Effects of supplemental organic carbon addition on sulfate reduction and zinc sulfide precipitation using microcosm and bench-scale constructed wetlands in cold climate regions
by Deborah Jean Borden

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University
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Abstract:
Two experimental constructed wetland (CW) systems were used to determine the effects of supplemental organic carbon addition on sulfate reduction and zinc precipitation over a simulated growing season.

The first research system consisted of sixteen 20x60-cm, batch-mode, subsurface CW microcosms (columns) that were filled with washed pea gravel and placed in a climate-controlled greenhouse. Eight of the columns were planted with Scirpus acutus (bulrush), four were planted with Typha latifolia (cattail) and four were left unplanted as controls. For one year, beginning in December 2000, all columns were used to treat a sulfate/zinc synthetic wastewater supplemented with two different concentrations of organic carbon. Four of the bulrush columns and all of the cattail and control columns were given 100 mg/L of supplemental organic carbon (sucrose); the remaining four bulrush columns were given 200 mg/L organic carbon. Two 20-day batch incubations were conducted at each of four temperature steps (4°C, 14°C, 24°C, 14°C) to simulate the seasons of the year. Samples were extracted from the sixteen columns on six occasions during each 20-day incubation. A second study, initiated in June 2000, consisted of eight bench-scale horizontal continuous-flow, subsurface wetlands. Three each were planted with cattail and bulrush and two were left unplanted as controls. A synthetic wastewater identical to the 100 mg/L organic carbon, batch-load system water was used. Samples of influent and effluent were analyzed once per hydraulic residence time, every five days. Samples from both systems were analyzed for sulfate (SO_4^{2-}) and chemical oxygen demand (COD). Periodically, samples were also taken for sulfide (S^{2-}), zinc (Zn^{2+}) and pH. In the batch-load system, redox potential (Eh) was automatically recorded in each column at three depths every four hours.

All treatments successfully removed sulfate and zinc in the batch-load system at warm temperatures (24°C). Bulrush plants had negative effects on sulfate and zinc removal at cold temperatures (4°C), due to root-zone oxidation. Cattail plants had similar but less dramatic negative effects on sulfate removal at cold temperatures, also likely due to root-zone oxidation. Bulrush columns with 200 mg/L organic carbon performed significantly better than all treatments with 100 mg/L organic carbon during the summer and fall (14°C). Bulrush columns with 200 mg/L organic carbon performed similarly to control columns with 100 mg/L organic carbon at cold temperatures. The batch-load system performed better than the continuous-flow system for COD and zinc removal. The continuous-flow system performed better than the batch-load system for sulfate removal.

EFFECTS OF SUPPLEMENTAL ORGANIC CARBON ADDITION ON SULFATE REDUCTION AND ZINC SULFIDE PRECIPITATION USING MICROCOSM AND BENCH-SCALE CONSTRUCTED WETLANDS IN COLD CLIMATE REGIONS

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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TABLE OF CONTENTS

LIST OF TABLES....................viii

LIST OF FIGURES....................ix

ABSTRACT....................xi

1. INTRODUCTION....................1

    Goals and Objectives....................4

2. BACKGROUND....................6

    Removal Pathways for Metals in Constructed Wetland....................6
        Exchange of Metals onto Organic Matter and Algae....................8
        Complexation of Metals with Larger Molecular Weight Organics....................9
        Adsorption of Metals to Precipitated Hydrous Oxides....................10
        Precipitation of Metals as Inorganic Oxyhydroxides....................11
        Precipitation of Metals as Insoluble Metal Sulfides....................12
        Uptake of Metals by Plants and Other Plant Effects....................14
    Alternate Electron Acceptors....................17
        Sulfate Reduction vs. Methanogenesis....................19
    Organic Matter Dynamics and Supplementation....................20
    Temperature and Seasonal Effects....................22
    Hydraulic Problems....................23
    Wetland Design for Metals Removal....................25

3. MATERIALS AND METHODS....................29

    Experimental Overview....................29
    Batch-Load System....................30
        Column Design and Construction....................30
        Wastewater Application, Sampling, and Analysis Procedures....................31
        Redox Measurement....................35
    Continuous-Flow Constructed Wetlands....................36
        Design and Construction....................36
        Wastewater Mixing and Delivery....................37

Sampling and Analysis Procedures....39
Statistical Analysis....40
Batch-Load System....40
Continuous-Flow System....41
Comparison of the Batch-Load and Continuous-Flow Systems....41

4. PLANT SPECIES AND CARBON LOAD EFFECTS IN THE BATCH-LOAD SYSTEM....42

Results....42
Plant Species Effects on Sulfate Removal....47
Carbon Load Effects on Sulfate Removal....48
Discussion....50
Plant Species Effects on Sulfate Removal....51
Carbon Load Effects on Sulfate Removal....54

5. TEMPERATURE AND SEASONAL EFFECTS IN THE BATCH-LOAD SYSTEM....61

Results....61
Sulfate Removal....61
Zinc Removal....65
Discussion....65

6. CONTINUOUS-FLOW SYSTEM RESULTS AND COMPARISONS TO THE BATCH-LOAD SYSTEM....73

Results....73
Discussion....81

7. CONCLUSIONS AND IMPLICATIONS....85

Conclusions....85
Plant Treatment Effects – Batch-Load System....85
Carbon Load Effects – Batch-Load System....85
Temperature and Seasonal Effects – Batch-Load System....86
Batch-Load vs. Continuous-Flow....86
Implications....87

CITED LITERATURE....88

APPENDIX A: WATER DELIVERY SYSTEM....95

Detailed Description of Water Delivery System....96

Circuit Diagram 99
Instructions for Timer Operation 100
Specifications on Centrifugal Pump 101

APPENDIX B: COD DATA 105

LIST OF TABLES

Table Page

1. Selected types of microbial oxidation-reduction reactions in wetlands..............18

2. Concentrations of significant wastewater constituents......................................33

3. Percent sulfate removal for Incubations 1-8...44

4. Within-incubation MANOVAR analysis for sulfate removal.............................46

5. Between-incubation MANOVAR analysis for sulfate removal..........................48

6. Percent sulfate and zinc removal and ANOVA analysis for the continuous-flow cells, overall, and in summer, fall and winter..........................76

LIST OF FIGURES

Figure Page

1. Schematic of column design and water delivery system (Allen, 1999) 32

2. Timeline and temperature steps for the eight incubations 34

3. Plan view schematic of a continuous-flow constructed wetland cell (Biederman, 1999) 38

4. Degradation of influent sulfate in the holding tanks over an eight-day emptying period 39

5. Sulfate concentrations over eight 20-day incubations 43

6. Sulfate concentrations and root-zone oxidation over 20-day incubations for spring, summer, fall and winter 52

7. COD concentrations used for sulfate reduction and other redox reactions on Day 5 for Incubations 1-8 in the bulrush-low and bulrush-high columns....58

