Sulfate reduction and carbon oxidation in model constructed wetlands for metal remediation
by Jason Richard Sturm

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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All cells showed decreasing DOC concentrations along the flow path. The control cells and one bulrush cell exhibited increasing DOC concentrations with depth, while the other cells had decreasing DOC concentrations with depth. Sulfate concentrations in the controls and bulrush decreased along the flow path, but increased along the flow path of the cattails. Average first-order DOC degradation constants for all cells ranged from 0.0033 hr^-1 to 0.0100 hr^-1. Average first-order SO42- degradation constants for all cells ranged from -0.0067 to 0.0142.

Wastewater using initial concentrations of 100 mg/L C and 200 mg/L SO42-became carbon limited. Wastewater with initial concentrations of 200 mg/L for both C and SO42- became sulfate limited, with severe limitation experienced below 0.1 mmol SO42-. Sulfate reduction contributed to less than 20% of the carbon oxidation within the cells. Over 95% of the influent zinc was sequestered.

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APPROVAL

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Jason Richard Sturm

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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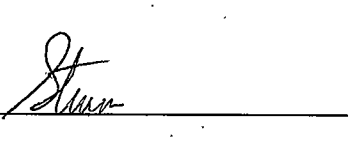
Signature 

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ABSTRACT

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In 1996, six model horizontal subsurface-flow constructed wetlands were constructed and housed in an environmentally-controlled greenhouse. Two were planted with cattail, two with bulrush, and two were left unplanted as treatment controls. From February to June 1998, a synthetic wastewater containing 200 mg/L SO_4^{2-} and 200 mg/L dissolved organic carbon (DOC) was applied to the system. A second wastewater with half the carbon load was run from June through September 1998. Samples were collected from the influent, effluent, and from ports placed within each wetland cell. Samples were analyzed for sulfate, DOC, and zinc concentrations. Change in sulfate and carbon concentration data along the flow path were fitted to a first-order model. Changes in the sulfate to DOC ratio were analyzed as a tool to gauge the impact of alternative electron acceptors. Zinc was tested to determine the effectiveness of the system to remove and retain a divalent metal.

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INTRODUCTION

The use of constructed wetlands is a relatively new tool for the remediation of heavy metal laden water. Although increasingly used as a low cost remedy for many kinds of metal laden waters, constructed wetlands are most commonly used in the treatment of acid mine drainage (AMD). AMD is the result of the oxidation of pyrite upon exposure to oxygen and water and can have disastrous impacts on natural waters. Constructed wetlands have shown an ability to increase pH and remove hazardous heavy metals from AMD, however methods for optimization of the chemical and microbial processes involved in heavy metal removal by constructed wetlands are not yet well understood. One process that appears to be important to heavy metal removal in constructed wetlands is microbially mediated sulfate reduction. Constructed wetlands are capable of providing an environment conducive to high levels of sulfate reduction. Sulfate reduction produces hydrogen sulfide gas that readily couples with many heavy metals to form highly insoluble metal precipitates, simultaneously raising the pH in the process. These precipitates remain in the wetland, effectively removing the metals from the aqueous environment.

Goals and Objectives

Initially, the goal of this research was to look at the effectiveness of the dissimilatory metal-reducing bacteria *Shewanella alga* BrY to reduce, precipitate, and remove metal contaminants from simulated, industrial waste streams within a constructed, vegetated sub-surface bed wetland system. Beginning in April 1997, and

continuing through September 1997, four attempts were made to induce the colonization of three model constructed wetland cells by *Shewanella alga* BrY. Each attempt failed to produce a sustainable population in the wetland cells. A typical graph showing the decreasing populations of the *Shewanella* with time is shown in the appendix. Since further work in this experiment was dependent on the *Shewanella alga* colonization, a change in research focus was required.

The new research objective was designed to contribute to the understanding of sulfate reduction as a means of removing heavy metals from wastewater in a constructed wetland. Six bench-scale constructed wetland cells containing cattail, bulrush, or no plants were supplied with a synthetic zinc-laden wastewater. Samples were taken to measure sulfate, carbon, and zinc concentrations, and the data were analyzed to determine the effect of carbon loading, plants, and other electron acceptor utilization on sulfate reduction and its accompanying zinc precipitation.

