulfate reduction (＜-250 mV).

Mass of arsenic retained in sediment ($M_{sed}$, Eq. 5) was greater in oxidizing series (809 and 541 mg in series 1 and 2, respectively) than in reducing series (270 and 326 mg in series 3 and 4, respectively). A greater percentage of inflow arsenic was removed in the first reactor of the oxidizing series amended with ZVI and the first reactor of the reducing series amended with ZVI (30 and 23 %, respectively) than in the first reactor of the unamended oxidizing and unamended reducing series (15 and 2 %, respectively). The first reactor of the oxidizing series amended with ZVI (series 1) removed the greatest percentage of inflow arsenic (30 %) compared to the first reactor of other series (2-23 %). In both oxidizing series, sediment redox potential and DO concentration were favorable for precipitation of iron oxyhydroxides (Table 1), which can remove arsenic from the aqueous phase via co-precipitation and sorption (Cheng, 2009), but not for dissimilatory sulfate reduction. In the current pilot-scale study, the removal rate coefficient for series 1 (0.13-0.77 d^{-1}) was within the range of removal rate coefficients from previous bench-
scale batch reactor studies with arsenic and ZVI under oxidizing conditions (Bang et al., 2005; Su and Puls, 2001b), where pseudo-first-order rate coefficients \(k = -d[As]/dt\) ranged from 0.09-0.84 d\(^{-1}\) depending on the speciation of arsenic, pH, and type of ZVI. The concentration of arsenic in the upper 6 cm of sediment was greater in the series amended with ZVI (23.1 mg As kg\(^{-1}\) sediment) than in the unamended series (12.8 mg As kg\(^{-1}\) sediment). ZVI provided a source of iron for precipitation of iron oxyhydroxides under oxidizing conditions and therefore improved the removal performance of series 1 compared to the unamended series (Table 3).

In both reducing series, sediment redox potential and DO concentration were favorable for dissimilatory sulfate reduction necessary for precipitation of arsenic sulfide and co-precipitation of arsenic with iron sulfide (Table 1), but not for the precipitation of iron oxyhydroxides. Throughout the experiment, AVS concentration was greater by approximately two orders of magnitude in reducing series 3 and 4 (100-643 mg L\(^{-1}\)) than in oxidizing series 1 and 2 (0-8 mg L\(^{-1}\)) (Figure 4). Greater AVS concentrations in reducing series compared to oxidizing series are attributed to dissimilatory sulfate reduction in the reducing series. AVS concentrations in the reducing series amended with ZVI (range from 101-643 mg L\(^{-1}\)) were greater than in the unamended reducing series (100-234 mg L\(^{-1}\)), which is attributed to the ZVI serving as an electron donor for dissimilatory sulfate reduction (Karri et al., 2005).

In all series, the concentration of arsenic in sediment was negatively correlated to sediment dry bulk density \(R^2 = 0.82, 0.82, 0.73\) and 0.56 for series 1, 2, 3, and 4, respectively) (Figure 5). It can be reasonably assumed, given that sediment dry bulk
density was approximately uniform (~1.3 g cm\(^{-3}\)) prior to amendment with organic matter or input of detritus, that sediment dry bulk density measurements during the experiment were proportional to organic matter content (Avnimelech et al., 2001). Therefore, the inverse relationship between sediment dry bulk density and arsenic concentration in sediment may indicate that sorption to organic matter is an important process for arsenic removal, especially in reducing series, which were amended with organic matter.

Between 1 and 12 % of inflow arsenic retained in the first reactor of each series \((M_{\text{sed}} + M_{\text{plant}}, \text{Eq. 5})\) partitioned to Typha latifolia. Concentrations of arsenic in Typha latifolia ranged from 6.0-15.5 mg As kg\(^{-1}\) plant (dry weight) among the four series initially, increased after 81 days to 65.1-200.2 mg As kg\(^{-1}\) plant, and decreased by the conclusion of the experiment (188 days) to 6.4-62.4 mg As kg\(^{-1}\) plant (Figure 6). At the conclusion of the experiment, the roots and submerged shoots accounted for 87-97 % of total plant-bound arsenic, with the remainder of plant-bound arsenic in the emergent shoots and tips. Throughout the experiment, arsenic concentrations were greater in the roots than in above-ground plant tissues (TF ≤ 1, Eq.3), indicating little translocation of arsenic from roots to shoots (Table 5). Sequestration of arsenic in the roots and submerged shoots of Typha latifolia is preferable to translocation to emergent shoots and tips in terms of mitigating risk of exposure to herbivores that could feed on the exposed portion of wetland plants.

In all plant samples, arsenic concentration was greater in the plant tissue than in the water (BCF > 1 L kg\(^{-1}\), Eq. 4), indicating partitioning of arsenic from water to Typha
Typha latifolia. BCF decreased in all series between 11/6/12 and 2/21/13 (Table 5). Plant shoots were green with new shoots emerging in each reactor during the 11/16/12 sampling period, whereas plant shoots were brown and dormant during the 2/21/13 sampling period. These observations are consistent with greater arsenic uptake by Typha latifolia during periods of active growth. Arsenate [As (V)] shares the same tetrahedral coordination as phosphate and can compete with phosphate for plant uptake (Carbonell et al., 1998; Li et al., 2011). Arsenite [As (III)], also tetrahedrally coordinated, is similar in size to silicic acid (H₂SiO₃) and is available for plant uptake as a substitute for silicon (Zhao et al., 2009).