8. Sulfate concentrations for all incubations on Days 1, 5 and 20 62

9. Zinc concentrations on Day 20 for Incubations 0 and 1 and on Day 5 for Incubations 2-8 63

10. Molar concentration of sulfate and zinc removed by Day 5 for Incubations 1-8 71

11. Mean influent and effluent sulfate concentrations for the continuous-flow cells 74

12. Mean effluent zinc concentrations for the continuous-flow cells......................75

13. Fraction of sulfate remaining on Day 5 for the CF system compared to the BL system for summer, fall and winter..78

14. Fraction of COD remaining on Day 5 for the CF system compared to the BL system for five incubations........79

15. Fraction of zinc remaining on Day 5 for the CF system compared to the BL system........80

16. COD concentrations over eight 20-day incubations........106

ABSTRACT

Two experimental constructed wetland (CW) systems were used to determine the effects of supplemental organic carbon addition on sulfate reduction and zinc precipitation over a simulated growing season.

The first research system consisted of sixteen 20x60-cm, batch-mode, subsurface CW microcosms (columns) that were filled with washed pea gravel and placed in a climate-controlled greenhouse. Eight of the columns were planted with *Scirpus acutus* (bulrush), four were planted with *Typha latifolia* (cattail) and four were left unplanted as controls. For one year, beginning in December 2000, all columns were used to treat a sulfate/zinc synthetic wastewater supplemented with two different concentrations of organic carbon. Four of the bulrush columns and all of the cattail and control columns were given 100 mg/L of supplemental organic carbon (sucrose); the remaining four bulrush columns were given 200 mg/L organic carbon. Two 20-day batch incubations were conducted at each of four temperature steps (4°C, 14°C, 24°C, 14°C) to simulate the seasons of the year. Samples were extracted from the sixteen columns on six occasions during each 20-day incubation. A second study, initiated in June 2000, consisted of eight bench-scale horizontal continuous-flow, subsurface wetlands. Three each were planted with cattail and bulrush and two were left unplanted as controls. A synthetic wastewater identical to the 100 mg/L organic carbon, batch-load system water was used. Samples of influent and effluent were analyzed once per hydraulic residence time, every five days. Samples from both systems were analyzed for sulfate (SO_4^{2-}) and chemical oxygen demand (COD). Periodically, samples were also taken for sulfide (S^{2-}), zinc (Zn^{2+}) and pH. In the batch-load system, redox potential (Eh) was automatically recorded in each column at three depths every four hours.

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CHAPTER 1

INTRODUCTION

Since the early 1980's, constructed wetlands have gained recognition as a cost effective, low maintenance alternative to traditional treatment of waters impacted by mining activities. These waters are often termed acid mine drainage (AMD) and are usually characterized by high concentrations of trace metals and sulfate, and almost always by a low pH. AMD is formed during coal and metal-mining where the disruption of soil and bedrock exposes pyrite-bearing strata to oxygen and water. Pyrite is an iron sulfide mineral (FeS_2) that forms in reducing environments. When exposed to oxygen and water, it will oxidize and release large quantities of iron and sulfate into solution. In addition, H^+ ions are liberated during the oxidation process causing an acidic solution that readily weathers other minerals, releasing other trace minerals into solution (Cohen and Gorman, 1991; Fennessy and Mitsch, 1989; Robb, 1994; Wieder, 1989; Wildeman *et al.*, 1993). The environmental impact of AMD can be severe. It is a major industrial water pollution problem in the coal mining regions of the U.S. and around the world. In the U.S., it impacts many miles of streams and rivers. Often, ferric hydroxide will coat the bottoms of streams and rivers causing serious damage to fish and aquatic plant communities (Robb, 1994). Under acidic conditions, metals may be transported downstream dissolved in water, and when pH changes to neutral or basic, the metals precipitate and end up in aquatic bottom sediments (Cohen and Gorman, 1991).

Remediation of AMD may involve active chemical treatment, such as liming, which increases the pH and subsequently precipitates metals. Chemical treatment requires significant amounts of materials and many hours of maintenance, which results in high operational costs. Alternatively, constructed wetlands can be a low cost, low maintenance treatment system proven to ameliorate AMD chemistry (Webb *et al.*, 1998). Wetlands used for AMD treatment are a fairly new technology and were first proven to improve AMD chemistry in the late 1970's and early 1980's (Huntsman *et al.*, 1978; Wieder and Lang, 1982). These findings led to an increased interest in the potential of constructed wetlands as an alternative to chemical treatment.

In the 1980's, extensive research was conducted to understand the biological and chemical processes in wetlands that may contribute to improvement of AMD chemistry (Stark *et al.*, 1995a; Wieder, 1989). Although research was beginning to explain the processes controlling the effectiveness of wetlands for AMD amelioration, results from wetland studies in the 1980's were highly variable (Hiel and Kerins, 1988; McIntire and Edenborn, 1990). This led many scientists to recommend additional research to improve the understanding of the factors controlling AMD chemistry in wetlands and to improve constructed wetland design for consistent treatment (Henrot and Wieder, 1990; Hiel and Kerins, 1988).

Considerable research continued through the 1990's at which time researchers suggested that sulfate reduction and subsequent metal sulfide precipitation was the predominant metal removal process in constructed wetlands after system start-up. Anaerobic sulfate reduction produces hydrogen sulfide, which chemically reacts with many trace metals to form highly insoluble metal precipitates that remain in the wetland. In addition, sulfate reduction and metal sulfide precipitation consume H^+ ions and raise the pH (Christensen *et al.*, 1996; McIntire *et al.*, 1990; Machemer, 1992; Machemer *et al.*, 1993; Webb *et al.*, 1998). In spite of a better understanding of the factors controlling AMD chemistry in constructed wetlands, treatment is still variable. Consequently, a Directive was developed in 1997 by the Office of Surface Mining, Reclamation and Enforcement (OSMRE) which states that wetlands are viewed as a viable AMD treatment alternative, but that treatment using this technology must be supported by an operable chemical or other conventional treatment system capable of consistently meeting water quality standards in the event that the wetland system does not adequately treat the discharge (OSMRE, 1997). This Directive significantly increases the costs of implementing a constructed wetland system. Future research may produce more consistent results, at which time, a conventional system may not be required.