Using two wastewater feeds of differing carbon loads, changes in sulfate and carbon concentrations were analyzed with respect to depth and longitudinal distance within the cells. Longitudinally, carbon oxidation and sulfate reduction were independently fitted to a first-order model. The effect of carbon loading, plants, and depth on the reduction rates of both constituents was studied. Alternative electron acceptor utilization was investigated by comparing depth-related changes in the sulfate/DOC ratio along the flow path. The effectiveness of sulfate reduction on heavy metal removal and retention was discerned from measured zinc concentrations.

BACKGROUND

Interest in using constructed wetlands as a wastewater treatment system has continually increased over the past several decades. Most of the focus has been on the usage of wetlands, and more specifically constructed wetlands (CW), for organic matter and nutrient removal. Around the early 1980s, interest was piqued in the possibility of CW being a viable treatment option for acid mine drainage (AMD) (Kittle *et al.*, 1995). Since then, CW have been tested and sometimes used successfully in the remediation of water containing many kinds of metals. Metals of concern include the heavy metals Al, Cd, Cu, Fe, Mn, Ni, Pb, and Zn, which can be toxic to humans or aquatic biota. From AMD to industrial discharge to even municipal wastes, CW have increasingly been used to facilitate removal of these metals.

The more conventional method of water treatment for metals removal includes an active, chemical treatment, such as liming (Webb *et al.*, 1997). By raising the pH of the water, the metals precipitate out as oxides or hydroxides (Stark *et al.*, 1995). However, chemical treatment can be expensive and require constant upkeep. The use of constructed wetlands is an alternative that can provide effective, passive treatment at much less cost. CW can also have the ancillary benefit of providing wildlife habitat or recreational areas (Makos and Hrncir, 1995).

A number of chemical, biological and physical processes occur in a wetland system. Mechanisms occurring in a wetland that aid in the removal of metals are precipitation, adsorption, and plant uptake (Crites *et al.*, 1997; Wildeman *et al.*, 1993; Reed *et al.*, 1995). Each will be examined more closely below.

Plant Uptake and Other Plant Effects

As an integral part of the mosaic that makes up a wetland system, aquatic plants affect the chemical and biological processes of the wetland in many ways. Common emergent plants used in constructed wetlands include cattails (*Typha sp.*), bulrush (*Scirpis sp.*), sedge (*Carex sp.*), and rush (*Juncus sp.*). Although many of the details of their role in treatment are not fully understood, it is clear that plants do impact the biological, chemical, and physical processes of a wetland. Direct plant uptake is a mechanism of metals removal that is probably minor in comparison to precipitation and adsorption, although early studies presumed plant uptake was an important removal process (Wildeman *et al.*, 1993). More recent studies, such as that done by Mitsch and Wise (1998), have found plant uptake to account for less than 5% of total metals retention.

Uptake of heavy metals can have deleterious effects on plants. Elevated concentrations of metals, especially Al, can be toxic to plants and have been found to weaken or kill off aquatic macrophytes (Wieder *et al.*, 1990; Schmidt, 1997; Taylor and Crowder, 1983). Most of the metals taken up by plants are stored in the roots (Taylor and Crowder, 1983; Kadlec and Knight, 1996). This can be significant, especially during senescence, because most roots remain alive while the leaves and stems die. With the eventual death and decomposition of the plant tissues, the metals can be returned to the aqueous solution (WPCF, 1990). For the above reasons, plant uptake and retention is not an effective removal process in the long term, or with waste streams with high metals loading.

Vegetation does affect removal in more indirect ways. Aquatic plants have the ability to transport oxygen to the roots from the stems and leaves (Reddy *et al.*, 1989). Oxygen transport seems to be a way to counteract the negative effects of an anoxic environment associated with living in a flooded environment (Moorhead and Reddy, 1988; Sand-Jensen *et al.*, 1981). Any excess oxygen not needed by the roots then may be transferred into the near-root aqueous solution. This released oxygen creates aerobic microenvironments (Reed and Crites, 1984; Sand-Jensen *et al.*, 1981), but does not appear to affect oxygen concentration of the overall solution, because microbial populations and chemical processes quickly consume any excess oxygen (Steinberg and Coonrod, 1994).

Evidence of oxygen transfer is given by the observation that the roots of wetland plants can retain metals through the formation of an absorptive plaque. Under near-neutral pH conditions, cattails are known to form iron hydroxide coatings (plaques) on their roots (Wieder *et al.*, 1990; Taylor *et al.*, 1984). These plaques are thought to be involved in the immobilization of Ni and Cu (Taylor and Crowder, 1983).