Mass of arsenic associated with Typha latifolia (Mplant, Eq. 5) was greater in series amended with ZVI (23 and 46 mg in series 1 and 4, respectively), than in series not amended with ZVI (10 and 3 mg in series 2 and 3, respectively). Both arsenate and arsenite have an affinity for iron oxyhydroxides, which have been observed as plaques associated with Typha latifolia roots (Blute et al., 2004). We hypothesized that amendment of ZVI to series 1 and 4 would increase the concentration of arsenic associated with Typha latifolia roots by promotion of iron oxyhydroxide plaque formation. Interestingly, the arsenic concentration associated with the roots was not consistently greater in series 1 and 4 (range from 5.5 - 155.0 and 3.2 - 40.4 mg As kg⁻¹ root, respectively) than in unamended series 2 and 3 (9.2-138.8 and 3.2-93.8 mg As kg⁻¹ root, respectively).

The minimal retention of arsenic in plants is consistent with other mass balance studies (Singhakhant et al., 2009; Ye et al., 2003). This finding could lead to the
conclusion that plants play only a minor role in the CWTS; however, the wetland plants have several benefits beyond direct arsenic uptake. Detritus from decaying plant material provides organic matter for dissimilatory sulfate reduction, binding sites for SRB, and sorption sites for arsenic. In addition, plants likely prevent resuspension of sediment by acting as a wind break. These effects may explain why previous studies (Rahman et al., 2011; Singhakhant et al., 2009) have observed greater arsenic removal performance in planted wetlands than unplanted wetlands. Plants also provide a source of detritus for accretion of wetland sediment. There is sometimes concern over arsenic accumulation in sediment (Christophoridis et al., 2009; Liber et al., 2011), however, sediment added internally to a wetland (accumulation of detritus and deposition of suspended solids) can result in sediment accretion ranging from mm year\(^{-1}\) to cm year\(^{-1}\) (Kadlec and Wallace, 2009), thus diluting concentration of COCs, such as arsenic, in the sediment.

Between 2 and 16 % of inflow arsenic was unaccountable (Table 4). It is possible for inorganic arsenic to undergo biologically mediated reduction to methylated arsines which can escape the system via volatilization (Frankenberger and Arshad, 2002).

Previous studies have attributed a portion of unaccountable arsenic to loss from volatilization (Rahman et al., 2011; Ye et al., 2003), however volatile arsines rarely occur in natural environments except under extremely reducing conditions (< -500 mV) (Sharma and Sohn, 2009). In the current study, the lowest sediment redox potential was -282 mV; therefore it is unlikely that arsenic was lost due to volatilization. Interestingly the highest percentages of unaccountable arsenic were in series 1 (10 %) and series 4 (15 %), both amended with ZVI. ZVI has a high affinity for arsenic with a sorption
capacity ranging from 732-1771 mg As kg\(^{-1}\) ZVI depending on the speciation of arsenic (Su and Puls, 2001b), and was heterogeneously distributed in the first reactor of series 1 and series 4. It is possible that unaccountable arsenic in series 1 and 4 was sorbed on the surface of ZVI.

4. Conclusions

A pilot-scale CWTS was designed and built to produce biogeochemical conditions that promoted targeted processes for the removal of arsenic from arsenic-contaminated water. Removal performance demonstrated that a CWTS could be used to decrease arsenic concentration in the aqueous phase to below the WHO water quality guideline of 10 µg L\(^{-1}\). The majority of arsenic removed from the aqueous phase during the 188-day experiment partitioned to sediment (88-99%), while the remainder partitioned to *Typha latifolia*. The percentage of inflow arsenic retained in the sediment of the first reactor of the two oxidizing series (20 and 13 % for series 1 and 2, respectively) was greater than the percentage of inflow arsenic retained in the sediment of the first reactor of the two reducing series (6 and 7 % for series 3 and 4, respectively). The addition of ZVI to oxidizing series and to reducing series enhanced arsenic removal from the aqueous phase (mean removal efficiency of 72 and 42 %, respectively) compared to unamended series (27 and 20 %, respectively) and increased the mass of arsenic retained in sediment. By the end of the 188-day experiment, a vertical concentration gradient had developed in the sediment, with 74-85 % of sediment-bound arsenic accumulated in the upper 6 cm and the remainder below 6 cm. The majority of arsenic retained in *Typha latifolia* was associated with the roots and submerged shoots.
(87-97 %), with little translocation to the emergent shoots and tips. The mass of arsenic associated with *Typha latifolia* was greater in series amended with ZVI (23 and 46 mg for series 1 and 4, respectively), than in series not amended with ZVI (10 and 3 mg for series 2 and 3, respectively). Results of this study demonstrate that a CWTS can decrease the concentration of arsenic in arsenic-contaminated water primarily by transferring arsenic from the aqueous phase to the sediment.
References


List of Figure Captions

**Figure 1.** Schematic diagram of pilot-scale constructed wetlands for treatment of arsenic-contaminated water. Series 1 and 2 were designed to promote oxidizing conditions, and series 3 and 4 were designed to promote reducing conditions. Series 1 and 4 were amended biweekly with 20 g ZVI per reactor. The third reactor in each of the two oxidizing series was unplanted and contained a 50-cm thickness of granitic gravel.

**Figure 2.** Profile of a pilot-scale CWTS reactor (not to scale). One sediment core was collected from the front (inflow area), one from the middle, and one from the back (outflow area) of the reactor and segmented into 6 cm depth intervals. One *Typha latifolia* plant was collected from the front and one from the back of the reactor. Each plant was rinsed with deionized water and segmented into roots, submerged shoots, emergent shoots, and tips.