There are various processes within a constructed wetland that can enhance or interfere with metals removal. Temperature and season, plants and organic matter availability all have significant effects on metals removal in constructed wetlands.

Goals and Objectives

Initially the purpose of this study was to look at the effectiveness of pre-established continuous-flow constructed wetlands for the treatment of a synthetic wastewater high in metals and sulfate without the addition of organic matter. It was hypothesized that internal carbon production would provide some sulfate reduction even without additional influent organic carbon. However, a trial in August 1999 using small concentrations of sulfate found higher sulfate concentrations in the effluent than in the influent, suggesting that added organic carbon is needed for successful sulfate reduction. Since organic carbon is necessary, a change in research focus was required.

The new purpose of the study was to evaluate the effects of plant species, organic carbon concentration, season and temperature on the treatment of a synthetic mine wastewater. The objectives were: (1) compare performance of two plant species and unplanted controls for removal of sulfate and zinc over one year, (2) compare performance of one plant species at two different concentrations of organic carbon, (3) quantify seasonal and temperature variation in removal of sulfate and zinc over one year, (4) compare performance of continuous-flow and batch-load systems.

Two systems were used to accomplish the objectives, a batch-load system and a continuous-flow system. Objectives 1, 2 and 3 were met using a batch-load system. Eight batch incubations were conducted over one year starting in December 1999 and ending in January 2001. Two incubations were conducted at each of four temperature steps simulating the four seasons of the year (4°C, 14°C, 24°C, 14°C). Objectives 1 and 3 were also addressed with the continuous-flow system. Objective 4 was met by comparing the batch-load and the continuous-flow system. The same synthetic wastewater recipe was used in each system to compare performance of sulfate and zinc removal. Due to mechanical problems, data from the continuous-flow system was taken only from June 2000 through the beginning of January 2001. Both systems were located in the same greenhouse and experienced identical environmental conditions.

Background, methods and conclusions for the entire study are presented in Chapters 2, 3 and 7, respectively. Chapter 4 discusses objectives 1 and 2 based on the batch-load system. Chapter 5 discusses objective 3 for the batch-load system. Chapter 6 discusses objective 1 for the continuous-flow system, as well as objective 4 comparing batch-load and continuous-flow systems.

CHAPTER 2

BACKGROUND

Removal Pathways for Metals in Constructed Wetlands

Trace and toxic metals resulting from mining activities may be successfully immobilized in constructed wetlands by a variety of processes. Metals generally enter the wetland environment in water-soluble form and are converted by physical, chemical and biological processes into less mobile forms that, depending on degree, can be classified as readily mobile and available, potentially mobile and available, and essentially immobile and unavailable (Gambrell, 1994). The goal of constructed wetland design is to adequately retain metals in a form that will not be easily released back into the environment.

Readily mobile and available metals consist of water-soluble metals and exchangeable (sorbed) metals. Water-soluble metals may be in the form of soluble free ions, soluble organic or soluble inorganic complexes. These forms are the most mobile and plant available. Exchangeable metals weakly bond to organic and inorganic surfaces and are temporarily immobilized in wetland substrates, but may be released from wetlands if there is a shift in pH or if adsorbed metals come into competition with metals that are more favorable for adsorption (Gambrell, 1994; Wildeman *et al.*, 1993).

The majority of removal pathways in wetlands retain metals in the potentially mobile and available form. These include:

1) Complexation with larger molecular weight organics (humic and fulvic acids)
2) Adsorption to precipitated hydrous oxides (Fe, Mn and Al oxides)
3) Precipitation as inorganic compounds (metal oxides, hydroxides or carbonates)
4) Precipitation as insoluble metal sulfides
5) Uptake by plants

Metals in these forms can be described as effectively immobilized but may potentially become available if there is a shift in pH or oxidation status (Gambrell, 1994; Wildeman *et al.,* 1993; Noller *et al.*, 1994).

Metals in the essentially immobile and unavailable form generally are bound within the crystalline lattice structure of primary minerals. These metals will bind by isomorphous substitution in clay minerals and will only become available due to weathering, generally over long periods of time (Gambrell, 1994).

The processes of exchange, complexation, precipitation and plant uptake that contribute to metals removal in wetlands will be discussed more specifically in the following text.

Exchange of Metals onto Organic Matter and Algae

Organics, generally humic and fulvic acids attached to soil surfaces, can temporarily retain metals in wetlands. During cation exchange processes, a pre-adsorbed cation will dissociate from the organic acid and be replaced with another by chemical binding (Wildeman *et al.*, 1993). Specific cations are preferentially adsorbed to organic matter. Machemer and Wildeman (1992) found that Fe and Cu adsorb more strongly than Zn which, in turn, adsorbs more strongly than Mn. An increase in total metal concentration decreased the percent of Mn and Zn adsorbed as Fe and Cu were preferentially bound. Sorption of metals also varies with pH (Machemer and Wildeman, 1992; Stumm and Morgan, 1996). At low pHs, H^+ ions are more preferentially sorbed than metals (Wildeman *et al.*, 1993).

There is a finite number of sites on organic matter where metals may be temporarily retained, known as the ion exchange capacity. It is defined as the number of moles of ion charge that can be adsorbed to a unit mass of soil under given conditions of temperature, pressure, soil solution composition and soil-solution mass ratio (Stumm and Morgan, 1996). When ion exchange sites are filled, the soil is considered saturated. During initial start-up of a wetland, sorption of metals can be the main removal pathway. Once the organic matter is saturated, this removal pathway is insignificant. Machemer and Wildeman (1992) found that it took approximately four months for their wetland to reach saturation. Once sorption sites were filled, metal sulfide precipitation became the dominant process for metal removal. Christensen *et al.* (1996) found similar results. Adsorption was the dominant metal removal process for the first three weeks after wetland start-up. After this time, metal sulfide precipitation became the dominant removal process. Wildeman *et al.* (1993) suggested that during wetland start-up, metal exchange onto organic matter could serve for temporary retention of metals until sulfate-reducing bacteria have a chance to establish and take over as the dominant metal removal mechanism.

There is growing evidence that algae also remove metals from mine drainage using ion exchange processes (Crist *et al.*, 1990; Wildeman *et al.*, 1993). This metal removal process is not considered significant, but may be considered important for short-term immobilization similar to ion exchange with organic matter (Kadlec and Knight, 1996). Harris and Ramelow (1990) found that two algaes, *Chlorella vulgaris* and *Scenedesmus quadricauda* were efficient at binding silver, copper, cadmium and zinc. Copper, cadmium and zinc binding was pH-dependent while silver was strongly bound over a wide pH range. Crist *et al.*, (1990) found that adsorption of Strontium (Sr) on *Vaucheria* released equivalent amounts of Ca and Mg. Release of protons was observed when Cu was adsorbed, demonstrating additional ion exchange characteristics. Algae have also been known to trap metal oxyhydroxide precipitates, further increasing adsorption of metals in wetlands (Wildeman *et al.*, 1993).