The most important role plants play in wetlands metal removal is as a long-term source of organic matter (OM). OM from decaying plant material serves two purposes. Firstly, the OM can adsorb metals. By adsorption onto OM, the metals are removed from the aqueous solution. This process was the design of many early constructed wetlands. Secondly, the OM is a source of organic carbon for microbial processes. This is particularly important for sulfate reducing bacteria and their ability to remove metals through sulfide production (Schmidt, 1997; Wildeman *et al.*, 1993). Mitsch and Wise

(1998) found sulfate concentrations in their vegetated CW plots to be lower than in the non-vegetated plots.

Adsorption

Adsorption is a mechanism that incorporates many elements of a wetland, and is a metal removal process that can be extremely effective (Mitsch and Wise, 1998). Organic matter, oxyhydroxides, and algae provide typical sorption sites. Many early wetlands constructed for metals removal, often using *Sphagnum* peat moss, were designed so that adsorption would be the dominant removal process (Wieder *et al.*, 1990; Vile and Wieder, 1993). Organic acids (humic and fulvic acids) from decaying organic matter are primarily responsible for the removal. Under the right conditions, the exchange of metals and acid functional groups on the humic and fulvic acids proceeds as:

$$RCOOH \leftrightarrow RCOO^- + H^+ \quad (2.1)$$

$$2\,RCOO^- + Me^{2+} \leftrightarrow Me(RCOO)_2 \quad (2.2)$$

where R is the inert portion of the organic acid RCOOH. Along with the dissociation of the carboxyl ion (COO^-) with the hydrogen ion, there is an exchange with a divalent or trivalent metal ion (Me) (Wildeman *et al.*, 1993; Kadlec and Knight, 1996).

Most heavy metals can be removed, with varying degrees of effectiveness, through adsorption. Makos and Hrncir (1995) found the most toxic form of chromium, Cr(VI) was reduced to Cr(III) and bound with the acid functional groups of humics and

fulvics. However, Wildeman *et al.* (1993) noted that organic adsorption may not be an effective removal process for Mn, Zn, and Cd. With aqueous solutions containing multiple metals, some metals are preferentially sorbed over others. When looking at Fe, Cu, Zn, and Mn from mine drainage water, Machemer and Wildeman (1992) found that initially all of the metals experienced a high level of removal. After a period of time, though, the concentrations of Zn and Mn in the effluent of their cells began to increase. After all the organic exchange sites were filled, the Cu and Fe began to replace the Zn and Mn already bound. There is a limit as to how many exchange sites are available and how much heavy metal can be adsorbed. When the sites are filled, adsorption loses its effectiveness as a metals removal mechanism; therefore, adsorption can not be relied on as a major removal process over the life of a constructed wetland (Machemer and Wildeman, 1992). Perhaps the most effective function of adsorption is to temporarily detain heavy metals thereby giving microbially mediated processes more time to operate (Wildeman *et al.*, 1993).

Adsorption can also occur with algae and oxyhydroxides. Fe(III), Al, and Mn(IV) oxyhydroxides are all capable of adsorbing metal on their surfaces. The oxyhydroxide precipitates are gelatinous and act as a weak acid which attracts hydroxide ions. The hydroxide ions make a negative surface which, in turn, attracts positive ions such as dissociated metals (Wildeman *et al.*, 1993).

Adsorption by algae is more variable. Some metals are toxic to algae, and different types of algae will react with metals in different ways (Kadlec and Knight, 1996). A study by Crist *et al.* (1990) found algae's adsorption of metals to be an ion-

exchange reaction. Two algae studied by Harris and Ramelow (1990), *Chlorella vulgaris* and *Scenedesmus quadricauda*, had similar binding efficiencies for four specific metals. They had the most affinity for Ag, followed by Cu, Cd, and then Zn. Algae also appear to aid in accelerating oxyhydroxide precipitation (Wildeman *et al.*, 1993).

All of the above processes are very much pH dependent. Removal efficiencies are usually directly related to pH. One benefit of these processes is an ability to raise pH. As metals are removed from the aqueous solution in an exchange with ions such as Ca^{2+} or Mg^{2+}, the pH is raised (Stumm and Morgan, 1996) leading to increased removal. Adsorption can act as an effective neutralization process with regards to acidity, but when the exchange sites fill, there will be no more reduction in acidity (Machemer and Wildeman, 1992; Vile and Wieder, 1993). If conditions change too much, such as lowering of pH, the metals will desorb back into solution.