**Figure 3.** Vertical distribution of arsenic in sediment in the first reactor of each series during the 8/17/12 (A), 11/6/12 (B), 1/5/13 (C), and 2/21/13 (D) sampling period. The initial concentrations of arsenic in sediment were within a narrow range among intervals (A), compared to later sampling dates (B-D), in which arsenic concentrations were significantly greater ($\alpha = 0.05$) from 0-6 cm than from 6-30 cm.

**Figure 4.** AVS concentration in the first reactor of each series. AVS concentrations in reducing series 3 and 4 are approximately two orders of magnitude greater than in oxidizing series 1 and 2. AVS concentration in the reducing series amended with ZVI (series 4) was greater than in the unamended series (series 3) during the 11/19/12, 1/4/13, and 2/21/13 sampling periods. Error bars represent standard deviation of three replicates.
**Figure 5.** Arsenic concentration in sediment vs. sediment dry bulk density in series 1 (A), series 2 (B), series 3 (C), and series 4 (D) (n = 15 for each series). Lines represent best fit with corresponding $R^2$ value. Arsenic concentration in sediment was negatively correlated to sediment dry bulk density. The sample with the lowest sediment dry bulk density and highest arsenic concentration corresponds to the sample with the highest organic matter content.

**Figure 6.** Distribution of arsenic in *Typha latifolia* in the first reactor of each series during the 8/17/12 (A), 11/6/12 (B), 1/5/13 (C), and 2/21/13 (D) sampling period. 8/17/12 is day 0 of the experiment, before arsenic was introduced, showing background concentration (scale expanded to show differences in concentrations between plant tissues). The concentration of arsenic in the roots and submerged shoots is greater than in the emergent shoots and tips.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.

A) As concentration (mg As kg\(^{-1}\)) vs. sediment dry bulk density (g cm\(^{-3}\)).

B) As concentration (mg As kg\(^{-1}\)) vs. sediment dry bulk density (g cm\(^{-3}\)).

C) As concentration (mg As kg\(^{-1}\)) vs. sediment dry bulk density (g cm\(^{-3}\)).

D) As concentration (mg As kg\(^{-1}\)) vs. sediment dry bulk density (g cm\(^{-3}\)).

Series 3: R\(^2\) = 0.73

Series 1: R\(^2\) = 0.8181

Series 4: R\(^2\) = 0.55

Series 2: R\(^2\) = 0.8174
Figure 6.
Table 1. Targeted biogeochemical conditions to promote processes for arsenic removal from arsenic-contaminated water in pilot-scale constructed wetland treatment system.

<table>
<thead>
<tr>
<th>Removal process</th>
<th>Sediment Redox potential (mV)</th>
<th>Dissolved Oxygen (mg L$^{-1}$)</th>
<th>pH (S.U.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-precipitation and sorption with Fe- oxyhydroxides</td>
<td>Oxidizing$^a$ (Eh &gt; -50)</td>
<td>≥ 2</td>
<td>4-9$^b$</td>
</tr>
<tr>
<td>Precipitation of As-S and co-precipitation of As with Fe-S</td>
<td>Reducing$^d$ (Eh -50 to -250)</td>
<td>≤ 2</td>
<td>5-8$^c$</td>
</tr>
</tbody>
</table>

$^a$ Buddhawong et al. (2005)  
$^b$ Cheng et al. (2009)       
$^c$ Lizama et al. (2011)      
$^d$ Rahman et al. (2011)
Table 2. Chemical characteristics of simulated arsenic-contaminated water (All units in mg L\(^{-1}\) unless noted)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Simulated water(^a)</th>
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<tbody>
<tr>
<td>As(_{\text{Total}})</td>
<td>0.30</td>
</tr>
<tr>
<td>As(_{\text{(III)}})</td>
<td>0.18</td>
</tr>
<tr>
<td>As(_{\text{(V)}})</td>
<td>0.12</td>
</tr>
<tr>
<td>Ca</td>
<td>55</td>
</tr>
<tr>
<td>Mg</td>
<td>50</td>
</tr>
<tr>
<td>Na</td>
<td>180</td>
</tr>
<tr>
<td>K</td>
<td>13</td>
</tr>
<tr>
<td>HCO(_3)</td>
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<td>220</td>
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</tr>
<tr>
<td>Si</td>
<td>15</td>
</tr>
<tr>
<td>P</td>
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<tr>
<td>Fe</td>
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<tr>
<td>pH (S.U.)</td>
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</tr>
<tr>
<td>Conductivity (µS cm(^{-1}))</td>
<td>890</td>
</tr>
</tbody>
</table>

\(^a\)Targeted values (after BGS, 2001)
Table 4. Distribution of arsenic in the first treatment reactor of each series at the conclusion of the experiment (188 days). All values are given as mg arsenic and a percentage of total inflow arsenic.

<table>
<thead>
<tr>
<th>Series</th>
<th>Inflow</th>
<th>Sediment</th>
<th>Plants</th>
<th>Outflow</th>
<th>Unaccountable</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4128.3 (100.0 %)</td>
<td>808.8 (19.6 %)</td>
<td>23.2 (0.6 %)</td>
<td>2904.2 (70.3 %)</td>
<td>392.2 (9.5 %)</td>
</tr>
<tr>
<td>2</td>
<td>4293.5 (100.0 %)</td>
<td>541.1 (12.6 %)</td>
<td>9.8 (0.2 %)</td>
<td>3661.9 (85.3 %)</td>
<td>80.7 (1.9 %)</td>
</tr>
<tr>
<td>3</td>
<td>4766.3 (100.0 %)</td>
<td>270.3 (5.7 %)</td>
<td>3.1 (0.1 %)</td>
<td>4694.6 (98.4 %)</td>
<td>-201.7 (-4.2 %)</td>
</tr>
<tr>
<td>4</td>
<td>4948.7 (100.0 %)</td>
<td>325.6 (6.6 %)</td>
<td>45.5 (0.9 %)</td>
<td>3793.7 (76.7 %)</td>
<td>783.8 (15.8 %)</td>
</tr>
</tbody>
</table>
Table 5. Arsenic bioconcentration factors (BCF, Eq. 4) and translocation factors (TF, Eq. 3) for *Typha latifolia* in the first treatment reactor of each series (dry weight).