Complexation of Metals with Larger Molecular Weight Organics

Metals complexed with larger molecular weight organics are more tightly bound and effectively immobilized in contrast to exchangeable metals. Complexation occurs by the same processes as adsorption, but complexation reactions are considerably more stable. Evidence suggests that metal complexation is more effective in anoxic and reducing conditions and long-term oxidation may result in a major release of complexed metals (Gambrell, 1994). Henrot and Wieder (1990) found that Fe binding to large molecular weight organics was one of the main removal mechanisms. Similar to adsorption of exchangeable cations, there are limited sites on large molecular weight organics for metal complexation (Henrot and Wieder, 1990).

Adsorption of Metals to Precipitated Hydrous Oxides

Fe(III) and Al hydroxide, as well as Mn(IV) oxyhydroxide precipitates have a strong capability of adsorbing other metal ions onto their surfaces (Stumm and Morgan, 1996). The surface of an oxyhydroxide operates as a weak acid, which attracts hydroxide ions creating a negative surface which, in turn, attracts metal cations. Adsorption to precipitated hydrous oxides can be used in a wetland as a polishing and buffering step to help remove residual metal contaminants (Wildeman *et al.*, 1993). Hydrous oxides form in aerobic environments. When exposed to anaerobic (i.e. low redox potential) environments, hydrous oxides become unstable which, in turn, causes a release of adsorbed metals. Because hydrous oxides are unstable at low redox potential, adsorption to hydrous oxides may only be important in the top few millimeters of wetland sediments, where aerobic conditions exist (Gambrell, 1994).

Precipitation of Metals as Inorganic Oxyhydroxides

Oxyhydroxide precipitation of Fe and Mn can be a major removal mechanism in aerobic environments of wetlands. Iron and manganese enter a wetland generally in the +2 state. Oxidation of Fe(II) and Mn(II) and subsequent hydrolysis create relatively insoluble metal oxyhydroxide precipitates (Wildeman *et al.*, 1993).

$$Fe^{2+} + \frac{1}{4} O_2 + H^{+} \rightarrow Fe^{3+} + \frac{1}{2} H_2O \qquad (1)$$

$$Fe^{3+} + 3\ H_2O \rightarrow Fe(OH)_3 + 3\ H^{+} \qquad (2)$$

Equations describing Mn oxidation and precipitation may also be found in Wildeman *et al.* (1993). Fe and Mn oxidation is pH dependent and uses oxygen as the electron acceptor (Henrot and Wieder, 1990; McIntire *et al.*, 1990). Fe(II) is autooxidized at pH values greater than 6, while in more acidic conditions, bacteria catalyze the reaction (McIntire *et al.*, 1990). Although Fe(II) may be oxidized to Fe(III) at pH values as low as 3.0, it will not oxidize at acceptable rates until the pH in the system nears 7.0. Mn (II) will not oxidize to Mn(IV) at acceptable rates for pH less than 9.0. Therefore, oxidation of Fe and Mn in low pH solutions, such as AMD, can be difficult and is the limiting step in the oxidation and precipitation sequence (Wildeman *et al.*, 1993). The oxidation and precipitation sequence of Fe and Mn produces H^+ ions, and therefore can be a self-limiting process (McIntire *et al.*, 1990; Stumm and Morgan, 1996; Wildeman *et al.*, 1993). Fe and Mn oxidation and precipitation is also dependent on other wetland parameters such as temperature, flow rate and total metal concentrations of Fe and Mn (Henrot and Wieder, 1990).

Precipitation of Metals as Insoluble Metal Sulfides

In the past ten years, many researchers have acknowledged sulfate reduction and subsequent metal sulfide precipitation as a major contributor to metals removal in wetlands (Christensen *et al.*, 1996; McIntire *et al.*, 1990; Machemer, 1992; Machemer *et al.*, 1993; Webb *et al.*, 1998). Provided conditions remain strongly reducing, metals can be immobilized in wetlands as highly insoluble metal sulfide precipitates (Gambrell, 1994). Mine waters generally contain high concentrations of sulfate and heavy metal cations. In an oxidation-reduction reaction, sulfate-reducing bacteria (SRBs) oxidize decayed organic matter (electron donor) and reduce sulfate (electron acceptor) to sulfide (Odom and Singleton, 1993; Stumm and Morgan, 1996). The produced sulfide will quickly react with trace and toxic divalent metals forming highly insoluble metal sulfide precipitates (Gambrell, 1994; Singh, 1992; Wildeman *et al.*, 1993).

Sulfate reducing bacteria are ubiquitous species. They will tolerate temperatures below -5°C and above 50°C. Their optimal pH range is 5.0 – 9.0, but they have been known to tolerate pH values as low as 2.6 and as high as 9.5. Their optimal oxidation-reduction potential (Eh) is less than –150 mV, but SRBs may be present in soils with Eh as high as –50 mV. The survival of SRBs in adverse conditions is probably due to their ability to form favorable microenvironments on surfaces and particulate material (Bolis *et al.*, 1991; Lyew and Shepard, 1999; Odom and Singleton, 1993; Wildeman *et al.*, 1993). Most SRBs utilize only simple organic substrates as electron donors. The most common of these include primary alcohols (such as ethanol), acetate, lactate, formate, pyruvate, glycerol and some dicarboxylic acids (such as malate and fumarate). They can also utilize

H_2 as an electron donor. Many SRBs cannot completely oxidize organic matter to CO_2 (Odom and Singleton, 1993). An equation for sulfate reduction is given below using lactate as the electron donor:

$$SO_4^{2-} + 2\text{ lactate} \rightarrow 2\text{ acetate} + H_2S + 2\ HCO_3^- \ (\text{pH} < 7.0) \quad (3)$$

The produced sulfide then reacts with a divalent metal (M) to for an insoluble metal sulfide precipitate:

$$H_2S + M^{2+} \rightarrow MS + 2H^+ \quad (4)$$

Metal sulfides precipitate out of solution in an order inverse to their solubility product (K_{sp}) values:

$$CuS < PbS < CdS < ZnS < NiS < FeS < MnS \quad (5)$$

Copper has the lowest solubility and will precipitate out of solution first, whereas Mn has a high solubility and does not readily form a metal sulfide precipitate (Christensen *et al.*, 1996; Machemer, 1992; Stumm and Morgan, 1996). Most metal sulfides have a lower solubility than their hydroxide counterpart (exceptions being Al, Fe and Mn) and can precipitate over a broad pH range (Dvorak *et al.*, 1991; Eger, 1992). They also have a higher sludge density than hydroxide precipitates, resulting in lower sludge handling costs (Christensen *et al.*, 1996).