Oxyhydroxide Precipitation

Oxyhydroxides not only remove metals passively through adsorption, but can also actively sequester metals by precipitation. Oxyhydroxide precipitation can be a dominant metals removal process in the aerobic portions of a CW (Webb *et al.*, 1998). The oxidation and precipitation of divalent metals typically occurs along the following reactions (Webb *et al.*, 1998; Stumm and Morgan, 1996; Wildeman *et al.*, 1993):

$$Me^{2+} + 2H_2O \rightarrow Me(OH)_2(s) + 2H^+ \quad (2.3)$$

$$Me^{2+} + H_2O \rightarrow MeO(s) + 2H^+ \quad (2.4)$$

$$4\ Me^{2+} + O_2 + 6H_2O \rightarrow 4MeOOH(s) + 8H^+ \qquad (2.5)$$

The precipitation of oxyhydroxides remove metals from solution, but at the same time produce H^+ ions. The production of H^+ ions thereby decreases the pH of the aqueous solution (Vile and Wieder, 1993; Wildeman *et al.*, 1993). With decreasing pH, the rate of oxyhydroxide precipitation decreases. Therefore, oxyhydroxide precipitation is a self-limiting process (Wildeman *et al.*, 1993). Vile and Wieder (1993) found iron oxyhydroxide deposition occurred more slowly at pH 3.5 than at pH 5.5. Significant iron oxidation occurs at pH values near 7, while manganese oxidation doesn't occur at acceptable rates for adequate removal until pH 9 (Wildeman *et al.*, 1993). Oxyhydroxides are highly soluble in acidic solutions. If changes occur in the wetland environment, metals precipitated as oxyhydroxides can be released back into the aqueous environment.

Sulfate Reduction and Sulfide Precipitation

Within the dynamic wetland system, there are many chemical and biological processes occurring. All of the previously mentioned removal mechanisms play a part in heavy metal sequestration, but for long-term removal of heavy metals by CW, the most effective process appears to be precipitation of metal sulfides. This is brought about by dissimilatory sulfate reduction coupled with the oxidation of an organic substrate occurring in an anaerobic environment. Two by-products of sulfate reduction are hydrogen sulfide and carbonate alkalinity (Schmidt, 1997). Hydrogen sulfide quickly

reacts with most dissolved divalent metals forming metal-sulfide precipitates. The basic equations governing this reaction are (White and Gadd, 1996; Christensen *et al.*, 1996; Wildeman *et al.*, 1993; Webb *et al*, 1998):

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (2.6)$$

$$H_2S \rightarrow H^+ + HS^- \quad (2.7)$$

$$Me^{2+} + HS^- \rightarrow MeS(s) + H^+ \quad (2.8)$$

where CH_2O is an idealized carbon source.

Carbonate alkalinity facilitates the neutralization of solution acidity. Possibly as important as carbonate alkalinity in reducing acidity is the release of H_2S gas to the atmosphere, thereby removing H^+ ions from the aqueous solution. Although Vile and Wieder (1993), found no correlations between changes in H^+ concentration and sulfate reduction in CW cells using *Sphagnum*, straw/manure, sawdust, and mushroom compost, many studies, such as those by Schafer & Associates (1994) and White and Gadd (1996), have found sulfate reduction to significantly increase pH.

Most heavy metals will be effectively removed by sulfide precipitation. This is a great advantage over other removal processes. Sulfide precipitates typically have lower solubilities over a broad pH range than precipitates of oxyhydroxides (Machemer and Wildeman, 1992). One exception to this is manganese. Manganese is a difficult metal to remove by any process, and sulfide precipitation is no exception, as Mn-sulfides are relatively soluble.

Sulfate reduction is an oxidation-reduction (redox) reaction. Redox reactions proceed according to the most favorable electron intensity or energy yield. Thermodynamically, the electron donor (carbon source) supplies electrons to the lowest unoccupied electron level (O_2) first, followed by other levels as electrons are available. This is parallelled biologically with an ecological succession of microorganisms (Stumm and Morgan, 1996). The procession of redox reactions are shown in Table 1.

Table 1. Organic matter decomposition reactions in order of decreasing energy yield.