<table>
<thead>
<tr>
<th>Series</th>
<th>8/31/12</th>
<th>11/6/12</th>
<th>1/5/13</th>
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<td>1</td>
<td>84.7</td>
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<td>885.7</td>
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<td>2</td>
<td>77.0</td>
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<td>871.3</td>
<td>172.4</td>
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<td>49.7</td>
<td>542.4</td>
<td>915.0</td>
<td>53.7</td>
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<tr>
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<td>53.4</td>
<td>1668.0</td>
<td>1529.7</td>
<td>520.4</td>
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</table>

<table>
<thead>
<tr>
<th>Plant translocation factor</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>4</td>
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</tbody>
</table>
CHAPTER FOUR

CONCLUSIONS

Constructed wetland treatment systems (CWTSs) may offer an effective approach to treating arsenic-contaminated groundwater. The major objectives of this study were to (1) assess treatment performance of a pilot-scale CWTS designed to decrease the concentration of arsenic in simulated Bangladesh groundwater, and (2) determine the fate and distribution of arsenic in a pilot-scale CWTS designed to target specific processes for arsenic removal.

1. **Assess treatment performance of a pilot-scale CWTS designed to decrease the concentration of arsenic in simulated Bangladesh groundwater**

The purpose of this study was to evaluate the performance of a CWTS for the removal of arsenic from simulated Bangladesh groundwater. The specific objectives of this study were to (1) design and construct a pilot-scale CWTS to compare the removal of arsenic in oxidizing series with the removal of arsenic in reducing series, and (2) assess arsenic removal performance in each series by determining removal extents, efficiencies, and rates. Two series were designed to promote co-precipitation and sorption of arsenic with iron oxyhydroxides under oxidizing conditions, and two series were designed to promote precipitation of arsenic with sulfide and co-precipitation of arsenic with iron sulfide under reducing conditions.

The CWTS was designed and built to produce biogeochemical conditions that promote targeted processes for the removal of arsenic from simulated Bangladesh
groundwater. Results indicate that the design of a full-scale CWTS for the effective treatment of arsenic-contaminated water would benefit from the incorporation of features from this pilot-scale study including oxidizing conditions and amendment with zero-valent iron (ZVI). The addition of ZVI enhances removal performance in a CWTS by providing a source of iron for sorption and co-precipitation of arsenic with iron oxyhydroxides and iron sulfide, and by providing an electron donor for sulfate reducing bacteria. Results indicate that a CWTS is suitable for the treatment of arsenic-contaminated water containing competitive anions. This pilot-scale study demonstrated that a CWTS could be used successfully to decrease the concentration of arsenic in simulated Bangladesh groundwater to below the World Health Organization (WHO) drinking water quality guideline of 10 µg L⁻¹.

2. **Determine the fate and distribution of arsenic in a pilot-scale CWTS designed to target specific processes for arsenic removal**

The purpose of this study was to determine the fate and distribution of arsenic in a pilot-scale CWTS. The specific objectives of this study were to (1) measure biogeochemical conditions in a CWTS designed to target specific processes for the removal of arsenic from arsenic-contaminated water, (2) assess arsenic removal from the aqueous phase in each series by determining removal extents, efficiencies, and rates, and (3) determine the fate and distribution of arsenic in each treatment series.

A pilot-scale CWTS was designed and built to produce biogeochemical conditions that promoted targeted processes for the removal of arsenic from arsenic-contaminated water. Removal performance demonstrated that a CWTS could be used to
decrease the concentration of arsenic in the aqueous phase from approximately 200 µg L\(^{-1}\) to below the WHO drinking water quality guideline of 10 µg L\(^{-1}\). The majority of arsenic removed from the aqueous phase during the 188-day experiment partitioned to sediment (88-99%), while the remainder partitioned to *Typha latifolia*. The addition of ZVI enhanced arsenic removal from the aqueous phase in both oxidizing series and reducing series and increased the concentration of arsenic in sediment. A vertical concentration gradient developed over time in the sediment, with the majority of sediment-bound arsenic (74-85 %) accumulated in the upper 6 cm. Results of this study demonstrate that a CWTS can be used successfully to decrease the concentration of arsenic in arsenic-contaminated water primarily by transferring arsenic from the aqueous phase to the sediment.

3. **Summary**

Results of this study indicate that a CWTS can be designed and built to produce biogeochemical conditions that promote targeted processes for the removal of arsenic from arsenic-contaminated groundwater. The design of a full-scale CWTS for the effective treatment of arsenic-contaminated groundwater would benefit from targeting sorption and co-precipitation of arsenic with iron-oxyhydroxides by incorporating features from this pilot-scale study including oxidizing conditions and amendment with ZVI. Results of this study demonstrate that a CWTS can be used successfully to decrease the concentration of arsenic in simulated Bangladesh groundwater to below the WHO
drinking water quality guideline primarily by transferring arsenic from the aqueous phase to the sediment.
APPENDICES
## Appendix A

Table A-1. Calculated bicarbonate concentration (mg L$^{-1}$) for each sampling date in which alkalinity was measured.