Generally, the sulfate reduction step is rate limiting. Although SRBs are ubiquitous and tolerate a wide range of environmental conditions, rates of sulfate reduction in wetlands have been found to be extremely variable and are dependent on many wetland factors (Webb *et al.*, 1998). These include pH, redox potential, temperature, concentration of dissolved sulfate, quantity and type of organic matter and the surface area available for SRB and fermentative bacteria attachment (Lyew and Sheppard, 1999; Westrich and Berner, 1988).

Uptake of Metals by Plants and Other Plant Effects

Initially thought to be a significant pathway, a number of results suggest that uptake of metals by plants accounts for removal of less than 1% of the total metal load applied to a wetland (Gersberg *et al.*, 1985; Hiel and Kerins, 1988; Mitsch and Wise, 1998; Sencindiver and Bhumbla, 1988; Wildeman *et al.*, 1993). Metal uptake in sufficient quantities can be toxic to plants. Plants will form iron oxide coatings on their roots to prevent excess metal uptake (Sencindiver and Bhumbla, 1988; Wildeman *et al.*, 1993).

There is contradictory evidence as to where plants store metals in their tissues. Taylor and Crowder (1983) and Hiel and Kerins (1988) suggested that plants retain most metals in the roots and not in the rhizomes or aboveground biomass. Sencindiver and Bhumbla (1988) suggested that Fe and Mn accumulate in the rhizomes and aboveground biomass and not in the roots. To explain these contradictions, Wildeman *et al.* 1993 suggested that as a survival mechanism, plants shift contaminants from the roots to the senescing leaves at the end of the growing season.

Although plants may not play a significant role in metals removal by uptake directly, they serve other important roles in the wetland environment. Plants provide the biomass necessary for other processes that can remove metals. Decayed plant materials break down by enzymatically catalyzed microbial processes to a soluble form of organic matter useable for sulfate reduction. The decaying organic matter also provides additional sorption sites for metals (Eger, 1992; Mitsch and Wise, 1998; Noller *et al.*, 1994; Wildeman *et al.*, 1993).

Plants can also provide oxygen to the substrate. Plant roots need oxygen for aerobic respiration and cannot obtain the oxygen they need from diffusion through the wetland surface (Reddy *et al.*, 1989). Instead, they must transport oxygen from the aboveground leaves to the roots through air-filled intercellular spaces (aerenchyma) that extend from foliar parts down to the roots (Armstrong, 1971). Many researchers have speculated that oxygen transported from aerial tissue into the root zone may exceed plant demands. If so, oxygen may diffuse out of submerged roots into wetland sediments (Callaway and King, 1995; Dunabin *et al.*, 1988; Flessa and Fisher, 1992; Moorhead and Reddy, 1988; Reddy *et al.*, 1989; Sorrell and Armstrong, 1994; Steinberg and Coonrod, 1994). It has been suggested by a number of researchers that the higher the sediment oxygen demand, the greater the sink for oxygen released from plant roots (Dunabin *et al.*, 1988; Reddy *et al.*, 1989; Sorrell and Armstrong, 1994).

For the purposes of metals removal, rhizosphere oxidation has advantages and disadvantages. Positively, rhizosphere oxidation detoxifies H_2S along with Fe and Mn, which may reach phytotoxic concentrations in waterlogged soils (Armstrong, 1971; Gambrell and Patrick, 1978; Reddy and D'Angelo, 1994). Aerobic microsites may allow for the oxidation of iron and manganese and subsequent precipitation as hydrous oxides, which have a high surface area and adsorptive capacity for phosphorus and metals (Patrick and Gambrell, 1976). Negatively, rhizosphere oxidation increases the redox potential of the wetland environment, which adversely affects anaerobic sulfate reduction/metal sulfide precipitation, the major pathway for metals removal in anaerobic wetlands (Cohen and Gorman, 1991; Wildeman *et al.*, 1993). Sulfate reducing bacteria are generally obligate anaerobes and cannot survive aerobic conditions (Odom and Singleton, 1993). In addition, oxygen is a more thermodynamically favorable electron acceptor than sulfate and will consume soluble organic compounds that would be used for sulfate reduction had oxygen not been present. The organic compounds oxidized by oxygen may limit the amount of organic compounds available for sulfate reduction, in effect, reducing the amount of metals removed in an anaerobic wetland. Cohen and Gorman (1991) and Wildeman *et al.* (1993) suggested that plants are not necessary or may be counter-productive for the purposes of sulfate reduction and metals removal in wetlands. Wildeman *et al.* (1993) suggested that for sulfate-reducing treatment systems, the most important role plants play is for aesthetic considerations.

Plants have several other advantages. They provide additional surface area for the attachment of microbial communities (Fennessy and Mitsch, 1989). They also act as a living filtration system for suspended solids and increase water retention time in wetlands, allowing for sedimentation processes to occur (Fennessy and Mitsch, 1989; Noller *et al.*, 1994; Reddy *et al.*, 1989). The many advantages and disadvantages of adding plants to a constructed wetland for mine water treatment can make it difficult to decide whether plants would be beneficial for metals removal.

Alternate Electron Acceptors

There are numerous oxidation-reduction (redox) reactions in a wetland that can utilize organic carbon in quantities that may limit the availability of organic carbon for sulfate reduction. Microbially mediated oxidation-reduction reactions oxidize organic matter using various inorganic electron acceptors such as O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-} and CO_2. The electron acceptors are used in a specific order based on the potential energy release from the reaction. Reactions that have greater energy release will be used first. Each redox reaction has a specific standard free energy change, $\Delta G°$, that describes how much energy is released. The more negative $\Delta G°$, the greater the energy release. The potential energy release from the oxidation of organic matter coupled with different electron acceptors can vary significantly as shown below in Table 1 (Reddy and D'Angelo, 1994; Snoeyink and Jenkins, 1980). Redox potential (Eh) (Table 1) is widely used as an indicator of the type of redox reactions occurring in a solution. It is measured as the potential of an electrode, usually in volts or millivolts. Eh is a function of $\Delta G°$ in which highly positive Eh values correspond to highly negative $\Delta G°$ values.