1. O_2 Reduction: $1/4CH_2O + 1/4O_2 \rightarrow 1/4CO_2 + 1/4H_2O$
2. Denitrification: $1/4CH_2O + 1/5NO_3^- + 1/5H^+ \rightarrow 1/4CO_2 + 1/2H_2O + 1/10N_2$
3. Mn Reduction: $1/4CH_2O + H^+ + 1/2MnO_2 \rightarrow 1/4CO_2 + 1/2Mn^{2+} + 1/8H_2O$
4. Fermentation: $3/4CH_2O + 1/4H_2O \rightarrow 1/4CO_2 + 1/2CH_3OH$
5. Fe Reduction: $1/4CH_2O + FeOOH + 2H^+ \rightarrow Fe^{2+} + 1/4CO_2 + 7/4H_2O$
6. SO_4^{2-} Reduction: $1/4CH_2O + 1/8SO_4^{2-} + 1/8H^+ \rightarrow 1/8HS^- + 1/4CO_2 + 1/4H_2O$
7. Methanogenesis: $1/4CH_2O \rightarrow 1/8CO_2 + 1/8CH_4$

In a plug-flow wetland, the above reactions will occur along the flow path based on the rate organic carbon is utilized. Dissolved oxygen will be the first terminal electron acceptor utilized. Aerobic respiring and faculatively aerobic, fermenting organisms will be dominant. As oxygen is depleted, the microbial population will begin to shift to obligately anaerobic organisms (Odom and Singleton, 1993). The faster the rate of consumption, the smaller each redox zone will be. Sulfate reduction is not a high energy-

yielding reaction. Only after the other higher energy electron acceptors have been consumed will sulfate reduction begin.

Most strains of sulfate reducing bacteria (SRB) utilize only simple organic substrates, and do not actually oxidize them completely to CO_2 (Feng and Hsieh, 1998). SRB utilize low molecular-weight organics, and do not degrade compounds such as proteins, lipids, or starches. They rely on other heterotrophic bacteria to provide the proper fermentation and degradation products (Feng and Hsieh, 1998; Odom and Singleton, 1993). SRBs are a dynamic group of organisms, though. Some will utilize organic acids and alcohols through anaerobic respiration coupled with sulfate to produce acetate, carbon dioxide, and sulfide, while other strains will oxidize acetate to carbon dioxide. Several strains of SRBs are even able to grow fermentatively in the absence of sulfate (Odom and Singleton, 1993).

A number of factors can affect the rate at which sulfate reduction occurs. These include pH, temperature and redox potential. Although SRB activity is greatest at moderate temperatures and near neutral pH, activity occurs at temperatures from 5° C to 50° C and at pH 3.0 to 9.5 (Gyure *et al.*, 1990; Wildeman *et al.*, 1993). Once the redox potential is low enough to facilitate sulfate reduction, the most limiting conditions for SRB activity appear to be the availability of sulfate and carbon. Feng and Hsieh (1998) found SRBs to be active with pore water sulfate concentrations as low as 5 mg/L, but sulfate levels below 30 mg/L seem to affect reduction rates.

In a study using column reactors and very high concentrations of organic carbon and sulfate, > 1200 mg/L and > 3900 mg/L, respectively, Lyew and Sheppard (1999)

measured sulfate reduction rates of between 0.83 mg/L/hr and 2.92 mg/L/hr. Hines *et al.* (1999) found sulfate reduction rates up to 14 mg/L/hr near the surface of marsh sediments. Below 5 cm from the surface, rates were around 2 mg/L/hr. This decrease in rate seems to be due to decreasing concentrations of available carbon with depth. The majority of sulfate reduction occurring during the growing season may be due to organic carbon supplied from the roots of marsh plants (Hines *et al.*, 1999).

Wetlands are a multifaceted system with great potential as a means of removing heavy metals from the aqueous environment. There are many studies looking at the overall effect a wetland has on water quality, but with the possibility of so many processes occurring simultaneously, it is often difficult to quantify what is actually happening within a wetland. With increased knowledge as to their inner-workings, constructed wetlands can be better designed to accentuate the significant chemical and biological processes to achieve the desired effect.

METHODS AND MATERIALS

Design of Wetland Cells

Six polypropylene boxes were constructed to serve as model plug-flow constructed wetlands. The wetland cells (boxes) were constructed of ⅝" (16-mm) opaque polypropylene plastic. Each cell was 91 centimeters long, 62 centimeters wide, and 53 centimeters deep. Attached to the inside front of each cell was a set of racks. The racks held an inlet manifold that could be set at three depths. Each manifold consisted of a piece of one-inch (25-mm) polyvinylchloride (PVC) pipe capped at each end. A half-inch (13-mm) nipple, from which Masterflex size 14 Norprene® tubing was attached, was screwed into each manifold for inflow. Outflow from the manifold was facilitated by a 3-mm slit cut along the length opposite the nipple. Within each cell, 12 centimeters from the front and another 10 cm from the rear, was a baffle to enhance plug flow through the middle of the system. The baffles consisted of a sheet of polypropylene plastic with 24, ¼" (6-mm) holes drilled into it. They also created open water sections at the front (forebay) and rear (afterbay) of the cell to act as complete-stir tank reactors (CSTR).