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Appendix B

Standard Operating Procedures for Water, Sediment, and Plant Analysis

The standard operating procedures used for (1) measurement of biogeochemical conditions in the CWTS (2) sampling of water, sediment, and plants in the CWTS, and (3) analysis of arsenic in water, sediment, and plant samples are listed below and found on the pages indicated.

General Water Chemistry Parameters................................................................. 88
Sediment Oxidation-Reduction Potential .............................................................. 91
Loading Simulated Water in a CWTS ................................................................... 94
Collecting Simulated Bangladesh Groundwater from a CWTS ............................. 96
Collecting Sediment and Plant Samples from a CWTS........................................ 98
Preparing Sediment and Plant Samples for Chemical Analyses.......................... 100
Sediment Dry Bulk Density .................................................................................. 102
Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Arsenic ............ 104
Acid Volatile Sulfide and Simultaneously Extracted Metal Concentration .......... 106
1.0 OBJECTIVE

The purpose of this protocol is to measure various general water quality parameters. Parameters such as pH, dissolved oxygen (DO), conductivity, temperature, alkalinity, and hardness are fundamental water quality parameters and are necessary for all water chemistry related studies.

2.0 HEALTH AND SAFETY

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

3.0 PERSONAL/TRAINING/RESPONSIBILITIES

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

4.0 REQUIRED MATERIALS

4.1 Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milli-Q water</td>
<td>all tests</td>
</tr>
<tr>
<td>pH buffers (4,7,&amp;10)</td>
<td>pH, alkalinity</td>
</tr>
<tr>
<td>0.02 N standard sulfuric acid solution (H$_2$SO$_4$)</td>
<td>alkalinity</td>
</tr>
<tr>
<td>Eriochrome Black T indicator</td>
<td>hardness</td>
</tr>
<tr>
<td>Standard EDTA titrant (0.01M, 0.02N)</td>
<td>hardness</td>
</tr>
<tr>
<td>Buffer solution (Reference Standard Methods 2340C)</td>
<td>hardness</td>
</tr>
</tbody>
</table>

4.2 Supplies

<table>
<thead>
<tr>
<th>Supply</th>
<th>Test:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graduated cylinder</td>
<td>all tests</td>
</tr>
<tr>
<td>100-mL beakers</td>
<td></td>
</tr>
<tr>
<td>Magnetic stir bar</td>
<td>alkalinity, hardness</td>
</tr>
</tbody>
</table>

Jeff Schwindaman, Brenda M. Johnson, Laura E. Ober, John H. Rodgers, Jr.
50-mL burette and stand
alkalinity, hardness

4.3 Equipment
Orion-model A325 pH Meter
Orion-model 420A pH Meter
YSI 55 Dissolved Oxygen Meter
YSI 30 Salinity, Conductivity, and Temperature Meter
Magnetic stir plate

5.0 PROCEDURE

5.1 pH
2. Rinse probe with milli-Q water to remove any prior contaminant.
3. Remove the small blue rubber stopper from the probe.
4. Submerge the tip of the probe in the sample and gently stir the sample with the probe.
5. When the pH reading has stabilized, record pH in S.U. to a tenth of a S.U.
6. Rinse probe with milli-Q water between measurements and return to holder when finished.

5.2 Dissolved Oxygen (DO)/Temperature
1. Calibrate the YSI 55 Dissolved Oxygen Meter.
2. Rinse probe with milli-Q water to remove any prior contaminant.
3. Completely submerge the tip of the probe in the sample.
4. When the DO reading has stabilized, record DO in mg/L. Also record the temperature to a tenth of a degree (i.e. 20.1°C).
5. Rinse probe with milli-Q water between measurements and return to holder when finished.

5.3 Conductivity
1. Turn on the YSI 30 Salinity, Conductivity, and Temperature Meter.
2. Rinse probe with milli-Q water to remove any prior contaminant.
3. Submerge the probe in the sample and gently stir the sample with the probe.
4. When the conductivity reading has stabilized the conductivity will record in (mS/cm and temperature in degrees Celsius.
5. Rinse probe with milli-Q water and return to holder.
6. When finished turn off the meter

5.4 Alkalinity
1. Using a graduated cylinder, measure 50 mL of sample water and pour it into a 100-mL beaker with a magnetic stir-bar.
2. Place sample beaker on magnetic stir-plate. Turn on stir-plate to begin mixing sample.
3. Calibrate Orion-model 420A pH meter. Place probe in the appropriate stand, with the tip completely submerged in the sample water. (Make sure the stir-bar does not hit the pH probe).
4. Record the initial level of titrant (0.02 N H$_2$SO$_4$) in the burette (fill burette as necessary).
5. Slowly drip titrant into the sample, allowing time for the pH meter to stabilize.
6. Titrate to pH 4.5.
7. Record the volume (mL) of titrant used to reach the pH endpoint (pH=4.5).
8. Calculate: Total Alkalinity (mg/L as CaCO$_3$) = col. Titrant (mL)x 20
9. Turn off stir-plate and discard sample.

5.5 Hardness
1. Using a graduated cylinder, measure 50 mL of sample water and pour it into a 100-mL beaker with a magnetic stir-bar. (Dilutions can be made to conserve EDTA titrant, be sure to calculate dilutions into the final equation.)
2. Add 2-5 mL of buffer solution (to give the sample a pH of 10.0-10.1).
3. Add 2-4 drops of Eriochrome Black T Indicator. Sample should turn pink.
4. Place sample beaker on magnetic stir-plate. Turn on plate to mix sample.
5. Record the level of titrant (EDTA) in the burette (fill burette as necessary).
6. Slowly drip titrant into the sample, allowing time for the color change to stabilize.
7. Titrate until pink turns to a blue-green color.
8. Record the volume of titrant (mL) used to reach the color change.
9. Calculate: Hardness (mg/L CaCO$_3$) = volume titrant (mL) x 20.
10. Turn off stir-plate and discard sample.