Aerobic decomposition using O_2 will proceed first because the reaction has the most negative $\Delta G°$ and therefore produces the highest energy release. Once O_2 is depleted, the microbial population will begin to shift to facultative and anaerobic organisms such as nitrate and manganese reducers (Odom and Singleton, 1993). The lower the energy release, the more slowly decomposition will occur (Reddy and D'Angelo, 1994). Sulfate reduction is not a high energy releasing reaction; all electron

Table 1. Selected types of microbial oxidation-reduction reactions in wetlands.

Process	Electron Acceptor (EA)	End Products	Moles of e^-/mole of EA	$\Delta G°$ (kJ/mole of electron)	Redox Potential - Eh (mV)
Aerobic respiration	O_2	H_2O, CO_2	4	-125.1	200 to 800
Nitrate reduction	NO_3^-	N_2, CO_2	5	-118.8	200 to 700
Manganese reduction	MnO_2	Mn^{2+}, CO_2	2	-94.5	200 to 500
Iron reduction	$Fe(OH)_3$	Fe^{2+}, CO_2	1	-24.3	0 to 300
Sulfate reduction	SO_4^{2-}	H_2S, CO_2	8	-25.4	-200 to -50
Methanogenesis	CO_2	CH_4, CO_2	8	-23.2	-200 to –100

acceptors with higher energy release will be used before sulfate. Diffusion of oxygen from the wetland surface and from plant roots, the presence of other electron acceptors as well as methanogenesis may deplete the amount of organic matter available for sulfate reduction. Burgoon *et al.* (1995) found that oxidation of organic matter by aerobic processes and methogenesis can be significant. They found that electron acceptors in the influent to a wetland (i.e. manganese, iron and sulfate) oxidized less than 2% of the organic matter. The remaining 98% was oxidized by electron acceptors found or produced in the wetland (i.e. oxygen and CO_2 addition and consumption by aerobes and methanogens).

Sulfate Reduction vs. Methanogenesis

Although the theoretical parameter, ΔG°, suggests that sulfate reduction is a slightly more favorable reaction than methanogenesis, SRBs and methane-producing bacteria (MPB) compete for simple organic carbon. The outcome of the competition is crucial for systems designed to remove metals using sulfate reduction. If MPBs outcompete SRBs, little sulfate reduction will occur and few metals will be removed from the influent waters. The outcome of the competition can be unpredictable and is dependent on many parameters. These include the carbon source available, the ratio of COD:S, pH, sulfide concentration and solids retention time (SRT) (Omil *et al.*, 1998). COD, chemical oxygen demand, is a measurement of the organic matter available for redox reactions. Fox and Ketha (1996) determined that the competition between SRBs and MPBs depends on the type of organic matter available. SRBs competed better for glucose and intermediates of isopropyl acetate (IPA). MPBs competed better for a fermentative pharmaceutical wastewater up to a concentration of 1:9 (wastewater:water) when the MPB population rapidly decreased from inhibition and SRBs became dominant. Research suggests that the COD:S ratio is very important in determining competition outcome. Generally, as the COD:S ratio decreases, the percentage of COD used for sulfate reduction increases (Fox and Ketha, 1996; Omil *et al*, 1998; Mizuno *et al.*, 1994; Vroblesky *et al.*, 1996) . Vroblesky *et al.* (1996) and Mizuno *et al.* (1994) found that at a COD:S ratio of 1.5, SRBs are dominant and at a COD:S ratio greater than 6.0, MPBs are dominant. Small concentrations of sulfide become toxic to MPBs before SRBs, therefore, when sulfide is present in quantities toxic to MPBs, SRBs will dominate (Capone *et al.*, 1983; Fox and Ketha, 1996; Mizuno *et al.*, 1998). As pH increases, SRBs outcompete MPBs. In a favorable environment for SRBs, as SRT decreases, the time for SRBs to outcompete MPBs decreases (Omil *et al.*, 1998).

Organic Matter Dynamics and Supplementation

Decomposition of organic matter can determine the rates of sulfate reduction in wetlands (Reddy and D'Angelo, 1994). The accumulation of organic matter in wetlands designed for sulfate reduction can be due, in part, to the decomposition of dead plant matter, which accumulates on the wetland surface and within the substrate. During decomposition, the plant matter is broken down into more simple, soluble forms by a number of processes, including leaching, fragmentation and consumption by fungi, bacteria and soil fauna. Fermentative bacteria produce extracelluar enzymes, which further decompose the plant matter to simple organic molecules which are readily used by aerobic and anaerobic microorganisms (Feng and Hsieh, 1998).

Rates of organic matter decomposition are dependent on the chemical makeup of the organic substrate, electron acceptor availability, temperature, pH, Eh and nutrient availability. Westrich and Berner (1988) suggested that the chemical makeup of the organic substrate is the main factor controlling the rate of decomposition. Plant matter is composed mainly of cellulose and lignin, two complex organic molecules. The cellulose fraction degrades more readily, therefore, substrate cellulose content decreases more rapidly during decomposition. The higher the cellulose fraction of the plant material, the faster decomposition will occur. Plant decomposition is also affected by the availability and type of electron acceptors. Greater concentrations of electron acceptors that produce lower energy-releasing oxidation-reduction reactions will decrease the Eh and, in turn, eventually decrease the rate of microbial activity responsible for decomposition (Table 1). A decrease in temperature or nutrient availability will also inhibit microbial activity and subsequently, decrease decomposition rates (Reddy and Patrick, 1975).

Commonly, the rate-limiting step for sulfate reduction is the rate at which fermentative bacteria decompose complex organic molecules to simple organic molecules (Westrich and Berner, 1988). Therefore, if organic matter from plants is the only carbon source, the chemical makeup of complex organics undergoing degradation is the main factor controlling sulfate reduction (Feng and Hsieh, 1998; Westrich and Berner, 1988).