The middle section of each cell was filled to a depth of 45 centimeters with washed ⅜" to ¾" (10-mm to 19-mm) gravel. The porosity was 0.40. In the middle of the gravel section was a 20-cm diameter, removable basket constructed of ¼" (6-mm) diamond-cut geotechnical screening. The removable basket allowed for internal inspection of the cell with minimal disturbance. Also within the gravel were two rows of

four sampling ports dividing the cells in thirds across the length and width. Each port consisted of a rod of polypropylene plastic with three lengths of ⅛" (3-mm) tubing attached. The tubing reached depths of 8, 23, and 38 centimeters below the gravel surface. At the bottom of the tail open water section was an outlet manifold. It was designed similarly to the inlet manifold, except instead of a nipple, there was a piece of one-inch (15-mm) PVC leading out to an 8" (20-cm) diameter PVC outflow box. Water left the cell through 25-mm plastic tubing maintained at a height of 44.5 centimeters to keep the phreatic water level just below the gravel. Each cell resided on a wooden pallet to facilitate moving if necessary. A schematic of components is shown in Figure 1.

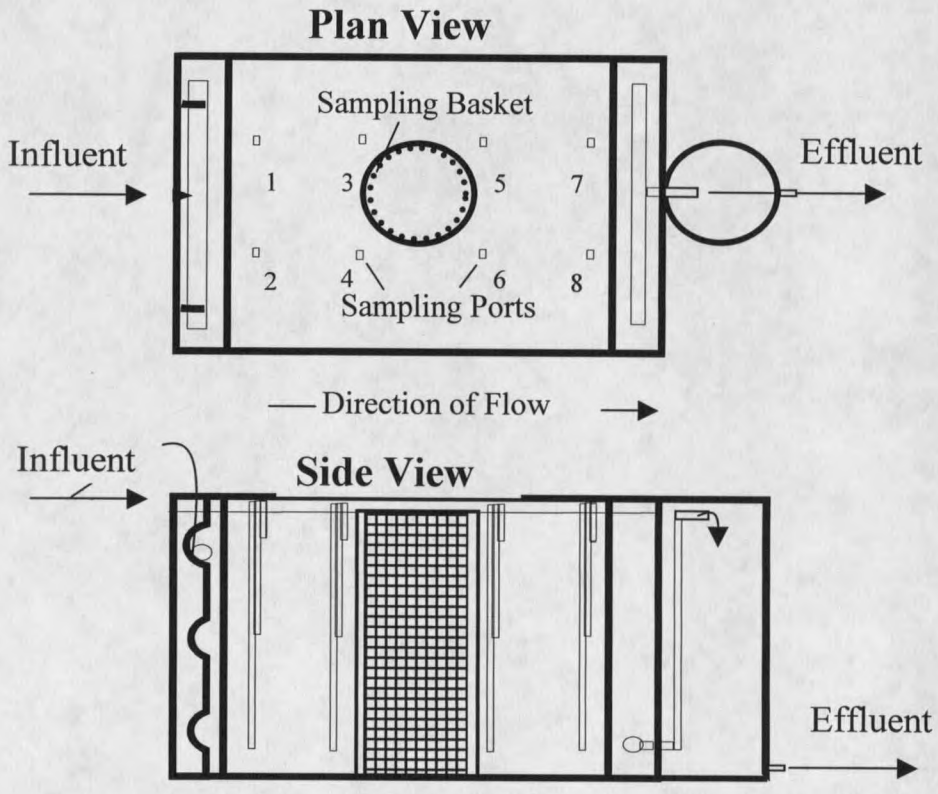

Figure 1. Schematic of constructed wetland cell. (Not to scale)

Experimental Design

Three treatment types were examined. Two cells were planted with cattails (*Typha sp.*), two with bulrush (*Scirpus sp.*), and two were left as unplanted controls. All plants were gathered from a natural wetland near Three Forks, Montana in the fall of 1996. They were transported and planted as root plugs. The plants were then given time to grow and mature as Peter's 50 PPM N 20-10-20 GP (The Scott's Company) fertilizer solution was fed into the cells.

The cells were housed in a greenhouse in the Plant Growth Center at Montana State University. The greenhouse was maintained at a temperature of 70° F (21° C) during the day and 60° (16° C) during the night. From October, 1997 through March, 1998, four hours of artificial light was used to maintain a growing season environment within the greenhouse. Thereafter, only natural light was used to facilitate growth.