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 HYDROSOIL IN A CONSTRUCTED WETLAND TREATMENT SYSTEM

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

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 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 the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED MATERIALS

4.1 Supplies

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

4.2 Equipment

pH or millivolt 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 Milli-Q water at 25°C. These measurements must be 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 equilibrium then record the reading to the nearest millivolt. If the reading is within ±10 mV from the theoretical redox standard value at 25°C (+183 mV), record the reading. The indicator electrode is ready for placement in the hydrosoil. If the reading is not within +10 mV, the indicator electrode must be remade.

Place the indicator electrode’s platinum tip into the sediment making certain it is not near the plant roots. Secure the electrode with cable ties. Allow the electrode to equilibrate for 24 hours prior to taking any readings. When measuring the redox potential of the hydrosoil place the reference electrode in the same water column as the probe. 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. Successive readings that vary less than +10 mV over 10 minutes are adequate for most purposes. Adjust the reading according to field corrections and electrode calibration corrections.

Example: The field measurement of a hydrosoil was -206 mV. When the electrode was initially calibrated in the lab, the redox reading was +193mV, which is +10mV different from the theoretical redox standard value of +183 mV. The field redox measurement must be corrected for this difference by subtracting 10 mV from -206 mV. This gives a redox measurement of -216 mV. The standard correction factor for field redox measurements for the millivolt reader is +240 mV. Therefore, this correction factor is added to the redox measurement of -2216 mV to yield a final redox measurement of +24 mV.

\[
E_{\text{h system}} = E_{\text{h observed}} - E_{\text{h reference observed}} + E_{\text{h field correction}}
\]

\[
E_{\text{h system}} = -206\text{mV} + 183\text{mV} - 193\text{mV} + 240\text{mV}
\]

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

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

7.0 REFERENCES

METHOD FOR SIMULATING AND LOADING SIMULATED BANGLADESH GROUNDWATER INTO A PILOT-SCALE CONSTRUCTED WETLAND TREATMENT SYSTEM (CWTS)

Jeff Schwindaman, Michael J. Pardue, Jennifer Horner, Brenda M. Johnson, Laura E. Ober, and John H. Rodgers Jr.

1.0 OBJECTIVE

The objective of this standard operating procedure (SOP) is to clearly outline and define the requirements of loading simulated Bangladesh groundwater to insure quality assurance and quality control measures.

2.0 HEALTH AND SAFTEY

Proper personnel protective equipment will be worn at all times.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

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

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies
   Hose
   1500 gallon detention basin
   Vinyl tubing (3/8 in. by 1/4 in.) (Outer diameter by inner diameter)
   4 Fluid Metering, Inc.® (FMI®) piston pumps
   1000 mL beaker
   200 mL graduated cylinder
   Salts (CaCl₂, MgCl₂, KCl, NaHCO₃, MgSO₄, FeSO₄, Na₂SiO₃, K₂PO₄, As₂O₃, As₂O₅)

5.0 PROCEDURE

Fill the detention basin with municipal water to 200 gal before adding salts. Keep the hose running while adding the desired concentrations (formulated from target constituent concentrations) of salts. Add the salts one at a time allowing for adequate mixing between additions. Dissolve salts in 500 mL of water before adding to the detention basin.

After the detention basin is adequately mixed the pumps to the CWTS can be turned on, the calibration of the FMI pumps must be verified. This is completed one at a time by
turning on the pumps, and measuring the collected volume in a 200 mL graduated cylinder over two minutes. If this volume is different than 256 mL, then the pumps must be adjusted accordingly to achieve the flow rate of 128 mL min$^{-1}$. After the pumps are calibrated, the pumps may be turned on to pump the simulated Bangladesh groundwater into the CWTS.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.
METHOD FOR SAMPLING SIMULATED BANGLADESH GROUNDWATER FROM A CONSTRUCTED WETLAND TREATMENT SYSTEM (CWTS) FOR MULTIPLE CHEMICAL ANALYSES

Jeff Schwindaman, Michael J. Pardue, Jennifer Horner, Brenda M. Johnson, Laura E. Ober, and John H. Rodgers, Jr.

1.0 OBJECTIVE

The objective of this standard operating procedure (SOP) is to clearly outline and define the requirements of aqueous sample collection of simulated Bangladesh groundwater to ensure quality assurance and quality control measures.

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 the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies
Centrifuge tubes (50 mL)

5.0 PROCEDURE

Simulated Bangladesh groundwater (loading predetermined) will be introduced into the pilot-scale CWTS starting at approximately time-0 hrs from the detention basin (1500 gallon carboy). CWTS influent should be sampled from the plastic tube delivering simulated Bangladesh groundwater to the first reactor in series (50 mL of water should be collected in a 50 mL centrifuge tube.

Water can be sampled along the flow path of the CWTSs at sampling ports (breaks in PVC pipes connecting microcosms). Water should be sampled after the first reactor (R1) 24 hours after the influent to the CWTS was sampled, assuming a 24-hr HRT per reactor. Water should be sampled after the second reactor (R2) in series 48 hours after the influent was sampled, continue for reactors 3 and 4. Depending on intended analyses 1-2 L of water should be collected, in addition to a 50 mL centrifuge tube.