In a metal treatment wetland, the organic matter supplied from decomposing plant material may not be sufficient to meet the carbon demand necessary for sufficient sulfate reduction to precipitate the metals of concern (Taritus and Unz, 1994). Adding a readily decomposable substrate (i.e. peat, mushroom compost, horse manure) to a constructed wetland will contribute to the organic carbon pool, increasing sulfate reduction rates. Taritus and Unz (1994) used decomposition kinetics to model the removal of mine water pollutants in constructed wetlands. Model simulations indicated that wetlands constructed with an organic substrate are initially capable of removing far greater amounts of metals than wetlands built with less biodegradable substrates (i.e. gravel). However, after three to five years of operation this difference becomes negligible. When the easily degradable fractions of the organic matter became exhausted, sulfate reduction became limited by the availability of simple organic substrate. They suggested that without regular carbon input, periodic additions of decomposable organic matter would be required for long-term metals removal using constructed wetlands. Several researchers have suggested that a regular input of soluble organic carbon with influent wastewater can significantly stimulate sulfate reduction and increase the longevity of a constructed wetland designed for metals removal (Christensen *et al.*, 1996; Dvorak *et al.*, 1991; McIntire *et al.*, 1990; Stark *et al.*, 1991; Stark *et al.*, 1995a; Stark *et al.*, 1995b). Stark *et al.* (1995a) recommend that soluble carbon supplementation should be seriously considered when designing a wetland for metals removal. Soluble forms of dairy whey and sodium lactate were both shown to stimulate sulfate reduction and subsequently remove metals when added to a wastewater influent.

Temperature and Seasonal Effects

Generally, metals removal efficiency decreases during the winter months of wetland operation. It is well known that, other factors being equal, biological processes, such as fermentative breakdown of organic matter and sulfate reduction, may slow down or stop as temperatures decrease. As a consequence, metals removal may be limited by the slowing of microbial activity. An experimental wetland in Fayette County, Pennsylvania demonstrated one to two orders of magnitude difference in sulfate reduction rates from summer to winter (McIntire and Edenborn, 1991). Wieder (1993) found that sulfate reduction occurred at substrate temperatures as low as 4°C, albeit at relatively slow rates. Henrot and Wieder (1990) demonstrated that iron removal efficiency in peat increased linearly with temperature over the 4 to 25°C range.

Plant processes that vary with temperature may also affect metals removal efficiency. Callaway and King (1996) demonstrated that oxygen leakage from plant roots of *Typha Latifolia* is greater in the winter than in the summer. This increased oxygen will promote aerobic processes, precipitating metals as oxides instead of sulfides. Plants may also supply more soluble organic carbon to the wetland during certain seasons of the year. Kadlec and Knight (1996) suggested that the organic carbon contribution from plants was significantly higher in the winter and spring compared to the summer and fall. Increased plant organic carbon in the spring and winter may increase microbial activity and subsequently increase metals removal.

In winter, wetlands may freeze if cold temperatures precede snow accumulation. If snow accumulates before cold temperatures, an ice layer will not tend to form because of the insulating properties of snow (Kadlec and Knight, 1996). Delivery systems, inlet and outlet structures are also subject to freezing. Wildeman *et al.* (1993) give wetland design guidelines for successful winter operation.

Hydraulic Problems

Rapid, catastrophic hydraulic failures have occurred in wetland systems due to clogging of pore spaces. Hydraulic conductivity, K, decreases over time due to substrate compaction and organic matter and precipitate accumulation (Drury and Mainhausen, 2000), resulting in short-circuiting, lowered retention times and subsequent decreases in metals removal (Hiel and Kerins, 1998). Experimental wetlands in Butte, Montana had a 90% decrease in hydraulic conductivity over a 2.5 year period (Drury and Mainhausen, 2000). Tanner and Sukias (1995) found that in four gravel-bed constructed wetlands, organic matter accumulation resulted in an average effective porosity reduction of 50% in the first two years of operation. Commonly used organic substrates have low hydraulic conductivities. For example, the K value for an experimental gravel wetland in Butte, Montana was 600 ft/d while the K value for a wetland with 50% compost was 12 ft/d (Drury and Mainhausen, 2000). The low hydraulic conductivity of organic substrates, intended to enhance treatment, may instead cause serious hydraulic problems. Wildeman *et al.* (1993) suggest that the permeability of a substrate is a critical design variable for successful operation. Higher hydraulic conductivity substrates such as gravel would be preferable to organic substrates because they are less susceptible to clogging. If lower hydraulic conductivity substrates must be used, Burgoon *et al.* (1995) recommend that periodic drain cycles may flush accumulated solids and extend the life of a wetland. Although this may decrease clogging problems, the drain/fill cycle can reoxidize sulfide and should be used as a last resort for anaerobic constructed wetlands for metals removal.

Accumulation of precipitates can be a major source of clogging problems. Fe oxides tend to form readily at the influent end of a wetland, lowering the hydraulic conductivity (McIntire and Edenborn, 1990; Sanders *et al.*, 1998; Wieder *et al.*, 1990; Wildeman *et al.*, 1993). Metal sulfides tend to cause much less clogging than metal oxide precipitates (Wildeman *et al.*, 1993). Sanders *et al.* (1998) suggested that to prevent clogging of wetlands by iron oxides, aerobic pretreatment of Fe might be necessary followed by a settling pond for precipitation of iron oxides. From the settling pond, AMD may flow to a subsurface anaerobic wetland for removal of other trace metals.

Wetland Design for Metals Removal

When constructed wetlands were first implemented for metals removal, they were designed to precipitate metals as oxyhydroxides. Shallow ponds were designed to aerobically precipitate Fe and Mn as oxyhydroxides. Problems with decreasing pH were solved by adding a base such as lime, limestone, sodium hydroxide or sodium carbonate. Although very effective at removing these and other metals, the labor and chemical expenses are high (Henrot and Wieder, 1990; Wieder, 1993). During the late 1980's, anaerobic wetlands gained recognition as an effective way to remove metals at significantly less cost. Anaerobic wetlands not only remove metals, but they also raise the pH (McIntire *et al.*, 1990). Design of anaerobic wetlands minimizes metal oxide precipitation, which is generally restricted to the upper sediments where oxygen can diffuse (Henrot and Wieder, 1990). Metal oxide precipitation may also occur in the plant rhizosphere where oxygen may diffuse from plant roots (Mitsch and Wise, 1998). In the presence of reducing conditions, metal oxides generally dissociate and can significantly increase soluble Fe and Mn in the effluent of anaerobic wetlands if Fe and Mn oxides are present in the influent (Taritus and Unz, 1995).

Today, most wetlands for metals removal are designed to promote sulfate reduction. Anaerobic wetlands can successfully remove metals to levels that meet water quality standards, and contrary to aerobic processes, anaerobic sulfate reduction generates alkalinity and can raise the pH of wetland effluent (Eger, 1992; Machemer, 1992; McIntire *et al.*, 1990; Webb *et al.*, 1998).