Two synthetic wastewater feeds were applied during the course of the experiment. Both feeds were loosely based on water from the Metro Storm Drainage (MSD) in Butte, Montana. The first feed ran from February 9, 1998 to June 5, 1998, while the second feed followed the first and ran from June 5, 1998 to September 24, 1998. Both feeds contained the same constituents with only the carbon concentration changing. The two wastewater feeds are described in Table 2 and Table 3.

Table 2. Wastewater chemicals.

Chemical	Formula
Sucrose	$C_{12}H_{22}O_{11}$
Sodium Sulfate	Na_2SO_4
Zinc Sulfate	$ZnSO_4 \cdot 7H_2O$
Potassium Phosphate	K_2HPO_4
Sodium Nitrate	$NaNO_3$

Table 3. Concentrations of significant wastewater constituents.

Constituent	Feed 1 (mg/l)	Feed 2 (mg/l)
SO_4^{2-}	200	200
C	200	100
Zn^{2+}	24	24
NO_3^-	20	20
P	1:10 (P:N)molar ratio	1:10 (P:N)molar ratio

The wastewater was held in a 230 gallon, polypropylene tank. The tank was connected to a distribution manifold by a length of hose. The wastewater was then supplied to the cells by a Masterflex L/S 6-600 rpm peristaltic, four-roller, cartridge pump (Cole-Parmer Insturment Co., Niles, IL) through Masterflex size 14 Norprene® tubing which ran from the distribution manifold, through the pump, and to the inlet manifold of the cells.

To achieve a desired mean hydraulic residence time of five days within the gravel section of each cell, the flow rate was set at 10 ml/min ±5%. Inflow rates were measured with a graduated cylinder over one minute intervals. Due to variability from many factors, the flow rate had to be recalibrated a minimum of four times a week.

Sampling

Samples were collected every five days for Feed 1, and every seven days for Feed 2. At each sampling date, samples were collected from the inflow line, outlet spout, and a set number of sampling ports. For Feed 1, samples were collected from all depths at ports 2, 3 and 8, positioned as shown in Figure 1, and at one depth for all other ports. This depth was rotated each sampling day. To get more data for a degradation analysis along the length of the cells, the sampling scheme for Feed 2 was altered. Every port was sampled at two depths. The specific depths were rotated on consecutive sampling days. Samples were only collected from the unplanted control cells, until the final three sampling periods of each feed. During the last three sampling times, samples were collected only from the planted cells. Table 4 lists the sampling locations from each sampling date. At the end of each wastewater run, samples were collected from both control cells, Cattail 2, and Bulrush 2 for zinc analysis.

Table 4. Sampling locations on each sampling day

Date	Cells	Longitudinal Depth (cm) All Ports	Vertical Sample Port #	Total Samples
2/24	Controls	38	2, 3, 8	28
3/1/98	Controls	23	2, 3, 8	28
3/6/98	Controls	8	2, 3, 8	28
3/11/98	Controls	38	2, 3, 8	28
3/16/98	Controls	23	2, 3, 8	28
3/21/98	Controls	8	2, 3, 8	28
3/31/98	Controls	38	2, 3, 8	28
4/5/98	Controls	23	2, 3, 8	28
4/10/98	Controls	8	2, 3, 8	28
4/15/98	Controls	38	2, 3, 8	28
4/20/98	Controls	23	2, 3, 8	28
4/30/98	Planted	8	2, 3, 8	56
5/4/98	Planted	23	2, 3, 8	56
5/11/98	Planted	38	2, 3, 8	56
6/10/98	Controls	8	2, 3, 8	28
6/18/98	Controls	23, 38	---	32
6/24/98	Controls	8, 38	---	32
7/1/98	Controls	8, 23	---	32
7/8/98	Controls	23, 38	---	32
7/15/98	Controls	8, 38	---	32
7/30/98	Controls	8, 23	---	32
8/19/98	Controls	8, 38	---	32
8/26/98	Controls	8, 23	---	32
9/2/98	Controls	23, 38	---	32
9/9/98	Planted	8, 23	---	64
9/16/98	Planted	23, 38	---	64
9/23/98	All	8, 38	---	96

Influent and effluent samples were collected by allowing the flow to drip into test tubes. Samples from the ports were collected by use of a 10cc syringe. First, 4 mL were extracted from the tube and wasted. A clean syringe was then used to remove approximately 9.5 mL of sample. All samples were then run through a sterile, Corning® 0.2-micron cellulose acetate membrane syringe filter into a clean, autoclaved, 15 mL test tube and refrigerated at 5° C until analysis. Samples used for zinc analysis were collected in the same manner as described above, but then 0.5 mL of concentrated hydrochloric acid was added to each sample.