All water samples will be immediately transported to the Ecotoxicology laboratory in Lehotsky Hall, room 228, and refrigerated at 4ºC until analyses can be conducted.
6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.
METHOD FOR EXTRACTING SEDIMENT GRAB SAMPLES, SEDIMENT CORES, AND PLANTS FROM A CONSTRUCTED WETLAND TREATMENT SYSTEM (CWTS) FOR MULTIPLE CHEMICAL ANALYSES

Jeff Schwindaman, Kristen Jurinko

1.0 OBJECTIVE

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

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 the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Supplies
Aluminum foil
1.91-cm chlorinated polyvinyl chloride (CPVC) pipe
Stainless-steel rod
#6 rubber stopper
Mallet
Stainless-steel spatula
50-mL polypropylene centrifuge tubes
Stainless-steel shears
Deionized water

5.0 PROCEDURE

5.1 Sediment Grab Sample
Collect sample of sediment (including detritus) from the surface water-detritus interface down to a depth of approximately 2 cm using an acid-washed stainless-steel spatula. Scoop sediment sample into a 50 mL centrifuge tube and cap underwater. Store sediment samples at 4°C prior to chemical analysis.

5.2 Sediment Core
Insert sharpened 1.91-cm diameter CPVC pipe with the aid of a mallet into hydrosoil until the tip contacts the bottom of Rubbermaid® tank (a depth of 30 cm). Insert rubber
stopper tightly into the top of CPVC pipe to create a vacuum. Gently twist the core and remove, sealing the bottom with one hand to prevent sediment from falling. Remove rubber stopper and use a metal rod to extrude the core onto a sheet of clean aluminum foil. Manually section the core into 6-cm intervals and place into labeled plastic bags. Store sediment samples at 4ºC prior to chemical analysis.

5.3 Plant
Dig beneath hydrosoil to uproot plant and remove it from reactor. Rinse sediment from roots with DI water. Section plant into roots, submerged shoots, emergent shoots, and tips with stainless-steel shears and place in labeled plastic bags. Store plant samples at 4ºC prior to chemical analysis.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

All procedures are subject to review by the Quality Assurance Unit.
METHOD FOR PREPARING PLANTS AND HYDROSOIL FOR MEASURING METAL CONCENTRATION

Jeff Schwindaman, Kristen N. Jurinko

1.0 OBJECTIVE

Metal concentration in the plants and hydrosoil provides a distribution of total metals sequestered in aquatic macrophytes and 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 the 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
Deionized water
Plastic bags

4.2 Equipment
2-mm sieve
Stainless-steel shears
Drying oven
CEM Microwave Sample Preparation System (includes turntable, pressure sensing line)
CEM Teflon vessels

4.3 Reagents
HNO₃, trace metal grade concentrated (67%) nitric acid

5.0 PROCEDURE

5.1 Standards
See the SOP for measurement of metals using an ICP-MS

5.2 Sample Preparation
Dry sediment at 100º C for at least 8 hours in a drying oven. Sieve sediment with a 2 mm sieve to remove large rock and mineral fragments and collect the fraction passed through the sieve in a labeled plastic bag.

Section plant into roots, submerged shoots, emergent shoots, and tips with stainless-steel shears Dry plant samples at 100º C for at least 8 hours in a drying oven and collect dried samples in labeled plastic bags.

5.3 Microwave Digestion

Weigh 0.5 g of sample into Teflon vessels. Add 10 mL of HNO₃ to each vessel and seal. Place all vessels into the turntable and place turntable into the system. Digest samples for 20 min at 170ºC (according to EPA method 3051B). Allow to cool to approximately 80ºC before opening vessels. In a fume hood, transfer digestate to a 50-mL centrifuge tube and bring to a volume of 25 mL with deionized water.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

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

7.0 REFERENCES


METHOD FOR MEASURING SEDIMENT DRY BULK DENSITY

Jeff Schwindaman

1.0 OBJECTIVE

Sediment dry bulk density is a measurement of the mass of sediment divided by the total volume. It can be multiplied by the volume of sediment in a CWTS reactor to determine the mass of sediment in the reactor.

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 the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.

4.0 REQUIRED MATERIALS

4.1 Supplies
50 mL glass graduated cylinder

4.2 Equipment
Drying oven
Analytical balance capable of weighing ± 0.1 mg

5.0 PROCEDURE

Dry sediment at 100° C for at least 8 hours in a drying oven. Weigh graduated cylinder with analytical balance and record mass to the nearest tenth of a mg. Tare the analytical balance and slowly add sediment to graduated cylinder, tapping lightly on the side to ensure settling of sediment. Record the volume of sediment in the graduated cylinder to the nearest mL. Weigh the sediment and graduated cylinder and record the mass to the nearest tenth of a mg. Sediment dry bulk density is equal to the mass of dry sediment divided by the total volume.

6.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA

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

7.0 REFERENCES
METHOD FOR MEASURING ARSENIC CONCENTRATION USING INDUCTIVELY COUPLED PLASMA MASS-SPECTROMETER (ICP-MS)

Jeff Schwindaman, Peter Van Heest, Dr. Brian Powell

1.0 OBJECTIVE

The purpose of this protocol is to measure total arsenic concentration in aqueous samples.

2.0 HEALTH AND SAFETY

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

3.0 PERSONAL/TRAINING/RESPONSIBILITIES

Any graduate research assistant familiar with the 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 plastic centrifuge tubes
- Pipette
- 1-L plastic Nalgene bottle
- Multi-element standards containing Ag, Au, 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.
- Trace-Metal Grade HNO₃
- Deionized water
- Tuning solution containing 10 ppb Li, In, Re, Ce, and Be
- Internal standard solution containing Ga and Y.