To promote anaerobic conditions, many wetland designs have incorporated some form of organic rich substrate such as peat, mushroom/municipal compost, horse manure or wood shavings. The organic substrate (1) rapidly depletes oxygen creating a reduced environment favorable for sulfate reduction and (2) provides the electron donor for SRB activity. It also may contain $CaCO_3$ (limestone) which can add to the neutralization potential of a wetland (Dvorak *et al.*, 1991; McIntire *et al.*, 1990). Additional alkalinity can be achieved by including crushed limestone along with an organic substrate. Alkalinity generation and pH increase in a wetland also depend on the influent AMD pH, alkalinity, flow rate and residence time (Wieder, 1993). An experimental anaerobic wetland studied by McIntire and Edenborn (1990) increased pH from 2.9 to 6.5 and alkalinity from 0 to 1077 ppm. Increases in pH, in turn, increased metal retention. Dunabin *et* al. (1988) found that metal retention in an anaerobic wetland increased from 49% to 99% when pH increased from 4.3 to 7.5.

Although organic substrates and limestone can add alkalinity, many researchers have determined that their neutralization capabilities become exhausted very quickly. McIntire *et al.* (1990) found that the neutralization potential of mushroom compost in their wetland was exhausted within three months. They suggested that sulfate reduction processes would be a more long-term solution for alkalinity generation than limestone and other alkalinity producing substrates. Wildeman *et al.* (1993) suggested that during wetland system start-up, organic substrate could add alkalinity in sufficient quantities until SRBs have a chance to colonize and start adding alkalinity themselves. Hedin *et al.*

(1994) suggested using an anoxic limestone drain (ALD) to raise the pH before entering an anaerobic wetland since SRBs tend to perform optimally at near neutral pH.

Problems can arise from attempting to raise the pH using sulfate reduction. In order for alkalinity to be generated, sulfate reduction must be in excess of metal precipitation. Metal precipitation produces 2 moles of H^+ counteracting the 2 moles of HCO_3^- produced from sulfate reduction (Dvorak *et al.*, 1991; Eger, 1992; Singh, 1992). Unfortunately, excess sulfate reduction produces excess sulfide that is not precipitated as a metal sulfide. The excess may accumulate to concentrations toxic to fish, macrophytes and bacteria. Free sulfide can also produce an undesirable rotten egg odor (Singh, 1992). To counteract sulfide toxicity, Singh (1992) suggests producing metal hydroxides instead of metal sulfides by raising the pH using lime.

There are advantages and disadvantages to designing wetlands for either metal hydroxide or metal sulfide precipitation. Metal sulfide precipitation seems to be gaining the most interest, but decisions as to the best removal process for design should be determined on a case-by-case basis.

Even though the understanding of mechanisms within wetlands for metals removal has improved significantly in recent years, performance continues to be inconsistent and, as a consequence, researchers have not come to any firm agreement on sizing criteria for a successful wetland, even at the same pH (Stark *et al.*, 1991). For this reason, sizing coefficients (which take the form of metal loading factors) should be used with caution. Physical, chemical and biological factors all play a role in the success of a wetland for metals removal. There are many recommendations in the literature to optimize metals removal in constructed wetlands but much more research must be conducted before design criteria can be used with confidence.

CHAPTER 3

MATERIALS AND METHODS

Experimental Overview

Two wetland experiments were conducted simultaneously in a single greenhouse unit of the Plant Growth Center at Montana State University in Bozeman, MT. Both experiments were conducted using a simulated mine-impacted water modeled after runoff/groundwater discharged to the Metro Storm Drain (MSD) in Butte, Montana. Contaminants in the MSD most likely originate from infiltration through areas of mine waste into the local alluvial aquifer. Typical of acid mine drainage, the water has high concentrations of metals and sulfate but atypically has a pH that is almost neutral (~ 6.6). The experiments were conducted to determine the effects of plant type, temperature and carbon concentration on sulfate reduction for the removal of zinc.

The first experiment consisted of sixteen microcosm subsurface constructed wetlands (columns) operated in batch-load mode. Eight of the columns were planted with *Scirpus acutus* (hardstem bulrush), four with *Typha latifolia* (broadleaf cattail) and four were left as unplanted controls. A series of eight 20-day incubations was conducted during a one year period. The second experiment consisted of eight horizontal continuous-flow constructed wetlands operated with a hydraulic residence time of five days. Three cells were planted with *Scirpus acutus,* three with *Typha latifolia* and two were left as unplanted controls. This experiment was conducted to compare continuous-flow and batch-load operation to determine the effect of hydraulic flow regime on various wastewater parameters.

Various wastewater parameters were analyzed in both systems to characterize each system's removal efficiency for sulfate and zinc. The greenhouse temperature was set at 24°C for the three summer months (June, July and August), 14°C for the three fall months (September, October and November) and three spring months (March, April and May) and 4°C for the three winter months (December, January and February). The batch-load study was initiated in December 1999 and concluded in January 2001. The continuous-flow study was initiated in June 2000 and concluded in January 2001. Natural variation in light and seasonal differences in temperatures were sufficient to produce typical patterns of plant dormancy in the winter and regrowth in the spring.

Batch-Load System

Column Design and Construction

Thirty-two constructed wetland microcosms "columns" were built in April 1997 (Allen 1999); sixteen of which were used in the current experiment. The columns, 60 cm tall, were constructed from 20 cm polyvinyl chloride (PVC) pipe capped on the bottom and filled to 50 cm with 3-13 mm diameter washed pea gravel. Porosity was 0.27 with a resulting pore volume of 4.3 L, and did not differ significantly among replicates (Allen, 1999). Three solution sampling ports (3-mm inner diameter vinyl tubing) and three access tubes (11-mm inner diameter PVC pipe) for platinum redox electrodes were installed vertically in the center of each column at three depths: 5, 15 and 30 cm. A water delivery system was used to replace evaporative losses and continuously maintain water levels just below the gravel surface through the addition of tap water to the bottom of the columns (Figure 1).

The columns had been used in a previous study looking at removal of organic carbon from a secondary wastewater source. The study was conducted for three years prior to the initiation of the current study. For details about plant collection and establishment within the columns, see Allen (1999).

Wastewater Application, Sampling, and Analysis Procedures

Two synthetic wastewater feeds, based on water from the MSD in Butte, Montana, were created by adding constituents to dechlorinated tap water and differed only in organic carbon concentration (Table 2). Organic carbon was supplied as sucrose. Zinc, supplied as $ZnSO_4 \circ 7H_2O$, was the only divalent metal introduced as representative of this class of contaminants. Remaining sulfate was supplied as Na_2SO_4. Ammonium chloride and potassium phosphate supplied appropriate nutrients for microbial and plant growth. The low-carbon wastewater was administered to four of the eight bulrush columns and all of the cattail and control columns. The high-carbon wastewater was administered to the remaining four bulrush columns to evaluate effects of carbon concentration.