Tracer Test

From 2/14/97 to 2/23/97, prior to the wastewater feeds, a tracer test was performed on the wetland cells to gain a better understanding of the internal hydraulics of the system. Each cell was filled with tap water. The tank was filled with a solution of 100 mg/L Br^- from KBr. The flow to the cells was set at 30 mL/min. Samples were taken from the inlet, to ensure uniform mixing in the tank, and from the outlet to gauge the response. After 70 hours, approximately one residence time within the entire cell, the bromide solution was stopped, and the tank was filled with clean water. Samples were taken according to a pre-set schedule. After the first 36 hours, in which samples were taken every 12 hours, sampling was done every 4 to 8 hours to get a good accounting of the breakthrough of bromide concentration. During the run, there were 31 sampling events. Samples were gathered and stored according to the methods previously described for the wastewater feeds.

Sample Analysis

All samples from both wastewater feeds were analyzed for sulfate (SO_4^{2-}), nitrate (NO_3^-), and dissolved organic carbon (DOC). Sulfate concentrations were determined using a Dionex ion chromatograph (IC) equipped with an anion column and suppressor. The IC was connected to a computer equipped with Dionex Peaknet® 4.3 workstation software and an autosampler. A schedule of samples and an analysis method were developed using the software. The schedule and method then controlled the autosampler and IC operations. Standard samples made from sodium sulfate (Na_2SO_4) and sodium nitrate (Na_2NO_3) were run to calibrate the IC. Samples were then run through a filter to remove solids and transferred to vials for analysis. For the bromide tracer test samples, standards were made from potassium bromide (KBr) and run in the same manner.

DOC concentrations were determined using a Dohrman DC-80 carbon analyzer. Samples were acidified with 20% phosphoric acid to a pH below 2, air sparged for six minutes to remove gaseous inorganic carbon and injected into the analyzer. A 1 mL, 10 ppm organic C standard was used to calibrate the machine. The DOC analyzer uses a one point calibration, so readings were kept near 10 for all injected samples. To achieve the target reading (mass), varying volumes of sample had to be injected. The actual DOC concentration was then calculated based on the machine reading and the volume of sample used.

After preparation, zinc samples were taken to the Department of Plant, Soil and Environmental Sciences at Montana State University in Bozeman, Montana for analysis using an inductively coupled plasma spectrometer (ICP).

Statistical Analysis

A paired t-test for means using Minitab® 12 was used to determine if side-to-side differences in concentrations within the cells were statistically significant. Depth and longitudinal differences in the data were analyzed by ANOVA and Tukey's method of pairwise comparisons for means using Minitab® 12 to determine statistical significance. All analyses were done the the 95% confidence level. A p-value less than 0.05 indicates there is a statistically significant difference in the data being tested. Linear regressions performed on longitudinal data sets were done within Excel® to obtain regression constants.

RESULTS AND DISCUSSION

Wetland Hydrodynamics

The bromide tracer test was begun on all six cells, but was finished on only four of the cells. During the run, Control 2 and Bulrush 2 experienced mechanical problems, and the test was discontinued. Because the test was run using only influent and effluent samples of the entire cell system, modeling was based on a complete-mix—plug-flow—complete-mix system described by Equation 4.1:

$$
\begin{aligned}
&C(t) = 0 && \text{for } t < \theta_G \\
&C(t) = C_o(1-e^{-(t-\theta G)(Q/Vf)})(1-e^{-(t-\theta G)(Q/Va)}) && \text{for } \theta_G \le t \le \theta_G + t_p \\
&C(t) = C(\theta_G + t_p)(e^{-(t-\theta G + tp)(Q/Vf)})(1-e^{-(t-\theta G)(Q/Va)}) && \text{for } t > \theta_G + t_p
\end{aligned}
\qquad (4.1)
$$

where t is time; θ_G is the hydraulic residence time within the plug-flow gravel section of the system; C_o is the influent concentration of the tracer; Q is the flow rate; V_f is the volume of the forebay; V_a is the volume of the afterbay; t_p is the total time of tracer input; $C(\theta_G + t_p)$ is the concentration at the time of $\theta_G + t_p$.