4.2 Instrumentation
- Thermo Scientific X Series 2 ICP-MS

5.0 PROCEDURE

5.1 Sample Prep
1. Centrifuge 25 mL sample at 8000 rpm for 20 minutes.
2. Pipette 1 mL of supernatant into a 15 mL centrifuge tube.
3. Bring volume to 10 mL with 2% trace-metal grade HNO₃ solution.

5.2 ICP-MS Analysis
1. Add 20 mL trace-metal grade HNO₃ to 1 L DI water contained in 1 L plastic Nalgene bottle
2. Create standards ranging from 0.005 to 100 µg/L by dilution of Multi-element standard in 2% HNO₃
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 Yttrium and Gallium as reference elements
9. In internal standard tab enter the calculated concentrations for each element in each internal standard
10. In sample list, enter the 9 standards followed by the samples
11. Enter a standard between approximately every 10 samples
12. Enter last 4 standards after the last sample on list
13. Put both intakes into the Nalgene bottle containing 2% HNO₃
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 settings to obtain As counts of about 10 cps. Settings 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 containing Y and Ga.
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 instrument.

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.
METHOD FOR DETERMINATION OF ACID-VOLATILE SULFIDES AND SIMULTANEOUSLY EXTRACTABLE METALS IN HYDROSOIL

Jeff Schwindaman and Kristen N. Jurinko

1.0 OBJECTIVE

Concentrations of AVS and SEM in the hydrosoil were measured by the modified diffusion method (Leonard et al., 1996). AVS is operationally defined by Leonard et al. (1996) as sediment sulfide that is liberated by treatment of the sediment with 1-N hydrochloric acid. AVS is a measure of reactive sulfide, which includes primarily free sulfides, amorphous iron monosulfide (FeS), and sulfides of other divalent metals (e.g. Cd, Cu, Ni, and Zn; Di Toro et al., 1992; Yu et al., 2001). Reactive metals liberated during AVS extraction are operationally defined as SEM (Di Toro et al., 1992; Allen et al., 1993; Leonard et al., 1996; Ankley et al., 1996; Yu et al., 2001).

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 the 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
100-mL volumetric flask
50-mL volumetric flasks (4)
45-µm Millipore membrane filter
500-mL filter flask
500-mL glass bottle (capped)
Parafilm®
Deionized water

4.2 Equipment
Magnetic stir bar
Magnetic stir plate
Mortar and pestle
Analytical balance capable of weighing ± 0.1 mg
ICP-MS
4.3 Reagents
De-aerated 1-N trace metal grade (37%) HCl
Sulfide Antioxidant Buffer (SAOB):
  2 M reagent grade NaOH to convert H₂S into S²⁻
  0.1 M reagent grade anhydrous ascorbic acid to prevent oxidation of S²⁻
  0.1 M reagent grade anhydrous EDTA to complex metals that may have catalyzed
  the oxidation of S²⁻

1.0 M Sulfide stock solution:
  Prepare using freshly washed sodium sulfide crystals to remove oxidized sulfide
  products & store at 4°C
  For calibration of the ion-selective electrode (ISE), prepare dilutions over the
  range 10⁻¹ to 10⁻⁴ M in SAOB solution.

5.0 PROCEDURE

5.1 MAKING STANDARDS

5.1.1 SAOB
To make 500 mL, add 100 mL of deionized water to a 500 mL glass bottle. Next, add 40
g NaOH and stir until NaOH completely dissolves to form a white liquid. Add 14.612g
EDTA and 8.8g ascorbic acid. Make to volume (500 mL) and cover with Parafilm® to
prevent oxidation. Store at 4°C. Use before solution turns dark brown.

5.1.2 Stock Standard
Set up vacuum filtration. Weigh and crush 2.4018 g NaS crystals with mortar and pestle
under fume hood. Rinse crystals with deionized water and vacuum filter water. Quickly
add NaS to 50 mL SAOB in a 100 mL volumetric flask and fill to volume with deionized
water. Stir with magnetic rod until crystals are dissolved. Cover with Parafilm® and
store at 4°C.

5.1.3 Standards & Calibration Curve
The stock solution prepared above is the 10⁻¹ M standard. To make 10⁻² M, mix 25 mL
SAOB with 5 mL stock solution and fill to volume with deionized water in a 50-mL
volumetric flask. Repeat making serial dilutions for 10⁻³ and 10⁻⁴ standards.

Clean and fill both the inner and outer filling solution of the reference probe. Place both
the sulfide probe and the reference probe in the standard poured into a small beaker.
Wait until the ISE reaches equilibrium; record mV readings to make a calibration curve.

5.2 Sample Preparation & Acidification
Add 50 mL of 1-N de-aerated trace metal grade (37%) HCl and magnetic stir bar to 500
mL glass bottle. Add 30 mL SAOB to a 50-mL centrifuge tube cut to fit inside of the
glass bottle.
Weigh and cap 5 g of wet hydrosoil in a 50-mL centrifuge tube. Quickly, place sample in bottom of the 500 mL glass bottle and cap. Stir for 60 minutes allowing it to be briskly stirred.

Pour SAOB into a small beaker for measurement of sulfide.

5.3 Measurement of Sulfide
Measure the sulfide generated and trapped in the SAOB using an ion-selective electrode. Rinse probes with deionized water between each measurement.

5.4 Simultaneously Extracted Metals
Vacuum-filter the HCl extract from the hydrosoil sample through a pre-cleaned 45 µm Millipore membrane filter into an acid cleaned 50-mL polypropylene centrifuge tube for metal analysis on an inductively coupled plasma mass spectrometer. See the SOP for measurement of arsenic and standards using an ICP-MS.

6.0 QUALITY CONTROL CHECKS
All procedures are subject to review by the Quality Assurance Unit.

7.0 REFERENCES


